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Research report

Effects of articaine on [^{3}H]noradrenaline release from cortical and spinal cord slices prepared from normal and streptozotocin-induced diabetic rats and compared to lidocaine

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ABSTRACT

Since a significant proportion of diabetic patients have clinical or subclinical neuropathy, there may be concerns about the use of local anaesthetics. The present study was designed to determine and compare the effects of articaine, a widely used anaesthetic in dental practice, and lidocaine on the resting and axonal stimulation-evoked release of [^{3}H]noradrenaline ([^{3}H]NA) in prefrontal cortex slices and the release of [^{3}H]NA in spinal cord slices prepared from non-diabetic and streptozocin (STZ)-induced diabetic (glucose level = 22.03 ± 2.31 mmol/l) rats. The peak of allodynia was achieved 9 weeks after STZ-treatment. Articaine and lidocaine inhibited the stimulation-evoked release in a concentration-dependent manner and increased the resting release by two to six times. These effects indicate an inhibitory action of these anaesthetics on Na^+- and K^+-channels. There was no difference in clinically important nerve conduction between non-diabetic and diabetic rats, as measured by the release of transmitter in response to axonal stimulation. The uptake and resting release of NA was significantly higher in the brain slices prepared from diabetic rats, but there were no differences in the spinal cord. For the adverse effects, the effects of articaine on K^+ channels (resting release) are more pronounced compared to lidocaine. In this respect, articaine has a thiophene ring with high lipid solubility, which may present potential risks for some patients.

1. Introduction

Diabetes mellitus (DM) is characterized by hyperglycaemia that is induced by defects in insulin secretion from pancreatic β-cells and/or the effects of insulin on target cells. After cardiovascular disease, DM is the third leading cause of death in the US, Europe (Veves et al., 2008) and Hungary (Jermendy et al., 2016; Vegh et al., 2017). It has been shown that the risk of neurotoxicity is higher in patients with diabetic peripheral neuropathy, which is a critical finding for clinicians who are involved in dental care or who apply local anaesthetics for surgery or for short- and long-term pain management.

All local anaesthetics that can inhibit both Na^+ and K^+-channels (Scholz, 2002) are potentially neurotoxic, as is the case for articaine (Bachmann et al., 2012b; Borchard and Drouin, 1980), which is a widely used anaesthetic in dental care (Snoeck, 2012). The inhibitory effects of these anaesthetics on Na^+ channels are responsible for the local anaesthetic effect (Karoly et al., 2010; Lenkey et al., 2011; Lenkey et al., 2010), whereas the inhibition of K^+ channels leads to adverse (Wolff et al., 2014) effects. Patients with diabetes may have peripheral neuropathy, which may have clinical implications for the use of regional nerve blocks. There is agreement among researchers that the prevalence and severity of periodontal diseases are greater in individuals with diabetes (Miley and Terezhalmy, 2005; Santos-Paul et al., 2015). Therefore, it is essential to be familiar with the possible complications that may occur during the treatment of diabetic patients.

Articaine is similar to lidocaine, and its use in clinical practice has shown that it is suitable and safe for clinical interventions that require a short duration and fast onset of action in dental practice and ambulatory spinal anaesthesia (Bachmann et al., 2012a; Snoeck, 2012). Although it has been shown that the risk of neurotoxicity with local

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anaesthetics is relatively small, there are still concerns about the safety of their use (Strichartz, 2010; Sircuta et al., 2016), especially among diabetic patients (Kroin et al., 2012).

In previous studies (Borbely et al., 2017; Sircuta et al., 2016), we showed that lidocaine and bupivacaine, due to their actions on Na^+ channels, inhibited the release of transmitters from frontal cortex and spinal cord slices in response to ongoing axonal activity and strongly enhanced the resting release induced by the inhibitory effects on K^+ channels. The effect on Na^+ channels was similar to that of tetrodotoxin, a selective Na^+ channel blocker, and the effect on K^+ channels was the same as that evoked by 4-aminopyridine, which is a selective K^+ channel blocker (Sircuta et al., 2016). Accordingly, in this study, we tested the effects of articaine on these two types of release using cortical and spinal cord slice preparations taken from non-diabetic and streptozotocin (STZ)-induced diabetic rats and compared them with the effects of lidocaine.

