Research Communication

Inhibition of Microsomal Cortisol Production by (–)-Epigallocatechin-3-Gallate Through a Redox Shift in the Endoplasmic Reticulum—a Potential New Target for Treating Obesity-Related Diseases

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Abstract

Conversion of cortisone to cortisol by 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) in the endoplasmic reticulum (ER) of the target cells is a major determinant of glucocorticoid action, and plays an important role in the development of obesity-related diseases. Inhibition of 11βHSD1 activity is, therefore, considered as a promising novel strategy for the treatment of metabolic syndrome and diabetes. Tea flavanols and their major representative, epigallocatechin gallate are known as antiobesity and antidiabetic agents. Their impacts on blood glucose level, hepatic glucose production, and insulin responsiveness resemble those observed on inhibition or depletion of 11βHSD1. We aimed to study the effect of epigallocatechin gallate on 11βHSD1 activity in ER-derived rat liver microsomes by measuring cortisone and cortisol with HPLC. Cortisol production was efficiently suppressed in a concentration dependent manner in intact microsomal vesicles. However, this effect was abolished by membrane permeabilization; and the three proteins involved in the overall process (11βHSD1, hexose 6-phosphate dehydrogenase, and glucose 6-phosphate transporter) were not or only mildly affected. Further investigation revealed the oxidation of luminal NADPH to $NADP^+$, which attenuates cortisone reduction and favors cortisol oxidation in this compartment. Such a redox shift in the ER lumen might contribute to the beneficial health effects of tea flavanols and should be regarded as a promising strategy for the development of novel selective 11βHSD1 inhibitors to treat obesity-related diseases. © 2013 BioFactors, 00(00):000–000, 2013

Keywords: *11β-hydroxysteroid dehydrogenase type 1; endoplasmic reticulum; redox shift; selective inhibitor; (–)-epigallocatechin-3-gallate; diabetes*

1. Introduction

Glucocorticoid hormones regulate the expression of several key enzymes of the intermediary metabolism and hence play a central role in the long-term control of fuel homeostasis [1]. Cortisol aids the mobilization of energy sources and maintenance of blood glucose level, which are essential for the adaptation to physical and emotional stress, including prolonged starvation [2]. Glucose producing capacity of the liver is largely affected by cortisol, which stimulates the gene expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase), two rate-limiting enzymes of hepatic gluconeogenesis [3]. The circulating cortisol levels are adjusted to the nutrient supply and energy demands by the hypothalamic–pituitary–adrenal axis. Insufficient cortisol production leads to enhanced stress sensitivity, while cortisol overproduction causes Cushing zczc syndrome, a complex metabolic disorder involving visceral obesity, dyslipidemia, hypertension, insulin resistance, hyperglycemia, and an increased risk of type 2 diabetes [4].

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It has become evident in the last decade that, besides circulating cortisol levels, prereceptor glucocorticoid activation in the target tissues is a major determinant of the cortisol effect. Moreover, local cortisol production was also shown to play a central role in preadipocyte differentiation [5]. Conversion of the prohormone, cortisone to cortisol is catalyzed by 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) in the lumen of the endoplasmic reticulum (ER) [6,7]. NADPH requirement of the reaction is met by the activity of hexose 6-phosphate dehydrogenase (H6PD) [8], which is not only functionally coupled but also physically associated to 11βHSD1. H6PD uses glucose 6-phosphate (G6P), which enters the ER lumen through glucose 6-phosphate translocase (G6PT) [9].

