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EFFECT OF OXYTOCIN HORMONAL IMPRINTING AND K-203 ACETYLCHOLINESTERASE REACTIVATOR ON BIOGENIC AMINES IN THE RAT CENTRAL NERVOUS SYSTEM

Ph.D Thesis

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2. Abbreviations

A = Adrenaline

ACh = Acetylcholine

AChE = Acetylcholinesterase

AChR = Acetylcholine receptor

AChERs = Cholinesterase Enzyme Reactivators

AChEI = Acetylcholinesterase Inhibitors

AMP = Adenosine Monophosphate

CB = Cerebellum

CNS = Central Nervous System

COMT = Catechol-*O*-Methyl-Transferase

CSF = Cerebrospinal Fluid

DA = Dopamine

DOPA = Dihydroxyphenylalanine

DOPAC = 3, 4-Dihydroxyphenylacetic Acid

FC = Frontopolar Cortex

GPCRs = G-protein Coupled Receptors

HC = Hippocampus

5-HIAA = 5-Hydroxyindoleacetic Acid

HPLC = High Performance Liquid Chromatography

HT = Hypothalamus

5-HT = Serotonin or 5-hydroxytryptamine

5-HTOL = 5-Hydroxytryptophol

HVA = Homovanillic Acid

LD_{50} = Lethal Dose

LOQ = Limit Of Quantification

MAO = Monoamine Oxidase

MO = Medulla Oblongata

MPOA = Medial Preoptic Area

3-MT = 3-MethoxyTyramine

Na_2HPO_4 = Disodium Hydrogen Phosphate

Na_2EDTA = Ethylenediaminotetraacetic Acid Disodium

NA = Noradrenaline

Np1 = Neurophysin 1

Ops = Organophosphates

OXT = Oxytocin

OXT-R = Oxytocin Receptor

OSA = Octane Sulfonic Acid

2-PAM = Pralidoxime

PCA = Perchloric Acid

PVN = Paraventricular Nuclei

RP-HPLC = Reversed Phase High Performance Liquid Chromatography

SC = Spinal Cord

SD = Standard Deviation

SON = Supraoptic Nuclei

ST = Striatum

TC = Truncus Cerebri

TMB-4 = Trimedoxime

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VTA = Ventral Tegmental Area

VX = O-ethyl S- [2-(diisopropylamino) ethyl] methylphosphonothioate

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3. Introduction

3.1. Oxytocin as a hormonal imprinter

Oxytocin (OXT) is a mammalian neurohypophysial hormone, which was discovered by British pharmacologist Sir Henry Dale in 1906 (Dale 1906; Mitchell and Schmid 2001). Its name is translated directly from the Greek language, and literally means ’fast birth’ (Dale 1906). The nonapeptide oxytocin was isolated and synthesised for the first time by the US Chemist Vincent du Vigneaud (Du Vigneaud et al. 1953). It was the first ever neuropeptide to be decoded and artificially reproduced again. Already in year 1960, oxytocin was available on the pharmaceutical market. Oxytocin is produced in the paraventricular nuclei of the hypothalamus and stored in the posterior part of the neurohypophysis, but also in neurons projecting from the paraventricular nucleus and surrounding structures to extra hypothalamic brain areas (i.e., the septum, the ventral tegmental area, the hippocampus, the amygdala, the medulla oblongata and the spinal cord) (Arias 2000; Mitchell and Schmid 2001). Oxytocin once thought to be limited to female smooth muscle reproductive physiology and neurotransmitter acting on its oxytocin receptor but recent studies have begun to investigate oxytocin’s role in various behaviors, including numerous central functions such as sexual, maternal behaviour, social recognition, anxiety, memory, learning, stress and social behaviors. Most of oxytocin’s roles are due to the cooperation with biogenic amines in different brain region (Melis et al. 1986; Pedersen et al. 1994; Arletti et al. 1995; Insel et al. 1997; Melis and; Waldherr and Neumann 2007; Wsol et al. 2008; Argiolas 2011; Tekes et al. 2011).

In psychiatric patients, oxytocin is emerging as one particular neural substance that may be influenced by the altered dopamine levels subserving neuropathologic related behavioral diseases (Carter 2007; Harony and Wagner 2010). It has emerged that disturbance in peripheral and central oxytocin levels have been detected in some patients with dopamine dependent disorders (Baskerville and Douglas 2008 and 2010). Thus, oxytocin is proposed to be a key neural substance that interacts with central dopamine systems (Mackenzie 2006). In addition oxytocin has recently been implicated in mediating mesolimbic dopamine pathways during drug addiction and withdrawal (Elliott et al. 2001; Arletti et al. 1993; Johns et al. 2004 and 2010).

Central oxytocin pathways may serve as a potential therapeutic target to improve mood (Arletti et al. 1995; Sole et al. 2015).

Serotonin plays an important role in regulating various physiological functions associated with neurological or psychiatric disorders (Veenstra-Vanderweele et al. 2000). Oxytocin effect on the mood is through its association with influence on the release of serotonin (Uher and McGuffin 2008), also stimulation of the hypothalamus by serotonin has been shown to lead to release of oxytocin (Lee et al. 2003). Galfi et al. in 2005 found that in rat oxytocin secretion was influenced directly by the serotonergic system (Jorgensen et al. 2003). The assessment of central biogenic amines functioning is a critical important in a wide range of neurochemical studies. Investigation of biogenic amines roles in the mechanism of drug action, their relation with oxytocin, their relations with the developing hormone receptors (hormonal imprinting), and their possible alteration in neuropsychiatric disorders all require an accurate determination of biogenic amines. The present study is concerned with the effect of oxytocin on the biogenic amine levels of the adult rat brain.

3.2. Pharmacology of organophosphate-intoxication

Organophosphates (OPs) are widely used all over the world in agriculture (pesticides, insecticides, acaricides) and in chemical industry (softeners, additives to lubricants) (Jeyaratnam 1990). In the terrorist attack were used as an organophosphate warfare agent at Tokyo metro station (Okumura et al. 1996) and during the Iraq-Iran war (McCauley et al. 2001), therefore organophosphate poisoning is a constant danger in the agriculture, giving hundreds of thousands of fatal cases in each year.

Organophosphates are esters, amides or thiol derivatives of phosphoric, phosphonic, phosphinic acids, and phosphorothioic or phosphonothioic acid. The phosphonic acids derivatives are more toxic than the phosphinic acids. They are very lipophilic agents and acute exposure results in acute cholinergic crisis at muscarinic and nicotinic acetylcholine receptors (AChR) both in the central and the peripheral nervous systems. Organophosphates cause irreversible inhibition of cholinesterases *via* a covalent reaction with the serine in the active center of the enzyme (Bajgar 2004; Bajgar et al. 2007; Kuca et al. 2006; Thiermann et al. 2007).

The therapy is known by the acronym "AFLOP" (atropine, fluid, oxygen and pralidoxime) (Petroianu and Kalasz 2007).

Pyridinium aldoximes such as pralidoxime and obidoxime are the only clinically available cholinesterase enzyme reactivators (AChERs) applied to organophoshates poisoned persons (Buckley et al. 2005; Eddleston at al. 2002). In the Department of Toxicology at the Faculty of Military Health Sciences, Defence University, Hradec Kralove, Czech Republic more than 500 asymmetric pyridinium aldoxime compounds (K-compounds, kukoximes) were synthesized. Following preliminary toxicological and *in vitro* effectiveness studies some of them were shown to be used as acetylcholinesterase reactivators for the treatment of intoxication following exposure to tabun, soman and certain organophosphate pesticides (Berend et al. 2008). Oximes are well known to reactivate the inhibited/phosphylated acetylcholinesterase but after so many years of the discovery, no oximes found to be a broad spectrum and effective against different groups of organophosphorus anticholinesterases therefore requesting more investigation. The present study is concerned with the effect of K-compounds on the biogenic amine levels of the adult rat brain but for the better understanding AChE, organophosphorus compounds and oximes are being discussed here.

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4. Hormonal imprinting

Hormonal imprinting is a basic biological phenomenon, which was first observed, named, and defined in 1980 by Csaba. Nowadays, thousands of web pages in Google Scholar and in Google can be found on the topic. The phenomenon means that in the developmentally critical periods, animals or their cells memorize normally or pathologically this first encounter with a given hormone or related structures, and this determines the receptor's later binding capacity as well as the reaction of the imprinted cell to the hormone for life (Csaba 1980, 1984, 2000 and 2008).

Hormonal imprinting can be observed already at a unicellular level, when the signal molecule and the ligand-binding membrane structure (to be receptor) meet each other and because of this meeting, the binding specificity and the response of the cell is altered. As a consequence of imprinting, receptor memory develops, which is transmitted to hundreds of progeny generations (Csaba 1980).

The process may have had evolutionary importance, since it helps to select the best molecules for being signals (hormones) and best protein configurations for being receptors. In addition, it helps to maintain the cell population (species), providing advantage by easier recognition and discrimination of useful or harmful materials (Csaba 1980).

Hormonal imprinting also takes place in mammals during the perinatal critical periods. Genetically determined hormone receptors are maturing in the presence of hormones, reaching binding capacity characteristics to the adult age. This process determines the lifelong binding capacity of the hormone receptor and, as a consequence, the physiological response of the cell (Csaba 2000).

During the time of physiological imprinting, molecules similar to the adequate hormone (members of the same hormone family, hormone analogues and environmental pollutants) can falsely imprint the receptors, resulting in disturbed hormone binding capacity, abnormal morphology and altered response (Csaba 2000).

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4.1. Oxytocin

4.1.1. Oxytocin structure

In humans, oxytocin is transcribed from a single copy gene on chromosome 20p13, composed of 3 exons and 2 introns. Exon 1 encodes the promoter region, a signal peptide, oxytocin, a tripeptide, and the first 9 amino acids of neurophysin 1 (Np1). Exons 2 and 3 encode the remaining conserved region and carboxyl terminal of Np1 respectively. The oxytocin protein is a 12.8kDa nonapeptide with a disulphide bridge between cysteines 1 and 6 forming a cyclical region, with a 3 amino acid carboxyl tail (Gimpl and Fahrenholz 2001) (Figure 1).

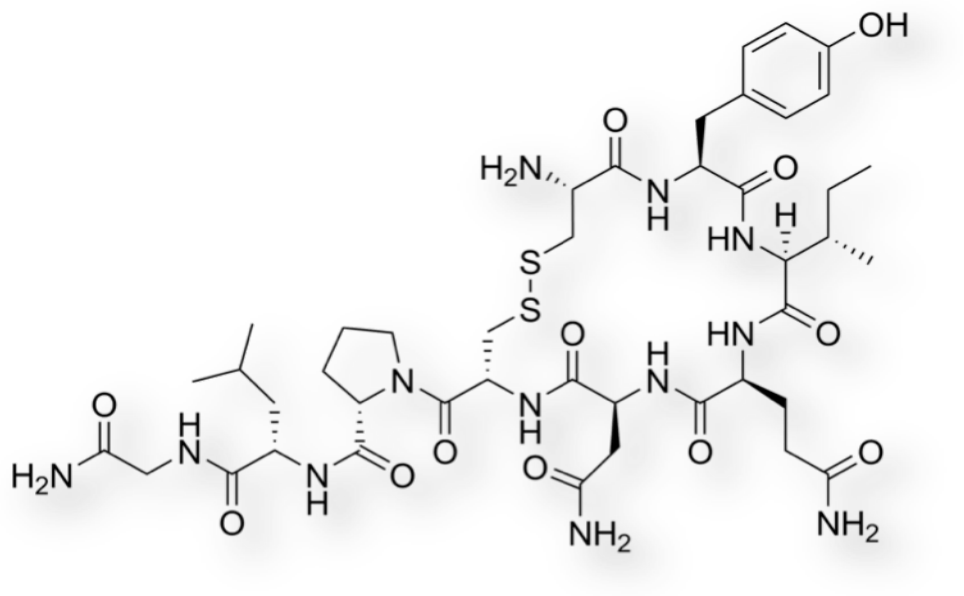

Figure 1. Oxytocin structure

4.1.2. Oxytocin central pathways

The oxytocin central pathways:

[1] The magnocellular oxytocin system originating in the supraoptic nuclei (SON) and hypothalamic paraventricular nuclei (PVN) can be further subdivided by its release characteristics in to axonal release (into the posterior pituitary), which regulates reproductive behavior and dendritic release (within the SON and PVN and may diffuse to other distant sites) to mediate oxytocin autoregulation (Baskerville and Douglas 2010) (Table 1 and Figure 2).

[2] The parvocellular oxytocin system originates in the parvocellular PVN and projects to numerous central nervous system (CNS) sites to regulate autonomic functions such as respiration and gastric reflexes (Baskerville and Douglas 2010) (Table 1 and Figure 2). Magnocellular and parvocellular oxytocin release into systemic circulation and CNS occurs via projections to the posterior pituitary and extrahypothalamic brain regions, respectively.

Table 1. Oxytocin central pathways
Abbreviation: SON, supraoptic nuclei; PVN, paraventricular nuclei.

Oxytocin systems	Origin	Projections	Functions
Magnocellular (axonal)	SON/PVN	Posterior pituitary	Parturition, uterine contractions, milk ejection reflex
Magnocellular (dendritic)	SON/PVN	SON/PVN, extrahypothalamic regions, Ventral tegmental area, hippocampus, brainstem, spinal cord	Autoregulation, endocannabinoid stimulation, Penile erection, ejaculation, gastric reflexes, respiration
Parvocellular	PVN		

Oxytocin release from axon terminals occurs in the classical manner where axonal terminal release is preceded by an influx of calcium into axonal terminals in response to an invading action potential. However, as first demonstrated by Moos et al. (Moos et al. 1984), oxytocin can also be released somatodendritically from magnocellular oxytocin neurons in the hypothalamic PVN and SON to regulate its own release. This finding was further substantiated in numerous *in vivo* studies using microdialysis to quantitatively measure oxytocin release in the plasma and the brain of parturient and lactating rats (Moos et al. 1989; Neumann et al. 1993).

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Unlike axonal release of oxytocin, dendritic release of oxytocin is triggered by release of calcium from intracellular stores and is generally electrically independent (Ludwig and Leng 2006; Ludwig et al. 2002).

Central (axon terminal) and peripheral (*via* hypophyseal secretion into circulation) oxytocin release from magnocellular cells can act synergistically to influence behavioral consequences. During various paradigms like suckling, there is a concomitant release of oxytocin into the bloodstream, SON and PVN (Moos et al. 1989; Neumann et al. 1993a).

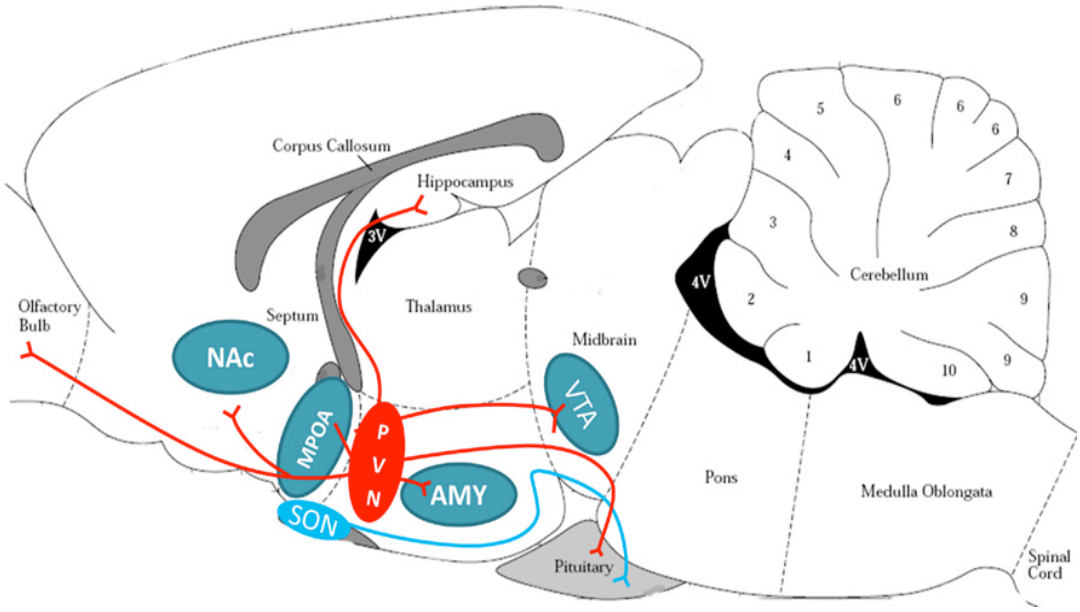

Figure 2. Oxytocinergic projections in the rodent brain

Abbreviation: PVN, paraventricular nucleus of hypothalamus; SON, supraoptic nucleus of hypothalamus; VTA, ventral tegmental area; NAc, nucleus accumbens; AMY, amygdala; MPOA, medial preoptic area of the hypothalamus (Paxinos and Watson 1998)

Such synergy between the central and peripheral oxytocin systems does not always exist and there can be an apparent dissociation between the two as seen during a psychosocial stressor such as social defeat (Bosch et al. 2004; Neumann et al. 2001).

Engelmann et al. demonstrated that whilst intra SON oxytocin release increased in response to social defeat, peripheral oxytocin release remained unaffected. Thus, it can be seen that during certain neuroendocrine mediated behaviors, centrally acting and peripherally acting oxytocin may act in union or independently to exert their behaviorally specific effects (Engelmann et al. 1999).

4.1.3. Oxytocin receptor

Oxytocin receptor (OXT-R) mRNA localization is in the adult rat brain (Yoshimura et al. 1993). OXT-R mRNA was detected in the regions where binding was reported such as the anterior olfactory nucleus, the bed nucleus of the stria terminalis, subiculum, paraventricular nucleus, the ventromedial hypothalamic nucleus, and the dorsal motor nucleus of the vagus nerve. Thus, in the adult brain, there was a high correlation between the localization of OXT-R mRNA and that of OXT-binding sites. This localization of mRNA and the binding site coincided well with the functions of oxytocin described above in memory, maternal and sexual behavior, social perception and autonomic function (Shapiro and Insel 1989; Tribollet et al. 1989; Melchers et al. 2015). The developmental expression profile of OTX-R mRNA could be divided into two types: transient type and constant type. During brain development, OXT-binding sites were observed more abundantly in the early postnatal rather than in the adult brain (Shapiro and Insel 1989; Tribollet et al. 1989).

Moreover, OXT-binding sites appeared as early as at day 14 of pregnancy**,** although the appearance of OXT-immunoreactive fibers was not observed until after birth (Buijs et al. 1980; Buijs 1992). The OTX-R is tied to Phospholipase C. Its genetic sequence was decoded in 1992 (Kimura et al. 1992). For the hormone to bond, magnesium (Pliska and Kohlhauf 1991) and cholesterol are necessary (Gimpl et al. 2000).

The encoded oxytocin receptor is a 389-amino acid polypeptide with seven transmembrane domains and is thus part of the G protein-coupled receptor family. When oxytocin binds to its receptor it initiates a cascade of intracellular events that culminate in a range of cellular responses including an increase in neuronal firing, neurotransmitter release, smooth muscle contraction and protein phosphorylation. In rats, peripheral expression of oxytocin receptors is concentrated (but not exclusively) in the male and female reproductive tract and in myoepithelial cells in mammary tissue (Gimpl and Fahrenholz 2001; Zhang et al. 2005).

Oxytocin receptors are found in both the brain and the periphery (Adan et al. 1995). In addition, oxytocin receptors are also abundantly expressed throughout the CNS and often exist in the same regions containing oxytocin fibers. In addition to their expression in the SON and PVN, oxytocin receptors are also found in the regions of the cortex,

hippocampus, limbic system, basal ganglia, MPOA, olfactory bulbs, amygdala, and the brain stem (Freundmercier et al. 1994; Yoshimura et al. 1993).

There is widespread distribution of oxytocin receptors in the thoracic and lumbosacral segments of the spinal cord, with the dorsal horn, dorsal gray commissure, intermediolateral cell column all possessing oxytocin receptors (Veronneau-Longueville et al. 1999). However, some brain areas show a distinct mismatch between oxytocin fiber distribution and oxytocin receptor expression, such as seen in the amygdala and olfactory bulbs where there is a significantly greater proportion of oxytocin receptors compared to oxytocin fibers that innervate these nuclei (Ferguson et al. 2001; Huber et al. 2005; Terenzi and Ingram 2005).

Such an anatomical mismatch gives rise to the possibility that centrally released oxytocin can diffuse to distant sites within the brain to exert its effects. Therefore, oxytocin in the brain is described as a neuromodulator and appears to have broad permissive actions. OXT-R is expressed in OXT-target tissues and is a class G-protein coupled receptors (GPCRs), which primarily activates the G-protein αq/11 upon oxytocin binding (Sanborn et al. 1995).

4.1.4. Oxytocin effects in the body

Oxytocin has a short half-life in the blood. It has between 3.5 (Fuchs 1984) and 15 minutes (Gonser 1995).

Oxytocin has many different effects. It seems to play a large part in the masculine sexuality. Oxytocin has a function in the ejaculation, in some mammals, changes the contractility in the tubuli seminiferi (Insel et al. 1997).

Oxytocin injections were able to release erections in animals (Melis et al. 1986). Oxytocin also modulates our experience of pain (Arletti et al. 1993).

Memory and mood are also influenced through its release (Arletti et al. 1995). Intranasal oxytocin can be positively affected on hippocampal learning and memory loss due to chronic restraint stress (Dayi et al. 2015).

Oxytocin has an important role in digestion and nutrient absorption. Oxytocin release activates the vagal nerve, which increases activity of the gut hormones (Uvnas-Moberg 1989 and 2003). Oxytocin actions also promote anabolic metabolism and release insulin (Uvnas-Moberg 1989 and 2003).

It was also shown that decreased oxytocin levels in the amygdala (Lubin et al. 2003) and the MPOA and VTA are associated with increased postpartum aggression and deficits in maternal care, respectively (Elliott et al. 2001; Johns et al. 2004).

Central oxytocin release is stimulated by multiple sensory signals, such as olfactory, auditory, visual, and physical inputs (Yoshimura et al. 1993) (Figure 3).

In particular, physiological stimuli are known to induce oxytocin system activation in mammals. When oxytocin release is increased in the central nervous system, many sensory, physiological, and behavioral functions are enhanced. Maternal as well as affiliative behaviors are enhanced by oxytocin.

Additionally, negative responses, such as pain, stress endocrine, and anxiety behaviors are diminished by oxytocin (Yoshimura et al. 1993) (Figure 3).

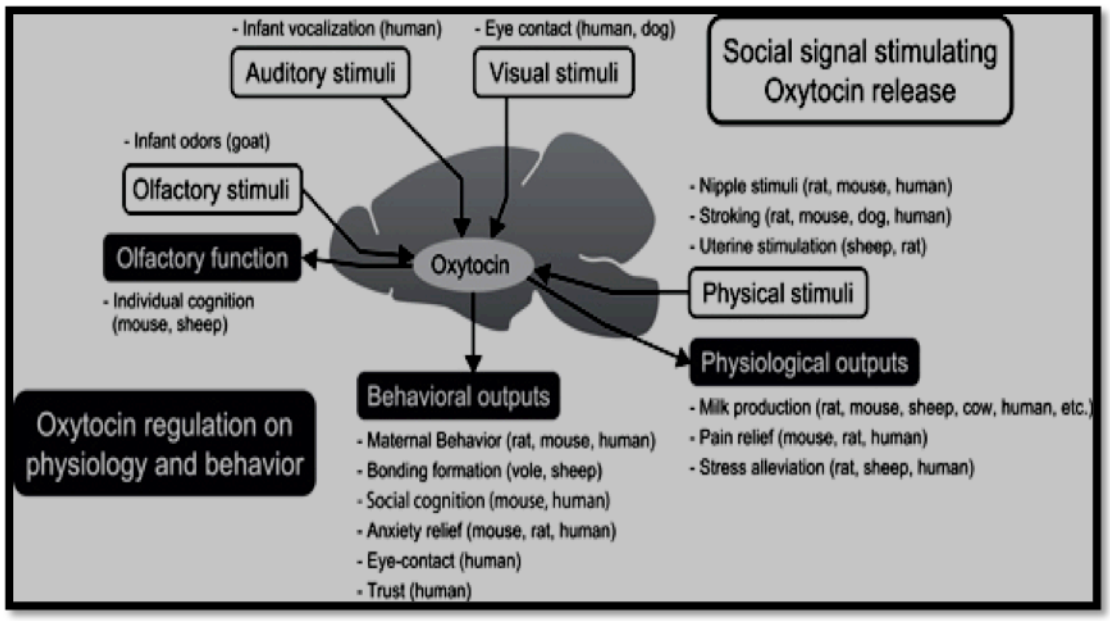

Figure 3. Summary of the role of the oxytocin system in reciprocal communication (Reproduced from Yoshimura et al. 1993)

4.2. Relation of hormonal imprinting to catecholamines and serotonin

4.2.1. Catecholamines

Biogenic amine neurotransmitters are biogenic substances with one or more amine groups (Purves et al. 2001). The amino acid tyrosine is the precursor for all three catecholamine neurotransmitters (Figure 5).

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Dopamine is produced in brain cells and adrenal cells by the action of DOPA decarboxylase and the enzyme aromatic L-amino acid decarboxylase on L-DOPA in the presynaptic terminals and after transported to synaptic sites and packaged into vesicles for release. After release, free dopamine is either reabsorbed into the presynaptic terminal for reuse or it is converted to DOPAC and 3-MT *via* the enzymes COMT and MAO. Homovanillic acid (HVA) is a result of the further degraded of DOPAC and 3-MT by COMT and MAO enzymes (Moron et al. 2002; Yavich et al. 2007) (Figure 4). Dopamine does not readily cross the blood-brain barrier (BBB). Dopamine acts by activating GPCRs, which exert their effects *via* complex second messenger systems (Purves et al. 2001). Dopamine receptors in mammals can be divided into two families, known as D1-like and D2-like. The effect of D1-like receptors (D1 and D5) can be excitation *via* opening of sodium channels or inhibition *via* opening of potassium channels and increase intracellular levels of cyclic AMP by activating adenylate cyclase. The effect of D2-like receptors (D2, D3, and D4) is usually inhibition of the target neuron and decrease intracellular levels of cyclic AMP by inhibiting adenylate cyclase (Grace 1991).

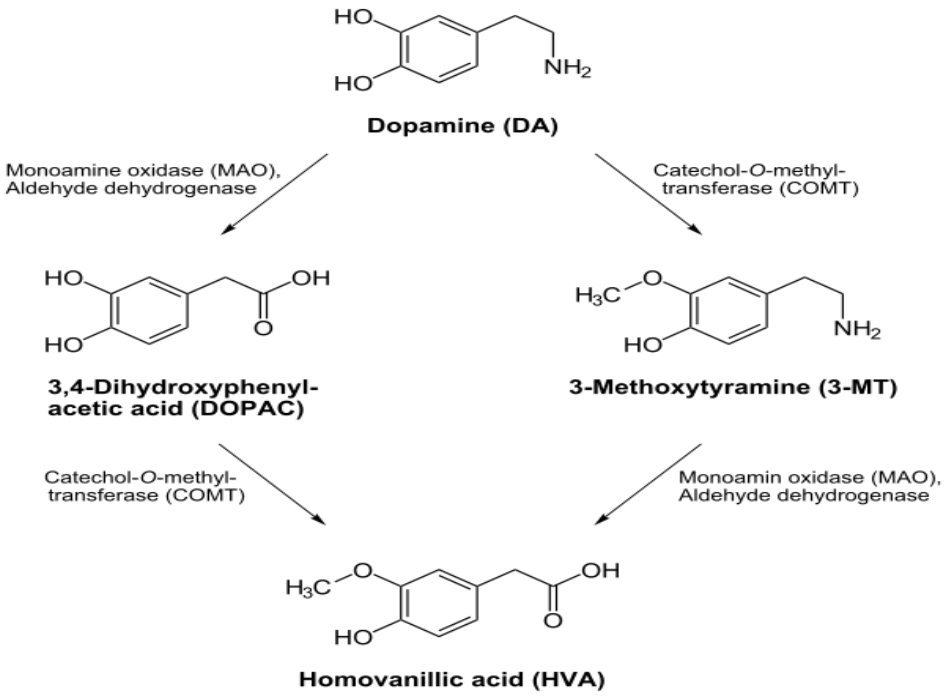

Figure 4. Dopamine metabolic pathway

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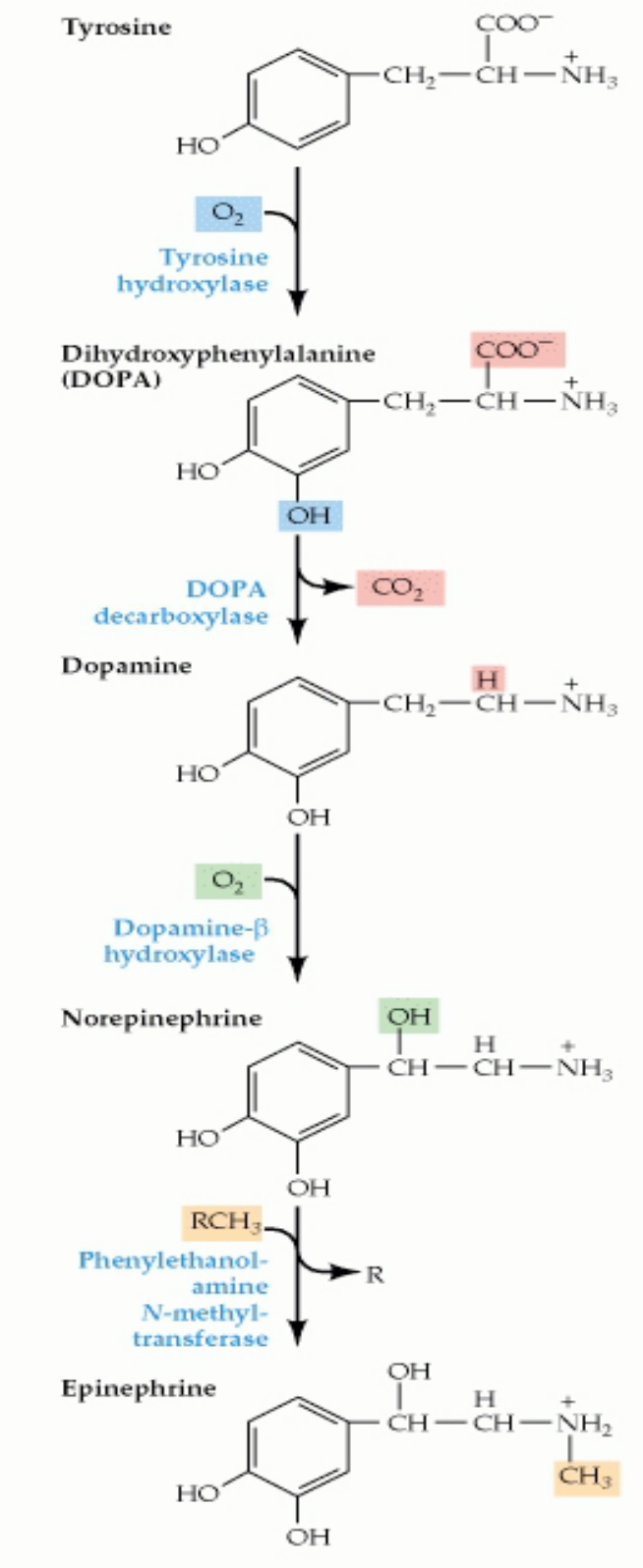

Figure 5. The biosynthetic pathway for the catecholamine neurotransmitters

Dopamine is also believed to be involved in motivation, reward, cognition, arousal and reinforcement, also plays a poorly understood role in lactation, sexual activity, and nausea (Purves et al. 2001). The major dopamine area of the brain is the substantia nigra (SN), a small midbrain area that forms a component of the basal ganglia (Figure 7) and their most important projections go to the striatum, globus pallidus, and subthalamic nucleus, which play important roles in motor control. Another large group of dopaminergic neurons are located in the VTA, which is a group of neurons located in the midbrain area (Björklund and Dunnett 2007). Central neurons system dopamine pathways, projections and functions are summarized in the Table 2 (Ben-Jonathan and Hnasko 2001; Sanghera et al. 1991; Bitran et al. 1988; Eriksson et al. 1996; Skoog and Noga 1995; Holstege et al. 1996).