2. Materials and methods

2.1. Animals

The experiments were carried out on male Wistar rats (200–300 g body weight). The rats were kept in cages with a mesh bottom placed over a metal plate lined with ground corn cob bedding, at room temperature (20 ± 2 °C) with relative humidity (60–70%) and a 12/12 h light/dark cycle. Rats were fed standard rodent food pellets and water *ad libitum*. The animals were housed in the local animal facility of the Department of Pharmacology and Pharmacotherapy (Semmelweis University, Budapest, Hungary) under animal housing and experimentation conditions approved by the ethical guidelines set by the Ethical Board of Semmelweis University, based on EC Directive 86/609/EEC. Permissions No. PEI/001/276-4/2013. The animals were sacrificed under slight (isoflurane) anaesthesia, their brains were quickly removed, and the prefrontal cortex or spinal cord was dissected.

2.2. Chemicals

All chemicals were obtained from Sigma-Aldrich Inc. (Budapest, Hungary). The radioactive compounds were purchased from American Radiolabeled Chemicals Inc. (USA). Lidocaine HCl (MWt. 288.81) and articaine HCl (MWt. 320.84) were dissolved in Krebs solution. Streptozotocin was obtained from Sigma Aldrich Inc. (Budapest, Hungary).

2.3. Streptozotocin (STZ) treatment

An intraperitoneal (i.p.) injection of STZ (60 mg/kg) at 2.5 ml/kg body weight was given as described previously (Courteix et al., 1993; Ganda et al., 1976). The STZ solution was made by dissolving 60 mg/2.5 ml cold distilled water (1–3 °C) in a relatively dark place prior to the injections and was used within 10 min to avoid any degradation. The age-matched control rats were injected with an equal volume of cold distilled water at the same temperature.

2.4. Blood glucose level measurements

Blood glucose level was measured 1 day prior to and 1 week following streptozotocin injection and after animal sacrifice on the day of the experiment. Briefly, the animals were slightly anaesthetized with 3% isoflurane in oxygen via a nose cone using a vaporizer (Eickemeyer Isoflo Vaporiser; Eickemeyer Veterinary Equipment Inc.). A small amount of blood (50 µl) was taken from rat tail veins and immediately placed on the test strip. The blood glucose was measured by an Accu-Chek Active blood glucose meter (Roche Diagnostics GmbH, Germany). The maximum measurable blood glucose level that could be detected in the applied test is 33.3 mmol/l. The animals were considered diabetic if the blood glucose concentration was higher than 14 mmol/l.

2.5. Nociceptive test (assessment of neuropathic pain)

Mechanical allodynia was determined on the day prior to STZ treatment and on the 3rd, 6th and 9th weeks after STZ treatment. Briefly, a metal filament with a 0.5 mm diameter was applied to the plantar surface of the animal paw. The threshold of hind paw withdrawal was determined using a Dynamic Plantar Aesthesiometer (DPA) (Ugo Basile, Italy) as described previously (de Novellis et al., 2012; Starowicz et al., 2013). Animals were placed in plastic cages with a perforated metal platform floor for the DPA once a day for 3 days ("handling") before STZ treatment. Animals were kept in these cages for at least 5 min before starting the measurement to habituate them. Increasing force was initiated when the tip of the metal filament touched the plantar surface of the rat's hind paw. The force was automatically set, and 50 g was the cut-off. The withdrawal threshold was measured and expressed in grams before and at the 3rd, 6th and 9th weeks after STZ treatment. The withdrawal threshold was determined alternately in each paw three times, and the average of the measurements was recorded for each animal. The animals were grouped as follows: control (weight-matched) group (n = 8), non-diabetic (vehicle treated) and diabetic rats (n = 11).

2.6. Tissue preparation

Frontal cortex slices were prepared from the prefrontal cortex (PFC) as described previously (Barth et al., 2008).