The metabolic syndrome, one of the fastest growing obesity-related diseases, shares the above-described symptoms of the Cushing zczc syndrome despite the absence of elevated circulating glucocorticoid levels [10]. Therefore, the role of enhanced prereceptor cortisol production by the G6PT-H6PD-11βHSD1 triad has been implicated in the pathomechanism of obesity-related metabolic diseases [11]. This theory is strongly supported by the studies using transgenic and knockout animals. Overexpression of 11βHSD1 in adipose tissue reproduces the metabolic syndrome in mice [12], while hepatic overexpression leads to similar metabolic abnormalities without obesity [13]. Both H6PD [14,15] and 11βHSD1 [16,17] knockout mice display fasting hypoglycemia, improved lipid and lipoprotein profile, insulin sensitivity, and glucose tolerance. They were also found to resist hyperglycemia provoked by obesity or stress, due to attenuated gluconeogenic responses [14,16]. Inhibition of 11βHSD1 activity is, therefore, considered as a promising novel strategy for the treatment of metabolic syndrome and diabetes [18].

Green tea consumption has several beneficial health effects, which attracted a remarkable scientific attention in the past decades [19]. Most of these observed protective and curative activities are attributed to polyphenolic molecules, which have long been considered as semiessential food components. Polyphenols represent 30% of the dry matter of green tea leaf, and these polyphenols are mostly flavanol (catechin) monomers, dominantly epigallocatechin, epicatechin, and their gallic esters [20].

Epigallocatechin gallate (EGCG) is the most widely studied tea flavanol. Its antiobesity and antidiabetic effects have been demonstrated in several studies (for a review see ref. 21). EGCG decreases body weight and fat mass [22], and it is preventive against the metabolic syndrome [23]. It was also shown to decrease hepatic glucose production by downregulating PEPCK and G6Pase at transcriptional level *in vivo* [24]. These findings suggest that EGCG might interfere with the activity of the G6PT-H6PD-11βHSD1 triad in the liver cells. Therefore, the aim of this study was to reveal any possible direct effects of EGCG on the cortisol producing machinery in the hepatic ER by using rat liver microsomes. The results support our hypothesis that decreased prereceptor glucocorticoid activation may contribute to the antiobesity and antidiabetic effects of EGCG and highlight the role of ER luminal NADP-NADPH pool as a potential drug target in these pathological conditions.

2. Materials and Methods

2.1. Materials

Alamethicin, cortisone, cortisol, EGCG, G6P, metyrapone, $NADP^+$, NADPH, and 4-morpholinepropanesulfonic acid (MOPS) were purchased from Sigma Chemical (St. Louis, MO). All other reagents and solvents were of analytical grade.

2.2. Preparation of Rat Liver Microsomes

Rat liver microsomes were prepared from male Wistar rats (200–250 g body weights) using fractional centrifugation [25]. Microsomes resuspended in MOPS-KCl buffer (100 mM KCl, 20 mM NaCl, 1 mM $MgCl_2$, and 20 mM MOPS, pH 7.2) were rapidly frozen and stored in liquid N_2 until used. Intactness of microsomal vesicles was checked by measuring the latency of mannose 6-phosphatase activity [26], which was greater than 92% in all the preparations used. Protein concentration of the microsomes was determined with Bio-Rad protein assay solution using bovine serum albumin as a standard according to the manufacturer zczc instructions.

2.3. Microsomal Cortisone–Cortisol Conversion

G6P-driven reduction of cortisone to cortisol was measured by incubating intact microsomes (0.5 mg/mL protein concentration) in MOPS-KCl buffer with 5 μM cortisone and 50 μM G6P in the final volume of 150 μL at 37°C for 30 min. The reaction catalyzed by 11βHSD1 was assessed directly in similar conditions by measuring $NADP^+$-driven cortisol oxidation after pretreatment of the microsomes with pore-forming alamethicin (0.1 mg/mg protein). These permeabilized vesicles (0.5 mg/mL protein) were incubated in MOPS-KCl buffer containing 5 μM cortisol and 50 μM $NADP^+$ in the final volume of 150 μL at 37°C for 30 min. In either case, the reaction was terminated with 150 μL ice-cold methanol, and the samples were stored at −20°C until HPLC analysis of cortisone and cortisol contents.