Table 2. Central nervous system dopamine pathways

Dopamine system	Origine	Projections	Functions
Nigrostriatal	SN (substantia nigra)	Striatum	Motility
Mesocortical	VTA (ventral tegmental area)	Cortex	Emotionality
Mesolimbic	VTA (ventral tegmental area)	Nucleus accumbens	Reward desired
Tuberoinfundibular	Arcuate Nucleus	Median eminence	Regulation of prolactine release
Incertohypothalamic	Zona incerta, Periventricular region	Various hypothalamic nuclei, Thalamus	Sexual arousal and copulation
Diencephalospinal	Hypothalamus	Spinal cord	Afferent stretch reflex, contraction of penile, striated muscle

All of these data show dopamine is an important neurotransmitter that exerts widespread effects in the CNS function such as social and sexual behavior and also known to be implicated in various neuropsychiatric behavioral disorders including autism and depression. Oxytocin has a substantial role in social attachment, affiliation and sexual behavior and it has emerged that disturbances in peripheral and central oxytocin levels have been detected in some patients with dopamine-dependent disorders therefore stimulation of central dopamine and oxytocin pathways are known to have similar effects on certain social behavioral paradigms and associated disorders such as sexual dysfunction, autism, anorexia and depression (Baskerville and Douglas 2008; Waldherr and Neumann 2007; Mackenzie 2006).

Norepinephrine (or noradrenaline in Europe) is produced by the action of dopamine β-hydroxylase on dopamine (Purves et al. 2001) (Figure 5). It is released from the adrenal medulla into the blood as a hormone, and is also a neurotransmitter in the CNS and the sympathetic nervous system (Purves et al. 2001).

Norepinephrine is also the transmitter used by the locus coeruleus, a brainstem nucleus that projects diffusely to a variety of forebrain targets including the cerebral cortex, limbic system, and the spinal cord, where it influences sleep and wakefulness, attention, and feeding behavior (Purves et al. 2001) (Figure 7).

Norepineprine and epinephrine performs their actions on the target cell by binding to and activating α- and -β adrenergic receptors that are G-protein-coupled (Dale Purves et al. 2001).

Norepinephrine plays a role in arousal, attention, fear, anxiety, learning, memory, (Barnes and Pompeiano 1991) and in aggressive behavior of males (Matsumoto et al. 1991) therefore alternation in the oxytocin system and norepinephrine has been associated with increased aggression in humans (Chichinadze et al. 2010; Bosch et al. 2005; Consiglio et al. 2005).

Epinephrine or adrenaline is produced by the action of phenylethanolamine-*N*-methyltransferase (PNMT) on norepinephrine, which utilizes S-adenosylmethionine as the methyl donor (Figure 5). Epinephrine-containing neurons in CNS are found in two groups in the rostral medulla (Figure 7). Epinephrine acts on the target cell by binding to and activating α- and -β adrenergic receptors (Purves et al. 2001).

Stress is a risk factor for a variety of illnesses, ranging from metabolic and cardiovascular disorders to mental illness. Stress induced increases in sympathoadrenal release of adrenaline and noradrenaline (Caldji et al. 2000), which increases heart and respiratory rate (Sabyasachi Sircar 2007).

Oxytocin administered cause reduces the rate and force of cardiac cells intrinsic contractions causing them to beat more slowly and contract less forcefully (Mukaddam-Daher 2001) therefore the connection between the catecholamines and oxytocin was always important (Wsol et al. 2008; Uvnas-Moberg and Petersson 2004).

4.2.2. Serotonin

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter, synthesized from the amino acid L-tryptophan by tryptophan hydroxylase and aromatic L-amino acid decarboxylase (Young 2007; Purves et al. 2001) (Figure 6).

Seven types of 5-HT receptors are known (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7). Most 5-HT receptors are metabotropic (G-protein coupled that activate an intracellular second messenger cascade), only the 5-HT_3 is a ligand-gated ion channel. Serotonin has been implicated in behaviors, including the emotions, circadian rhythms, motor behaviors, and state of mental arousal. Impairments in the function of these receptors have been implicated in numerous psychiatric disorders, such as depression, anxiety disorders, and schizophrenia (Purves et al. 2001, Veenstra-VanderWeele et al. 2000).

Serotonin is located in groups of neurons in the raphe region of the pons and upper brainstem, which have widespread projections to the forebrain and have been implicated in the regulation of sleep and wakefulness (Hutson et al. 1986) (Figure 7). The serotonergic system may be important in this regard through, its potential influence on the release of oxytocin. Stimulation of the hypothalamus by serotonin has been shown to lead to release of oxytocin as a precursor molecule (Lee et al. 2003).

Galfi et al. (2005) and Jorgensen et al. (2003) reported that in the rats, oxytocin secretion was influenced directly by the serotonergic system (Galfi et al. 2005; Jorgensen et al. 2003) and also administration of 5-HT antagonists blocked stress-induced increases in oxytocin secretion (Jorgensen et al. 2002).

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The serotonergic system may however be important for responding sensitively to the partner as well as to the offspring, partly through it's influence on the release of oxytocin. The assessment of central biogenic amines functioning is critically important in a wide range of neurochemical studies.

Investigation of biogenic amine neurotransmitters roles in the mechanism of drug action, their roles in a variety of normal processes, their relation with oxytocin, their relations with the developing hormone receptors (hormonal imprinting), and their possible alteration in neuropsychiatric disorders all require an accurate determination of biogenic amine neurotransmitters.

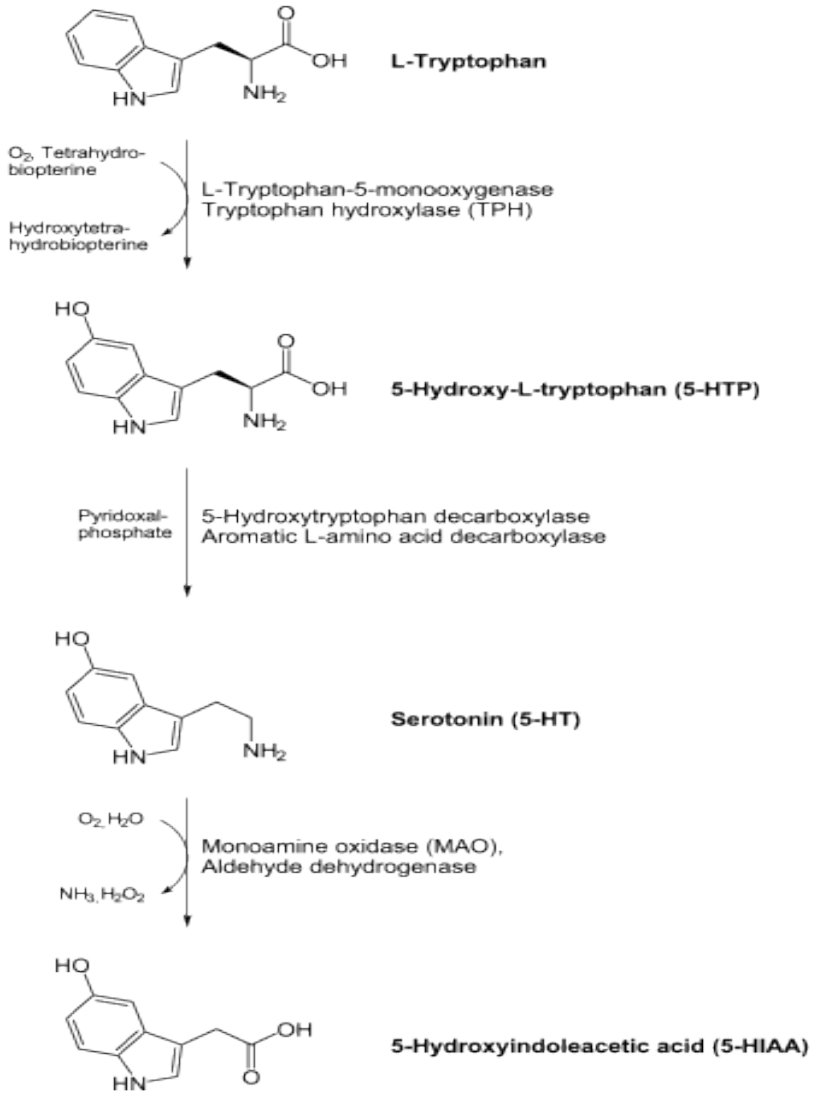

Figure 6. Serotonin biosynthesis and metabolism

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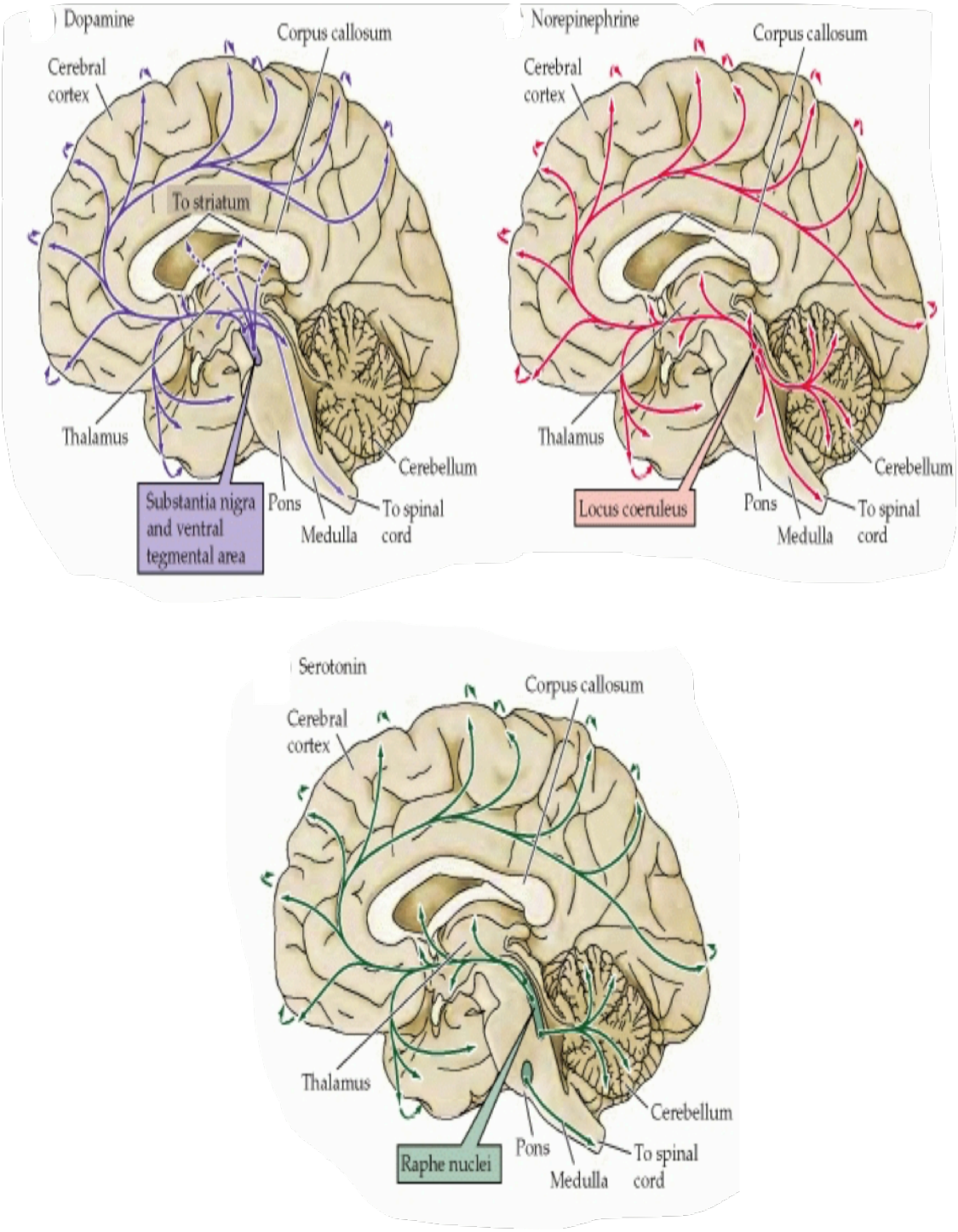

Figure 7. The distribution in the human brain of neurons and their projections (arrows) containing biogenic amine neurotransmitters (Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia AS, McNamara JO and Williams SM. (2001) The Biogenic Amines. Neuroscience, 2nd edition)

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5. Acetylcholinesterase inhibiton and reactivation

Acetylcholinesterase (AChE) is a globular protein and belongs to the family of carboxylesterases. It is an important part of the cholinergic nervous system taking place in the central and peripheral nervous system, where it stops neurotransmission by hydrolysis of the neurotransmitter ACh. There are different types of cholinesterases in the human body. The principle ones are AChE (EC 3.1.1.7, AChE) found in the nervous system and also present in the outer membrane of red blood cells and other one is plasma cholinesterase (EC 3.1.1.8, BuChE). The acute toxicity of organophosphorus compound is due to inhibition of AChE (EC 3.1.1.7, AChE), which belongs to serine esterase family (Miroslav et al. 2013).

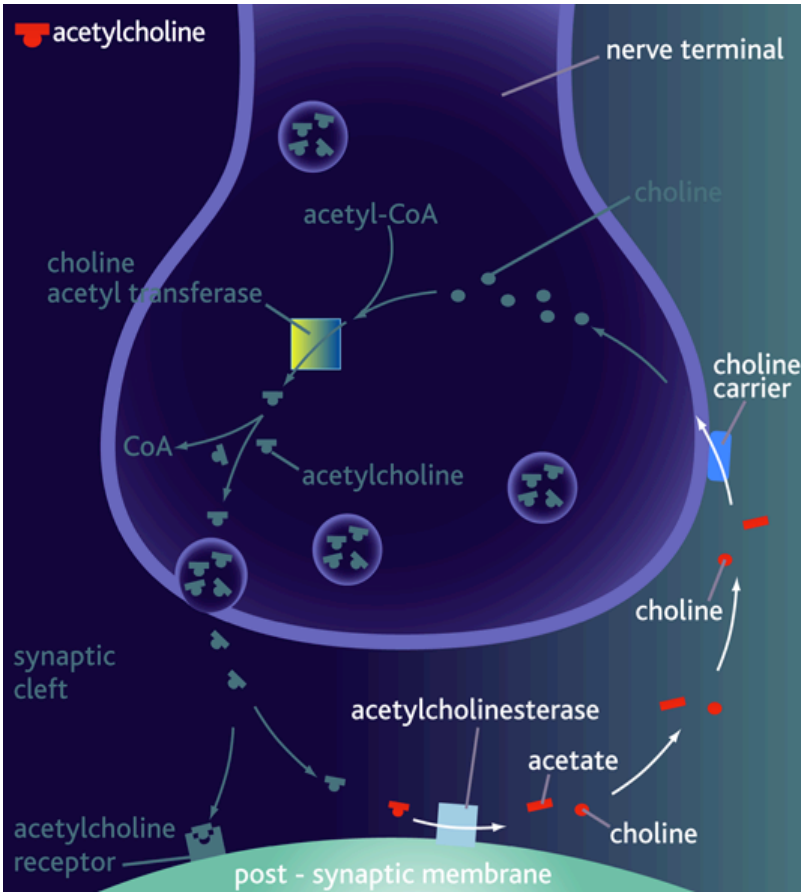

Figure 8. The mechanism of action of acetylcholinesterase (Introduction to autonomic pharmacology. In: Basic and clinical pharmacology, Katzung 2001**)**

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Acetylcholine mediates messages between the nerves, which is responsible for muscle contraction. When ACh is released from the nerve into the synaptic cleft, it got recognised by its receptors present on the postsynaptic membrane, which further transmits signal. Along with the acetylcholine receptors, AChE is also present on the postsynaptic membrane, which helps in the termination of the signal transmission by hydrolyzing ACh. On hydrolysis, ACh split into two products one is choline and the other is acetic acid. Choline and acetic acid are recycled by the body to form again ACh to maintain the reserves of the neurotransmitter therefore they can be used by the body again during the time of need (Boublik et al. 2002) (Figure 8).

Acetylcholinesterase contains one catalytic center which has two active sites: anionic and esteratic. The anionic site, positions the ACh in the active site through an electrostatic interaction with the quaternary nitrogen in choline. The hydroxyl group forms a serine residue in the esteratic site then covalently binds to the ester carbonyl group. This forms an unstable tetrahedral intermediate, which rapidly decomposes, liberating choline and leaving the enzyme covalently bound to acetate. A water molecule spontaneously hydrolyzes this bond, regenerating the free enzyme (Wiener et al. 2004).

Figure 9 shows the positively charged nitrogen in the ACh molecule that is attracted to the ionic site on AChE, and hydrolysis is catalyzed at the esteric site to form choline and acetic acid (Wiener et al. 2004).

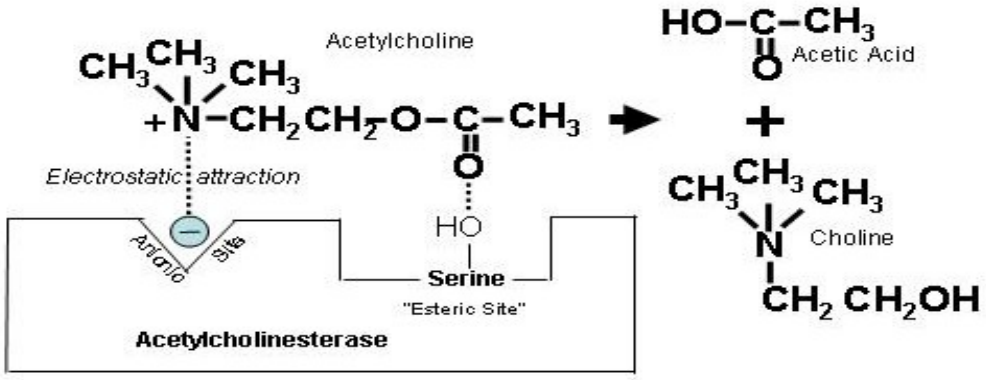

Figure 9. Break down of acetylcholine (Wiener et al. 2004)

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5.1. Acetylcholinesterase inhibitors (AChEI)

Acetylcholinesterase inhibitors being very lipophilic compounds (organophosphates) may be rapidly absorbed via dermal, conjunctival, respiratory, and gastrointestinal routes. The inhibition of the AChE leads to accumulation of ACh in the synaptic cleft resulting in over stimulation of nicotinic and muscarinic ACh receptors, which cause acute poisoning with AChEI (Erdman 2004; Costa 2008) (Table 3).

Table 3. The signs and symptoms of acetylcholinesterase inhibitors (AChEI) poisoning (Erdman 2004; Costa 2008)

Site Affected		Cholinergic Receptor	Manifestation
1	Bladder	Muscarinic	Urinary frequency, urinary incontinence
2	Cardiovascular system	Muscarinic	Bradycardia, bradydisrhythmias, hypotension
3	Eyes	Muscarinic	Blurred vision, lacrimation, miosis
4	Exocrine glands	Muscarinic	Increased salivation, perspiration
5	Gastrointestinal tract	Muscarinic	Abdominal cramps, diarrhea, nausea, vomiting
6	Respiratory system	Muscarinic	Bronchoconstriction, increased bronchial secretion, rhinorrhea
7	Central nervous system	Muscarinic and/or nicotinic	Agitation, anxiety, coma, confusion, convulsion depression of respiratory and circulatory centers, dizziness, fatigue, hallucination, headache, lethargy, seizures, somnolence
8	Cardiovascular system	Nicotinic	Tachycardia, transient hypertension
9	Skeletal muscle	Nicotinic	Cramps, flaccid paralysis, generalized weakness, muscle fasciculation, twitching

According to the mode of action, acetylcholinestrase inhibitors can be divided into two groups:

1. Reversible AChEI play an important role in pharmacological manipulation of the enzyme activity. These inhibitors include compounds with different functional groups (carbamate, quaternary or tertiary ammonium group), and have been applied in the diagnostic and/or treatment of various diseases such as: myasthenia gravis, Alzheimer's disease, post-operative ileus, bladder distention, glaucoma, as well as antidote to anticholinergic overdose (Aldridge and Davison 1953; Boublik et al. 2002).

2. Irreversible AChEI (e.g. the insecticide: carbofuran, nerve agents: sarin, soman, tabun, OPs and VX) inhibit AchE by alkyl phosphorylation of a serine hydroxyl group at the esteratic site of the enzyme. The inactive phosphorylated enzyme is very stable and AChEI eventually loses an alkyl side chain and the stability of the enzyme-nerve agent complex is enhanced, thereby increasing both the level and duration of the neurotransmitter acetylcholine at nicotinic, muscarinic and central nervous synapses. This process is known as ageing which varies in half time (time for half of involved cholinesterase to age) from 2 min for soman, to > 40 h for VX and tabun (Aldridge and Davison 1953; Boublik et al. 2002).

5.1.1. Organophosphate type inhibitors

An organophosphate or phosphate ester is the general name for esters of phosphoric acid. Phosphates are probably the most pervasive organophosphorus compounds. The first synthesized organophosphorus compound was a mono ester named tetraethylpyrophosphate (TEPP) and the process was first published in 1854 by Philippe de Clermont (Antonijevic and Stojilkovic 2007; Karczmar 1970).

organophosphates cause irreversible inhibition of cholinesterases *via* a covalent reaction with the serine in the active center of the enzyme (Bajgar 2004; Bajgar et al. 2007; Kuca et al. 2006; Thiermann et al. 2007).

5.1.2. General structure of organophosphates

Organophosphates are esters or thiols derived from phosphoric, phosphonic, phosphinic or phosphoramidic acid. The basic chemical structure of all OPs is described by Schrader's formula (Figure 10).

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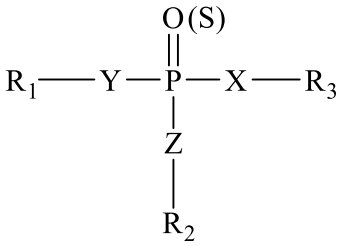

Figure 10. General structure of organophosphates (OPs)

R1 and R2 are aryl or alkyl groups that are bond to the phosphorus atom either directly (forming phosphinates), or through an oxygen or sulphur atom (forming phosphates or thiophosphates). In some cases, R1 is directly bonded to the phosphorus atom, and R2 is bonded to an oxygen or sulphur atom (forming phosphonates or thiophosphonates). In phosphoramidates, at least one of these groups is –NH2 (un-, mono- or bi-substituted), and the atom double-bonded with phosphorus is either oxygen or sulphur (Bajgar 2004; Sogorb and Vilanova 2002; Smulders et al. 2004).

The –XR3 group, also binding to the phosphorus atom through oxygen or sulphur atom, may belong to a wide range of halogen, aliphatic, aromatic or heterocyclic groups. This leaving group is released from the phosphorus atom when the organophosphate is hydrolyzed by phosphotriesterases or upon interaction with protein targets (Bajgar 2004; Sogorb and Vilanova 2002; Smulders et al. 2004).

Phosphorothionate esters (P=S) are generally poor anticholinesterases. The poor AChE activity of P=S esters is explained on the basis of their relatively low reactivity, attributed to the smaller extent to which the P=S bond is polarized compared to the P=O, owing to the lower electronegativity of sulfur compared to oxygen. Polarization of the P=O linkage results in a more electropositive phosphorus atom, which facilitates attack on phosphorus by nucleophilic agents, e.g. the serine hydroxyl of AChE. Organophosphorus esters containing the P=S moiety are less reactive and more stable to hydrolytic degradation than the corresponding P=O esters. Investigations on the metabolism and mode of action of organophosphorus compounds revealed that the toxicity of a P=S ester is attributed to the corresponding P=O esters, formed by metabolic oxidation of P=S to P=O (Gage 1953; Dauterman 1971).

5.1.3. Mechanism of toxicity

There are various groups of organophosphorus compounds, which are structurally and toxicologically different.

The OPs and their active metabolites are electrophilic molecules with moderate to high potency for phosphylation (denotes both phosphorylation and phosphonylation) of the serine hydroxyl group located at the active site of AChE. This phosphylation occurs by the loss of the leaving group and the establishment of a covalent bond with AChE through the serine hydroxyl. The resultant phosphylated AChE is typically very stable and is only slowly regenerated by spontaneous hydrolysis of the phosphate ester. While the AChE remains phosphylated, its enzyme activity is inhibited and therefore ACh accumulates in the synaptic clefts of muscles and nerves, leading to over-stimulation of cholinergic receptors that is essentially poisoning by endogenous ACh (Marrs 1993; Pope 1999).

Additionally, OPs may also interact (inhibit) with other serine esterases, (Casida and Quistad 2004 and 2005) may have a direct action on muscarinic and nicotinic receptors binding to (with high/low affinity) and modulating the function of these receptors, (Bakry et al. 1988) and may induce specific organ lesions (Cao et al. 1999).

Propyl, isopropyl, butyl, and higher alkyl phosphates and phosphonates are more likely than methyl or ethyl analogs to act with secondary targets (Cao et al. 1999).

The concentrations of organophosphorus compounds required to act directly on nicotinic receptors are much higher than those on muscarinic receptors, suggesting that muscarinic receptors are more important as secondary targets in organophosphorus compound action. Combination of possible interactions will produce resultant toxic effect(s) for the particular organophosphorus compound. The spectrum of effects is further modulated by various toxicokinetic factors (Karalliedde et al. 2003).

There are four stages of interaction of OPs with AChE:

Stage 1: Partially electropositive phosphorus of the organophosphorus compounds (ex. nerve agent) is attracted to partially electronegative serine hydroxyl group on AChE and a complex forms (Figure 11). In the transition state showing which bonds break and which ones form (Johnson et al. 2000; Wiener and Hoffman 2004) (Figure 12).

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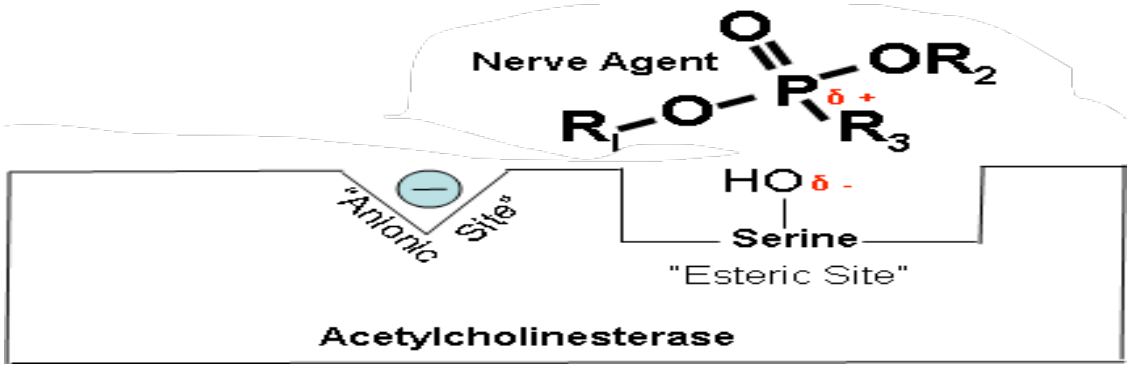

Figure 11. Partially electropositive phosphorus is attracted to partially electronegative serine. δ + Indicates that phosphorus is partially electropositive. δ – Indicates that oxygen is partially electronegative (Wiener and Hoffman 2004).

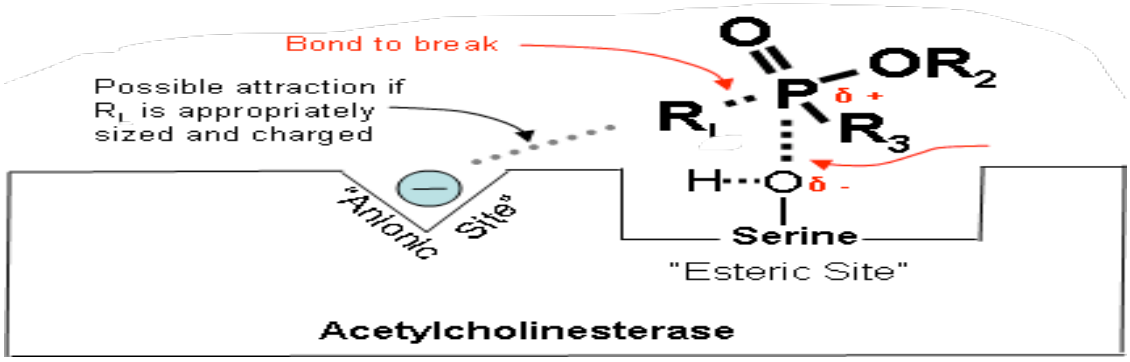

Figure 12. Transition state showing which bonds break and which ones form (Wiener and Hoffman 2004)

Stage 2: The formation of a stable covalant bond between organophosphate compound and serine group of AChE. In this step organophosphorylation of the enzyme (esterase) takes place (Johnson et al. 2000; Wiener and Hoffman 2004) (Figure 13).

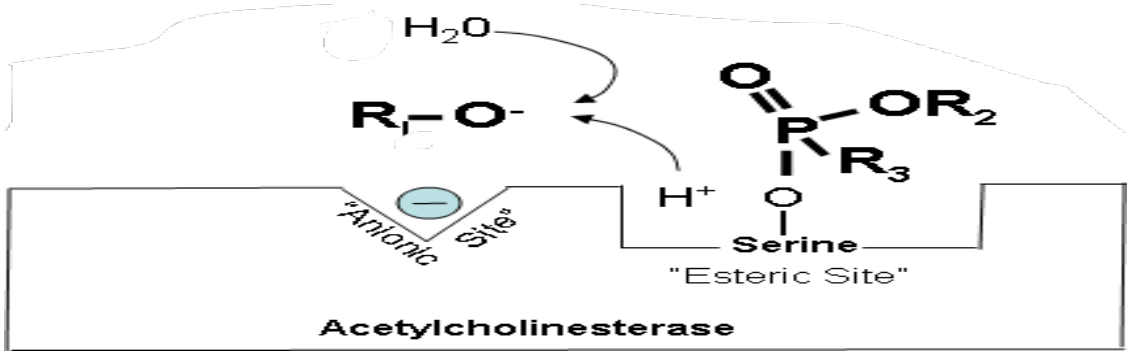

Figure 13. Organophosphates attached to acetylcholinesterase preventing the attachment of acetylcholine (Wiener and Hoffman 2004)

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Stage 3: Cholinesterase is blocked, but it can hydrolyze to original state (slow) or regenerate with an oxime (fast) or ageing can appear which cannot regenerate (Johnson 2000; Wiener and Hoffman 2004) (Figure 14).