Caudal spinal cord segments L5-S_1 were prepared using a previously described technique (Borbely et al., 2017; Umeda et al., 1997). Spinal cord segments were removed and placed into ice-cold modified Krebs solution of the following composition (mmol/l): NaCl, 113; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 2.5; glucose, 11.5; ascorbic acid, 0.3; and Na_2EDTA, 0.03) aerated with carbogen (95% O_2 ± 5% CO_2). The spinal cord segments were isolated and cut into 400-µm slices using a Mcllwain tissue chopper. The average weight of the PFC slices was 24.61 ± 1.21 mg, (n = 60), and the average weight of the spinal cord slices was 10.88 ± 0.28 mg, (n = 72).

2.7. Release of [^{3}H]noradrenaline

The slices were loaded with Levo-[7-^{3}H]noradrenaline ([^{3}H]NA; 5 µCi/ml, sp. activity: 14.8 Ci/mmol; NET 377, American Radiolabeled Chemicals Inc., USA) for 45 min at 37 °C in modified Krebs solution of above mentioned composition (mmol/l): NaCl, 113; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 2.5; glucose, 11.5; ascorbic acid, 0.3; and Na_2EDTA, 0.03) and bubbled with carbogen. After loading, the tissue was transferred to a microvolume (100 µl) four-channel perfusion system (Vizi et al., 1985) to measure the release of [^{3}H]NA from slices at rest and in response to electric field stimulation. The preparations were perfused with Minipuls 3 multi-channel peristaltic pump (Gilson Inc., Middleton, WI, USA) at 37 °C at a rate of 0.7 ml/min for 60 min with Krebs solution and continuously bubbled with carbogen and the effluent used for washing was discarded. During the experiment, the effluent was collected in 3-min fractions, and the radioactivity was measured. The preparations were stimulated via platinum electrodes using a Grass S88 stimulator (Astro-Med Inc., West Warwick, RI, USA). For PFC slices, supramaximal (20 V) field stimuli of 2-ms pulse durations were applied at 2 Hz for 90 s (Σ 180 shocks). For spinal cord slices, supramaximal (40 V) field stimuli of 1-ms pulse duration were applied at 3 Hz for 80 s (Σ 240 shocks). This type of stimulation excited noradrenergic axons and resulted in release of NA in a tetrodotoxine-sensitive manner (Vizi et al., 1997). The amount of the release induced by the first stimulation (S_1) was used as an internal standard. Local anaesthetics were applied 21 min before the second stimulation (S_2) was applied to the preparations and kept in the solution

continuously afterwards. After the fractions were collected, 0.5 ml of each perfusate solution was mixed with 2 ml of liquid scintillation cocktail. The radioactivity was determined as described in the following section.

At the end of the experiments, tissues were homogenized in 0.5 ml of 10% trichloroacetic acid for 30 min. Aliquots (500 µl) of the superfusate and aliquots (100 µl) of the tissue supernatant were added to 2 ml of Packard Ultima Gold scintillation cocktail. Released [^{3}H]NA was counted with a Packard-Canberra TR 1900 liquid scintillation counter, and the radioactivity was expressed as d.p.m./g tissue (Bq/g). The fractional release (FR) was expressed as a percentage of the total tissue tritium content at the onset of the collection period. The fractional release represents the percentage of the total [^{3}H]NA content present at the beginning of the fraction collection period that was released from the tissue in a specific fraction. The transmitter release collected in four fractions following electrical stimulation (fractions 3–6 and 13–16) was considered to be the release due to neuronal activity (FRS_1 and FRS_2). The release in the two subsequent consecutive fractions (7–8 and 17–18) was considered the resting release.

2.8. Statistical analysis

Radioactivity values corresponding to [^{3}H]NA uptake and release were statistically analysed using one or two-way ANOVA with Graphpad-Prism 7.0 statistical software. The data are presented as the means ± SEM. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Streptozotocin-induced neuropathic pain in diabetic rats

The rats treated with streptozotocin became diabetic (blood glucose level were > 14 mmol/l) (Fig. 1A) and displayed pronounced tactile allodynia (Fig. 1B), as indicated by a significant decrease in paw pressure thresholds (unpaired *t*-test, $p < 0.05$). The peak of allodynia was achieved 9 weeks after STZ treatment (Fig. 1B). The paw pressure threshold was (g) 40.88 ± 2.74 (n = 7) for non-diabetic rats and 32.02 ± 1.95 (12) for diabetic rats.