2.4. Fluorimetric Detection of NADPH; Measurement of H6PD and 11βHSD1 Activities

NADPH was detected by fluorimetry at 350 nm excitation and 500 nm emission wavelengths using a Cary Eclipse spectrofluorimeter (Varian, Woburn, MA). Endogenous NADPH content of intact microsomes was monitored at 1 mg/mL protein concentration in MOPS-KCl buffer. Oxidation and reduction of the luminal pyridine nucleotide pool was induced by metyrapone (5 μM) and G6P (100 μM), respectively. H6PD activity was assessed by real-time detection of NADPH production. Intact microsomes (1 mg/mL protein) were incubated in a fluorimetric cuvette in MOPS-KCl buffer containing 2 mM $NADP^+$ and 100 μM G6P at 37°C to record a baseline. The reaction was initiated by eliminating the membrane barrier using alamethicin (0.1 mg/mg protein) to allow free access of the substrates

to the intraluminal enzyme. 11βHSD1 activity was measured similarly but G6P was replaced with cortisol (100 μM) in the incubation medium. Fluorimetric detections were finished with NADPH standard (0.5 μM) addition in both cases.

2.5. Endogenous Reducing/Oxidizing Capacity of the Microsomal Vesicles

Intrinsic cortisone reducing or cortisol oxidizing capacity of the microsomes was analyzed by incubating intact microsomal vesicles (0.5 mg/mL protein concentration) in MOPS-KCl buffer at 37°C for 2 h in the presence of 10 μM cortisone or cortisol, respectively. The reactions were terminated by adding equal volume of ice-cold methanol. Samples were stored at −20°C until HPLC analysis of cortisone and cortisol contents.

2.6. HPLC Analysis

The methanol-treated samples were centrifuged (20,000*g* for 10 min at 4°C) and the protein-free supernatants were analyzed to measure cortisone and cortisol simultaneously. Compounds were separated by HPLC (Alliance 2690; Waters, Milford, MA) in a Nucleosil 100 C18 column (5 μm, 25 × 0.46) (Teknokroma, Barcelona, Spain) with isocratic methanol-water (58:42, vol/vol) at 0.7 mL/min flow rate; and the absorbance in the eluate was detected at 245 nm wavelength (Waters Dual λ Absorbance Detector 2487). Retention times of cortisone and cortisol were determined by injecting 10 μM standards.

2.7. Lipid Peroxidation

Oxidative damage of the membrane lipids was tested by measuring the formation of thiobarbituric acid reactive substances (TBARS). Microsomal vesicles (1 mg protein/mL) were incubated in MOPS-KCl buffer for 5 min at 37°C, in the absence or presence (50 or 100 μM) of EGCG. Lipid peroxidation was triggered by the combination of 100 μM Fe^{2+} and 1 mM ascorbate as a positive control. TBARS were detected in samples prepared by the addition of trichloroacetic acid (5% final concentration) as described earlier [27].

2.8. Statistical Analysis

Experiments were performed in triplicate, with each of the values of a single set of experiments corresponding to the mean of a minimum of two to three determinations. Data are presented as mean ± SEM. The results were analyzed by one-way ANOVA and Tukey-Kramer Multiple Comparison Test using GraphPad Prism® software. A *P* value below 0.05 was considered as a significant difference.

3. Results

3.1. Concentration-Dependent Inhibition of Microsomal Cortisol Production by EGCG

The possible interference of EGCG with the overall activity of the G6PT-H6PD-11βHSD1 triad (*i.e.*, the cortisone reducing machinery of the ER) was tested first. Intact rat liver microsomal vesicles (0.5 mg/mL) were incubated in the presence of cortisone (5 μM) and G6P (50 μM) substrates for 30 min, and the amount of cortisol was measured with HPLC. Cortisol production was significantly inhibited by EGCG at 25 μM level; and the effect increased in a concentration-dependent manner (Fig. 1). The rate of cortisone–cortisol conversion was less than half of the control value at 25 μM and hardly detectable above 50 μM concentrations of the flavanol.