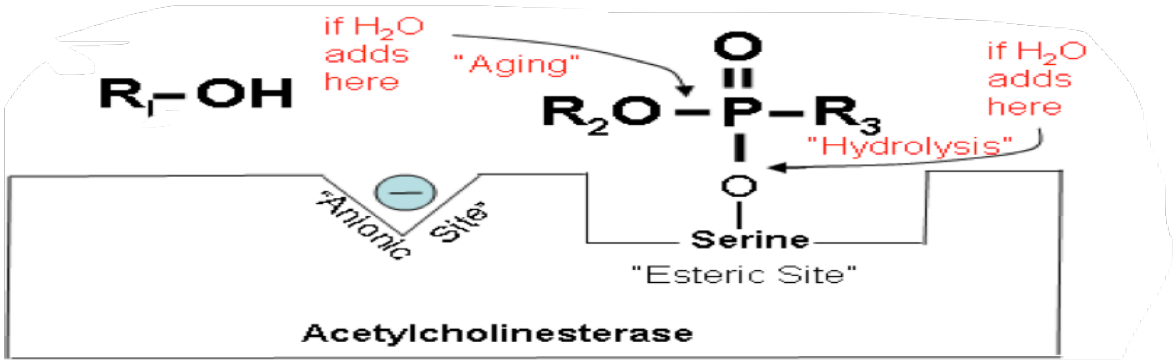

Figure 14. Blocking of cholinesterase by organophosphates
(Wiener and Hoffman 2004)

Stage 4: Ageing. Over time, a functional group leaves the nerve agent, strengthening the bond and making it permanent. When this occurs, the enzyme-nerve agent complex is said to have aged (Johnson 2000; Wiener and Hoffman 2004) (Figure 15).

Figure 15. The "aged" bond (Wiener and Hoffman 2004)

5.1.4. Application of organophosphates

Organophosphates are widely used in agriculture (pesticides, insecticides), in chemical industry (softeners, additives to lubricants) (Jeyaratnam 1990) and some of them are extremely toxic chemical nerve gas warfare agents (tabun, sarin, cyclosarin, soman, VR, VX) that pose potential neurotoxic threat to both military and civilian populations, as evidenced in recent terrorist attacks (Okumura et al. 1996).

The developing and production of these extremely toxic nerve agents started in the 1930s, and later used in wars and by terrorists on several occasions. As chemical weapons, they are classified as weapons of mass destruction by the United Nations, and their production and stockpiling was outlawed by the chemical weapons convention. In 1994, sarin was used in terrorist attacks in Japan, indicating that these compounds constitute a clear terrorist threat (Weiner and Hoffman 2004).

Several Middle-Eastern nations are believed to have developed and stockpiled nerve agents, notably Libya and Iraq. In 1984, it was confirmed that Iraq had used nerve agents in its war against Iran (Sidell and Borak 1992; McCauley et al. 2001).

In 1949, Dr. Ranajit Ghosh, a chemist working in England, was working on insecticide synthesis and discovered an extremely potent nerve agent that later came to be known as VX (Somani et al. 1992). Based on the acute toxicity, VX is the most toxic compound among all the nerve agents (Gupta 2006).

According to the WHO statistics more than 2 million pesticide poisonings occur every year and the rate of death is alarming mainly in the developing countries. Organophosphate compounds may be used in the therapy of neurological damages such as Alzheimer's disease. The example is trichlorfon (metrifonate) that used to be applied as a pesticide, and has medicine implementation analogous to the carbamate rivastigmine (Cummings et al. 2001).

5.2. Acetylcholinesterase reactivation : Oximes

The current treatment of organophosphate compounds poisoning includes three stages:

1. The use of an anticholinergic drug (e.g., atropine)
2. Cholinesterase-reactivating agents (e.g., oximes)
3. Anticonvulsant drugs (e.g., benzodiazepines)

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Atropine, a parasympatholytic alkaloid isolated from *Atropa belladonna* by the German pharmacist Heinrich F. G. Mein in 1831, first successfully used by Thomas Richard Fraser, as an antidote for SLUDGE symptoms (salivation, lacrimation, urination, diaphoresis, gastrointestinal motility, emesis) caused by organophosphate poisoning (Karczmar 1970). The most important anticonvulsant is diazepam (Lipp 1972; Sellström 1992) and the combination of atropine and diazepam is more effective than atropine or oxime alone in reducing mortality (McDonough et al. 1989). In the cholinergic nervous system, diazepam probably decreases the synaptic release of ACh (Shih 1991), therefore diazepam has benefit in organophosphate poisoned patients by reducing anxiety, restlessness and muscle fasciculations, terminating convulsions, reducing morbidity and mortality when used in conjunction with atropine and oxime. Diazepam should be given to patients poisoned with organophosphorus compound whenever convulsions or pronounced muscle fasciculation are present. In severe poisoning, diazepam administration should be considered even before these complications develop (Marrs 2003). Oximes (cholinesterase-reactivating agents) are the nitrogen containing organic compounds drived from ketones or aldehydes by condensing them with hydroxylamine, with the general formula $RR'C{=}NOH$, where R is an organic side chain and R' may be hydrogen, forming an aldoxime (Figure 16), or another organic group, forming a ketoxime (Dawson 1994) (Figure 16).

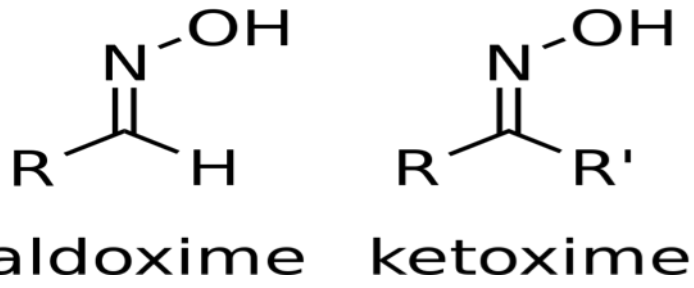

Figure 16. structure of oximes (aldoxime, ketoxime)

Oximes are strong nucleophilic type compounds, comprised oxime moiety attached to a quaternary nitrogen pyridinium ring or imidazolium ring or quinuclidinium ring or other modified structure with basic oxime moiety to enhance the nucleophilicity.

However, therapeutically available oximes for organophosphate compounds antidote are only pyridinium aldoximes: Pralidoxime or 2-PAM, obidoxime, trimedoxime, and HI-6 (Dawson 1994).

Pralidoxime (2-PAM)

Pralidoxime (pyridinium-2-aldoxime or 2-PAM) was synthesised by Wilson and Ginsburg in the USA and Childs et al. in the UK in 1955 (Wilson and Ginsburg 1955). Chemically, pralidoxime is a (2-hydroxyimino-methyl-1-methylpyridinium chloride). As a quaternary pyridinium salt, 2-PAM does not penetrate the blood-brain barrier (BBB), and because of that pro-2-PAM was synthesised as a pro-drug of 2-PAM that can access to the CNS. Unexpectedly, pro-2-PAM turned out to be even less effective than 2-PAM against experimental poisoning with paraoxon (Boškovic et al. 1980). However, it appears that in organophosphate compounds poisoning 2-PAM can pass BBB at higher concentrations when given with atropine. There are four salts of pralidoxime namely 2-PAM Cl, methiodide, methysulphate and mesylate, that were investigated and introduced into practice which 2-PAM Cl is used all over the world where mesylate is used in UK only (Bismuth et al. 1992). 2-PAM is very efficient in reactivating AChE inhibited with sarin or VX (Johnson and Stewart 1970; Nozaki and Aikawa 1995), but is not successful in reactivation with tabun or soman (Inns and Leadbeater 1983; Koplovitz and Stewart 1994).

Trimedoxime

Trimedoxime (TMB-4 Cl_2) was synthesised in the USA in 1957. Chemically, trimedoxime is a 1,3-bis (4-hydroxyimino-methyl-1-pyridinium) propane-dibromide (Poziomek et al. 1958). It is the only of the major bispyridinium oximes with a propylene bridge between the two-pyridinium rings. Experiments have shown that TMB-4 is a more potent reactivator of the AChE than 2-PAM (Hobbiger and Sadler 1958) and LüH-6 in case of tabun inhibition (Hobbiger and Vojvodic 1966).

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Trimedoxime was the first oxime that was efficient in the treatment of animals intoxicated with tabun (Schoene and Oldiges 1973) and at the same time, shown in mice that its median lethal dose (LD_{50}) is 3, 4 and 8 times lower than respective LD_{50} of LüH-6, 2-PAM and HI-6 (Clement 1981) therefore TMB-4 is the most toxic oxime among the others (Bokonjic et al. 1993).

Obidoxime

Obidoxime or LüH-6 Cl_2 was named in honour of Lüttringhaus and Hagedorn who synthesised it in Germany and introduced into medical practice in 1964. Chemically, obidoxime is a [1,1-bis (4-hydroxyimino-methyl-1-pyridinium)-2-oxapropane] dichloride (Lüttringhaus and Hagedorn 1964). Obidoxime showed a significant potential, as antidote in organophosphorus compound poisoning and up to now, it is one of the most active reactivator (Erdmann and Engelhard 1964). Obidoxime was more efficient than TMB-4 as antidote against tabun poisoning (Heilbronn and Tolagen 1965).

HI-6

The HI-6 was synthesised in 1966 and given the code name HI-6, after the last name of Ilse Hagedorn and the first name of her student Irmo Stark, chemists who synthesised it in Freiburg, Germany in 1966 (Schoene 1967; Hagedorn and Gündel 1967). Chemically it is [1-(2-hydroxyimino-methyl-1-pyridinium)-3-(4-carbamoyl-1-pyridinium)-2-oxapropane] dichloride. It was the first oxime that could reactivate soman-inhibited AChE and it was more potent than obidoxim against poisoning with sarin and VX (Clement 1982; Inns and Leadbeater 1983). The only drawback of HI-6 was that this oxime could not reactivate tabun-inhibited AChE (Clement 1982, Cetkovic 1984). The intrinsic toxicity of HI-6 is lowest among the aforementioned oximes, with the LD_{50} values as high as 781.3 mg/kg in rats (Dawson 1994; Rousseaux and Dua 1998).

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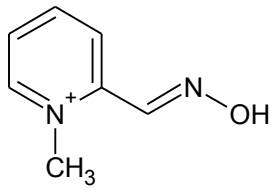
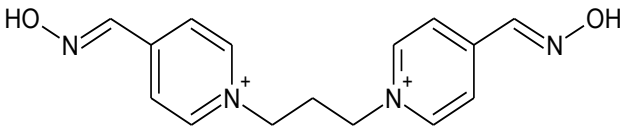
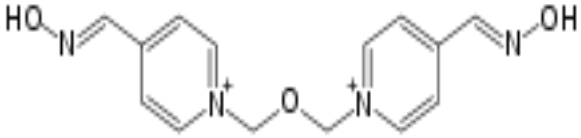
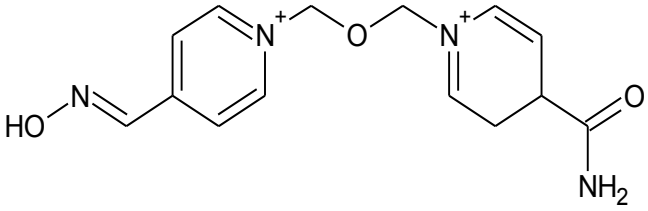
Table 4. Name, chemical structure, calculated logP and total polar surface area (TPSA) of classic pyridinium aldoximes

Compound	Chemical structure	LogP	TPSA ($Å^2$)
Pralidoxime (2-PAM)		-2.38	36.47
Trimedoxime		-2.65	72.94
Obidoxime		-3.04	82.17
HI-6		-3.16	92.6

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5.2.1. Mechanism of acetylcholinesterase reactivation

Cholinesterases have two binding sites: (1) catalytic site (anionic and esteratic) and (2) allosteric site. The allosteric site is catalytically inactive. Oximes bind to cholinesterases either at the catalytic site or at the allosteric site, or at both sites of the enzymes (Primozic et al. 2004).

Figures 17 and 18 below explain that electropositive nitrogen on the oxime (2-PAM) is attracted to electronegative anionic site on cholinesterase. Then the oxime is oriented proximally to exert a nucleophilic attack on the phosphorus and form the enzyme-inhibitor complex.

The enzyme-inhibitor-oxime complex is then split off, leaving the regenerated enzyme. However, this cannot happen after aging has occurred (de Jong and Ceulen 1978).

Phosphylated oximes formed during the reactivation process might be potent inhibitors of cholinesterases, which could cause re-inhibition of the previously reactivated enzyme. Re-inhibition of AChE can be faster than reactivation in case when a phosphylated oxime inhibits the enzyme at a rate higher than that of its elimination or decay to non-toxic products (Thiermann et al. 1999).

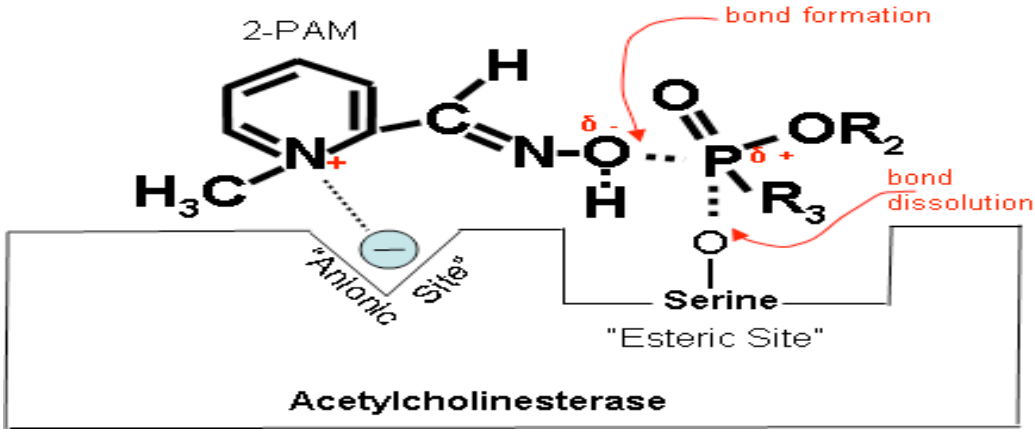

Figure 17. Partially electropositive nitrogen on the oxime (2-PAM) is attracted to electronegative anionic site on cholinesterase. δ + Indicates that phosphorus is partially electropositive. δ – Indicates that oxygen is partially electronegative (Wiener and Hoffman 2004)

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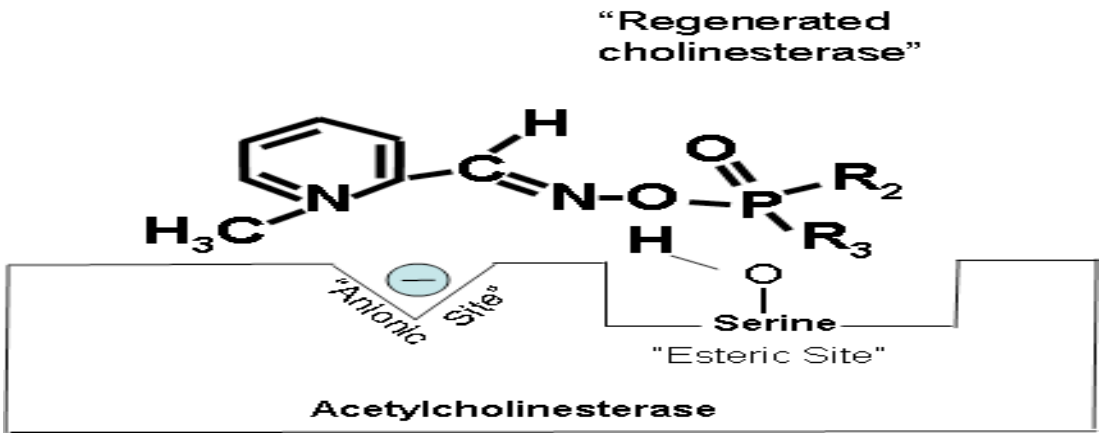

Figure 18. Regenerated cholinesterase (Wiener and Hoffman 2004)

5.2.2. Application of acetylcholinesterase reactivators

Clinical view shows of oximes as adjuncts in the therapy of organophosphates poisoning. There are many factors that influence the beneficial role and efficacy of oximes for therapy therefore there would be very limited basis for choosing an effective oxime.

1. An oxime may be effective against a specific organophosphate and ineffective for others (Skrinjaric et al. 1973). For example 2-PAM is very efficient in reactivating AChE inhibited with sarin or VX (Nozaki and Aikawa 1995) but is inefficient in the reactivation of the tabun-inhibited and soman-inhibited enzyme. TMB-4 is a powerful reactivator and was the first oxime efficient in the treatment of animals intoxicated with tabun (Bokonjic et al. 1993) also effective against sarin or VX poisoning but is totally ineffective against soman (Inns and Leadbeater 1983).

2. Oximes are not equally effective and rank order of effectiveness changes with the organophosphate compound involved. Even if the same dosage regimen of an oxime is administered, due to interindividual variations, different plasma concentrations will be obtained therefore dosing and time of treatment plays also vital role for successful treatment (Skrinjaric et al. 1973). HI-6 is that this oxime cannot reactivate tabun-inhibited AChE (Clement 1982).

3. Acetylcholinesterase inhibited by OPs may undergo a secondary reaction in the different time period, i.e., spontaneous dealkylation through alkyl-oxygen bond scission (aging), resulting in an irreversibly inactivated enzyme and cannot be reactivated by oximes (Skrinjaric et al. 1973).

5.2.3. K-compounds

In the Department of Toxicology at the Faculty of Military Health Sciences, Defence University, Czech Republic several promising asymmetric pyridinium aldoxime compounds were synthesized by Kamil Kuca and Kamil Musilek and these compound were named K-oximes (Kuca et al. 2003b; Musilek et al. 2005).

These AChE reactivators are nucleophilic compounds and react with the nerve agent after it has been bound to the cholinesterase. K-oximes were basically targeted for tabun intoxication. More than 500 structurally different K-oximes have been synthesized since 2003 (Kassa et al. 2008).

The reactivating potency of the newly synthesized pyridinium aldoximes (K-027 and K-203), were compared with that of the classic mono- and bis-aldoximes. The oximes K-075 and K-203 showed higher potency to reduce tabun-induced acute lethal toxicity than obidoxime or HI-6 (Kassa et al. 2008; Musilek et al. 2007c).

The most promising among K-compounds are K-027, K-048 and K-203, which worked well against pesticide poisoning as well. The efficiency of treatment decreases in the following order: K-027 > K-048 > K-203 > methoxime > BI-6 > pralidoxime (Petroianu et al. 2007).

Structurally all the K-oximes are either asymmetrical or symmetrical bispyridinium aldoximes with changes in the position of functional aldoxime as well as in some cases changes in linker chain. Pyridinium aldoximes are polar organic compounds with large negative lipophilicity (logP) values and it has been widely demonstrated that they are highly hydrophilic, therefore can hardly penetrate in the BBB (Kalasz et al. 2015).

The chemical structure and values of lipophilicity of novel pyridinium aldoximes synthesized by Kuca's group: K-027 (Kuca et al. 2003a); K-048 (Kuca et al. 2003b); K-074 and K-075 (Kuca et al. 2005); and K-203 (Musilek et al. 2007a, 2007b, 2007c) are shown in Table 5.

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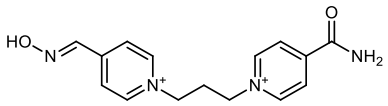
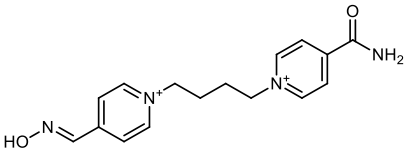
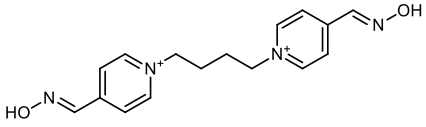
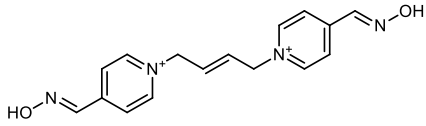
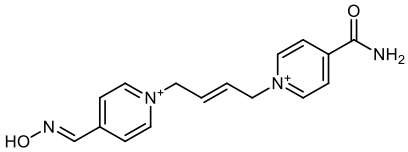
Table 5. The chemical structure, calculated logP and total polar surface area (TPSA) of some promising synthesized pyridinium aldoximes

№	Name	Chemical structurs	LogP	TPSA (Å^2)
1	K-027		-3.03	83.44
2	K-048		-3.02	83.44
3	K-074		-2.60	72.94
4	K-075		-2.71	72.94
5	K-203		-3.11	83.44

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6. Aims and objectives

6.1. Optimize a sensitive bioanalytical method according to the validation requirements for determination of the biogenic amines and their metabolites by HPLC-EC

6.2. Determine the possible hormonal imprinting effect of oxytocin and examine it's effect on the biogenic amine and their metabolite levels of the adult rat brain

6.3. Determine the effect of K-203 (AChER), a potential antidote in OPs intoxication on the biogenic amine and their metabolite levels of the adult rat brain

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7. Materials and Methodes

7.1. Materials

7.1.1. Experimental animals

During all the experiments, the guiding principles in the care of and use of laboratory animals have been observed. All experimental procedures conformed to 86/509/EEC regulation on the well-being of experimental animals, and the experimental protocol was approved by the local ethical committee (permission No: 1806/007/2004 ANTSZ, Budapest, Hungary).

Wistar rats of our Charles River originated closed breeding colony (Toxicoop, Budapest, Hungary) were housed in polypropylene cages (43 x 22.5 x 20.5 cm) at room temperature (22-24 °C), humidity (55±6%), and 12h light-dark cycle with light on at 7.00 am. Standard laboratory food and tap water were available *ad libitum.* Male Wistar rat's average weights in the K-203 experiments were 196±1.9g (Toxicoop, Budapest, Hungary).

7.1.2. Chemicals

Oxytocin acetate salt hydrate ($C_{43}H_{66}N_{12}O_{12}S_2$), dopamine hydrochloride (3,4-dihydroxyphenethylamine), DOPAC (3-4-dihydroxyphenylacetic acid), homovanillic acid (HVA), serotonin hydrochloride (5-HT), 5-hydroxy-3-indole acetic acid (5-HIAA), 5-hydroxytriptophol (5-HTOL) and phosphoric acid (H_3PO_4), disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 12H_2O$), citric acid monohydrate, l-octane sulfonic acid sodium salt, ethylenediaminotetraacetic acid disodium salt dihydrate (Na_2EDTA) and perchloric acid 70% (PCA) in the best available quality for HPLC were from Sigma-Aldrich (Steinheim, Germany). Acetonitrile was from Merck (Darmstadt, Germany).

K-203 (E-1-(4-carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide) was synthesized and kindly donated by K. Kuca (Department of Toxicology, Faculty of Military Health Sciences, Defence University, Hradec Kralove, Czech Republic).

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7.1.3. Instrumentation and chromatographic conditions

Samples were analyzed by reversed phase high-performance liquid chromatography with amperometric/electrochemical detection (HPLC–EC) consisting from a Jasco pump (PU1580, Tokyo, Japan) equipped with a DG-2080-54 four-line degasser, an AS 2057 Plus Automatic injector and connected to an Intro digital amperometric (Antec, Leyden, Zoeter-woude, Netherlands) detector operated at E_{ox}=+0.65 V with a sensitivity of 1-10 nA/V with a time filter of 1.0 sec, and JMBS Hercule 2000 Chromatography Interface (Le Fontanil, France). Standard temperature of the column was 25±0.15 °C. Chromatograms were electronically stored and evaluated using Borwin 1.50 Chromatography Software (JMBS, Le Fontanil, France). The separations were done using a Zorbax RX-C18 4.6x12.5 mm (5-µm) pre-column and a Zorbax RX-C18 4.6x250 mm, (5-µm) octadecyl silica column (Agilent Technologies, supplied by Kromat Kft, Budapest, Hungary).

For the serial determinations of the biogenic amines the mobile phase contained 56.2 mmol/L Na_2HPO_4, 47.9 mmol/L citric acid, 0.027 mmol/L Na_2EDTA, 0.925 mmol/L octane sulfonic acid sodium and 75:925 mL acetonitrile/phosphate buffer for hormonal imprinting oxytocin treatment and 65:935 mL acetonitrile/phosphate buffer for K-203 treatment. The pH was adjusted to 3.7 with 85% phosphoric acid (H_3PO_4) (inoLab pH Level 2, WTW GmbH, Germany). The flow rate of the mobile phase was 1 mL/min.

During the optimization we fully adapted the guideline for bioanalytical method validation of the FDA (Food and Drug Administration, Guidance for Industry - Bioanalytical Method Validation, 2001).

During the validation process, the following parameters were determined:

1) Calibration curve and Linearity: the calibration sequences from stock solutions were prepared at a concentration of at least 8 and measurements were replicated 3 times. Linearity was determined by calculation of a regression line from the peak area versus concentrations of standard solutions using the equation $y = mx + c$. A value of ≥0.98 would be considered acceptable for the correlation coefficient.

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2) Limit of detection and Limit of quantification: the limit of detection (LOD) is defined as the lowest concentration of the analyte giving a signal to noise ratio of 3:1. The limit of quantitation (LOQ) of the assay is the smallest concentration that can be quantitated reliably with a specified level of accuracy and precision, giving a signal to noise ratio of 10:1. The LOD and LOQ values were determind of the samples from at least six different animals. The limit of quantitation (LOQ) was determined at a signal to noise ratio of 10. The limit of detection (LOD) was determined at a signal to noise ratio of 3.

7.2. Methodes

7.2.1 Animals treatment

1) Oxytocin hormonal imprinting treatment: offspring of four females and two males were used in the experiment. Three females (185±12 g) were co-housed with one male for 8 days for mating and following this period females were housed separatedly during the 21 day period of pregnancy (number of offspring and treatment is shown in Table 6). Offsprings were treated subcutaneously (sc.) with a single dose of 5 mg/kg oxytocin (dissolved in saline) when they were one day old. At the end of the suckling period (in age of 21 days) offspring was housed separatedly from the mother. The 21 days old rats were selected according to their sex and were grown in standard cages.

Table 6. Number of offspring and treatment of rats

Abbreviation: **(**sc., subcutaneously)

Serial number of the mother	**Number of offspring**	**Treatment** (in the age of 24 h)
1	17	saline (sc. 0.1 ml)
2	16	saline (sc. 0.1 ml)
3	15	oxytocin (sc. 5 mg/kg, 0.1 ml)
4	22 (2 offspring dropped off by the end of the first day)	oxytocin (sc. 5 mg/kg, 0.1 ml)

No death of the treated rats was observed during the 4 months of the experiment. Weight gain of the rats was recorded weekly. The numbers of rats were 68, divided to four groups (14 control females, 19 control males, 17 oxytocin treated females and 18 oxytocin treated males).

When the offspring were fourth months old, they were exsanguinated through the canthus under ether anesthesia. Data are summarized in Table 7. The eight regions of the brain (hippocampus, HC; hypothalamus, HT; medulla oblongata, MO; spinal cord, SC; frontal cortex, FC; cerebellum, CB; striatum, ST; truncus cerebri, TC) were dissected on 0 °C aluminum surface according to the method of Paxinos G. and Watson C. (1998) and Palkovits M. (2001). The samples were kept frozen at -80 °C.

2) K-203 treatment: rats were injected in 0.2 mL volume intramuscularly (i.m) with 50 μmol of K-203 freshly dissolved in saline. The control group received an equal volume (0.2 mL) of solvent treatment. Five rats were used for each data point. Rats were sacrificed by decapitation 15 or 60 min following treatment. Seven regions of the brain (FC, HT, HC, ST, MO, CB and SC) were dissected and immediately placed on an ice-cold aluminum surface according to the method of Paxinos G. and Watson C. (1998) and Palkovits M. (2001). All samples were kept frozen at -80 °C until direct analysis.

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Table 7. Data of weight gain of the rats from weeks 3rd to 16th

Group of rats		Age (weeks)						
		3	4	5	6	7	8	9
		Weight (g± SD)						
Control	male (n=19)	55 ±0.8	81 ±0.7	114 ±0.9	138 ±1.0	198 ±1.9	234 ±1.4	256 ±3.1
	female (n=14)	54 ±0.5	78 ±0.8	100 ±0.9	119 ±2.1	129 ±2.3	136 ±3.1	141 ±3.9
Oxytocin imprinted	male (n=18)	55 ±0.6	79 ±0.8	113 ±1.4	137 ±1.6	199 ±2.3	231 ±2.6	258 ±3.0
	female (n=17)	55 ±0.5	80 ±0.7	101 ±1.1	120 ±1.2	131 ±1.6	135 ±2.3	139 ±2.3

Group of rats		Age (weeks)						
		10	11	12	13	14	15	16
		Weight (g± SD)						
Control	male (n=19)	271 ±2.3	320 ±5.2	329 ±4.6	332 ±4.1	349 ±5.8	342 ±6.9	359 ±7.4
	female (n=14)	156 ±5.1	174 ±4.8	228 ±5.3	238 ±5.2	236 ±4.9	239 ±6.0	254 ±5.6
Oxytocin imprinted	male (n=18)	272 ±3.5	322 ±3.2	331 ±4.1	334 ±4.2	348 ±3.9	356 ±6.0	358 ±7.4
	female (n=17)	152 ±2.7	168 ±2.9	219 ±3.2	229 ±2.9	241 ±4.6	243 ±4.5	258 ±6.9

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7.2.2. Samples preparation

1) Sample preparation in the oxytocin-experiment: The brain samples were homogenized in 5 volumes of 0.8 mol/L ice cold PCA and serum samples were homogenized in 10 volumes of 0.8 mol/L PCA by an Ultra Turrax T-25 Janke&Kunkel homogenizer (IKA Labortechnik, Staufen, Germany) at 20,000 rpm for 20 seconds then the brain samples were centrifuged in an Eppendorf centrifuge (A. Hettich, Tuttlingen, Germany) at 14,000 rpm for 10 min at 4 °C and the serum samples for 20 min at 4 °C. The supernatants gained were used for HPLC analysis. Samples were injected directly using a 50 μL loop for the HPLC. Samples were kept at -80 °C before their analysis.

2) Sample preparation in the K-203-experiment: Brain samples were homogenized in 4 volumes of 0.8 mol/L ice cold PCA by an Ultra Turrax T-25 Janke&Kunkel homogenizer (IKA Labortechnik, Staufen, Germany) at 20,000 rpm for 20 seconds and after centrifuged in an Eppendorf centrifuge (A. Hettich, Tuttlingen, Germany) at 14,000 rpm for 10 min at 4 °C and the supernatants were used for HPLC analysis. Samples were injected directly using a 50 μL loop for the HPLC. Samples were kept at -80 °C before their analysis.

7.2.3. Statistical evaluation

In the oxytocin-hormonal imprinting experiment the groups were compared by unpaired Student t-test. The significance level was set to 0.01 to decrease the type I error inflation due to the multiple testing.

In the K-203 experiment for each data point five rats were used. Comparisons were made using two-tailed Student test.

Data are given in nanogram per gram (ng/mg) wet tissue or in nanogram per milliliter (ng/mL) serum ± standard deviation (± SD), as indicated.

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8. Results

8.1. Calibration curve determination

The calibration sequences from stock solutions (100 μg/mL) were prepared at a concentration of at least 8 and measurements were replicated 3 times in the range of 2-200 ng/mL for oxytocin hormonal imprinting experiment and in the range of 10-200 ng/mL for K-203 experiment. Solvent used in the sample preparation was 0.8 M PCA solution and for spiked chromatograms the supernatant originating from the brain and serum samples gained from vehicle-treated controls were used. From all the areas of the peaks as a function of concentration the least squares method (Microsoft Excell 2003) was used.

8.1.1. Calibration curve determination for oxytocin imprinted rats

Samples were injected directly using a 10 μL, 20 μL, 50 μL and 95 μL loop for calibration curve. We had high peak in some brain areas from biogenic amines and their metabolites therefore we injected 10 μL and 20 μL instead of 50 μL for the brain samples (Tables 8 and 9). For the serum we injected 20 μL instead of 95 μL because of high peak in some biogenic amines and their metabolites (Tables 8 and 9). Calibration curves for all noradrenaline, adrenaline, DA, DOPAC, 5-HIAA, HVA, 5-HT, and 5-HTOL were linear in the range of 2-200 ng/mL (Figures 19 to 26).

The result of calibration curve equations and correlation coefficients associated values are shown in Table 10.