3.2. Uptake and release of [^{3}H]NA measured in PFC and spinal cord slices prepared from non-diabetic and diabetic rats

In 19 experiments, the PFC slices prepared from non-diabetic rats contained 354.90 ± 21.41 kBq/g radioactivity after 45 min of loading with [^{3}H]NA and subsequent washing (Table 1). Over a 3-min collection period at rest, the average release from the PFC slices was 4.02 ± 0.46 kBq/g (1.24 ± 0.14% of the total radioactive content, n = 19). In response to electrical field stimulation, the release (S_1) was 10.87 ± 1.24 kBq/g (n = 19), which was 2.63 ± 0.29% of the radioactivity present in the PFC tissue at the time of stimulation. When we compared the uptake and resting release of [^{3}H]NA measured in non-diabetic and diabetic brain slices, a significantly higher amount of [^{3}H]NA was taken up by the slices prepared from diabetic rats (Table 1). Similarly, the release at rest was higher in diabetic preparations (Table 1). However, there was no significant difference in stimulation-evoked releases.

Table 1
The uptake and release (at rest and stimulation-evoked) of [^{3}H]NA from PFC slices prepared from non-diabetic and diabetic rats.

	Non-diabetic	Diabetic	Significance
Uptake (kBq/g)	354.90 ± 21.41 n = 19	439 ± 28.23 n = 41	$p < 0.05$
R_1 resting release (kBq/g)	4.02 ± 0.46 n = 19	5.38 ± 0.30 n = 41	$p < 0.05$
S_1 stimulation-evoked release (kBq/g)	10.87 ± 1.24 n = 19	13.19 ± 1.04 n = 41	n.s.

Stimulation: 2 Hz, 2 ms impulse duration, 20 V supramaximal field stimuli for 90 s, Σ 180 shocks. Two-tailed Welch corrected *t*-test. The stimulation-evoked release of [^{3}H]NA was expressed in kBq/g measured on the top of resting release.

Table 2
The uptake and release (resting and stimulation-evoked) of [^{3}H]NA from spinal cord slices prepared from non-diabetic and diabetic rats.

	Non-diabetic	Diabetic	Significance
Uptake (kBq/g)	235.0 ± 15.17 n = 24	261.4 ± 13.36 n = 48	n.s.
R_1 resting release (kBq/g)	3.11 ± 0.18 n = 24	3.55 ± 0.17 n = 24	n.s.
S_1 evoked release (kBq/g)	5.31 ± 0.46 n = 24	5.00 ± 0.28 n = 24	n.s.

Stimulation: 3 Hz, 1 ms impulse duration, 40 V supramaximal field stimuli for 80 s, Σ 240 shocks. Two-tailed Welch corrected *t*-test. The stimulation-evoked release of [^{3}H]NA was expressed in kBq/g measured on the top of resting release.

After the tissue had been loaded, the average uptake of radioactivity in the spinal cord slices from the control (non-diabetic) group was 235.0 ± 15.2 kBq/g (Table 2, n = 24), and failed to differ from uptake measured in diabetic rats (Table 2, n = 48). The average resting release was 3.11 ± 0.18 kBq/g, 1.69 ± 0.09% of the total radioactivity content of the tissue (FRR_1) (n = 24) and was not significantly different from the release measured in diabetic slices. In response to electrical stimulation (S_1), the release was 5.31 ± 0.46 kBq/g, 2.65 ± 0.18% (n = 24) of the total radioactivity present in the slices.