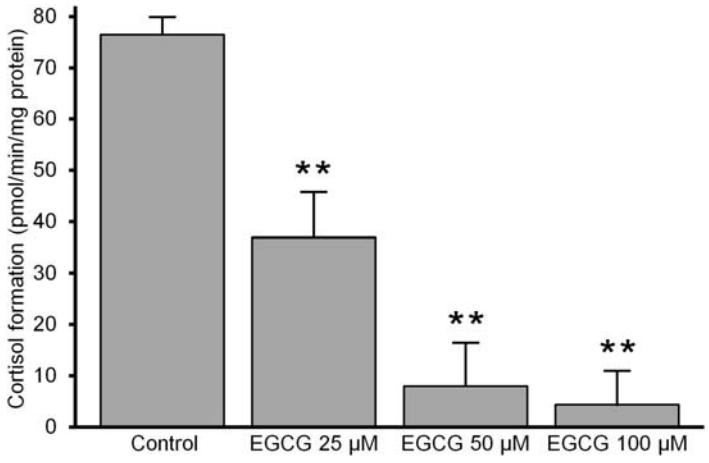

FIG 1 *Microsomal cortisol production. Intact rat liver microsomal vesicles (0.5 mg/mL) were incubated at 37 °C for 30 min in MOPS-KCl buffer supplemented with cortisone (5 μM), glucose 6-phosphate (50 μM), and EGCG in different concentrations as indicated. The amount of cortisol was then measured with HPLC. The rate of cortisol production was calculated and shown as mean + SEM,* n = *3, **P < 0.01 versus control.*

3.2. Effect of EGCG on 11βHSD1 and H6PD Activities Separately

Hindrance of G6P-driven cortisol production observed in intact microsomes can be due to either direct inhibition of 11βHSD1 or decreased luminal NADPH generation. In other words, all the three members of the G6PT-H6PD-11βHSD1 triad are potential targets of EGCG action. However, attenuation of G6P uptake into rat liver microsomal vesicles, that is, inhibition of G6PT by EGCG was ruled out in our previous study [28] but the two luminal dehydrogenases remain to be investigated. The pore forming alamethicin (0.1 mg/mg protein) uncouples H6PD and 11βHSD1 activities by providing free access of the enzymes to NADP(H) cofactors added into the incubation medium. Therefore, the two enzymes can be separately studied in permeabilized microsomes.

H6PD and 11βHSD1 activities were assessed by incubation of rat liver microsomes in the presence of $NADP^+$ and real-time fluorescent detection of G6P-dependent (Fig. 2) or cortisol-dependent (Fig. 3) NADPH production, respectively. Both enzyme activities proved to be completely latent, that is, they only started upon membrane permeabilization due to luminal