The mobile phase contained 10 g $Na_2HPO_4.2H_20$, 10 g citric acid, 10 mg Na_2EDTA, 200 mg octane sulfonic acid sodium and 60 mL acetonitrile + 940 mL distilled water for serum samples and 58 mL acetonitrile + 942 mL distilled water for brain samples. The pH was adjusted to 3.7 with 85% phosphoric acid.

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Table 8. Calibration curve determinations for oxytocin imprinted rats (DOPAC, noradrenaline, adrenaline and dopamine)

Noradrenaline	Area			
Conc. (ng/mL)	10 µL	20 µL	50 µL	95 µL
2	347740	-	3979563	3872746
5	803080	1704877	17396588	9889143
7	1097753	2612529	26116487	13954540
10	1828770	4055843	36711537	20317633
20	3311193	7912907	65987432	40195194
50	8435771	21503941	146869989	108179304
70	11756153	28778324	-	153024403
100	17122600	44850304	-	213643193
200	-	88628444	-	-
Adrenaline	**Area**			
Conc. (ng/mL)	10 µL	20 µL	50 µL	95 µL
2	-	-	5989652	3536062
5	-	2114252	13946355	9859454
7	-	3020085	21058413	13796064
10	-	4307792	30868993	20279713
20	-	7685563	-	39389388
50	-	21288509	-	107878837
70	-	28778324	-	145689723
100	-	42366348	-	202546081
200	-	83449402	-	-
Dopamine	**Area**			
Conc. (ng/mL)	10 µL	20 µL	50 µL	95 µL
2	1046587	-	12097886	5871884
5	1653538	2943208	29257089	15734738
7	1961428	4193768	43603394	21749401
10	3146756	6034794	59636600	31652916
20	6116403	12212677	-	65396102
50	14212353	31894370	-	167418773
70	19260885	40030902	-	-
100	28287880	61996559	-	-
200	-	126591755	-	-
DOPAC	**Area**			
Conc. (ng/mL)	10 µL	20 µL	50 µL	95 µL
2	245672	-	6606578	6315702
5	929731	2845308	14765523	16835756
7	1250539	4625997	22314477	24448377
10	1454890	6562438	33721468	34773460
20	2633253	14907509	53826426	77001416
50	7424476	36057070	128492991	186401307
70	10401240	47271076	-	-
100	13872860	72360535	-	-
200	-	143910013	-	-

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Table 9. Calibration curve determinations for oxytocin imprinted rats (5-HTOL, HVA, 5-HIAA and 5-HT)

5-HTOL	Area			
Conc. (ng/mL)	10 µL	20 µL	50 µL	95 µL
2	-	-	1832116	8326554
5	-	2639804	5063336	22377548
7	-	3035219	7982309	29276601
10	-	6535108	16525623	42710064
20	-	13963392	32464321	86273585
50	-	39196473	90142401	226421418
70	-	54281977	124088118	-
100	-	93829363	-	-
200	-	180636710	-	-
HVA	Area			
Conc. (ng/mL)	10 µL	20 µL	50 µL	95 µL
2	-	-	2225631	2869916
5	-	1106199	4218750	8267649
7	-	1676413	6224386	11810074
10	-	3061351	7879345	17860998
20	-	5936534	14739990	35476910
50	-	16325251	41814169	95911677
70	-	21488066	59166731	131089980
100	-	33169362	-	196047808
200	-	66464422	-	-
5-HIAA	Area			
Conc. (ng/mL)	10 µL	20 µL	50 µL	95 µL
2	703380	-	4189217	7771509
5	1075486	2644487	9182564	21860230
7	1296880	3583342	12929432	27993598
10	2050516	7365025	25690616	43796736
20	4494202	14795599	65783489	89367798
50	11896241	43758599	134190427	245411910
70	16216966	67845633	175984938	339146090
100	23695864	99836711	311037747	593470823
200	-	198994923	-	-
5-HT	Area			
Conc. (ng/mL)	10 µL	20 µL	50 µL	95 µL
2	-	-	5196918	4951718
5	-	2426641	14018901	19970029
7	-	4632881	18248287	31121121
10	-	7619882	32808296	44236314
20	-	17970476	64789273	99426648
50	-	49815291	148015974	269566345
70	-	70328209	189023829	392068457
100	-	113562312	339247379	571460053
200		222437360	-	-

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Tabale 10. Equations and correlations coefficient of the biogenic amines and their metabolites for oxytocin hormonal imprinting

Compound	Injections	Equation of the line	Correlation coefficient (R^2)
Noradrenaline	10 µL	y = 17037x- 34458	0.9990
	20 µL	y = 446524x – 780873	0.9993
	50 µL	y = 2865219x + 4382640	0.9978
	95 µL	y = 2163995x – 973203	0.9997
Adrenaline	20 µL	y = 418432x + 29494	0.9998
	50 µL	y = 3051840x - 201847	0.9963
	95 µL	y = 2059163x - 111872	0.9990
Dopamine	10 µL	y = 277901x + 261290	0.9993
	20 µL	y = 631215x - 715457	0.9988
	50 µL	y = 5991196x + 241594	0.9983
	95 µL	y = 3365100x - 1263972	0.9999
DOPAC	10 µL	y = 140328x + 171561	0.9970
	20 µL	y = 720752x - 527060	0.9994
	50 µL	y = 2538992x + 1963307	0.9998
	95 µL	y = 3766358x - 1236482	0.9995
5-HIAA	10 µL	y = 238933x - 267761	0.9997
	20 µL	y = 1015476x - 3869707	0.9994
	50 µL	y = 3102032x - 6369242	0.9962
	95 µL	y = 4930624x - 4753491	0.9995
HVA	20 µL	y = 334860x - 684757	0.9994
	50 µL	y = 838771x - 72735	0.9985
	95 µL	y = 1950838x - 1804389	0.9993
5-HT	20 µL	y = 1127273x - 4691714	0.9993
	50 µL	y = 3358820x - 4133180	0.9965
	95 µL	y = 5758899x - 10227901	0.9994
5-HTOL	20 µL	y = 925980x - 3306865	0.9985
	50 µL	y = 1821326x - 2374027	0.9995
	95 µL	y = 4536407x - 1646641	0.9996

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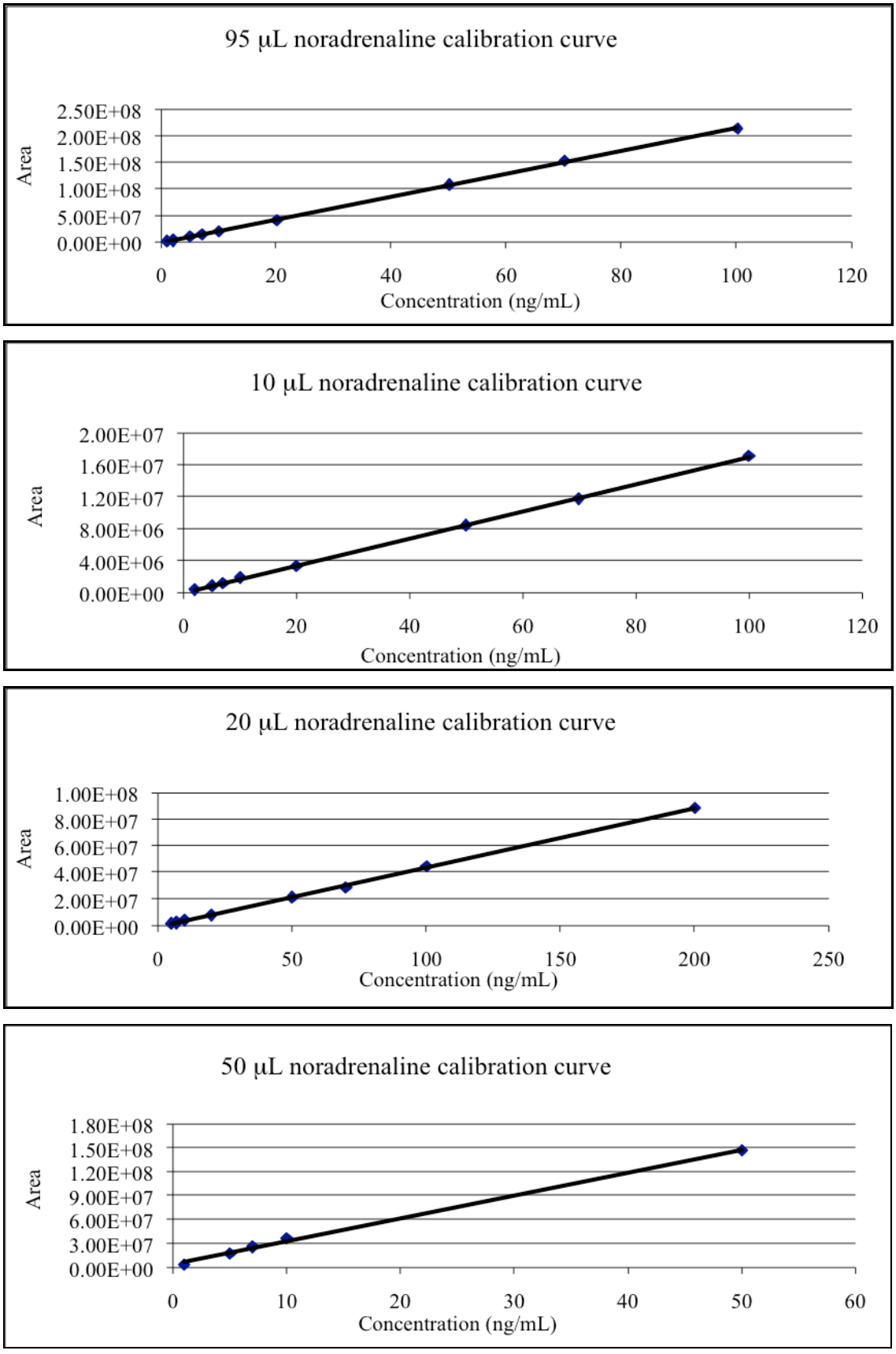

Figure 19. Calibration curves of the noradrenaline

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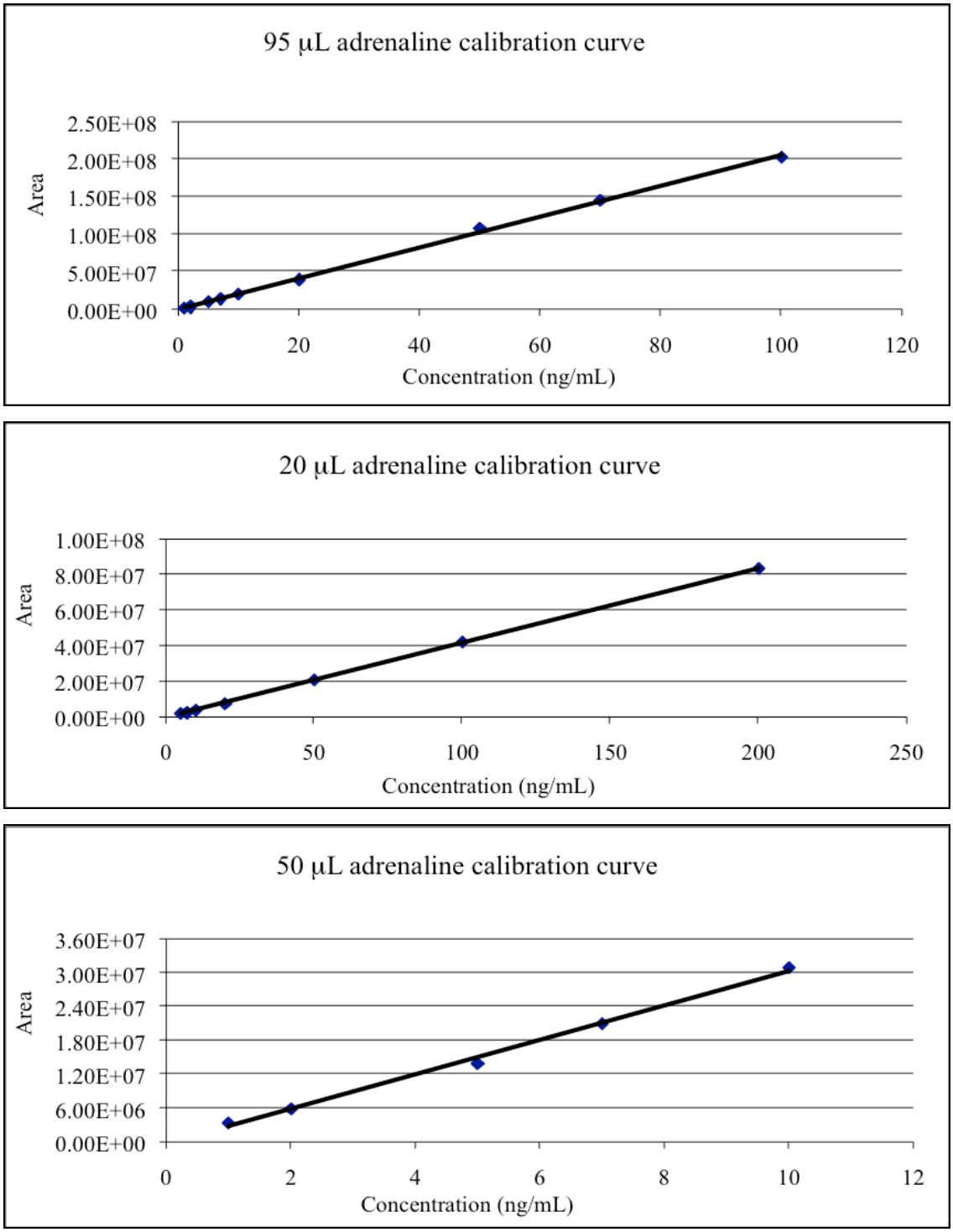

Figure 20. Calibration curves of the adrenaline

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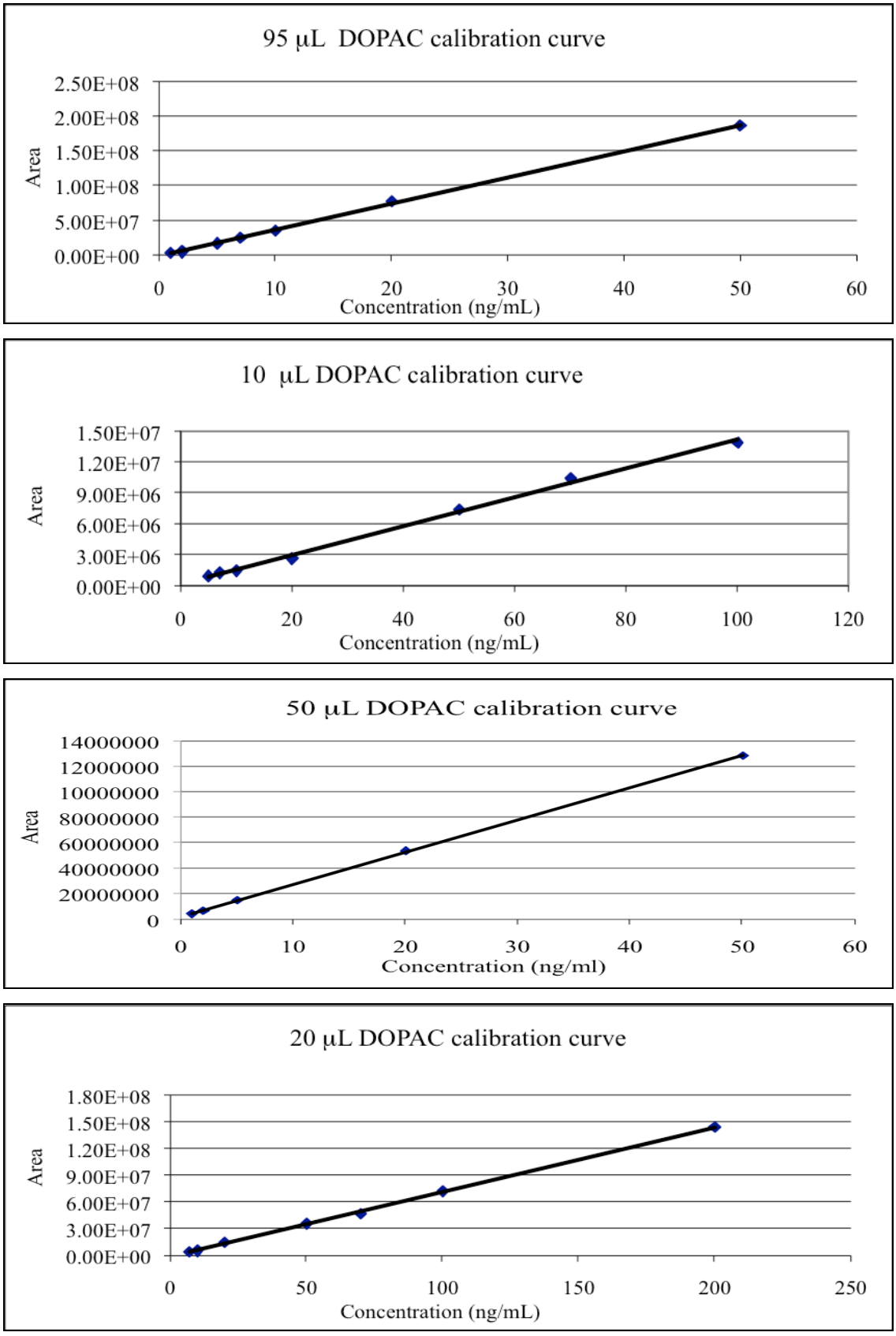

Figure 21. Calibration curves of the DOPAC

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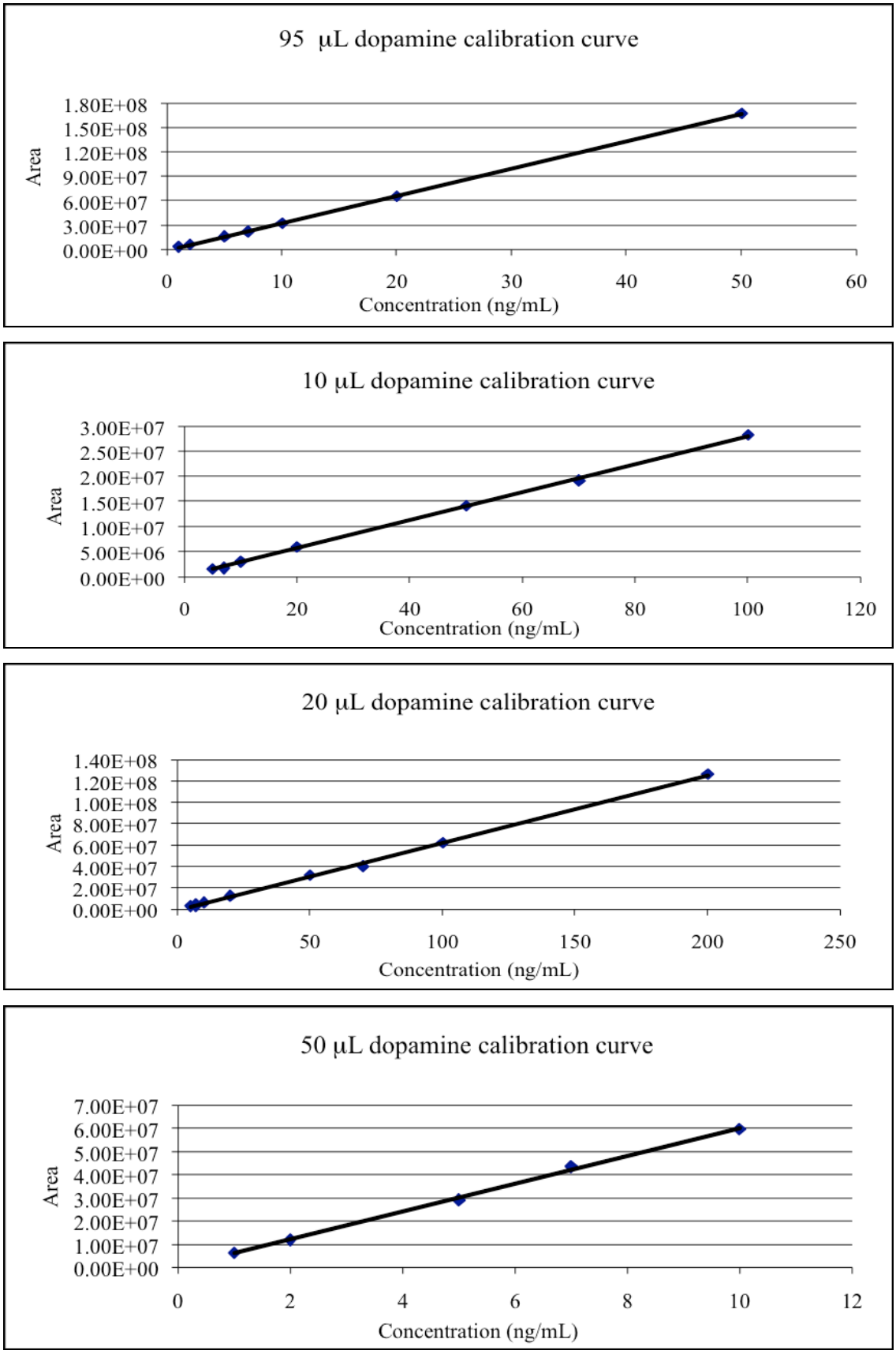

Figure 22. Calibration curves of the dopamine

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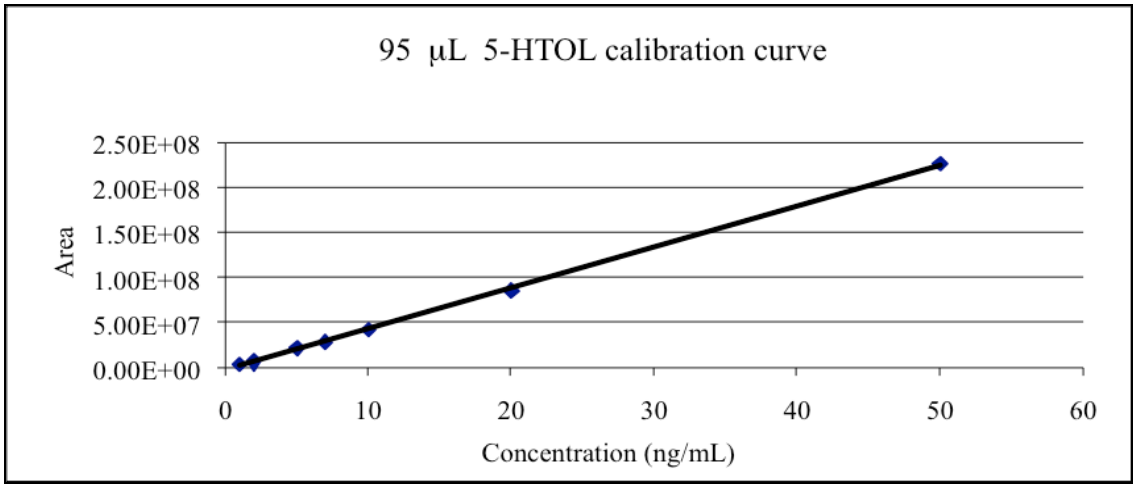

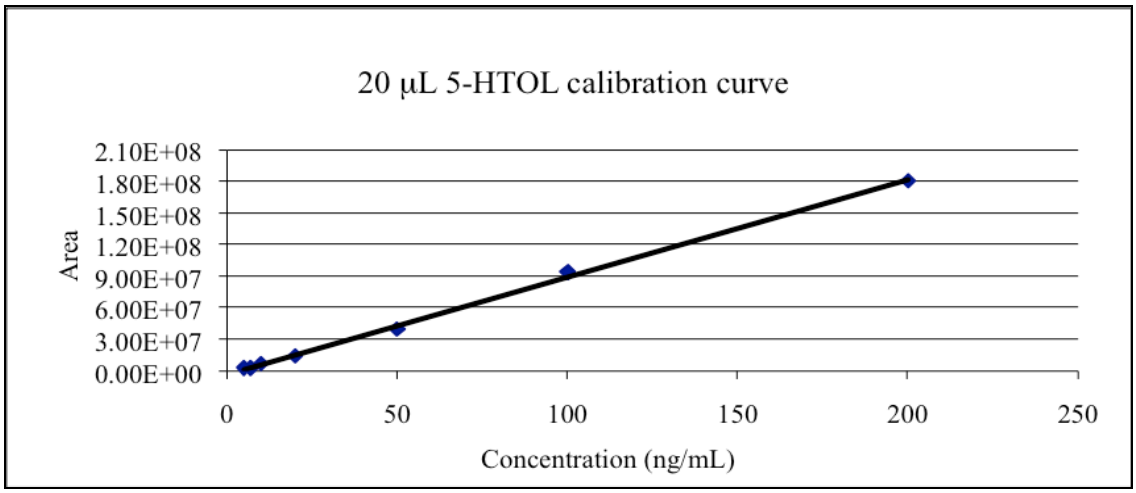

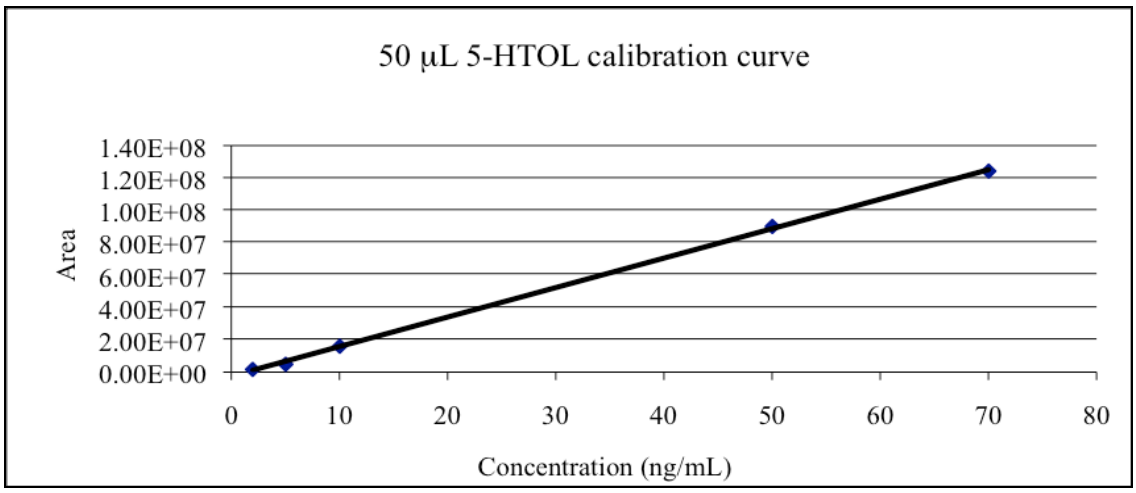

Figure 23. Calibration curves of the 5-HTOL

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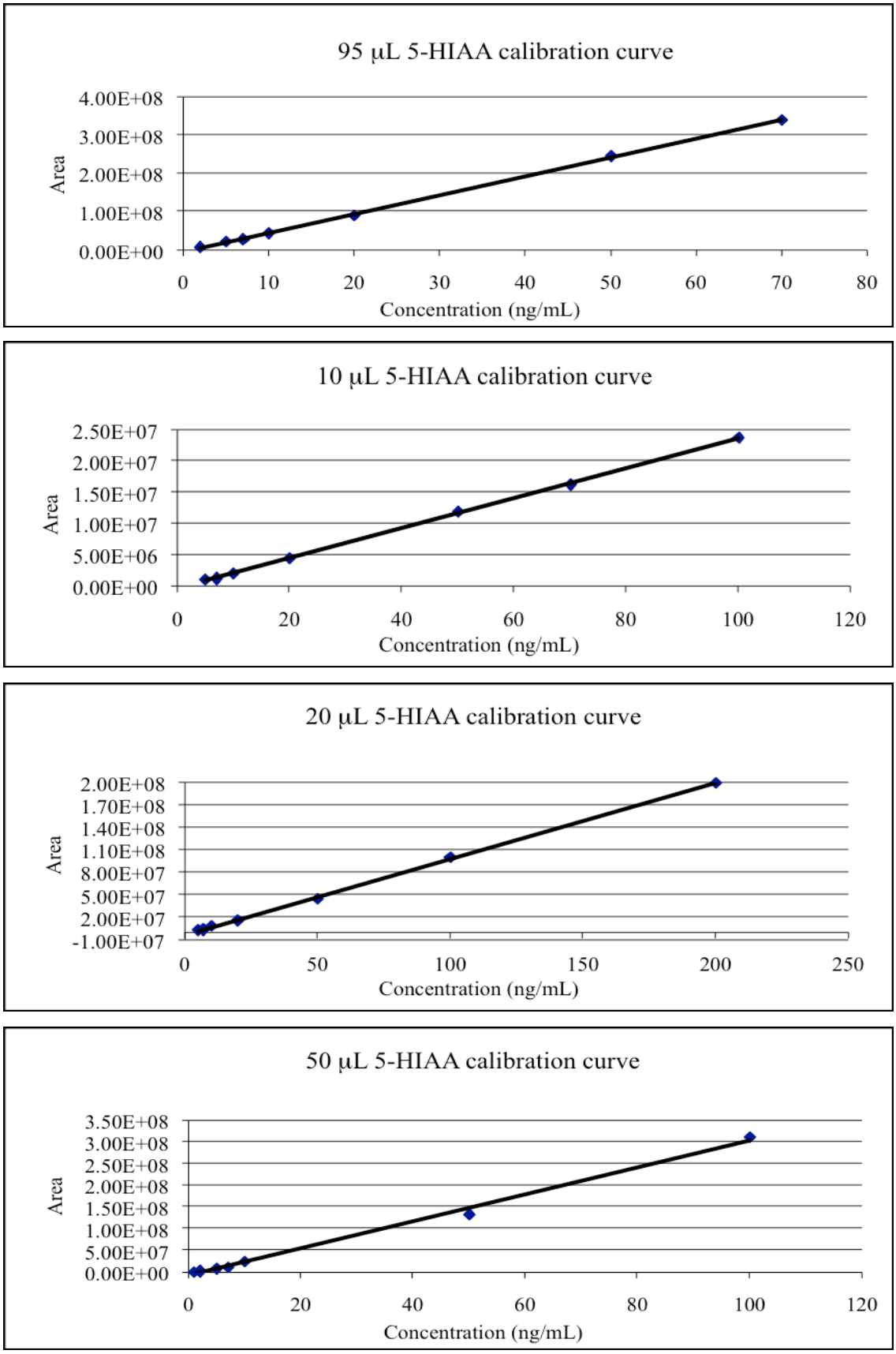