3.3. Effects of articaine and lidocaine on [^{3}H]-NA release from PFC and spinal cord slices prepared from non-diabetic and diabetic rats

The effects of articaine compared to lidocaine on [^{3}H]NA release from PFC (Table 1) and spinal cord slices (Table 2) at various concentrations (0.3–10 mM) (Table 3) were studied and were likely reached following injection into the tissue. In PFC preparations at rest, 0.7 ± 0.05% of the radioactive content was released during the 3-min collection period. The change in resting release over a 30-min period was always compared to the control value (FRR_1), determined during

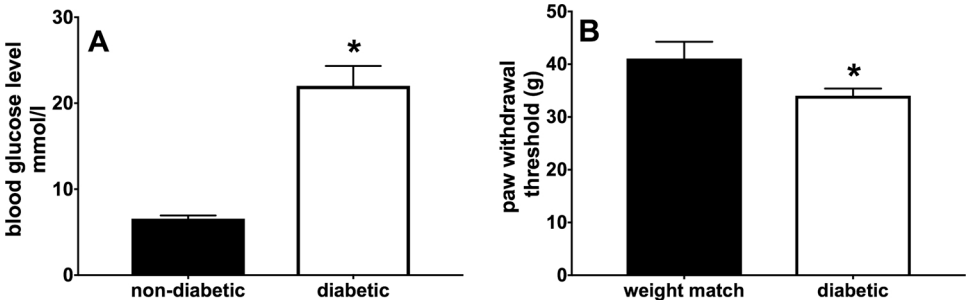

Fig. 1. Effects of streptozotocin treatment on blood glucose level and nociceptive test (for details, see Materials and Methods).
(A) Blood glucose level: non-diabetic rats (6.57 ± 0.37 mmol/l, n = 6) and diabetic rats (22.03 ± 2.31 mmol/l, n = 12). The difference is significant, p = 0.003, unpaired *t*-test. (B) Assessment of neuropathic pain measured by Dynamic Plantar Aesthesiometer and expressed as paw threshold (g). Vertical lines show SEM.

Table 3
Effects of articaine and lidocaine on stimulation-evoked and resting release of [^{3}H]NA from cortex slice preparations of non-diabetic rats.

	Concentrations	FRS_2/FRS_1	Significance	FRR_2/FRR_1	Significance
Control		0.84 ± 0.09 (6)		0.87 ± 0.06 (6)	
Articaine	0.3 mM	1.22 ± 0.10 (6)	n.s.	1.40 ± 0.08 (6)	n.s.
	3 mM	0,02 ± 0.11 (7)	$p < 0.01$	3.89 ± 0.46 (7)	$p < 0.05$
	10 mM	release fully inhibited	–	13,49 ± 0.77 (4)	$p < 0.01$
Lidocaine	0.3 mM	1.00 ± 0.05 (6)	n.s.	0.79 ± 0.02 (6)	n.s.
	3 mM	0,41 ± 0.23 (6)	n.s.	2,46 ± 0.27 (6)	n.s.
	10 mM	0,01 ± 0.01 (6)	$p < 0.01$	4,62 ± 0.01 (6)	n.s.

the 3-min collection period (FRR_2/FRR_1 = 0.87 ± 0.06, n = 6) (Fig. 2A). In samples that underwent electrical field stimulation, [^{3}H]NA was released (S_1 = 7.4 ± 0.9 kBq/g, n = 6), and the FRS_2/FRS_1 was 0.84 ± 0.09 (n = 6). At a concentration of 3 mM, articaine resulted in a four-fold increase in the resting release (Fig. 2C), whereas lidocaine resulted in a two and a half-fold (Fig. 2B) increase. While 3 mM articaine completely blocked the stimulation-evoked release (Fig. 2C), lidocaine only reduced this release. However, 10 mM of lidocaine completely blocked the stimulation-evoked release and enhanced the resting release by more than five times (Table 3). Articaine at the 0.3 mM concentration induced a significant 1.4-fold increase in resting release and failed to reduce the stimulation-evoked release. No differences were observed in the effects of local anaesthetics on the resting release measured in non-diabetic and diabetic slices; both conditions enhanced the release to the same extent (Table 2).

In spinal cord slices, both lidocaine and articaine significantly increased the resting release (Table 2, Fig. 2B and C, E and F), and their effects on the release of NA from slices prepared from non-diabetic and diabetic animals differed.