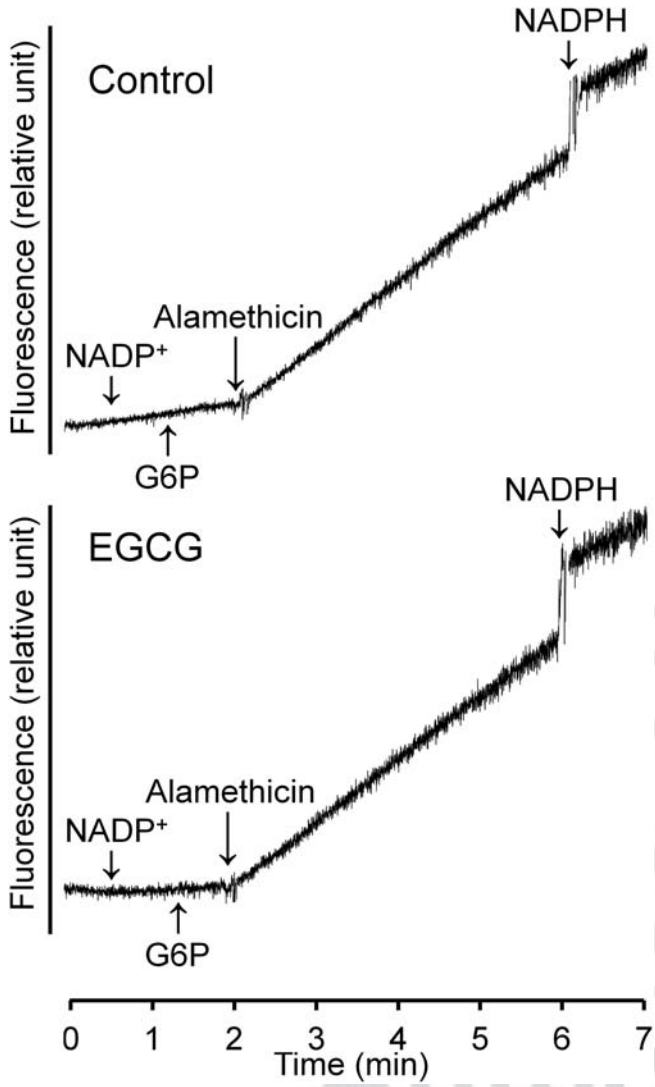

FIG 2 *Hexose 6-phosphate dehydrogenase activity. Intact microsomes (1 mg/mL protein) were incubated in a fluorimetric cuvette in MOPS-KCl buffer containing 2 mM $NADP^+$ and 100 μM G6P at 37 °C in the absence (top panel) or presence of 100 μM EGCG (bottom panel). The baseline fluorescence of NADPH was recorded at 350-nm excitation and 500-nm emission wavelengths. H6PD activity was initiated by permeabilizing the membranes with alamethicin (0.1 mg/mg protein) and the reaction was assessed by real-time detection of NADPH production. Detections were finished with the addition of NADPH standard (0.5 μM). Typical traces of three independent experiments are shown.*