Figure 24. Calibration curves of the 5-HIAA

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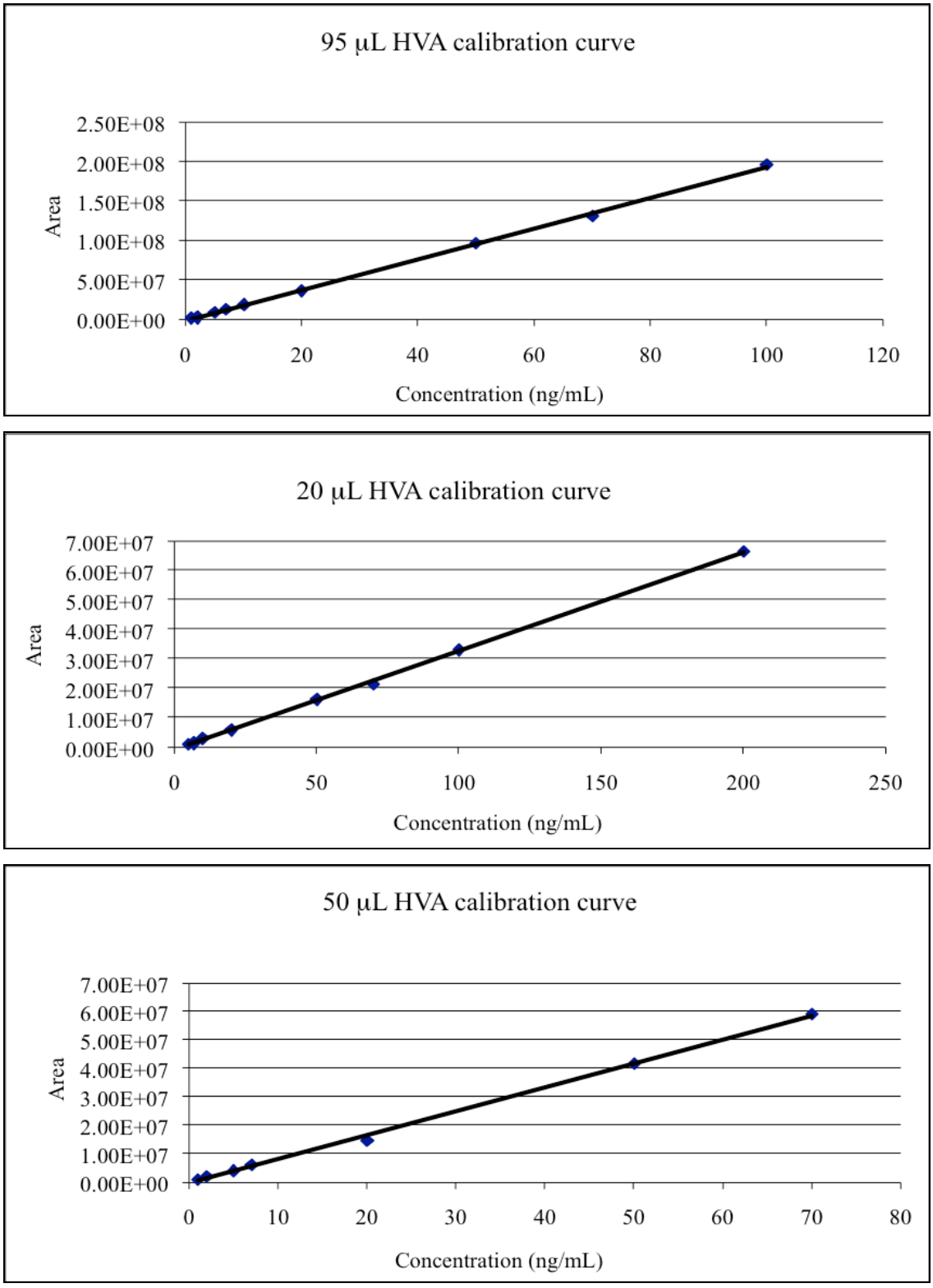

Figure 25. Calibration curves of the HVA

DOI:10.14753/SE.2015.1814

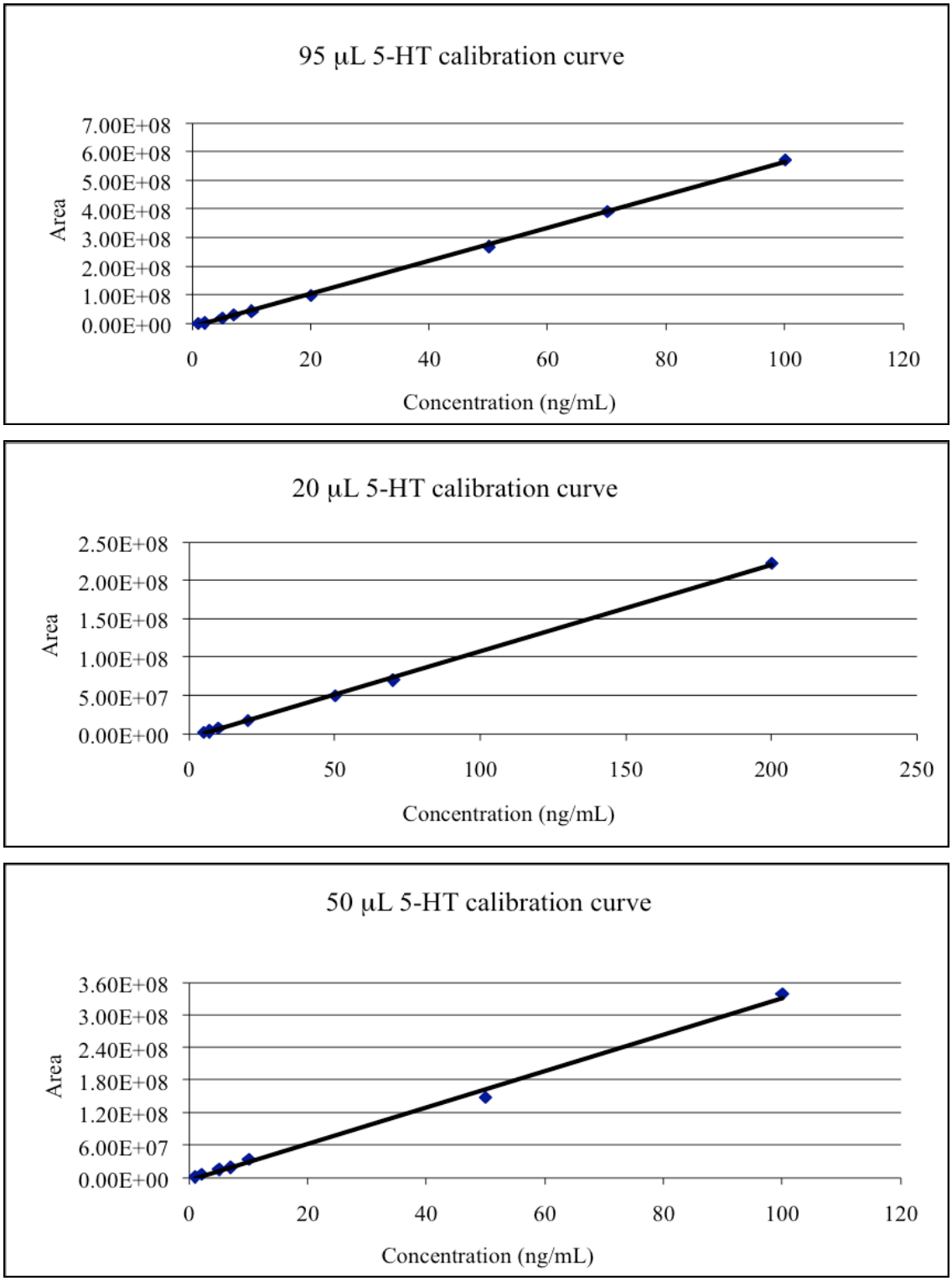

Figure 26. Calibration curves of the 5-HT

8.1.2. Calibration curve determination for K-203 experiment

Calibration curves for 5-HIAA, DA, 5-HT and HVA were linear in the range of 10-200 ng/mL (Table 11), (Figure 27 and 28). The mobile phase contained 10 g $Na_2HPO_4.2H_2O$, 10 g citric acid, 10 mg Na_2EDTA, 200 mg octane sulfonic acid sodium and 58 mL acetonitrile + 942 mL distilled water. The pH was adjusted to 3.7 with 85% phosphoric acid. The result of calibration curve equations and correlation coefficients associated values are shown in Table 12.

Table 11. Calibration curve determinations for K-203 experiment

Conc. (ng/mL)	Area				
	DOPAC	**DA**	**5-HIAA**	**HVA**	**5-HT**
10	2107237	1299503	3199622	1851745	4342112
20	3551228	2922396	4966639	3142909	8778696
50	9776046	8544953	15868174	7833025	24515830
70	13996648	12207819	22886880	10398717	32957089
100	20001750	18104646	33398157	15325711	47721998
150	30601423	27002304	52307823	23117444	73756397
200	41175108	36374237	71146700	31643179	101311988

Table 12. Equations and correlation coefficients of the biogenic amines and their metabolites for K-203

Compound	Equation of the line	Correlation coefficient (R^2)
5-HT	y = 507487x - 1586890	0.9991
5-HIAA	y = 361591x - 1882938	0.9990
Dopamine	y = 18515x - 66216	0.9990
HVA	y = 156414x - 76493	0.9990
DOPAC	y = 20698x - 42606	0.9990

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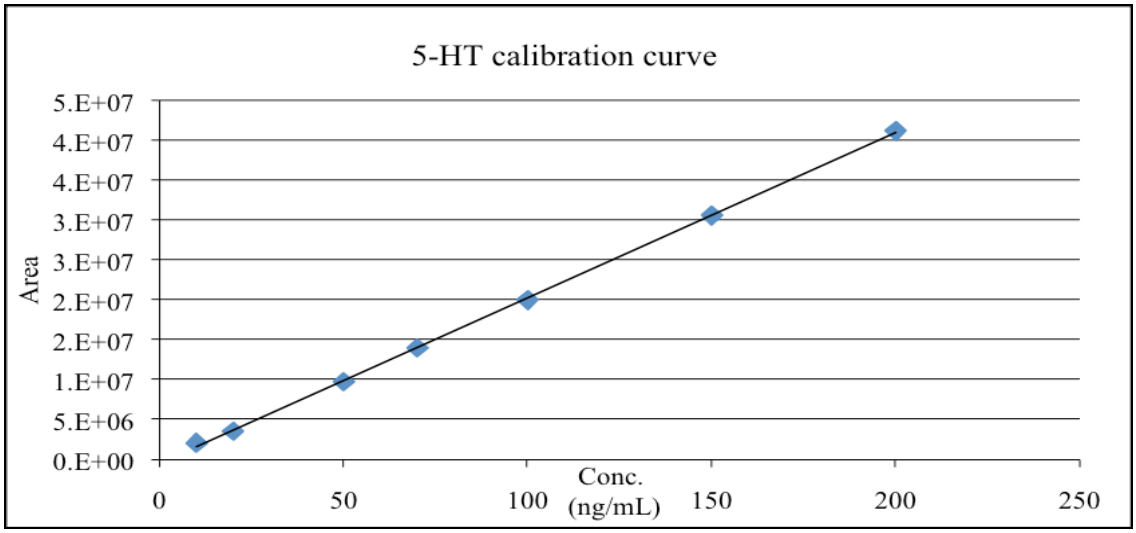

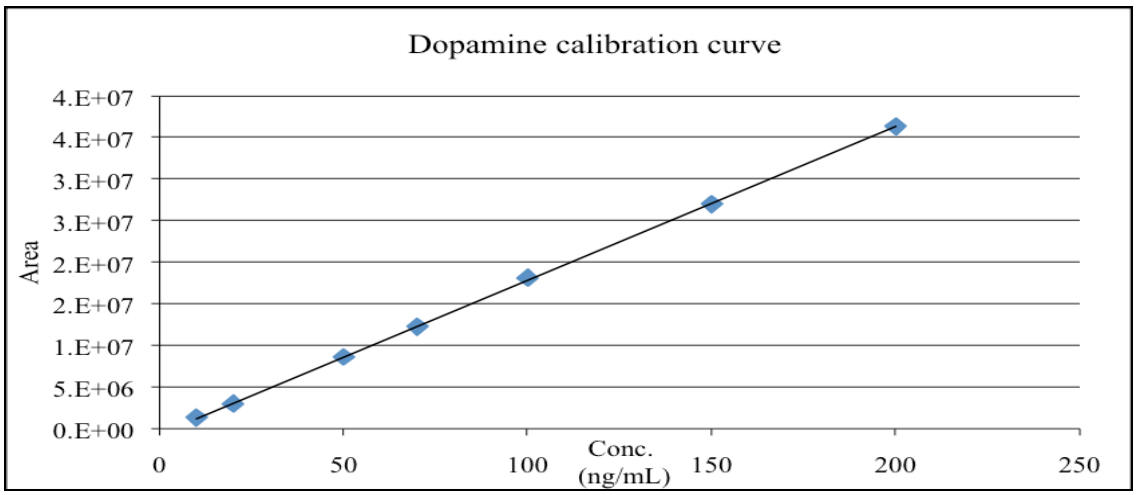

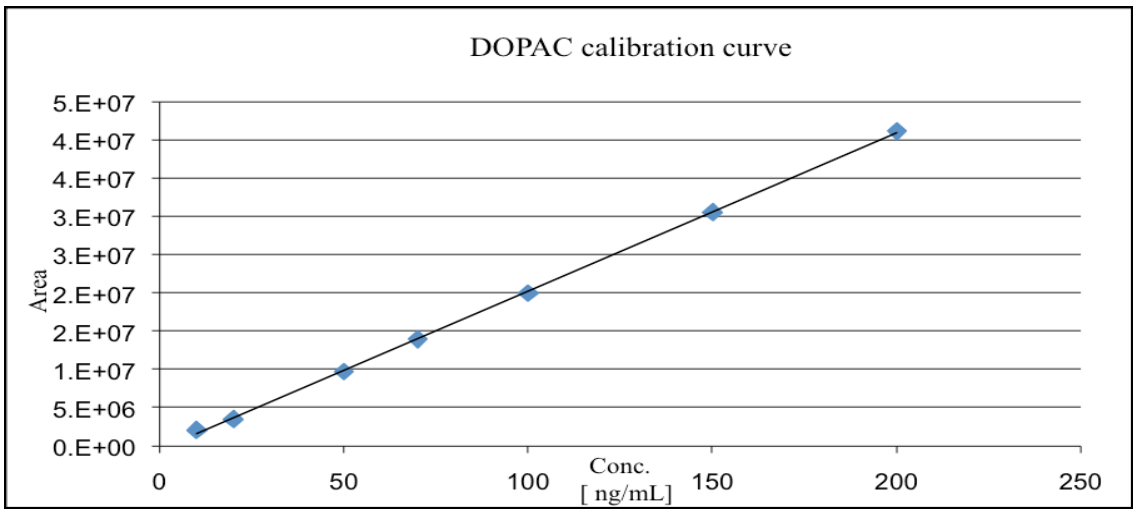

Figure 27. Calibration curves of the 5-HT, dopamine and DOPAC

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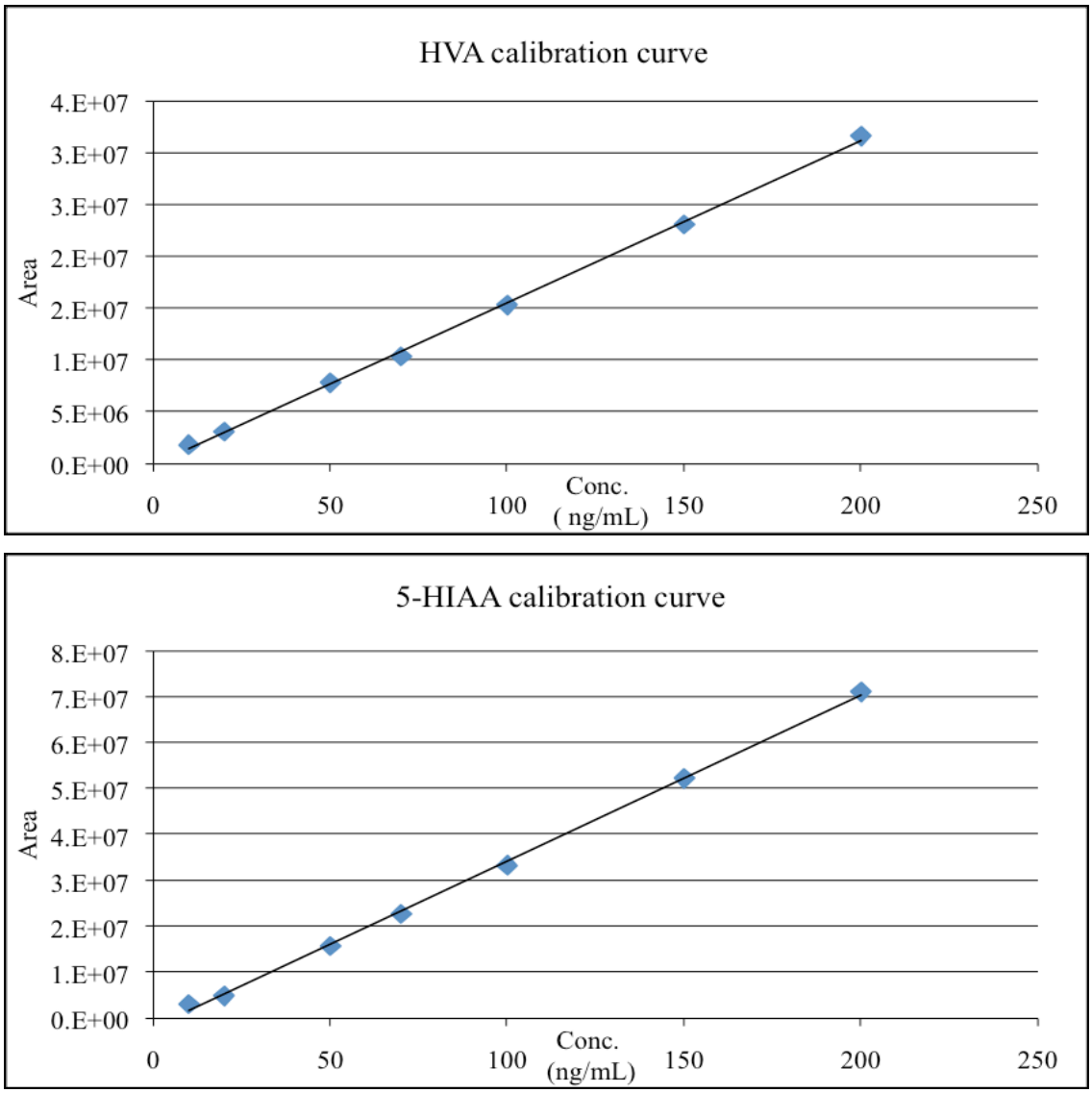

Figure 28. Calibration curves of the HVA and 5-HIAA

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8.2. Oxytocin hormonal imprinting measurement

Figure 29 and 30 show the representative chromatograms of biogenic amines (DA and 5-HT) and their metabolites (DOPAC, HVA and 5-HIAA) determinations from the oxytocin hormonal imprinted rat brain and serum. The following concentrations were used for spiking of the rat brain: 100 ng/g for DOPAC, 3 ng/g for dopamine, 100 ng/g for 5-HIAA, 100 ng/g for HVA and 5 ng/g for 5-HT (Figure 33).

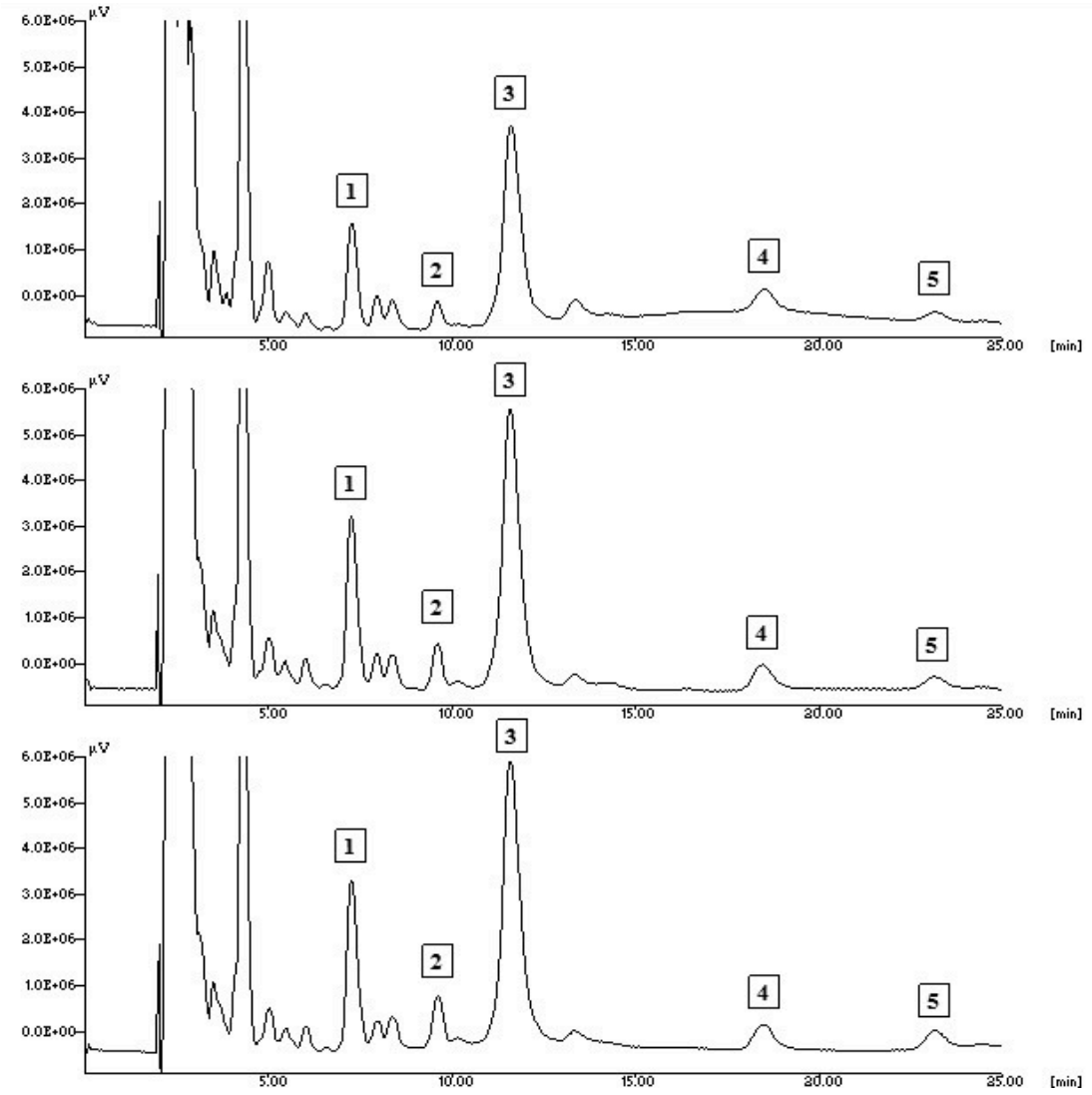

Figure 29. Representative chromatograms of biogenic amines and their metabolites (1: DOPAC; 2: dopamine; 3: 5-HIAA; 4: HVA and 5: 5-HT) tissue level from the rat brain (upper chromatogram: control animal, on middle chromatogram: spiked and on bottom chromatogram: oxytocin treated rats).

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The following concentrations were used for spiking of the rat serum: 2 ng/mL for DOPAC, 20 ng/mL for DA, 10 ng/mL for 5-HIAA, 50 ng/mL for HVA and 100 ng/mL for 5-HT (Figure 30).

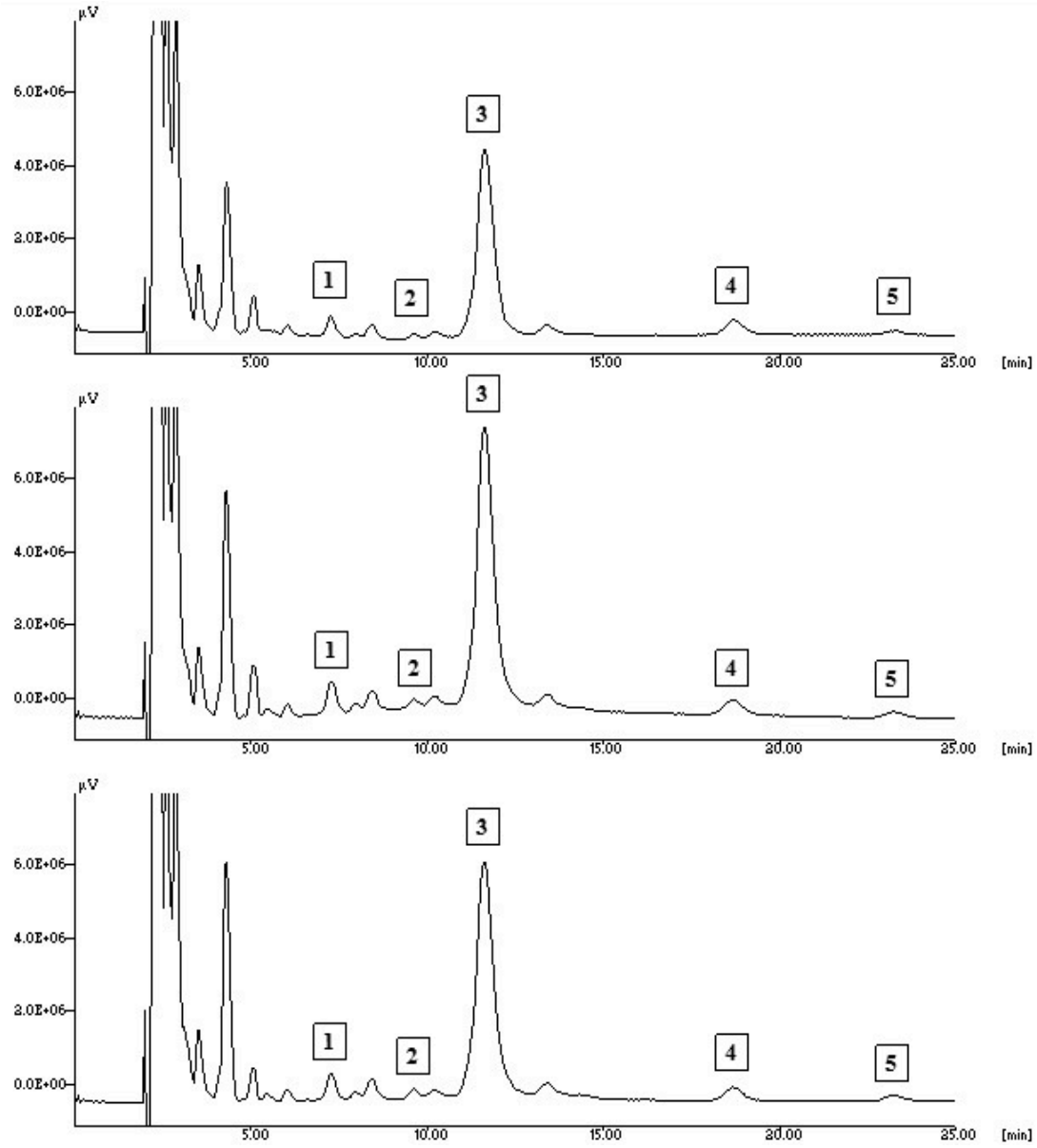

Figure 30. Representative chromatograms of biogenic amines and their metabolites (1: DOPAC; 2: dopamine; 3: 5-HIAA; 4: HVA and 5: 5-HT) tissue level from the rat serum (upper chromatogram: control animal, on middle chromatogram: spiked and on bottom chromatogram: oxytocin treated rats).

The results of the measurements from serum and eight regions of the brain parts (HC; HT; MO; SC; FC; CB; ST; TC) are shown in Tables 13 to 20.

Epinephrine

There is no significant difference in epinephrine tissue levels in the blood serum and different parts of brain regions between the control and oxytocin-treated animals (Table 13).

Table 13. The effect of neonatal oxytocin hormonal imprinting on the epinephrine tissue level in blood serum and brain areas of rats (Hashemi et al. 2013)

Group of Rats	Control		Oxytocin imprinted	
	Male	Female	Male	Female
Serum (ng/mL ± SD)	7.41 ± 1.4	10.54 ± 5.2	13.35 ± 1.25	10.44 ± 3.85
HT (ng/g ± SD)	5.46 ± 2.4	5.09 ± 2.6	5.55 ± 1.92	5.36 ± 2.51
MO (ng/g ± SD)	19.56 ± 5.18	20.64 ± 8.90	17.62 ± 3.19	17.22 ± 2.70
ST (ng/g ± SD)	11.30 ± 1.43	13.88 ± 4.46	10.40 ± 3.53	10.51 ± 3.35

Norepinephrine

There is a significant difference in norepinephrin tissue levels between the control males and females in case of the blood serum, HC, and FC in favour of the females. Oxytocin-treated male samples contain higher norepinephrine neurotransmitter levels in the FC and CB, while there is a decrease in the MO (Table 14).

Table 14. The effect of neonatal oxytocin hormonal imprinting on the norepinephrine tissue levels in blood serum and brain areas of rats ([a] P<0.05) (Hashemi et al. 2013)

Group of Rats	Control		Oxytocin imprinted	
	Male	Female	Male	Female
Serum (ng/mL ± SD)	227.86 ± 51.27[a]	269.32 ± 49.68[a]	248.15 ± 74.65	286.18 ± 88.91
HC (ng/g ± SD)	4.32 ± 1.06[a]	8.27 ± 2.85[a]	9.36 ± 5.65	17.85 ± 10.55
HT (ng/g ± SD)	41.23 ± 19.87	35.81 ± 12.58	43.28 ± 12.58	45.85 ± 11.66
MO (ng/g ± SD)	328.13 ± 67.67	343.01 ± 75.73	247.96 ± 68.66[a]	305.99 ± 92.77
SC (ng/g ± SD)	524.08 ± 43.86	422.16 ± 105.8	493.37 ± 60.65	417.93 ± 66.02
FC (ng/g ± SD)	56.34 ± 15.6[a]	84.14 ± 21.0[a]	186.79 ± 94.72[a]	125.21 ± 60.03
CB (ng/g ± SD)	15.23 ± 3.52	12.00 ± 3.6	17.09 ± 3.43[a]	15.96 ± 6.52
ST (ng/g ± SD)	2.24 ± 2.6	1.39 ± 1.6	5.24 ± 4.31	3.31 ± 1.19
TC (ng/g ± SD)	77.55 ± 18.6	91.92 ± 20.9	77.51 ± 31.79	59.98 ± 14.63

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Dopamine

There is a significant difference between the male and female blood serum dopamine content in favour of males. Treated males serum contains significantly less dopamine, then the control ones. However significantly elevated dopamine level is present in the FC in oxytocin-treated females (Table 15).

Table 15. The effect of neonatal oxytocin hormonal imprinting on the dopamine tissue levels in blood serum and brain areas of rats (^{a} P<0.05. ^{b} P<0.01), (Hashemi et al. 2013)

Group of Rats	Control		Oxytocin imprinted	
	Male	Female	Male	Female
Serum (ng/mL±SD)	164.84 ± 52.47^{b}	101.47 ± 44.43^{b}	116.35 ± 43.93^{b}	79.17 ± 33.35
HT (ng/g ± SD)	4.64 ± 0.54	4.45 ± 0.47	4.25 ± 2.21	7.58 ± 3.16
MO (ng/g ± SD)	1.94 ± 0.37	2.05 ± 0.70	2.32 ± 1.42	3.01 ± 1.21
SC (ng/g ± SD)	4.01 ± 0.72	3.70 ± 1.78	3.96 ± 0.94	3.86 ± 1.10
FC (ng/g ± SD)	0.96 ± 0.47	1.84 ± 1.45	1.97 ± 1.04	4.43 ± 2.15^{b}
ST (ng/g ± SD)	1451.5 ± 612.9	1181.95 ± 665.0	1628.69 ± 1003.9	1789.66 ± 917.31
TC (ng/g ± SD)	5.09 ± 7.99	9.51 ± 4.12	9.01 ± 8.57	4.31 ± 4.27^{a}

DOPAC (3, 4-Dihydroxyphenyl acetic acid)

There is a significant difference between the male and female DOPAC content in the HT in favor of males. Less DOPAC was found in male and female serum, HT, SC, male ST, and female TC oxytocin-treated rats compared to the control ones (Table 16).