4. Discussion

In dental care and during spinal and epidural anaesthesia, the neurons are exposed to relatively high concentrations of local anaesthetics that diffuse directly into the tissue (Strichartz, 2010) and beside their therapeutical action may cause adverse effects. Since a significant proportion of diabetic patients have clinical or subclinical neuropathy, there may be concerns about the use of local anaesthetics. For epidural anaesthesia and in dental care, local anaesthetics are applied at concentrations of 1.5–2% (lidocaine, 55.2-69.25 mM) or 1–4% (articaine, 31.41–124.67 mM) for dental analgesia (Haase et al., 2008). Therefore, the present study was designed to determine and compare the effects of articaine and lidocaine on the resting and axonal stimulation-evoked release of [^{3}H]NA in prefrontal cortex and spinal cord slices prepared from non-diabetic and streptozotocin-induced diabetic rats. We failed to observe differences between the axonal stimulation-evoked releases measured in slices obtained from non-diabetic and diabetic rats. These findings indicate that there is no impairment in nerve conduction. The uptake and resting release of NA were higher in brain slices prepared from diabetic rats but not in preparations obtained from the spinal cord. Any inhibitory effect of local anaesthetics on the release of transmitter

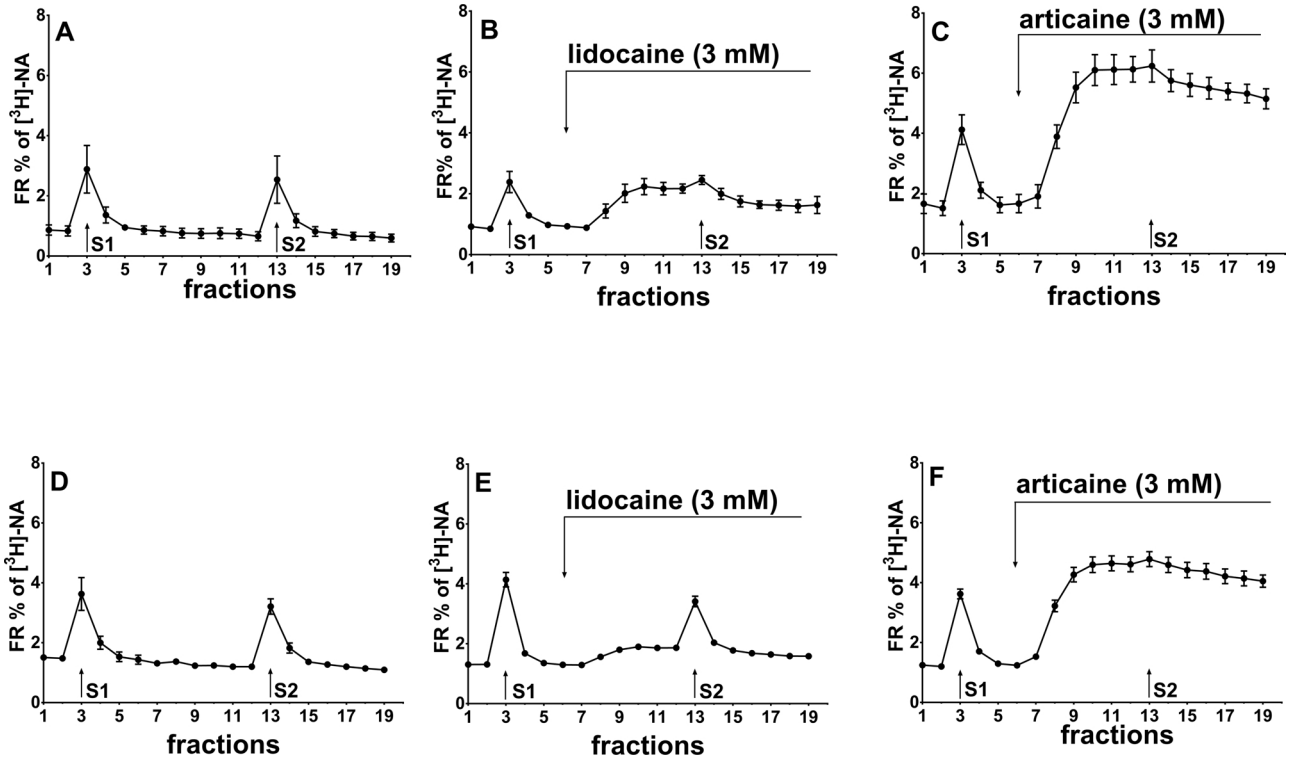

Fig. 2. Effects of lidocaine (B and E) and articaine (C and F) on the fractional release of [^{3}H]noradrenaline (FR% of [^{3}H]NA) in prefrontal cortex slices prepared from non-diabetic (A,B,C) and diabetic (D,E,F) rats. A and D show control experiments (n = 6). The slices were stimulated twice (S_1 and S_2), as indicated. Vertical lines represent the SEM. FR, fractional release (n = 6-6). Administration of local anaesthetics are indicated. The resting releases in the 2nd (R_1) and 13th (R_2) collection periods were compared for significance. The release of [^{3}H]NA was increased by both local anaesthetics in non-diabetic and diabetic preparations and was highly significant, ($p < 0.05$). For PFC slices, supramaximal (20 V) field stimuli of 2-ms pulse durations were applied at 2 Hz for 90 s (Σ 180 shocks).