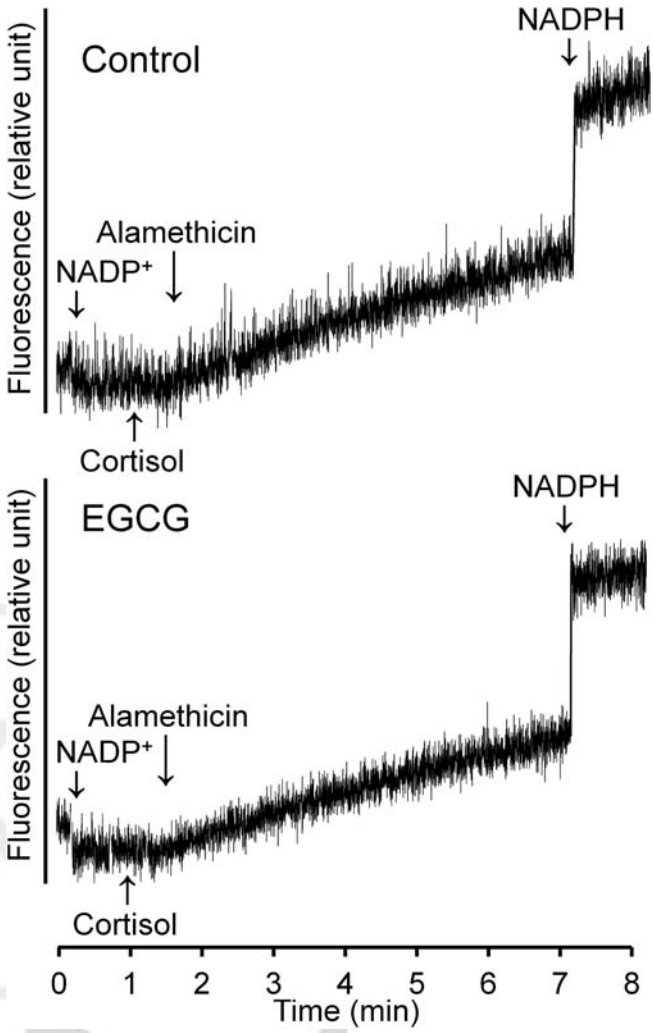

FIG 3 *11β-Hydroxysteroid dehydrogenase type 1 activity. Intact microsomes (1 mg/mL protein) were incubated in a fluorimetric cuvette in MOPS-KCl buffer containing 2 mM $NADP^+$ and 100 μM cortisol at 37 °C in the absence (top panel) or presence of 100 μM EGCG (bottom panel). The baseline fluorescence of NADPH was recorded at 350-nm excitation and 500-nm emission wavelengths. 11βHSD1 activity was initiated by permeabilizing the membranes with alamethicin (0.1 mg/mg protein) and the reaction was assessed by real-time detection of NADPH production. Detections were finished with the addition of NADPH standard (0.5 μM). Typical traces of three independent experiments are shown.*

localization of the enzymes and impermeability of the ER membrane to pyridine nucleotides. This also approves membrane integrity and excludes the interference of extravesicular activities with our measurements. The linear increment of the fluorescent signal corresponds to H6PD or 11βHSD1-mediated NADPH generation. H6PD activity (Fig. 2) was not affected by EGCG even at 100 μM concentration of the flavanol (5.76 ± 0.65 nmol/mg vs. 5.77 ± 0.41 nmol/mg control value, mean ± SEM; $n = 3$); whereas 11βHSD1 activity was only slightly inhibited (0.68 ± 0.05 nmol/mg vs. 0.82 ± 0.03 nmol/mg control value, mean ± SEM; $n = 3$; $P < 0.05$). This effect of EGCG on 11βHSD1 activity was also tested by HPLC measurement of cortisol–cortisone conversion in permeabilized microsomes (Fig. 4). In line with the fluorimetric experiments, the F4

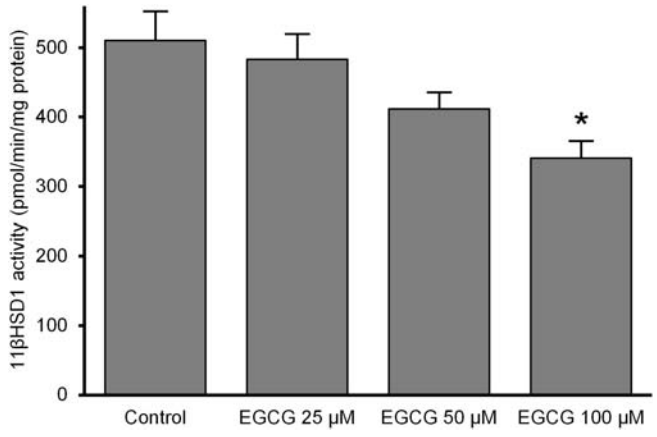

FIG 4 *NADP$^+$-driven cortisol oxidation in permeabilized microsomes. 11βHSD1 activity was assessed separately on the basis of cortisol–cortisone conversion. Microsomes (0.5 mg/mL protein) permeabilized with alamethicin (0.1 mg/mg protein) were incubated at 37 °C for 30 min in MOPS-KCl buffer supplemented with cortisol (5 μM) and NADP$^+$ (50 μM) and EGCG in different concentrations as indicated. The amount of cortisone was then measured with HPLC. The rate of cortisone production was calculated and shown as mean + SEM,* n = *3,* *P < *0.05 versus control.*