Table 16. The effect of neonatal oxytocin hormonal imprinting on the DOPAC tissue levels in blood serum and brain areas of rats ([a] P <0.05. [b] P<0.01. [c] P<0.001), (Hashemi et al. 2013)

Group of Rats	Control		Oxytocin imprinted	
	Male	Female	Male	Female
Serum (ng/mL ± SD)	7.72 ± 1.8	8.75 ± 1.9	5.52 ± 0.73[c]	5.70 ± 1.31[c]
HC (ng/g ± SD)	5.01 ± 2.32	11.13 ± 6.8	4.68 ± 1.20	6.61 ± 2.66
HT (ng/g ± SD)	109.18 ± 13.2	131.81 ± 10.4	84.94 ± 15.6[a]	90.92 ± 31.0[a]
MO (ng/g ± SD)	47.51 ± 11.0	46.77 ± 8.17	41.28 ± 18.39	42.66 ± 11.15
SC (ng/g ± SD)	21.22 ± 7.7	17.41 ± 2.4	14.52 ± 2.17[a]	11.92 ± 1.27[b]
FC (ng/g ± SD)	55.18 ± 19.2	54.35 ± 11.1	80.58 ± 31.34	50.12 ± 9.29
ST (ng/g ± SD)	5859.4 ± 779.7	5782.6 ± 771.5	4578.05 ± 670.79[a]	4894.02 ± 1281.95
TC (ng/g ± SD)	148.28 ± 74.2	91.27 ± 54.9	75.69 ± 67.9	42.51 ± 27.8[a]

HVA (homovanillic acid)

There is a significant difference in homovanillic acid tissue levels between the control and oxytocin-treated animal serum, male HC, HT, ST, and TC. Control female truncus contains less homovanillic acid, than control male (Table 17).

Table 17. The effect of neonatal oxytocin hormonal imprinting on the HVA tissue levels in blood serum and brain areas of rats (^{a} P<0.05. ^{b} P < 0.01. ^{c} P<0.001), (Hashemi et al. 2013)

Group of Rats	Control		Oxytocin imprinted	
	Male	Female	Male	Female
Serum (ng/mL ± SD)	225.16 ± 24.8	242.18 ± 37.9	175.92 ± 20.04^{c}	197.25 ± 23.87^{c}
HC (ng/g ± SD)	48.54 ± 4.9	48.75 ± 1.6	40.79 ± 1.29^{c}	50.42 ± 3.01
HT (ng/g ± SD)	116.4 ± 13.3	125.61 ± 7.12	101.84 ± 16.73^{b}	115.89 ± 20.31
MO (ng/g ± SD)	160.32 ± 46.3	151.97 ± 7.0	149.73 ± 16.7	141.52 ± 19.6
SC (ng/g ± SD)	148.12 ± 28.0	22.71 ± 18.1	112.38 ± 18.8	111.16 ± 26.1
FC (ng/g ± SD)	1021.7 ± 111.8	1019.61 ± 00.8	920.791 ± 19.5	1038.291 ± 58.2
ST (ng/g ± SD)	278.07 ± 23.3	284.89 ± 30.8	234.98 ± 19.87^{b}	285.02 ± 34.03
TC (ng/g ± SD)	1672.05 ± 381.7^{a}	1155.26 ± 217.0^{a}	1209 ± 196.0^{a}	1124.58 ± 84.3

5-HIAA (5-hydroxy indole acetic acid)

There is a significant difference in 5-HIAA tissue levels between the male and female control sera in favor of females. Oxytocin-treated male 5-HIAA values are less in HC, HT, SC and ST as well as in female serum, HT, and MO. The female serum value is higher than male in the controls (Table 18).

Table 18. The effect of neonatal oxytocin hormonal imprinting on the 5-HIAA tissue levels in blood serum and brain areas of rats (^{a} P < 0.05. ^{b} P<0.01. ^{c} P<0.001), (Hashemi et al. 2013)

Group of Rats	Control		Oxytocin imprinted	
	Male	Female	Male	Female
Serum (ng/mL ± SD)	35.11 ± 7.39^{c}	69.00 ± 9.94^{c}	47.00 ± 12.31^{b}	56.37 ± 8.75^{c}
HC (ng/g ± SD)	151.74 ± 16.6	151.8 ± 11.9	134.40 ± 15.0^{a}	162.63 ± 21.8
HT (ng/g ± SD)	359.3 ± 48.25^{a}	414.27 ± 40.51^{a}	302.17 ± 26.1^{b}	302.10 ± 36.4^{c}
MO (ng/g ± SD)	948.6 ± 163.0	949.56 ± 159.0	797.61 ± 120.6	712.42 ± 76.4^{b}
SC (ng/g ± SD)	715.13 ± 159.5	621.1 ± 82.2	548.19 ± 53.19^{a}	564.57 ± 102.1
FC (ng/g ± SD)	836.8 ± 267.5	995.9 ± 175.7	850.37 ± 112.9	783.03 ± 57.93
CB (ng/g ± SD)	33.95 ± 5.82	28.36 ± 3.52	31.42 ± 3.99	27.59 ± 7.49
ST (ng/g ± SD)	177.05 ± 18.3	182.82 ± 9.3	159.31 ± 10.08^{b}	167.9 ± 313.16
TC (ng/g ± SD)	842.49 ± 267.5	995.6 ± 175.7	850.37 ± 112.49	783.03 ± 57.93

5-HTOL (5-hydroxytryptophol)

There is a significant decrease in 5-HTOL, tissue level in the HT in both males and females in oxytocin-treated animals and a lower level 5-HTOL in the male oxytocin-treated in MO (Table 19).

Table 19. The effect of neonatal oxytocin hormonal imprinting on the 5-HTOL tissue levels in blood serum and brain areas of rats ([a] P<0.05.[b] P<0.01), (Hashemi et al. 2013)

Group of Rats	Control		Oxytocin imprinted	
	Male	Female	Male	Female
HC (ng/g ± SD)	10.26 ± 0.78	10.53 ± 0.53	9.76 ± 0.90	10.45 ± 0.62
HT (ng/g ± SD)	11.31 ± 1.07	13.02 ± 1.54	10.02 ± 1.25[b]	9.94 ± 1.61[a]
MO (ng/g ± SD)	15.07 ± 1.54	15.22 ± 3.46	12.39 ± 1.72[a]	13.20 ± 1.84
SC (ng/g ± SD)	17.94 ± 2.99	15.72 ± 4.43	13.39 ± 1.29	19.57 ± 3.92
FC (ng/g ± SD)	45.67 ± 4.53	40.20 ± 4.23	38.94 ± 7.58	38.20 ± 10.88
TC (ng/g ± SD)	43.22 ± 11.05	39.90 ± 9.22	34.25 ± 9.12	43.01 ± 7.82

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Serotonin (5-HT)

There is a significant difference in the serotonin tissue levels between the oxytocin-treated male and female sera in favor of females. In the HT and the ST of females, the serotonin tissue level was higher than the female control values (Table 20).

Table 20. The effect of neonatal oxytocin hormonal imprinting on the 5-HT tissue levels in blood serum and brain areas of rats ([a] P<0.05. [c] P < 0.001), (Hashemi et al. 2013)

Group of Rats	Control		Oxytocin imprinted	
	Male	Female	Male	Female
Serum (ng/mL ± SD)	1299.25 ± 348.9	1399.38 ± 477.0	1306.59 ± 228.0	1561.43 ± 225.7[a]
HT (ng/g ± SD)	9.82 ± 1.52	8.69 ± 0.40	9.24 ± 2.18	14.78 ± 5.10[a]
MO (ng/g ± SD)	13.55 ± 2.72	15.68 ± 2.38	14.67 ± 2.48	19.93 ± 5.44
SC (ng/g ± SD)	30.43 ± 5.89	32.22 ± 11.11	25.75 ± 7.26	34.55 ± 7.68
ST (ng/g ± SD)	10.33 ± 1.44	12.13 ± 1.78	14.09 ± 5.33	18.77 ± 2.88[c]
TC (ng/g ± SD)	39.77 ± 16.6	41.54 ± 9.58	34.01 ± 13.30	35.3 ± 515.73

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8.3. K-203 treated rats

Figure 31, 32 and 33 show the representative chromatograms of biogenic amines (DA and 5-HT) and their metabolites (DOPAC, HVA and 5-HIAA) tissue levels that were obtained from the K-203 treated rats brain, CSF and serum.

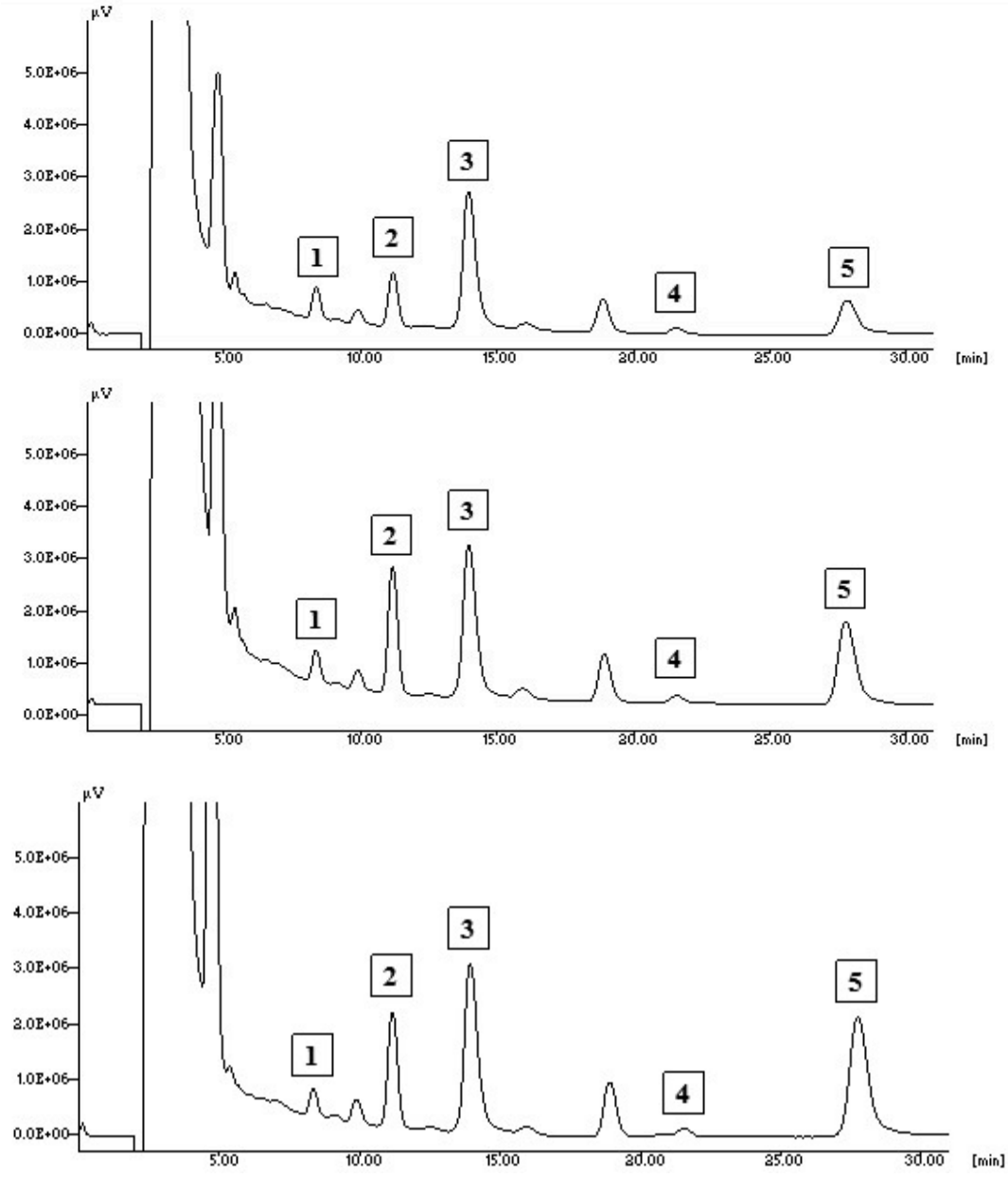

Figure 31. Representative chromatograms of biogenic amines and their metabolites (1: DOPAC; 2: dopamine; 3: 5-HIAA; 4: HVA and 5: 5-HT) tissue level from the rat brain (upper chromatogram: control animal, on middle chromatogram: spiked and on bottom chromatogram: K-203 treated rats).

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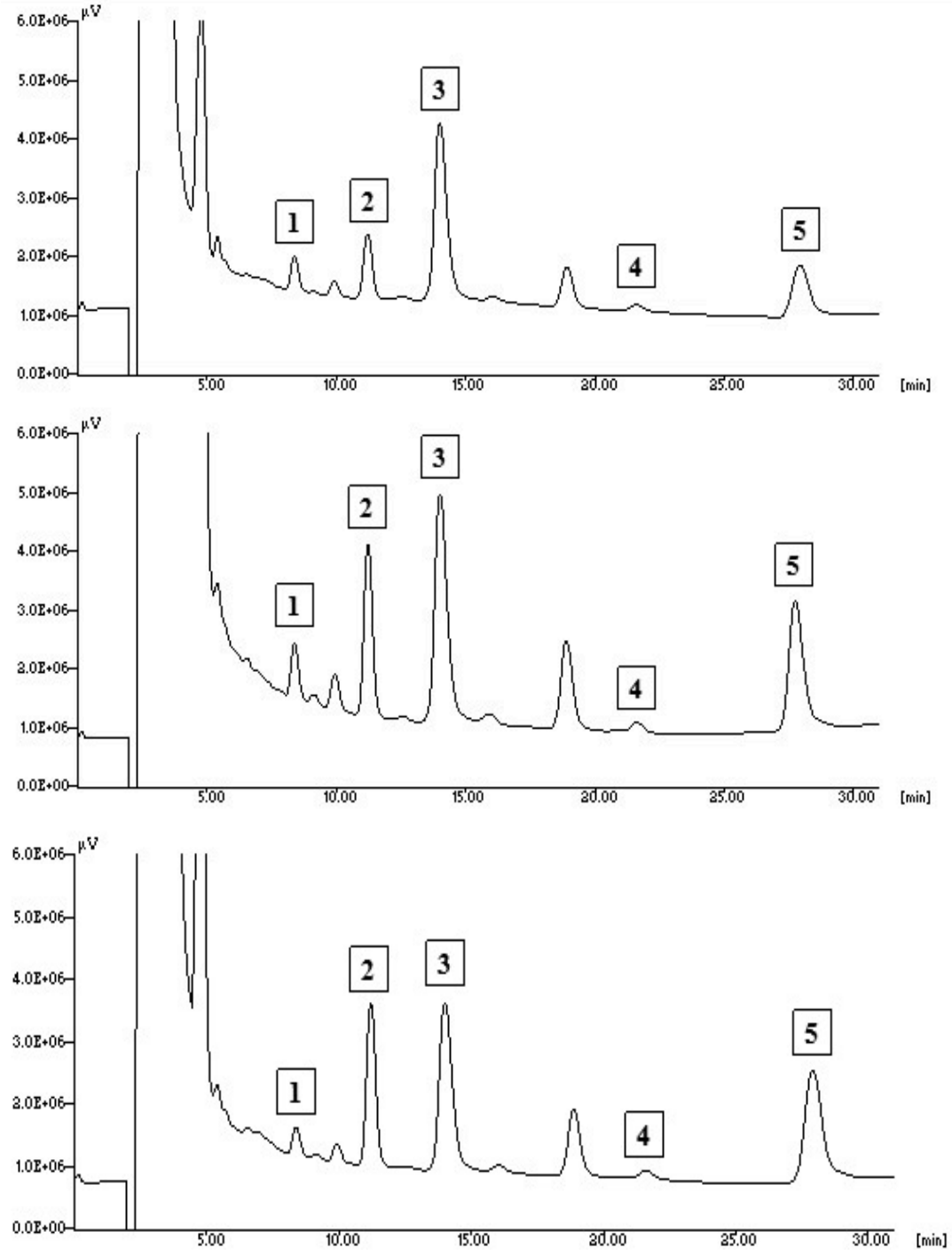

Figure 32. Representative chromatograms of biogenic amines and their metabolites (1: DOPAC; 2: dopamine; 3: 5-HIAA; 4: HVA and 5: 5-HT) tissue level from the rat CSF (upper chromatogram: control animal, on middle chromatogram: spiked and on bottom chromatogram: K-203 treated rats).

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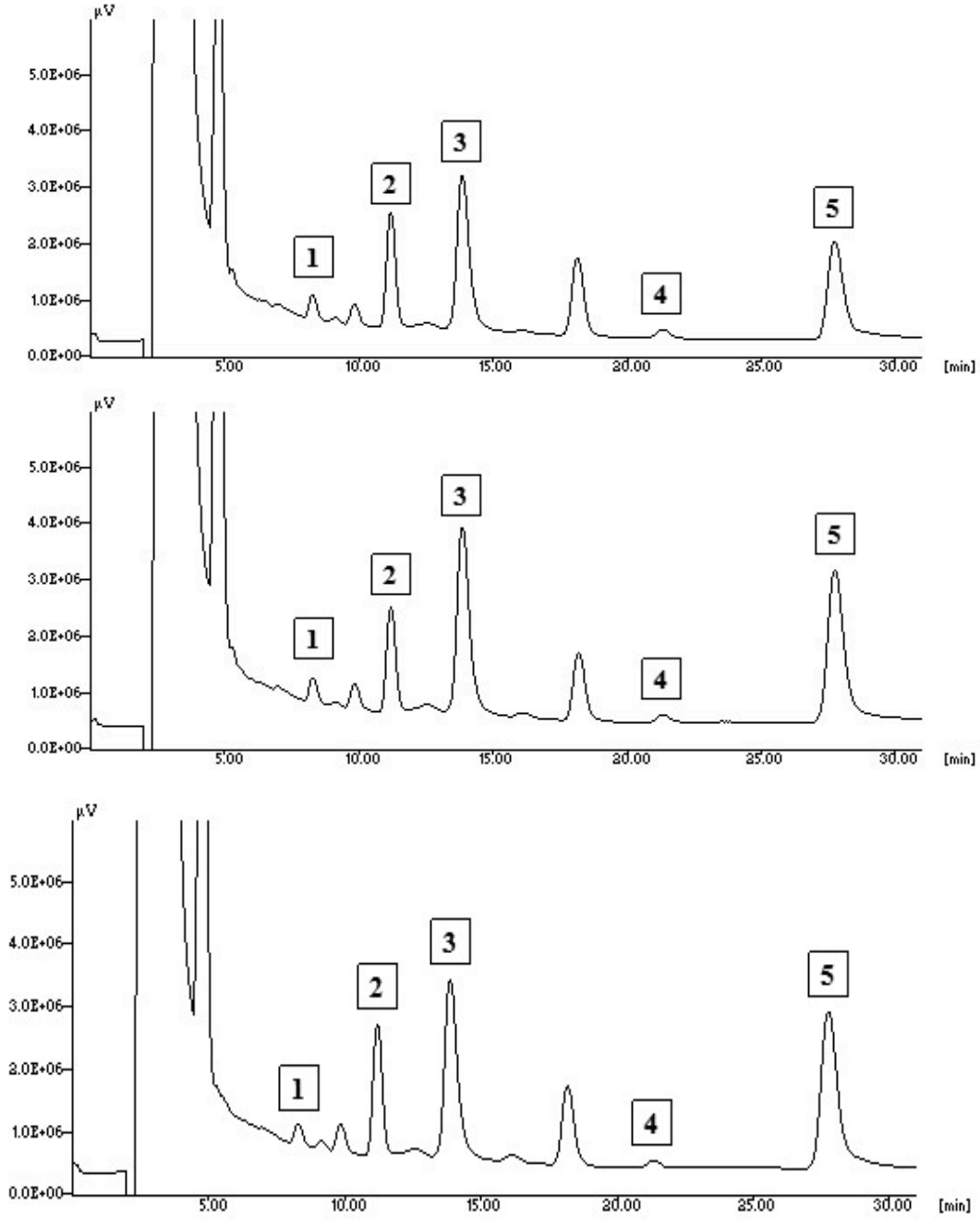

Figure 33. Representative chromatograms of biogenic amines and their metabolites (1: DOPAC; 2: dopamine; 3: 5-HIAA; 4: HVA and 5: 5-HT) tissue level from the rat serum (upper chromatogram: control animal, on middle chromatogram: spiked and on bottom chromatogram: K-203 treated rats).

Figures 34 and 35 show representative chromatograms of biogenic amines (DA and 5-HT) and their metabolites (5-HIAA) tissue levels in the spinal cord following 15 and 60 min of 50-μmol intramuscular (i.m) K-203 treated of rats.

Figure 36 show representative chromatogram of dopamine tissue level in the striatum following 60 min of 50-μmol intramuscular (i.m) K-203 treated of rats.

Table 21 summarizes biogenic amines (DA and 5-HT) and their metabolites (HVA and 5-HIAA) tissue levels in the different brain areas (CB, SC, HC, HT, ST, MO, FC) studied 15 and 60 min following 50-μmol intramuscular (i.m) K-203 injections.

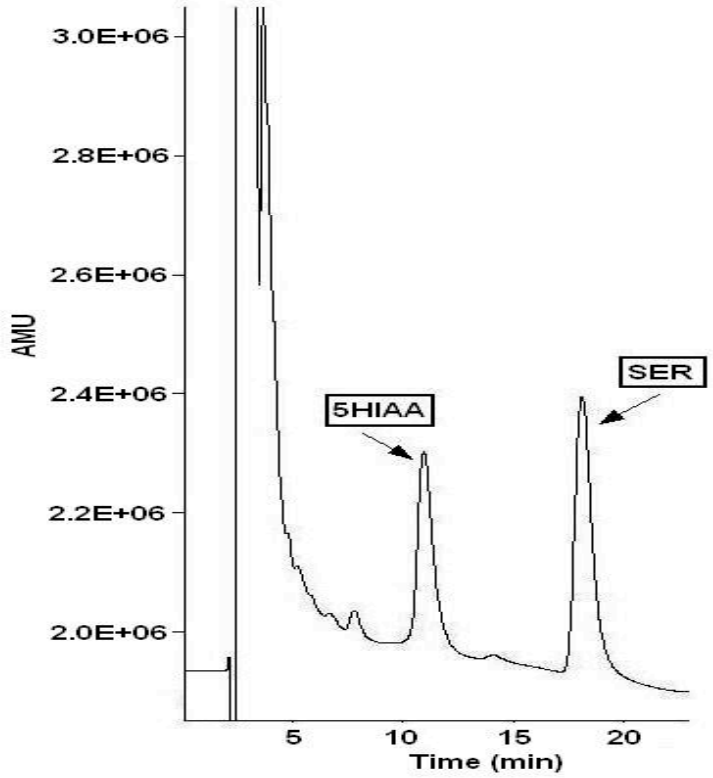

Figure 34. Representative chromatogram of serotonin (SER) and 5-HIAA levels in the spinal cord sample of rat following 15 min of K-203 (50-μmol, i.m) treatment

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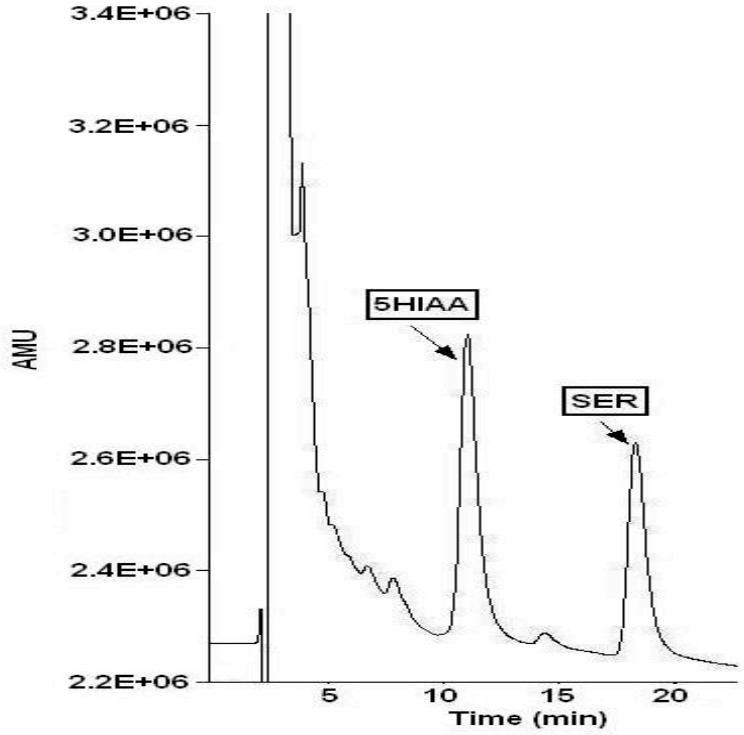

Figure 35. Representative chromatogram of serotonin (SER) and 5-HIAA levels in the spinal cord sample of rat following 60 min of K-203 (50-µmol, i.m) treatment

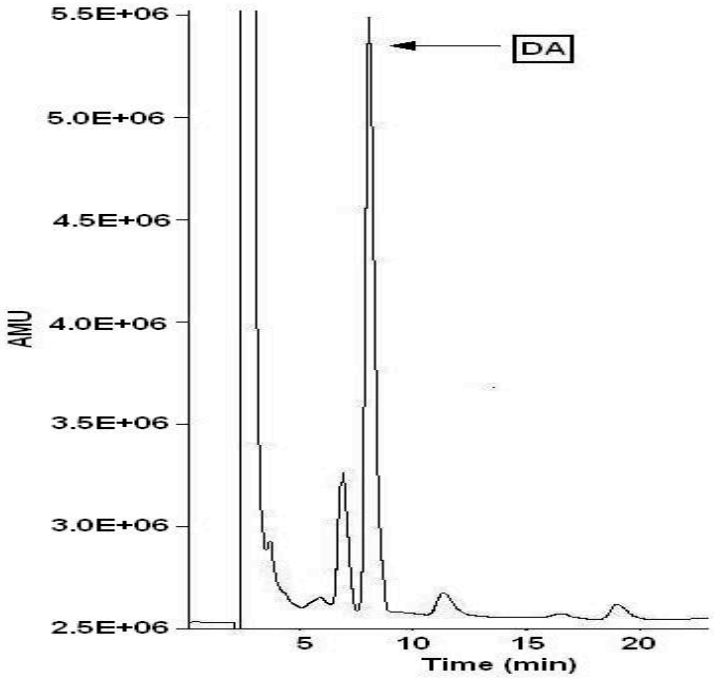

Figure 36. Representative chromatogram of dopamine level in the striatum sample of rat brain following 60 min of K-203 (50-µmol, i.m) treatment

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Table 21. Effect of K-203 on the biogenic amine and their metabolites tissue levels in rat brain areas. Each value represents data from five animals (n=5). (Hashemi et al. 2013)

	Tissue level			
Rat brain area	**5-HT (ng/g ± SD)**	**5-HIAA (ng/g± SD)**	**DA (ng/g± SD)**	**HVA (ng/g± SD)**
Cerebellum				
Control	21.33±4.24	114.1±8.97	16.23±0.62	< LOQ(1ng/g)
K-203				
15 min	42.82±11.0	125.1±11.1	17.43±1.09	
60 min	24.62±6.87	197.0±26.7	16.16±1.55	
Spinal cord				
Control	489.4±70.8	342.7±19.5	30.03±4.88	< LOQ(1ng/g)
K-203				
15 min	620.9±31.4	358.1±24.4	36.07±4.28	
60 min	440.1±11.1	426.2±62.1	33.85±2.35	
Hippocampus				
Control	32.17±5.65	170.4±34.3	13.04±0.31	< LOQ(1ng/g)
K-203				
15 min	52.08±8.55	177.1±10.8	14.07±0.82	
60 min	37.50±10.4	186.5±7.60	14.11±0.94	
Hypothalamus				
Control	108.2±10.3	263.0±64.1	65.24±8.52	< LOQ(1ng/g)
K-203				
15 min	170.9±35.7	265.7±19.5	91.98±28.5	
60 min	132.8±37.4	260.0±50.6	63.34±26.7	
Striatum				
Control	61.64±23.2	173.3±39.3	1493±529	285.9±69.3
K-203				
15 min	86.47±14.0	179.9±12.9	1844±136	220.0±17.0
60 min	70.79±27.4	193.6±9.77	1683±413	277.8±59.6
Medulla oblongata				
Control	145.5±22.3	201.8±53.42	22.25±2.56	< LOQ(1ng/g)
K-203				
15 min	167.1±29.5	194.6±29.4	24.68±4.36	
60 min	121.0±35.1	184.2±38.7	21.76±3.15	
Frontal cortex				
Control	22.46±9.04	154.2±23.5	15.56±1.36	< LOQ(1ng/g)
K-203				
15 min	51.71±15.6	146.4±5.57	16.83±1.83	
60 min	52.59±37.7	155.0±17.8	23.48±19.1	

As serotonin and 5-HIAA tissue levels showed a consistent changing tendency, we calculated the 5-HIAA/5-HT ratios (known as a measure for turnover) in all the brain areas (Table 22).

Table 22. Serotonin turnover in rat brain areas following K-203 50-μmol i.m administration. The P< 0.05 was considered significantly different. *: compared to control; +: compared to 15 min value. Each value represents data from five animals (n=5). (Hashemi et al. 2013)

	5-HIAA/5-HT (mean ± SD)			
Rat brain area	**Control**	**15 min**	**60 min**	**P-value**
Cerebellum	11.26 ± 4.20	4.73 ± 1.73*	10.36 ± 4.23	0.015
Spinal cord	0.84 ± 0.22	0.70 ± 0.05	0.97 ± 0.26	0.206
Hippocampus	9.08 ± 3.73	4.90 ± 0.69*	8.37 ± 2.94	0.040
Hypothalamus	3.13 ± 0.74	2.05 ± 0.67*	2.60 ± 0.56	0.043
Striatum	4.39 ± 2.04	2.79 ± 0.43	4.39 ± 2.09	0.126
Medulla oblongata	1.78 ± 0.58	1.47 ± 0.16	2.44 ± 1.38	0.238
Frontal cortex	12.09 ± 6.16	3.57 ± 0.63*	4.41 ± 1.77+	* 0.036 + 0.031

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9. Discussion

In our work we wanted to optimize a sensitive, cost-effective and robust bioanalytical method for serial determinations of biogenic amines and their main metabolites from different rat tissues.We have chosen HPLC that is available in many laboratories and is sensitive enough for these determinations.

In line with the aim of the work we have optimized a bioanalytical method using HPLC with electrochemical detection to measure biogenic amines (dopamine, norepinephrine and serotonin) and their metabolites (HVA, DOPAC, 5-HIAA, 5-HTOL) in different brain areas, CSF and blood serum of rats. As the different tissues measured contain these endogenous compounds concentrations in a large scale we had to use such calibration curves that fit both the endogenous tissue levels and changes of these different concentrations following the different animal treatments. The method developed was appropriate to determine the compounds in question. Identification of the peaks in the samples was carried out by spiking. No annoying background peaks could be seen neither in oxytocin nor in K-203 treatment experiments.

The biogenic amines and their metabolites calibration curves obtained (Figures 19 to 26 for oxytocin hormonal imprinting) (Figure 27 and 28 for K-203) are considered to be linear within the measuring ranges studied, with reflect values of the regression coefficients for each curves (R^2: 0.9994 - 0.9999).

9.1. Effect of the oxytocin hormonal imprinting on the biogenic amine levels

It was demonstrated that brain is very sensitive to hormonal imprinting (Csaba 2000, Csaba et al. 2003a and 2003b; Tekes et al. 2007, 2009a, 2009b and 2011) and the neonatal period has an organizational time frame in the central nervous system.

Hormonal imprinting may lead to certain functional changes, for example, in sexual behavior (Csaba et al. 2003a and 2003b; Melis et al. 1986; Melis and Argiolas 2011), in social behavior (Uher and McGuffin 2008; Eaton et al. 2012) including adult pair bonding and parental behavior (Insel et al. 1997 and Bales et al. 2007) as well as in vocalization (Kramer et al. 2003) are deeply touched.