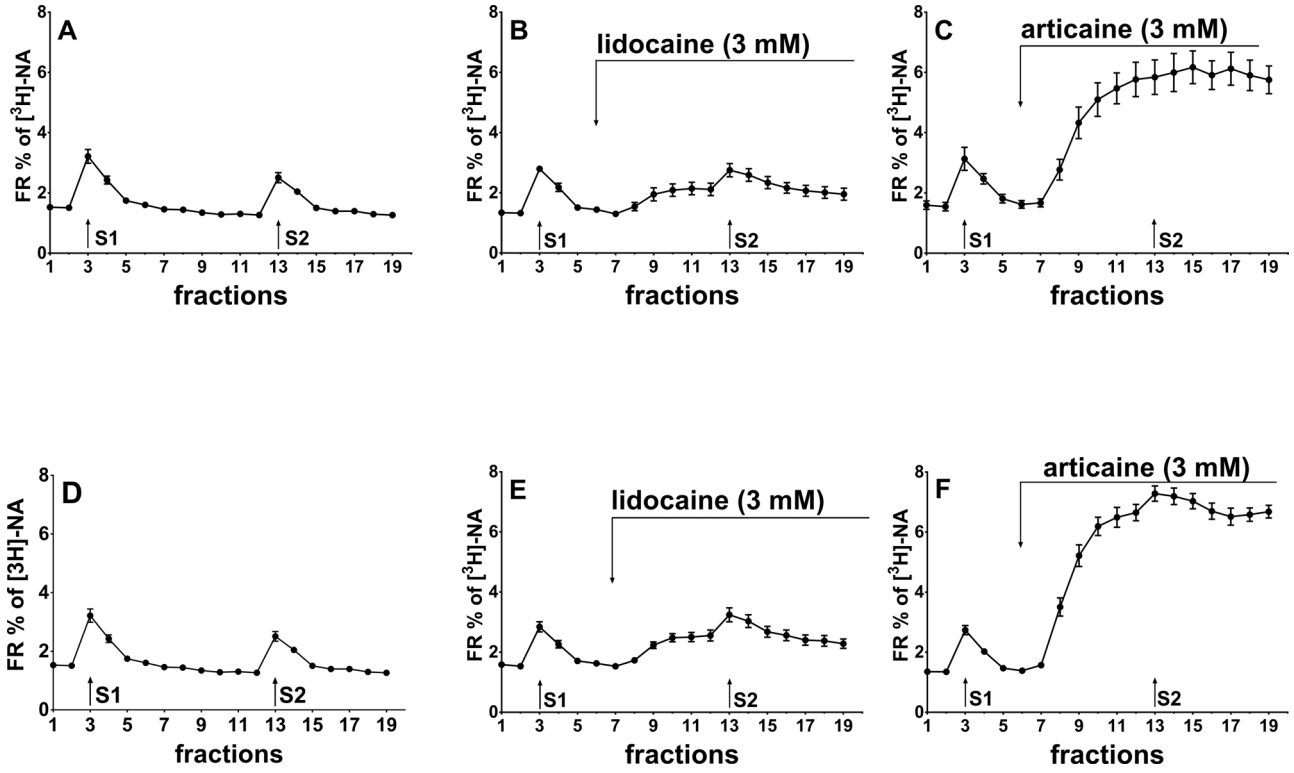

Fig. 3. Effect of lidocaine (B and E) and articaine (C and F) on resting and stimulation-evoked fractional release of [^{3}H]noradrenaline (FR% of [^{3}H]NA) in spinal cord slices prepared from non-diabetic (A,B,C) and diabetic (D,E,F) rats. A and D show control experiments. Stimulation (S_1 and S_2). Vertical lines represent the SEM. The resting releases in the 2nd (R_1) and 13th (R_2) collection periods were compared for significance. The release of [^{3}H]NA was increased by both local anaesthetics in non-diabetic and diabetic preparations and was highly significant, ($p < 0.05$). For further details, see Fig. 2. (n = 6–6). For spinal cord slices, supramaximal (40 V) field stimuli of 1-ms pulse durations were applied at 3 Hz for 80 s (Σ 240 shocks).

from nerve terminals in response to axonal activity or an increase in release at rest (defined as when there is no axonal firing) indicates a Na^+-channel- or K^+-channel-dependent action. The former effect results in conduction blockage and numbing of pain, and the latter effect may produce adverse reactions.

It should be mentioned that in clinical studies investigating local anaesthetics, an increased duration of sensory block in diabetic vs control patients was observed (Echevarria et al., 2008). These findings are consistent with the results observed in rats (Kalichman and Calcutt, 1992; Kroin et al., 2012). When the therapeutic efficacy of articaine was compared (Snoeck, 2012) to that of other local anaesthetics (lidocaine, prilocaine and chloroprocaine), the studies failed to show any evidence of clinical advantages of any anaesthetic over another.

Our findings with articaine and lidocaine administered at concentrations of 0.3–3 mM were that the stimulation-evoked releases were inhibited in a concentration-dependent manner, with the resting release increasing by two to six times (Table 3), depending on the concentration applied. This indicated the inhibitory action of these anaesthetics on Na^+- and K^+-channels (Fig. 3A–D). In clinical practice, these concentrations were easily reached and could inhibit both the Na^+ and K^+ channels. As far as adverse effects are concerned, the effects on K^+ channels seem to