Oxytocin is a mammalian neurohypophysial hormone with role in various behaviors, including numerous central functions such as sexual, maternal behaviour, social recognition, anxiety, memory, learning, stress and social behaviors. Most of oxytocin roles are due to the cooperation with biogenic amines in different brain region (Melis et al. 1986; Pedersen et al. 2004; Insel et al. 1997; Melis and Argiolas 2011; Tekes et al. 2011; Waldherr and Neumann 2007; Wsol et al. 2008; Arletti et al. 1995).

Results of our present experiments unanimously show that treatment of neonate rats with a single dose of oxytocin (5mg/kg, s.c.) results in an effective hormonal imprinting effect.

We could demonstrate, that 4 months following neonatal hormonal imprinting with oxytocin the adult levels of biogenic amines in different brain regions (Tables 13 to 20) were significantly and permanently influenced in a region-specific manner. As the summarizing data constructed from these Tables, it can be evidenced, that most sensitive brain region to imprinting was the hypothalamus, where DOPAC, 5-HIAA and 5-HTOL tissue levels were decreased in both sexes, while in case of HVA only males showed decreased tissue levels and females showed increased 5-HT levels (Table 23). It is interesting, that in males more brain areas showed decreased DOPAC levels (HT, SC and ST), decreased HVA (HC, HT, ST and TC), decreased 5-HTOL (HT and MO) and NA (MO) tissue levels (Table 23). In females DOPAC levels were decreased only in HT, SC and TC and a decrease in 5-HIAA levels was observed only in the HT and the MO (Table 23). It is worth to mention, that females produced an increased 5-HT level in HT and ST, while in males significantly elevated NA levels were seen in FC and CB (Table 23).

The neonatally oxytocin imprinted animals showed an important deficiency in dopamine metabolism in the brain as DOPAC is a direct and main metabolite of dopamine in rodents, (Wallace and Traeger 2012) transformed by monoamino-oxydase, and HVA is transformed from DOPAC by catechol-O-methyl transferase. These metabolites are indicators of central dopaminergic activity (Revin and John 2012).

Impairment of serotonin metabolism was indicated by the decreased 5-HIAA concentrations. There are several diseases that are in close connection with the metabolism of dopamine and serotonin in the hypothalamus and striatum (Hornykiewicz 1975).

One of the most studied is Parkinson disease, deduced to perinatal chemical injuries (Muthian et al. 2010).

Table 23 Summarized data on the effect of oxytocin hormonal imprinting on biogenic amines and their metabolites level:

Table 23. Effect of Oxytocin hormonal imprinting on biogenic amines and their metabolites level. meaning of abbreviations: f - female, m - male, ↑- significant ($p< 0.05$) increase, ↓- significant ($p< 0.05$) decrease, ns – no significant change, nda – no data available.

Biogenic Amine		Serum	HC	HT	MO	SC	FC	CB	ST	TC
DA	m	↓	nda	ns	ns	ns	ns	nda	ns	ns
	f	ns	nda	ns	ns	ns	↑	nda	ns	↓
DOPAC	m	↓	ns	↓	ns	↓	ns	nda	↓	ns
	f	↓	ns	↓	ns	↓	ns	nda	ns	↓
HVA	m	↓	↓	↓	ns	ns	ns	ns	↓	↓
	f	↓	ns	ns	ns	ns	ns	ns	ns	ns
5-HIAA	m	↑	↓	↓	ns	↓	ns	ns	↓	ns
	f	↓	ns	↓	↓	ns	ns	ns	ns	ns
5-HTOL	m	nda	ns	↓	↓	ns	ns	nda	nda	ns
	f	nda	ns	↓	ns	ns	ns	nda	nda	ns
5-HT	m	ns	nda	ns	ns	ns	nda	nda	ns	ns
	f	↑	nda	↑	ns	ns	nda	nda	↑	ns
NA	m	ns	ns	ns	↓	ns	↑	↑	ns	ns
	f	ns	ns	ns	ns	ns	↑	ns	ns	ns

The pervasive developmental disease (eg, autism) and several neuropsychiatric disorders (eg, schizophrenia) are also characterized by significantly disturbed dopaminergic and serotonergic functionings (Wyatt et al. 1995; Sharma et al. 1998).

Aggressive behavior is also influenced by brain serotonin metabolism (Veenstra Vanderweele et al. 2000; Stanley et al. 2000, Csaba et al. 2003b). It is also known that the effect of neonatal oxytocin treatment in men could be manifested already at infant age in the screaming children syndrome (Plothe 2009), later hypertention or hypotention, aggressivity, phobies in puberty and adult age, appear with higher propability than in control population.

It is supposed that oxytocin is a key molecule, which interacts with the brain dopamine and serotonin system (Arletti et al. 1995; Csaba et al. 2003a; Thackare et al. 2006; Baskerville and Douglas 2010) influencing emotional and social behavior and this exerts heavy impact on the development of such diseases, as autism spectrum disorder (Harony and Wagner 2010). Considering the strong and late manifesting effect of imprinting, the single oxytocin treatment of neonates may indicate increased vulnerability to these disorders in oxytocin-imprinted subjects.

Although the overall tables (Tables 13 to 20) show the disturbed metabolism of biogenic amines in the brain, there are sometimes quantitative differences between the values of males and females in the control as well as in the treated animals. This is not surprising, as gender differences are known in the structure, function, and chemistry of the brain (Cosgrove et al. 2007), and some psychiatric diseases also appear in different frequences in the two genders (Carter 2007).

Summarizing the results, it can be established that single neonatal oxytocin treatment (hormonal imprinting) seems to be a serious factor in the formation of the emotional and social life. Oxytocin imprinting could significantly influence the dopamine and serotonin metabolism in the brain of the experimental animals.

However, in men the oxytocin imprinting (in the most sensitive critical periods of life) is not applied experimentally, but widely used in the practice done by oxytocin induced parturitions for about 50% of labors in the United States (Moleti 2009) and about 20% in the United Kingdom (Mackenzie 2006), and it is also very frequent in other developed countries.

It is a striking statistical data that the incidence rate of diseases connected to disturbed dopamine and serotonin system (e.g. autism and other pervasive developmental disorders) is continually growing (Newschaffer et al. 2007; Keyes et al. 2011).

The casual effect of oxytocin induced labor and hormonal imprinting has to be considered (Wahl 2004). The situation may be even more serious in the light of the transgenerational effect of hormonal imprinting (Tekes et al. 2009; Csaba 2007 and 2008).

It is worth to mention that the administration of oxytocin as a therapy for autism is also used in the last time (Modi and Young 2012; Matsuzaki et al. 2012; Angelidou et al. 2012; Peñagarikano et al. 2015). This also calls the attention to the importance of oxytocin system in this disease.

However, a substance, which is a medicament for the matured or maturing organism, could be a dangerous imprinter in a critical period of development, in the prenatal and in the early postnatal periods, shown in the current study results.

Considering the frequency of oxytocin-induced parturitions and the diseases mentioned, it is out of question that more inductions are done, than such disorders develop. However, it is characteristic to the imprinting that it is manifested rarely in disease but alters such different parameters that influence receptor binding, hormone levels, and behavior.

These factors appear as outside variants however seem to be inside the normal limits, (Csaba 1980, 1984, 2007, and 2008). Nevertheless, it can be supposed that misimprinted organisms cannot sufficiently tolerate the extreme loadings, and this could provoke the development of the disease.

If the mechanism of oxytocin imprinting is similar in human beings, as it is in rat, it can provoke pervasive developmental disorders or could participate in the emergence of them.

9.2. Effect of K-203 on the biogenic amine levels in rat CNS

Organophosphate poisoning is a constant danger, giving hundreds of thousands of fatal cases in each year, as organophosphates are widely used all over the world in agriculture, in chemical industry (Jeyaratnam 1990), and were used in terrorist attack (Okumura et al. 1996), and also in the Iraq-Iran war (McCauley et al. 2001).

The widely accepted therapy is known by the acronym “AFLOP” (atropine, fluid, oxygen and pralidoxime) (Petroianu and Kalasz 2007). Oximes such as pralidoxime and obidoxime are the only clinically available AChERs applied to organophoshates poisoned persons (Buckley et al. 2005; Eddleston at al. 2002).

However, the mechanism of action of oximes is relatively well characterized in theory, their practical value remains uncertain and oximes have disappointed clinically (Buckley et al. 2005; Antonijevic and Stojilkovic 2007).

In search of more effective broad-spectrum oximes, new asymmetric bispyridinium oximes have recently been synthesized (Kuca et al. 2003; Berend et al. 2008; Kassa et al. 2008). The K-203 is a newly synthesized bispyridinium monoaldoxime type antidote with low toxicity and superior *in vitro* potency for use in organophosphate poisoning, especially in case of tabun-poisoned (Musilek et al. 2007).

There is a clear demand for new, clinically effective cholinesterase reactivators with high efficacy and activity against of a large scale of poisonings with different OPs. In spite of the fact, that these K-compounds as AChERs are highly hydrophilic remarkable amounts (1–10%) penetrate into the central nervous system (CNS) resulting in detectable brain levels for several 10 minutes (Csermely et al. 2008).

Early results suggested that AChERs might influence brain neurotransmitter functions on their own (Kuca et al. 2003; Musilek et al. 2007; Berend et al. 2008; Kassa et al. 2008).

When calcium-dependent, potassium-evoked 3H-acetylcholine release from rat brain slices was examined bisquaternary pyridinium oximes (TMB-4, HGG-42, HGG-12) were shown to have dual function in cholinergic transmission by acting as presynaptic agonists and postsynaptic antagonists (Kloog et al. 1986).

Loke et al. (2005) demonstrated that in rats pretreated with the O-benzyl derivative of pralidoxime, soman-induced increase of both the dopamine and its metabolite HVA efflux and that of the 5-HT and its metabolite 5-HIAA releases could be prevented (Loke et al. 2005). It was also shown by microdialysis technique, that the intramuscularly injected AChER HI-6 (in higher than 50 mg/kg) almost completely prevented the increase in brain dopamine level observed following soman administration (Cassel et al. 1997). In mice poisoned with soman the increase of brain dopamine level was fully antagonized by HI-6 (Reithmann et al. 1988).

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Requirements for an optimal antidote include: a) its penetration into all body compartments where the poisonous compound can reach effective level, b) lack of own pharmacological effect, c) its complex with the poisonous compound should be more stable than that of the poison at the endogenous target, d) the antidote-poison complex should be hydrophilic enough to be excreted.

In our experiments we aimed to find out whether K-203, one of the most promising newly developed AChER can modify dopamine and 5-HT neurotransmissions by using RP-HPLC technique and to measure the effect of the K-203 on the tissue levels of biogenic amines (dopamine and 5-HT) and their metabolites (HVA and 5-HIAA) in the different brain areas (CB, SC, HC, HT, ST, MO and FC) following 15 and 60 min of 50-μmol intramuscular K-203 treated of rats.

The dose and way of application was chosen according to the LD_{10} of K-203 (Petroianu and Kalasz 2007) and to the potential administration as an antidote.

Tables 21 and 22 summarize the tissue levels of biogenic amines (dopamine and 5-HT) and their metabolites (HVA and 5-HIAA) of seven brain areas of the rat brain and show no significant change following 50-μmol intramuscular (i.m) administration of K-203. However, in some of the brain areas studied (CB, HC, HT and FC) a significant decrease in 5-HT turnover could be seen 15 min following K-203 treatment, while 5-HT turnover was not influenced by the AChE reactivator in the SC, ST and MO.

Tissue levels may mirror the immediate (15 minutes following treatment) and acute reaction (60 minutes after treatment) changes as a secondary response to the effect of K-203.

The parallel determination of 5-HT and its metabolite 5-HIAA gave the possibility for the 5-HT turnover calculations. Significant decrease in 5-HT turnover found in the CB (especially important in the pain sensation, motor activity and arousal), in the HC (where the regulation of memory and movement coordination is located), in the HT (that regulates hormonal levels of the organism) and in the FC.

However no change in 5-HT turnover was found in the SC (with particular role in pain sensation as well as in the regulation of the tone of blood vessels), in the ST (which plays an essential role in movement coordination) and in the MO (the main center of respiratory and vasomotor control).

According to basic pharmacokinetic principles, the blood-brain barrier (BBB) is relatively impermeable to highly hydrophilic drugs such as oximes due to their quaternary structure and hydrophilic oxime group but the concentrations reached in the brain are only about 4-10% of the plasma level (Terasaki and Ohtsuki 2005).

Brain area-specific 5-HT turnover decrease observed may also indicate (indirectly) the K-203 penetration through the blood-brain-barrier as was shown earlier (Tekes et al. 2006; Szegi et al. 2010).

Our data clearly show that intramuscularly (i.m) administered K-203 enter the central nervous system, and results in a short-term decrease in 5-HT turnover of some brain areas. Data suggests that limited brain penetration of K-203 is actually desirable. One possible explanation for this unexpected conclusion is the formation of phosphylated oximes, which are generated by the reaction of oximes with organophosphates-inhibited enzymes and which are highly toxic (Becker et al. 2010).

An "ideal" oxime must not only be non-toxic itself, but should also yield non-toxic products after phosphylation.

Moreover, these phosphylation products should ideally be very hydrophilic, thus barely entering the brain. K-203 might come closer to this ideal than the established oximes. The physiological meaning of these significant changes needs further studies, however demonstrates that K-203 is a safe potential antidote. This seems to be supported by the fact that following 60 minutes decreased serotonin turnover could only be observed in FC.

Recent investigations, showed a strong defensive effect of K-203 in organophosphate intoxications (especially in tabun intoxication), much higher effectiveness than any other pyridinium aldoximes used hitherto. The results underlined the stronger effects and milder side-effects of K-203 than other pyridinium aldoxime (Musilek et al. 2007; Kassa et al. 2008).

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10. Conclusion

The following conclusions may be drown from the present research work:

>> The optimized RP-HPLC method using electrochemical (EC) detection is a valuable bioanalytical method to determine biogenic amines and their metabolites from different rat brain areas following neonatal oxytocin hormonal imprinting and a single dose of K-203 treatment.

>> The single dose of 5 mg/kg oxytocin treatment (hormonal imprinting) of neonates, after 4 months, strongly, permanently and in brain region specific manner influenced the adult level of biogenic amines (noradrenaline, adrenaline, dopamine, 5-HT) and their metabolites (DOPAC, 5-HIAA, HVA and 5-HTOL). The hypothalamus and striatum are the most sensitive to the effect of neonatal oxytocin imprinting.

>> Oxytocin is a strong imprinter, which interacts with the brain dopamine and serotonin systems influencing emotional and social behavior. Our data may indicate impact of oxytocin on the development of such diseases, as autism spectrum disorder. Considering the strong and late manifesting effect of hormonal imprinting, the single oxytocin treatment of neonates may explain the symptoms that are characteristic to diseases.

>> Studying the effect of K-203, a potential antidote in OP poisoning on the biogenic amines and their metabolites levels in seven brain areas of rat in a dose (50-μmol) and by the proposed type of administration (i.m) it can be concluded, that K-203 is a safe potential antidote. Its effect on the 5-HT and dopamine metabolism is in brain-area specific and transient. In line with literature data it can be summarized, that K-203 has much higher antidotal effectiveness than any other pyridinium aldoximes used hitherto and milder side effects than other pyridinium aldoximes.

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11. Summary

1. Oxytocin is a mammalian neurohypophysial hormone and has role in various behaviors, including numerous central nervous system functions such as sexual, maternal behaviour, social recognition, anxiety, memory, learning, stress and social behaviors. Most of oxytocin's roles are due to the cooperation with biogenic amines in different brain regions.

Our results show that the single dose of 5mg/kg oxytocin treatment to rat neonates has a significant hormonal imprinting effect resulting in strongly and permanently influenced adult level of biogenic amines in different brain regions.

The hypothalamus is the most sensitive to imprinting followed by striatum.

Literature data unanimously show that there are diseases that are in close connection with the metabolism of dopamine and serotonin in the hypothalamus and striatum (Parkinson disease, autism, schizophrenia), and aggressive behavior is influenced by brain serotonin metabolism. It is supposed that oxytocin is a key molecule, which interacts with the brain dopamine and serotonin system, therefore it can be established that perinatal oxytocin treatment seems to be a serious factor.

2. As organophosphate poisoning is a constant danger and the therapeutic usefulness of currently available antidotes is low and is still a matter of controversy. There is a permenant need for more effective broad-spectrum antidotes. K-203 is a newly synthesized bispyridinium monoaldoxime type antidote with low toxicity and superior *in vitro* potency for use in organophosphate poisoning, especially in the case of tabun-poisoned.

Measuring the effect of K-203 on the biogenic amines and their metabolites in the different brain areas of the rat brain in a dose (50-μmol) and by the proposed type of administration (i.m) we can conclude that the effect of K-203 on the 5-HT and dopamine metabolism is in brain-area specific and transient. K-203 can be evaluated as an effective and safe antidote in organophosphate intoxication with effective acetylcholinesterase reactivator activity.

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11.1. Összefoglalás

1. Az oxytocin, mint a neurohipofízis nonapeptid hormonja emlősökben számos viselkedési forma (nemi funkciók, anyai gondoskodás, a fajtársak felismerése, félelmi reakciók, memória, tanulási képesség, stressz-válasz, társas kapcsolatok) központi idegrendszeri szabályozásában is fontos szerepet játszik.Ezek a sokrétű hatások döntően a biogénaminok anyagcseréjének befolyásolása útján valósulnak meg. Vizsgálatainkban kimutattuk, hogy újszülött patkányok egyszeri 5 mg/kg oxytocinnal történő kezelése jelentős hormonális „imprinting" hatású, mellyel tartós és jelentős valamint régiószelektív változást okoz a felnőttkori agyi biogénamin anyagcserében. A hormonális imprintig a nyolc vizsgált agyterület közül legerőteljesebben a hypothalamus és a striatum dopamin és szerotonin anyagcseréjét befolyásolja. Irodalmi adatok egyértelműen bizonyítják, hogy a hypothalamus és a striatum dopamin és szerotonin anyagcseréjének zavarával jellemezhetőek olyan betegségek, mint pl. a Parkinson kór, autizmus, skizofrénia valamint a szerotonin anyagcsere zavara fontos szereppel bír az aggresszív viselkedésforma létrejöttében. A kísérletes adatok tükrében az oxytocin kulcsszerepet játszik a dopamin és a szerotonin anyagcsere szabályozásában, ezért perinatális oxytocin-kezelés esetén súlyos következményekkel lehet számolni.

2. Az organofoszfátokkal történő véletlen és szándékos mérgezések súlyossága és gyakorisága valamint a klinikai gyakorlatban hozzáférhető antidótumok csekély terápiás értéke miatt az új, nagyhatékonyságú és az organofoszfátok széles skálájával szemben is alkalmazható acetilkolineszteráz- reaktivátor antidótumok kutatása nagy erőkkel folyik. A többszáz közelmúltban szintetizált vegyület közül a bispyridinium monoaldoxim szerkezetű K-203 kiemelkedő *in vitro* hatékonyságot mutat különösen tabun-mérgezéssel szemben és csekély önálló toxicitású. Kimutattuk, hogy a K-203 további előnyös tulajdonságai közé tartozik az is, hogy terápiásan hatékony mennyiségben bejut a központi idegrendszerbe is. Vizsgálatainkban a terápiás alkalmazást modellezve (50-μmol, i.m.) megállapítottuk, hogy a K-203 a központi idegrendszeri dopamin és szerotonin anyagcserére csak átmeneti és gyenge hatást fejt ki. A K-203 vizsgálataink tükrében egy igen hatékony és biztonságos acetilkolineszteráz reaktiváló antidótumnak minősül organofoszfátok okozta mérgezés esetén.

12. References

Adan RA, Van Leeuwen FW, Sonnemans MA, Brouns M, Hoffman G, Verbalis JG and Burbach JP. (1995) Rat oxytocin receptor in brain, pituitary, mammarygland, and uterus: partial sequence and immunocytochemical localization. Endocrinol, 136: 4022-4028.

Aldridge WN and Davison AN. (1953) The mechanism of inhibition of cholinesterase by organophosphorus compounds. Biochem J, 55: 763-766.

Amico JA, Tenicela R, Johnston J and Robinson AG. (1983) A time-dependent peak of oxytocin exists in cerebrospinal fluid but not in plasma of humans. J Clin Endocrinol Metab, 57: 947-951.

Angelidou A, Asadi S, Alysandratos KD, Karagkouni A, Kourembanas S and Theoharides TC. (2012) Perinatal stress, brain inflammation and risk of autism-review and proposal. BMC Pediatr, 12: 89.

Antonijevic B and Stojilkovic MP. (2007) Unequal efficacy of pyridinium oximes in acute organophosphate poisoning. Clin Med Res, 5: 71-82.

Arias F. (2000) Pharmacology of oxytocin and prostaglandins. Clin Obstet Gynecol, 43(3): 455-68.

Arletti R, Benelli A and Bertolini A. (1993) Influence of oxytocin on nociception and morphine antinociception. Neuropeptides, 24:125-129.

Arletti R, Benelli A, Poggioli R, Luppi P, Menozzi B and Bertolini A. (1995) Aged rats are still responsive to the antidepressant and memory-improving effects of oxytocin. Neuropeptides, 29: 177-182.

Bajgar J. (2004) Organophosphates/nerve agent poisoning: Mechanism of action, diagnosis, prophylaxis, and treatment. Adv Clin Chem, 38: 151-216.

Bajgar J, Fusek J, Kuca K, Bartosova L and Jun D. (2007) Treatment of organophosphate intoxication using cholinesterase reactivators: facts and fiction. Mini Rev Med Chem, 7: 461-466.

Bakry NM, el-Rashidy AH, Eldefrawi AT and Eldefrawi ME. (1988) Direct actions of organophosphate anticholinesterases on nicotinic and muscarinic acetylcholine receptors. J Biochem Toxicol, 3: 235-259.

Bales KL, van Westerhuyzen JA, Lewis-Reese AD, Grotte ND, Lanter JA and Carter CS. (2007) Oxytocin has dose-dependent developmental effects on pair bonding and alloparental care in female prairie voles. Horm Behav, 52(2): 274-279.

Barnes CD and Pompeiano O. (Eds.) Progress in Brain Research. Neurobiology of the Locus Coeruleus, Elsevier Science Publishers BV, Amsterdam, 1991: 88.

Baskerville TA and Douglas AJ. (2008) Interactions between dopamine and oxytocin in the control of sexual behaviour. Prog Brain Res, 170: 277-290.

Baskerville TA and Douglas AJ. (2010) Dopamine and oxytocin interactions underlying behaviors: potential contributions to behavioral disorders. CNS Neurosci Ther, 16(3): 92-123.

Ben-Jonathan N and Hnasko R. (2001) Dopamin as a prolactin (PRL) inhibitor. Endocrinol Rev, 22 (6): 724-763.

Becker C, Worek F and John H. (2010) Chromatographic analysis of toxic phosphylated oximes (POX): a brief overview. Drug Test Anal, 2: 460-468.

Berend S, Vrdoljak AL, Radic B and Kuca K. (2008) New bispyridinium oximes: in vitro and in vivo evaluation of their biological efficiency in soman and tabun poisoning. Chem Biol Interact, 175: 413-416.

Bismuth C, Inns RH and Marrs TC. Efficacy, toxicity and clinical use of oximes in anticholinesterase poisoning. In: Ballantyne B, Marrs TC, editors. Clinical and experimental toxicology of organophosphates and carbamates. Butterworth-Heinemann, Ltd, Oxford, 1992: pp. 555-577.

Bitran D, Hull EM, Holmes GM and Lookingland KJ. (1988) Regulation of Male-rat copulatory-behavior by preoptic incertohypothalamic dopamine neurons. Brain Res Bull, 20: 323-331.

Björklund A and Dunnett SB. (2007) Dopamine neuron systems in the brain: an update. Trends Neurosci. 30(5): 194-202.

Bokonjic D, Stojiljkovic MP, Stulic D, Kovacevic V and Maksimovic M. (1993) Application of response surface modelling for evaluation of the efficacy of a HI-6/trimedoxime mixture against tabun and soman poisoning in rats. Arch Toxicol Kinet Xenobiot Metab, 1: 223-232.

Bosch OJ, Kromer SA, Brunton PJ and Neumann ID. (2004) Release of oxytocin in the hypothalamic paraventricular nucleus, but not central amygdala or lateral septum in lactating residents and virgin intruders during maternal defence. Neuroscience, 124: 439-448.

Bosch OJ, Meddle SL, Beiderbeck DI, Douglas AJ and Neumann ID. (2005) Brain oxytocin correlates with maternal aggression: link to anxiety. J Neurosci, 25: 6807-6815.

Boškovic B, Tadic V and Kušic R. (1980) Reactivating and protective effects of pro-2-PAM in mice poisoned with paraoxon. Toxicol Appl Pharmacol, 55: 32-36.

Boublik Y, Saint-Aguet P, Lougarre A, Arnaud M, Villatte F, Estrada-Mondaca S and Fournier D. (2002) Acetylcholinesterase engineering for detection of insecticide residues. Protein Engineering, 15: 43-50.

Buckley NA, Eddleston M and Szinicz L. (2005) Oximes for acute organophosphate pesticide poisoning. Cochrane Database Syst Rev, 25(1): CD005085.

Buijs RM, Velis DN and Swaab DF. (1980) Ontogeny of vasopressin and oxytocin in the fetal rat: early vasopressinergic innervation of the fetal brain. Peptides 1: 315-324.

Buijs RM. (1992) The development of vasopressin and oxytocin system in the brain. In: Handbook of Chemical Neuroanatomy, Vol: Ontogeny of Transmitters and Peptides in the Central Nervous System 10: 547-571. Eds: A. Bjoerklund, T. Hoekfelt and M. Tohyama.

Cao CJ, Mioduszewski RJ, Menking DE, Valdes JJ, Katz EJ, Eldefrawi ME and Eldefrawi AT. (1999) Cytotoxicity of organophosphate anticholinesterases. In Vitro Cell Dev Biol Anim, 35: 493-500.

Carter CS. (2007) Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders. Behav Brain Res, 176(1): 170-186.

Cassel G, Karlsson L, Waara L, Ang KW and Goransson-Nyberg A. (1997) Pharmacokinetics and effects of HI 6 in blood and brain of soman-intoxicated rats: a microdialysis study. Eur J Pharmacol, 332: 43-52

Casida JE and Quistad GB. (2004) Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets. Chem Res Toxicol, 17: 983-998.

Casida JE and Quistad GB. (2005) Serine hydrolase targets of organophosphorus toxicants. Chem Biol Interact, 157-158: 277-283.

Cetkovic S. (1984) Effect of PAM-2Cl, HI-6 and HGG-12 in poisoning by tabun and its thiocholine analog in the rat. Fundam Appl Toxicol, 4(2): 116-122.

Chichinadze KN, Domianidze TR, Matitaishvili TT, Chichinadze NK and Lazarashvili AG. (2010) Possible relation of plasma testosterone level to aggressive behavior of male prisoners. Bull Exp Biol Med, 149: 7-9.

Caldji C, Diorio J and Meaney MJ. (2000) Variations in Maternal Care in Infancy Regulate the Development of Stress Reactivity. Biol Psychiatry, 48(12): 1164-1174.

Clement JG. (1981) Toxicology and pharmacology of bispyridinium oximes–insight into mechanism of action vs soman poisoning in vivo. Fundam Appl Toxicol, 1: 193-202.

Clement JG. (1982) HI-6: reactivation of central and peripheral acetylcholinesterase following inhibition by soman, sarin and tabun in vivo in the rat. Biochem Pharmacol, 31: 1283-1287.

Consiglio AR, Borsoi A, Pereira GA and Lucion AB. (2005) Effects of oxytocin microinjected into the central amygdaloid nucleus and bed nucleus of stria terminalis on maternal aggressive behavior in rats. Physiol Behav, 85: 354-362.

Costa LG. Toxic effects of pesticides. Casarett and Doull's Toxicology: The Basic Science of Poisons, 7th ed.; Klaassen CD, Ed., McGraw Hill Medical, New York, 2008: pp. 883-930.

Cosgrove KP, Mazure CM and Staley JK. (2007) Evolving knowledge of sex differences in brain structure and chemistry. Biol Psychiat, 62(8): 847-855.

Cummings JL, Nadel A, Masterman D and Cyrus PA. (2001) Efficacy of metrifonate in improving the psychiatric and behavioral disturbances of patients with Alzheimer's disease. J. Geriatr. Psychiat Neurol, 14: 101-108.

Csaba G. (1980) Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. Biol Rev, 55: 47-63.

Csaba G. (1984) The present state in the phylogeny and ontogeny of hormone receptors. Horm Metab Res, 16: 329-335.

Csaba G. (2000) Hormonal imprinting: its role during the evolution and development of hormones and receptors. Cell Biol Int, 24: 407-414.

Csaba G, Knippel B, Karabélyos C, Inczefi-Gonda A, Hantos M and Tekes K. (2003a) Impact of single neonatal serotonin treatment (hormonal imprinting) on the brain serotonin content and sexual behavior of adult rats. Life Sci, 73(21): 2703-2711.

Csaba G, Knippel B, Karabélyos C, Inczefi-Gonda A, Hantos M and Tekes K. (2003b) Effect of neonatal beta-endorphin imprinting on sexual behavior and brain serotonin level in adult rats. Life Sci, 73(1): 103-114.

Csaba G. (2007) Thoughts on the cultural evolution of man. Developmental imprinting and transgenerational effect. Riv Biol, 100(3): 461-474.

Csaba G. (2008) Hormonal imprinting: phylogeny, ontogeny, diseases and possible role in present day human evolution. Cell Biochem Funct, 26: 1-10.

Csermely T, Kalasz H, Petroianu G A, Kuca K, Darvas F, Ludanyi K, Mudhafar A A, Tekes K. (2008) Analysis of pyridinium aldoximes - a chromatographic approach. Curr Med Chem, 15: 2401-2418

Dale HH. (1906) On some physiological actions of ergot. J Physiol (Lond), 34: 163-206.

Dayi A, Cetin F, Sisman AR, Aksu I, Tas A, Gönenc S and Uysal N. (2015) Conditioned same-sex partner preference in male rats is facilitated by oxytocin and dopamine: Effect on sexually dimorphic brain nuclei. Med Sci Monit, 21:69-75.

Dauterman WC. (1971) Biological and nonbiological modification of organophosphorus compound. Bull World Health Organization, 44: 133-150.

Dawson RM. (1994) Review of oximes available for treatment of nerve agent poisoning. J Appl Toxicol, 14: 317- 331.

De Jong LP and Ceulen DI. (1978) Anticholinesterase activity and rate of decomposition of some phosphylated oximes. Biochem Pharmacol, 27: 857-863.

Du Vigneaud V, Ressler C and Trippett S. (1953) The sequence of amino acids in oxytocin, with a proposal for the structure of oxytocin. J Biol Chem, 205: 949-957.

Eddleston M, Szinicz L, Eyer P and Buckley N. (2002) Oximes in acute organophosphorus pesticide poisoning: a systematic review of clinical trials. Q J M, 95(5): 275-283.

Eaton JL, Roache L, Nguyen KN, Cushing BS, Troyer E, Papademetriou E and Raghanti MA. (2012) Organizational effects of oxytocin on serotonin innervation. Dev Psychobiol, 54(1): 92-97.

Elliott JC, Lubin DA, Walker CH and Johns JM. (2001) Acute cocaine alters oxytocin levels in the medial preoptic area and amygdala in lactating rat dams: implications for cocaine-induced changes in maternal behavior and maternal aggression. Neuropeptides, 35: 127-134.

Engelmann M, Ebner K, Landgraf R, Holsboer F and Wotjak CT. (1999) Emotional stress triggers intrahypothalamic but not peripheral release of oxytocin in male rats. J Neuroendocrinol, 11: 867-872.

Erdman AR. Insecticides. Medical Toxicology. Dart RC, Lippincott Williams & Wilkins, 3rd ed., Philadelphia, 2004: pp. 1475-1496.

Erdmann WD and Engelhard H. (1964) Pharmacological toxicological research with the dichloride of Bis (4-hydroxyimminomethyl-pyridinium-(1)-methyl) ether, a new esterase reactivator. [Pharmakologisch toxikologische Untersuchungen mit dem Dichlorid des Bis (4-hydroxyimminomethyl-pyridinium-(1)-methyl) äthers, einem neuen Esterase-Reaktivator, in German]. Arzneimittelforschung, 14: 5-11.

Eriksson J, Olausson B and Jankowska E. (1996) Antispastic effects of L-dopa. Exp Brain Res, 111: 296–304.

Ferguson JN, Aldag JM, Insel TR and Young LJ. (2001) Oxytocin in the medial amygdala is essential for social recognition in the mouse. J Neurosci, 21: 8278-8285.

Freundmercier MJ, Stoeckel ME and Klein MJ. (1994) Oxytocin receptors on oxytocin neurons-Histoautoradiographic detection in the lactating rat. J Physiol, 480: 155-161.

Fuchs AR and Fuchs F. (1984) Endocrinology of human parturition: a review. Br J Obstet Gynaecol, 91(10): 948-967.

Gage JC. (1953) A cholinesterase inhibitor derived from OO-diethyl O-p-nitrophenyl thiophosphate in vivo. Biochem J, 54: 426-430.

Galfi M, Radacs M, Juhasz A, Laszlo F, Molnar A and Laszlo FA. (2005) Regul Pept. Serotonin-induced enhancement of vasopressin and oxytocin secretion in rat neurohypophyseal tissue culture. 127: 225-231.

Gimpl G, Burger K, Politowska E, Ciarkowski J and Fahrenholz F. (2000) Oxytocin receptors and cholesterol: interaction and regulation. Exp Physiol, 85: 41S-49S.

Gimpl G and Fahrenholz F. (2001) The oxytocin receptor system: structure, function, and regulation. Physiol Rev, 81: 629-683.

Gonser M. (1995) Labor induction and augmentation with oxytocin: pharmacokinetic consider- ations. Arch Gynecol Obstet, 256(2): 63-66.

Grace AA. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the eitiology of schizophrenia. Neuroscience, 41(1): 1-24.

Gupta RC. Toxicology of organophosphate and carbamate compounds. Academic Press, Elsevier, Amsterdam, 2006: pp. 347-360.

Hagedorn I and Gündel WH. (1969) Reactivation of the phosphorylated acetylcholinesterase with oximes: contribution to the study of the reactivation process [Schoene K. Reaktivierung phosphorylierter Acetylcholin-Esterase mit Oximen: Beitrag zum Studium des Reaktionsablaufes, in German]. Arzneimittelforschung, 19: 603-606.

Harony H and Wagner S. (2010) The contribution of oxytocin and vasopressin to mammalian social behavior: potential role in autism spectrum disorder. Neurosignals, 18(2): 82-97.

Heilbronn E and Tolagen B. (1965) Toxogonin in sarin, soman and tabun poisoning. Biochem Pharmacol, 14: 73-77.

Hobbiger FW and Sadler PW. (1958) Protection by oximes of bis-pyridinium ions against lethal diisopropyl phosphonofluoridate poisoning. Nature, 182: 1672.

Hobbiger FW and Vojvodic V. (1966) The reactivating and antidotal actions of N,N'-trimethylenbis (pyridinium-4-aldoxime) (TMB-4) and N,N'-oxydimethylenbis (pyridinium-4- aldoxime) (toxogonin), with particularly reference to their effect on phosphorylated acetylcholinesterase in the brain. Biochem Pharmacol, 15: 1677-1690.

Holstege JC, VanDijken H, Buijs RM, Goedknegt H, Gosens T and Bongers CMH. (1996) Distribution of dopamine immunoreactivity in the rat, cat, and monkey spinal cord. J Comparat Neurol, 376: 631-652.

Hornykiewicz O. (1975) Brain monoamines and Parkinsonism. Natl Inst Drug Abuse Res Monogr Ser, 3: 13-21.

Huber D, Veinante P and Stoop R. (2005) Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. Science, 308: 245-248.

Hutson PH, Sarna GS, Sahakian BJ, Dourish CT and Curzon G. (1986) Monitoring 5HT metabolism in the brain of the freely moving rat. Ann NY Acad Sci. 473: 321-336.

Inns RH and Leadbeater L. (1983) The efficacy of bispyridinium derivatives in the treatment of organophosphate poisoning in the guinea pig. J Pharm Pharmacol, 35: 427.

Insel TR, Young L and Wang Z. (1997) Central oxytocin and reproductive behaviours. Rev Reprod, 2(1): 28-37.

Introduction to autonomic pharmacology. In: Basic and clinical pharmacology, 8th ed., Katzung BG. The McGraw Hill Companies Inc, USA, New York, 2001: pp. 75-91.

Jeyaratnam J. (1990) Acute pesticide poisoning: a major global health problem. World H Stat Q, 43(3): 139-144.

Johns JM, Lubin DA, Walker CH, Joyner P, Middleton C, Hofler V and McMurray M. (2004) Gestational treatment with cocaine and fluoxetine alters oxytocin receptor number and binding affinity in lactating rat dams. Int J Dev Neurosci, 22: 321-328.

Johnson DD and Stewart WC. (1970) The effects of atropine, pralidoxime and lidocaine on nerve muscle and respiratory function in organophosphate treated rabbits. Can J Physiol Pharmacol, 48: 625-630.

Johnson MK, Jacobsen D, Meredith TJ, Eyer P, Health AJ, Ligtenstein DA, Marrs TC, Szinics L, Vale JA and Haines JA. (2000) Evaluation of antidotes for poisoning by organophosphorus pesticides. Emerg Med, 12: 22-37.

Jorgensen H, Knigge U, Kjaer A and Warberg J. (2002) Serotonergic involvement in stress-induced vasopressin and oxytocin secretion. Eur J Endocrinol, 147: 815-824.

Jorgensen H, Riis M, Knigge U, Kjaer A and Warberg J. (2003) Serotonin receptors involved in vasopressin and oxytocin secretion. J Neuroendocrinol, 15: 242-249.

Karalliedde LD, Edwards P and Marrs TC. (2003) Variables influencing the toxic response to organophosphates in humans. Food Chem Toxicol, 41: 1-13.

Karczmar AG. History of research with anticholinesterase agents. In: Karczmar AG, Usdin E, Wills JH (Eds.) Anticholinesterase agents. Pergamon Press, Oxford, 1970: pp. 1-44.

Kalász H, Nurulain SM, Veress G, Antus S, Darvas F, Adeghate E, Adem A, Hashemi F and Tekes K. (2015) Mini review on blood-brain barrier penetration of pyridinium aldoximes. J Appl Toxicol, 35(2):116-123.

Kassa J, Kuca K, Karasova J and Musilek K. (2008) The development of new oximes and the evaluation of their reactivating, therapeutic and neuroprotective efficacy against tabun. Mini Rev Med Chem, 8(11): 1134-1143.

Keyes KM, Susser E, Cheslak-Postava K, Fountain C, Liu K and Bearman PS. (2011) Cohort effects explain the increase in autism diagnosis among children born from 1992 to 2003 in California. Int J Epidemiol, 41(2): 495-503.

Kimura T, Tanizawa O, Mori K, Brownstein MJ and Okayama H. (1992) Structure and expression of a human oxytocin receptor. Nature, 356: 526-529.

Kloog Y, Galron R and Sokolovsky M. (1986) Bisquaternary pyridinium oximes as presynaptic agonists and postsynaptic antagonists of muscarinic receptors. J Neurochem, 46: 767-772

Koplovitz I and Stewart JR. (1994) A comparison of HI-6 and 2- PAM against soman, tabun, sarin and VX in the rabbit. Toxicol Lett, 70: 269-279.

Kramer KM, Cushing BS and Carter CS. (2003) Developmental effects of oxytocin on stress response: single versus repeated exposure. Physiol Behav, 79: 775-782.

Kuca K, Bielavsky J, Cabal J and Bielavska M. (2003a) Synthesis of a potential reactivator of acetylcholinesterase 1- (4-hydroxyimino-methyl-pyridinium)- 3-(carbamoylpyridinium)-propane dibromide. Tetrahedron Lett, 44: 3123-3125.

Kuca K, Bielavsky J, Cabal J and Kassa J. (2003b) Synthesis of a new reactivator of tabun-inhibited acetylcholinesterase. Bioorg Med Chem Lett, 13: 3545-3547.

Kuca K, Juna D and Musilek K. (2006) Structural requirements of acetylcholinesterase reactivators. Mini Rev Med Chem, 6: 269-77.

Lee R, Garcia F, Van de Kar LD, Hauger RD and Coccaro EF. (2003) Plasma oxytocin in response to pharmaco-challenge to D-fenfluramine and placebo in healthy men. Psychiat Research, 118: 129-136.

Lipp JA. (1972) Effect of diazepam upon soman-induced seizure activity and convulsions. Electroencephalogr Clin Neurophysiol, 32: 557-560.

Loke WK, Sim MK and Go ML. (2005) Novel neuroprotective effects with o-benzyl derivative of pralidoxime in soman-intoxicated rodents. Eur J Pharmacol, 521: 59-69.

Lubin DA, Elliott JC, Black MC and Johns JM. (2003) An oxytocin antagonist infused into the central nucleus of the amygdala increases maternal aggressive behavior. Behav Neurosci, 117: 195-201.

Ludwig M, Sabatier N, Dayanithi G, Russell J A and Leng G. (2002) The active role of dendrites in the regulation of magnocellular neurosecretory cell behavior. Vasopressin Oxytocin: Genes Clinl Appl, 139: 247-256.

Ludwig M and Leng G. (2006) Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci, 7: 126-136.

Lüttringhaus A and Hagedorn I. (1964) Quaternary hydroxyiminomethylpyridinium salts. [Quärtare Hydroxyiminomethylpyridinium Salze, in German]. Arzneimittelforschung, 14: 1-5.

Mackenzie IZ. (2006) Induction of labour at the start of the new millenium. Reproduction, 131(6): 989-998.

Matsuzaki M, Matsushita H, Tomizawa K and Matsui H. (2012) Oxytocin: a therapeutic target for mental disorders. J Physiol Sci, 62(6): 441-444.

Marrs TC. (1993) Organophosphate poisoning. Pharmacology and therapeutics 58(1): 51-61.

Marrs TC. (2003) Diazepam in the treatment of organophosphorus ester pesticide poisoning. Toxicol Rev, 22: 75-81.

Matsumoto K, Cai B, Satoh T, Ohta H and Watanabe H. (1991) Desipramine enhances isolation-induced aggressive behavior in mice. Pharmacol. Biochem Behav, 39: 167-170.

McCauley LA, Rischitelli G, Lambert WE, Lasarev M, Sticker DL and Spencer PS. (2001) Symptoms of Gulf War veterans possibly exposed to organophosphate chemical warfare agents at Khamisiyah, Iraq. Int J Occup Environ Health, 7: 79-89.

McDonough JH Jr, Jaax NK, Crowley RA, Mays MZ and Modrow HE. (1989) Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. Fundam Appl Toxicol, 13: 256-276.

Melchers M, Montag C, Felten A and Reuter M. (2015) The oxytocin receptor gene and social perception. Soc Neurosci, 10(4): 345-353.

Melis MR, Argiolas A and Gessa GL. (1986) Oxytocin-induced penile erection and yawning: site of action in the brain. Brain Res, 398: 259-265.

Melis MR and Argiolas A. (2011) Central control of penile erection: A re-visitation of the role of oxytocin and its interaction with dopamine and glutamic acid in male rats. Neurosci Biobehav Rev, 35(3): 939-955.

Miroslav P, Vojtech A and Rene K. (2013) An acetylcholinesterase-based chronoamperometric biosensor for fast and reliable assay of nerve agents. Sensors, 13: 11498-11506.

Mitchell BF and Schmid B. (2001) Oxytocin and its receptor in the process of parturition. J Soc Gynecol Investig, 8(3): 122-133.

Modi ME and Young LJ. (2012) The oxytocin system in drug discovery for autism: animal models and novel therapeutic strategies. Horm Behav, 61(3): 340-350.

Moos F, Freundmercier MJ, Guerne Y, Guerne JM, Stoeckel ME and Richard P. (1984) Release of oxytocin and vasopressin by magnocellular nuclei in vitro Specific facilitatory effect of oxytocin on its own release. J Endocrinol, 102: 63-72.

Moos F, Poulain DA, Rodriguez F, Guerne Y, Vincent JD and Richard P. (1989) Release of oxytocin within the supraoptic nucleus during the milk ejection reflex in rats. Exp Brain Res, 76: 593-602.

Moron JA, Brockington A, wise RA, Rocha BA and Hope BT. (2002) Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. J Neurosci, 22(2): 389-395.

Moleti CA. (2009) Trends and controversies in labor induction. MCN Am J Matern Child Nurs, 34(1): 40-47.

Mukaddam-Daher S, Yin YL, Roy J, Gutkowska J and Cardinal R. (2001) Negative inotropic and chronotropic effects of oxytocin. Hypertension, 38(2): 292-296.

Musilek K, Kuca K, Jun D, Dohnal V and Dolezal M. (2005) Synthesis of a novel series of bispyridinium compounds bearing a xylene linker and evaluation of their reactivation activity against chlorpyrifos-inhibited acetylcholinesterase. J Enz Inhib Med Chem, 20(5): 409-415.

Musilek K, Holas O, Jun D, Dohnal V, Gunn-Moore F, Opletalova V, Dolezal M and Kuca K. (2007a) Monooxime reactivators of acetylcholinesterase with (E)- but-2-ene linker: preparation and reactivation of tabun and paraoxon inhibited acetylcholinesterase. Bioorg Med Chem, 15: 6733-6741.

Musilek K, Holas O, Kuca K, Jun D, Dohnal V, Opletalova V and Dolezal M. (2007b) Novel series of bispyridinium compounds bearing a (Z)-but-2-ene linker synthesis and evaluation of their reactivation activity against tabun and paraoxon-inhibited acetylcholinesterase. Bioorg Med Chem Lett, 17: 3172-3176.

Musilek K, Jun D, Cabal J, Kassa J, Gunn-Moore F and Kuca K. (2007c) Design of a potent reactivator of tabun-inhibited acetylcholinesterase-synthesis and evaluation of (E)-1-(4-carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (K203). J Med Chem, 50: 5514-5518.

Muthian G, Mackey V, King J and Charlton CG. (2010) Modelling a senzitization stage and a precipitation stage for Parkinson's disease using prenatal and postnatal 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine administrations. Neuroscience, 169(3): 1085-1093.

Nakamura H, Itakuara A, Okamura M, Ito M, Iwase A, Nakanishi Y, Okada M, Nagasaka T and Mizutani S. (2000) Oxytocin stimulates the translocation of oxytocinase of human vascular endothelial cells via activation of oxytocin receptors. Endocrinology, 14(12): 4481-4485.

Neumann I, Ludwig M, Engelmann M, Pittman QJ and Landgraf R. (1993a) Simultaneous microdialysis in blood and brain Oxytocin and vasopressin release in response to central and peripheral osmotic stimulation and suckling in the rat. Neuroendocrinology, 58: 637-645.

Neumann I, Russell JA and Landgraf R. (1993b) Oxytocin and vasopressin release within the supraoptic and paraventricular nuclei of pregnant, parturient and lactating rats. A microdialysis study. Neuroscience, 53: 65-75.

Neumann ID, Toschi N, Ohl F, Torner L and Kromer SA. (2001) Maternal defence as an emotional stressor in female rats: Correlation of neuroendocrine and behavioural parameters and involvement of brain oxytocin. Eur J Neurosci, 13: 1016-1024.

Nozaki H and Aikawa N. (1995) Sarin poisoning on the Tokyo subway. Lancet, 345: 1446-1447.

Okumura T, Takas N, Ishimatsu S, Miyanoki S, Mitsuhashi A, Kumada K, Tanaka K and Hinohara S. (1996) Report on 640 victims of the Tokyo subway sarin attack. Ann Emerg Med, 28(2): 129-135.

Palkovits M. (2001) Útmutató és atlasz patkányagy sejtcsoportjainak izolált kivételére. Akadémiai Kiadó.

Paxinos G. and Watson C. The Rat Brain in Stereotaxic Coordinates, 6th ed., Academic Press, San Diego, 1998.

Pedersen CA, Caldwell JD, Walker C, Ayers G and Mason GA. (1994) Oxytocin activates the post-partum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. Behav Neurosci, 108: 1163-1171.

Peñagarikano O, Lázaro MT, Lu XH, Gordon A, Dong H, Lam HA, Peles E, Maidment NT, Murphy NP, Yang XW, Golshani P and Geschwind DH. (2015) Exogenous and evoked oxytocin restores social behavior in the Cntnap2 mouse model of autism. Sci Transl Med, 7(271): 271-278.

Petroianu GA and Kalasz H. (2007) Comparison of the ability of pyridinium aldoximes to reactivate human RBC cholinesterase's inhibited by ethyl- and methyl-paraoxon. Curr Org Chem, 11: 1624-1634.

Petroianu GA, Hasan MY, Nurulain SM, Nagelkerke N, Kassa J and Kuca K. (2007) New K-Oximes (K-27 and K-48) in Comparison with Obidoxime (LuH-6), HI-6, Trimedoxime (TMB-4), and Pralidoxime (2-PAM): Survival in Rats Exposed IP to the Organophosphate Paraoxon. Toxicol Mech Methods, 17: 401-408.

Pliska V and Kohlhauf AH. (1991) Effect of Mg2+ on the binding of oxytocin to sheep myometrial cells. Biochem J, 277: 97-101.

Plothe C. (2009) The perinatal application of oxytocin and its potential influence on the human psyche. Prenatal and Perinatal Psychol and Med, 21(3/4): 181-198.

Pope CN. (1999) Organophophorus pesticides: Do they all have same mechanism of toxicity? J Toxical Environ Health (Part B), 2(2): 161-181.

Poziomek EJ, Hackley BE Jr and Steinberg GM. (1958) Pyridinium aldoximes. J Org Chem, 23: 714-717.

Primozic I, Odzak R, Tomic S, Simeon-Rudolf V and Reiner E. (2004) Pyridinium, imidazolium and quinucludinium oximes: synthesis, intractions with native and phosphylated cholinesterases and antidotes against organophosphorus compounds. J Med Chem Def, 2: 1-29.

Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia AS, McNamara JO and Williams SM. The Biogenic Amines. 2nd ed., Neuroscience, Sunderland (MA), 2001.

Reiner E, Radic Z and Rudolf VS. (2007) Mechanism of Organophosphate and Detoxification with emphasis on studies in crotia. Arh Hig Toksiko, 58: 329-338.

Reithmann C, Arbogast H, Hallek M, Auburger G, Szinicz L. (1988) Studies on the role of central catecholaminergic mechanisms in the antidotal effect of the oxime HI 6 in soman poisoned mice. Arch Toxicol, 62: 41-44

Revin SB and John SA. (2012) Simultaneous determination of two important dopamine metabolites at physiological pH by voltammetry. Anal Methods, 4(2): 348-352.

Rousseaux CG and Dua AK. (1989) Pharmacology of HI-6, an H-series oxime. Can J Physiol Pharmacol, 67: 1183-1189.

Sabyasachi Sircar. (2007) Medical Physiology. Thieme Publishing Group, pp. 536.

Sanborn BM, Qian A, Ku CY, Wen Y, Anwer K, Monga M and Singh SP. (1995) Mechanisms regulating oxytocin receptor coupling to phospholipase C in rat and human myometrium. Adv Exp Med Biol, 395: 469-479.

Sanghera MK, Anselmofranci J and Mccann SM. (1991) Effect of medial zona incerta lesions on the ovulatory surge of gonadotropins and prolactin in the rat. Neuroendocrinology, 54: 433-438.

Schoene K and Oldiges H. (1973) The action of pyridinium salts against poisoning with tabun and sarin in vivo and in vitro. [Die Wirkungen von Pyridiniumsalzen gegenüber Tabun- und Sarinvergiftungen in vivo und in vitro, in German]. Arch Int Pharmacodyn Ther, 204: 110-123.

Schoene K. Presentation and investigation of a new acetylcholinesterase reactivator. [Darstellung und Untersuchung neuer Acetylcholinesterase reaktivatoren, Dissertation in German]. University of Freiburg, Germany, Freiburg, 1967.

Sellström Å. Anticonvulsants in anticholinesterase poisoning. In: Ballantyne B, Marrs TC (Eds.). Clinical and experimental toxicology of organophosphates and carbamates. Butterworth-Heinemann, Oxford, 1992: pp. 578-586.

Shapiro LE and Insel TR. (1989) Ontogeny of oxytocin receptor in rat forebrain: quantitative study. Synapse, 4: 259-266.

Sharma RP, Javaid JL, Davis JM and Janicak PG. (1998) Pretreatment plasma homovanillic acid in schizophrenia and schizoaffective disorder: the influence and inpatient drug-free period. Biol Psychiatry, 44(6): 488-492.

Shih TM. (1991) Cholinergic actions of diazepam and atropine sulfate in soman poisoning. Brain Res Bull, 26: 565-573.

Sidell FR and Borak J. (1992) Chemical warfare agents: II. Nerve agents. Ann Emerg Med, 21: 865-871.

Skládal P, Nunes GS, Yamanaka H and Ribeiro ML. (1997) Detection of Carbamate Pesticides in Vegetable Samples Using Cholinesterase-Based Biosensors. Electroanalysis, 9: 1083-1087.

Skoog B and Noga BR. (1995) Dopaminergic control of transmission from group-Ii muscle afferents to spinal neurons in the cat and Guinea-Pig. Exp Brain Res, 105: 39-47.

Skrinjaric SM, Simeon V and Reiner E. (1973) Spontaneous reactivation and aging of dimethylphosphorylated acetylcholinesterase. Biochim Biophys Acta, 315: 363-369.

Smulders CJ, Bueters TJ, Vailati S, van Kleef RG and Vijverberg HP. (2004) Block of Neuronal Nicotinic Acetylcholine Receptors by Organophosphate Insecticides. Toxicol Sci, 82: 545-554.

Sogorb MA and Vilanova SE. (2002) Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicol. Lett. 128: 215-228.

Solé B, Jiménez E, Martinez-Aran A and Vieta E. (2015) Cognition as a target in major depression: New developments. Eur Neuropsychopharmacol, 25(2): 231-247.

Somani SM, Solana RP and Dube SN. Toxicodynamics of nerve agents. In: Somani SM, ed. Chemical Warfare Agents. Academic Press, San Diego, 1992: pp. 67-123.

Stanley B, Molcho A, Stanley M, Winchel R, Gameroff MJ, Parsons B and Mann JJ. (2000) Association of aggressive behavior with altered serotonergic function in patients who are not suicidal. Am J Psychiat, 157(4): 609-614.

Szegi P, Kalasz H, Laufer R, Kuca K and Tekes K. (2010) Pyridinium aldoxime analysis by HPLC: the method for studies on pharmacokinetics and stability. Anal Bioanal Chem, 397: 579-586.

Terasaki T and Ohtsuki S. (2005) Brain-to-blood transporters for endogenous substrates and xenobiotics at the blood–brain barrier: an overview of biology and methodology. Neurotherapeutics, 2: 63-72.

Terenzi MG and Ingram CD. (2005) Oxytocin-induced excitation of neurones in the rat central and medial amygdaloid nuclei. Neuroscience, 134: 345-354.

Tekes K, Hasan MY, Sheen R, Kuca K, Petroianu G, Ludanyi K and Kalasz H. (2006) High performance liquid chromatographic determination of the plasma concentration of K-27, a novel oxime-type cholinesterase reactivator. J Chromatogr A, 1122: 84-87.

Tekes K, Gyenge M, Hantos M and Csaba G. (2007) Effect of beta-endorphin imprinting during late pregnancy on the brain serotonin and plasma nocistatin levels of adult male rats. Horm Metab Res, 39(7): 479-481.

Tekes K, Gyenge M, Sótonyi P and Csaba G. (2009a) Effect of neonatal nociceptin or nocistatin imprinting on the brain concentration of biogenic amines and their metabolites. Brain Dev, 31(4): 282-287.

Tekes K, Gyenge M, Hantos M, Csaba G. (2009b) Transgenerational hormonal imprinting caused by vitamin A or vitamin D treatment of newborn rats. Alterations in the biogenic amine content of the adult brain. Brain Dev, 31(9): 666-670.

Tekes K, Szegi P, Laufer R, Hantos M and Csaba G. (2011) Effect of perinatal stress on the biogenic amine neurotransmitter level of the adult rat's brain. Int J Dev Neurosci, 29(2): 171-175.

Thibonnier M, Conarty D, Preston J, Plesnicher C, Dweik R and Erzurum S. (1999) Human vascular endothelial cells express oxytocin receptors. Endocrinology, 140 (3): 1301-1309.

Thiermann H, Szinicz L, Eyer F, Worek F, Eyer P, Felgenhauer N and Zilker T. (1999) Modern strategies in therapy of organophosphate poisoning. Toxicol Lett, 107: 233-239.

Thiermann H, Szinicz L, Eyer P, Felgenhauer N, Zilker T and Worek F. (2007) Lessons to be learn from organophosphorus pesticide poisoning for the treatment of nerve agent poisoning. Toxicology, 233: 145-154.

Tribollet E, Charpak S, Schmit A, Dubois-Dauphin M and Dreifuss JJ. (1989) Appearance and transient expression of oxytocin receptors in fetal, infant, and peripubertal rat brain studied by autoradiography and electrophysiology. J Neurosci, 9: 1764-1773.

Turdean GL, Popescu IC, Oniciu L and Thevenot DR. (2002) Sensitive detection of organophosphorus pesticides using a needle type amperometric acetylcholinesterase-based bioelectrode. Thiocholine electrochemistry and immobilised enzyme inhibition, Journal of Enzyme Inhibition and Medicinal Chemistry 17: 107-115.

Uher R and McGuffin P. (2008) The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. Molecular Psychiatry, 13: 131-146.

Uvnas-Moberg K. (1989) Physiological and psychological effects of oxytocin and prolactin in connection with motherhood with special reference to food intake and the endocrine system of the gut. Acta Physiol Scand Suppl, 583: 41-48.

Uvnas-Moberg K. The Oxytocin Factor. Da Capo Press, Cambridge (MA), 2003.

Uvnas-Moberg K and Petersson M. (2004) Oxytocin-biochemical link for human relations. Mediator of antistress, well-being, social interaction, growth, healing. Lakartidningen, 101(35): 2634-2639.

Veenstra-VanderWeele J, Anderson GM and Cook EH Jr. (2000) Pharmacogenetics and the serotonin system: initial studies and future directions. Eur J of Pharmacol, 410: 165-181.

Veronneau-Longueville F, Rampin O, Freund-Mercier MJ, Tang Y, Calas A, Marson L, McKenna KE, Stoeckel ME, Benoit G and Giuliano F. (1999) Oxytocinergic innervation of autonomic nuclei controlling penile erection in the rat. Neuroscience, 93: 1437-1447.

Wahl RU. (2004) Could oxytocin administration during labor contribute to autism and related behavioral disorders? – A look at the literature. Med Hypotheses, 63(3): 456-460.

Wallace LJ, Traeger JS. (2012) DOPAC distribution and regulation in striatal dopaminergic varicosities and extracellular space. Synapse, 66(2): 160-173.

Waldherr M and Neumann ID. (2007) Centrally released oxytocin mediates mating-induced anxiolysis in male rats. Proc Natl Acad Sci, USA, 104: 16681-16684.

Wiener SW and Hoffman RS. (2004) Nerve Agents: A Comprehensive Review. J intensive care med, 19: 22-37.

Wilson IB and Ginsburg S. (1995) A powerful reactivator of alkylphosphate inhibited acetylcholinesterase. Biochim Biophys Acta, 18: 168-170.

Wsol A, Cudnoch-Jedrzejewska A, Szczepanska-Sadowska E, Kowalewski S and Puchalska L. (2008) Oxytocin in the cardiovascular responses to stress. J Physiol and Pharmacol, 59(8): 123-127.

Wyatt RJ, Karoum F and Casanova MF. (1995) Decreased DOPAC in the anterior cingulate cortex of individuals with schizophrenia. Biol Psychiat, 38(1): 4-12.

Yavich L, Forsberg MM, Karayiorgou M, Gogos JA and Mannisto PT. (2007) Site-specific role of catechol-O-methyltransferase in dopamine overflow within prefrontal cortex and dorsal striatum. J Neurosci, 27(38): 10196-10209.

Yoshimura R, Kiyama H, Kimura T, Araki T, Maeno H, Tanizawa O and Tohyama M. (1993) Localization of oxytocin receptor messenger-ribonucleic-acid in the rat-brain. Endocrinology, 133: 1239-1246.

Young SN. (2007) How to increase serotonin in the human brain without drugs. J Psychiat neurosci, 32(6): 394-399.

Zhang XH, Filippi S, Vignozzi L, Morelli A, Mancina R, Luconi M, Donati S, Marini M, Vannelli GB, Forti G and Maggi M. (2005) Identification, localization and functional in vitro and in vivo activity of oxytocin receptor in the rat penis. J Endocrinol, 184: 567-576.

13. Publications

13.1. Publications related to the thesis

1. Kalasz H, Nurulain SM, Veress G, Antus S, Darvas F, Adeghate E, Adem A, **Hashemi F** and Tekes K. (2015) Mini review on blood-brain barrier penetration of pyridinium aldoximes. Journal of Applied Toxicology, 35(2): 116-123. **IF: 3.174**

2. **Hashemi F**, Laufer R, Szegi P, Csomor V, Kalasz H and Tekes K. (2014) HPLC determination of brain biogenic amines following treatment with bispyridinium aldoxime K203. Acta Physiologica Hungarica, 101(1): 40-46. **IF: 0.747**

3. **Hashemi F**, Tekes K, Laufer R, Szegi P, Tothfalusi L and Csaba G. (2013) Effect of a Single Neonatal Oxytocin Treatment (Hormonal Imprinting) on the Biogenic Amine Level of the Adult Rat Brain: Could Oxytocin-Induced Labor Cause Pervasive Developmental Diseases? Reproductive Sciences, 20(10): 1255-1263. **IF: 2.179**

4. Nurulain S, Kalász H, Szegi P, Kuca K, Adem A, Hasan M, **Hashemi F** and Tekes K. (2013) HPLC analysis in drug level monitoring of K027. Acta Chromatographica, 25(4): 703-710. **IF: 0.485**

13.2. Other publications

1. Tekes K, Tariq S, Adeghate E, Laufer R, **Hashemi F**, Siddiq A and Kalasz H. (2013) Nociceptinergic system as potential target in Parkinson's disease. Mini-Reviews in Medical Chemistry, 13(10): 1389-1397. **IF: 3.186**

2. Tekes K, Szegi P, **Hashemi F**, Laufer R, Kalasz H, Siddiq A and Ertsey C. (2013) Medicinal Chemistry of Antimigraine Drugs. Current Medicinal Chemistry, 20(26): 3300-3016. **IF: 3.715**

3. Ram N, Kalász H, Adeghate E, Darvas F, **Hashemi F** and Tekes K. (2012) Medicinal Chemistry of Drugs with Active Metabolites (N-, O-, and S-desalkylation and Some Specific Oxidative Alterations). Current Medical Chemistry, 19(33): 5683-5704. **IF: 4.070**

4. Csomor V, **Hashemi F**, Laufer R, Szegi P, Hantos M, Kalász H and Tekes K. (2012) Gyógyszerek okozta ízérzékelési zavarok. Gyogyszereszet, 56(11): 645-649.

5. Tekes K, **Hashemi F**, Szegi P, Sótonyi P, Laufer R and Kalász H. (2011) Prodrugs and active metabolites among antidepressive compounds. Neuropsychopharmacologia Hungarica, 13(2): 103-110.

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15. List of figures and tables

15.1. List of figures

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15.2. List of tables

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