be more important. The inhibitory effects on K^+ channels result in the release of large amounts of transmitters, including NA and glutamate, into the extracellular space, where their metabolites (DOPAL and DOPEGAL) (Burke et al., 2004) and glutamate itself could be neurotoxic. In this respect, articaine has a thiophene ring with high lipid solubility (Snoeck, 2012), which may confer a potential risk for some patients. Neurotoxic injuries have been reported after local anesthetics, in humans even an overrepresentation of neurosensory disturbance associated with 4% formulations of articaine used in dentistry (Garisto et al., 2010). In addition a concentration-dependent neurotoxicity was show in animal experiments after 4% rather than 2% of articaine (Hillerup et al., 2011). However, articaine does display an advantage of negligible cardiotoxic effects compared to other local anaesthetics (Szabo et al., 2007).

The intrathecal and epidural administration of α_2-adrenoceptor agonists (e.g. clonidine) are widely used for spinal analgesia for intractable pain (Rauck et al., 1993). Accordingly, the NA-releasing effect of anesthetics (lidocaine and bupivacaine, (Borbely et al., 2017; Sircuta et al., 2016) and articaine (this paper) may be involved in their pain killing effects.

Epidural infusion of local anesthetic alone may be applied for postoperative analgesia. It has been shown, that articaine (50 mg) used for spinal anesthesia (Bachmann et al., 2012a) for knee arthroscopy (Hendriks et al., 2009) migh result in more than 2 mM concentration in lumbo sacral cerebrospinal fluid (~60 ml). In our experiments articaine (inhibited NA release in response to field stimulation) produces a complete block of nerve conduction, but also results in high ambient concentration of NA in the extraneuronal space, therefore we conclude, that NA released by anesthetics are involved in their pain-killing effects via stimulation of α_2-adrenoceptors.

Our findings may have implications for the appropriate dosages of local anaesthetics in diabetic patients undergoing regional analgesia with nerve blocks either in dental treatment or in spinal anaesthesia.

Conflicts of interest

None.

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