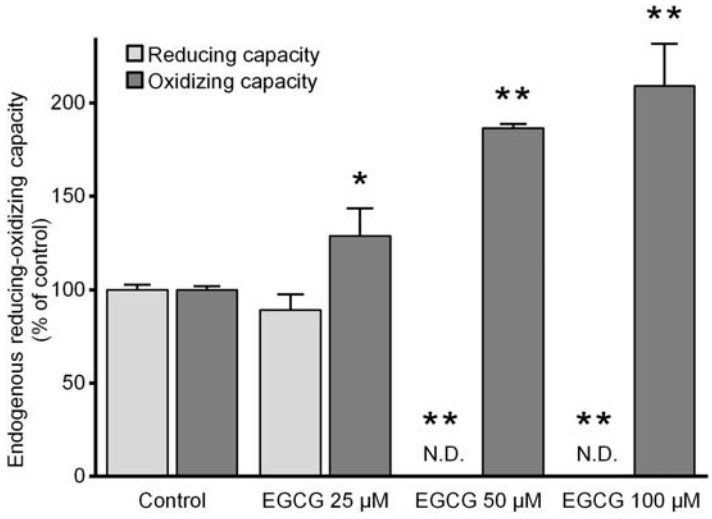

FIG 5 *Endogenous reducing and oxidizing capacities of the liver microsomes. Intrinsic cortisone reducing or cortisol oxidizing capacity of the microsomes was analyzed by incubating intact microsomal vesicles (0.5 mg/mL protein concentration) in MOPS-KCl buffer at 37 °C for 2 h in the presence of 10 μM cortisone or cortisol, respectively. The amounts of cortisol and cortisone were then measured with HPLC. Data are expressed as % of the control values (cortisone reduction: 0.55 ± 0.04 pmol/min/mg protein; cortisol oxidation: 2.37 ± 0.31 pmol/min/mg protein) and shown as mean + SEM,* n = *3,* *P < *0.05,* **P < *0.01 versus control. ND: not detectable (below 1% of control).*

alternative approach also revealed only a small inhibition. Statistically significant, ~30% reduction of 11βHSD1 activity was found only at the highest (100 μM) EGCG concentration (0.34 ± 0.02 nmol/mg vs. 0.51 ± 0.04 nmol/mg control value, mean ± SEM; $n = 3$; $P < 0.05$). It can be concluded that the remarkable suppression of microsomal cortisol production observed in intact microsomes (Fig. 1) cannot be attributed to direct inhibition of either protein component involved in the process.

3.3. Luminal Redox Shift in EGCG-Treated Microsomes

The above results show that intact membrane barrier and retained compartmentation are prerequisites for an efficient inhibition of the cortisol producing machinery by EGCG. This strongly suggests that the existence of a separate NADP$^+$-NADPH pool plays a key role in the mechanism of action, and oxidation of luminal NADPH might underlie the attenuation of cortisone reduction, as it was reported earlier in case of metyrapone [5].

Alteration in the redox state of luminal NADP$^+$-NADPH pool was first studied by estimating the endogenous reducing-oxidizing capacity of the microsomes, that is, their intrinsic ability to convert cortisone to cortisol and vice versa. The relevant enzyme activities were essentially not affected by EGCG; therefore, they could be reliably utilized in such an indirect analysis. Intact microsomes were incubated with either cortisone or cortisol and the rate of cortisol or cortisone production (driven merely by endogenous reducing or oxidizing power) was measured by HPLC. Cortisone reducing power of the microsomes (control activity: 0.55 ± 0.04 pmol/min/mg protein; mean ± SEM; $n = 3$) was remarkably suppressed by EGCG in a concentration-dependent manner (Fig. 5). The effect was already significant at 25 μM level, and cortisol production was not detectable in the presence of the flavanol at 50 μM concentration or above. At the same time, cortisol oxidation (control activity: 2.37 ± 0.31 pmol/min/mg protein; mean ± SEM; $n = 3$) was largely enhanced by EGCG treatment (Fig. 5). The effect was saturable: the rate of cortisone production could be more than doubled upon addition of 50 μM EGCG and did not further increase at higher concentrations of the flavanol.

Shift in the endogenous cortisol–cortisone conversion toward cortisone production is likely due to oxidation of luminal pyridine nucleotides. Therefore, changes in NADPH content of the ER vesicles were also assessed directly by fluorimetry. Luminal NADPH can be detected as stable baseline fluorescence in intact microsomal vesicles. Addition of EGCG caused a remarkable decrement of this signal, which could be partially reverted by the addition of G6P (Fig. 6). This phenomenon corresponds to an EGCG-induced conversion of luminal NADPH to NADP$^+$ and a concomitant G6P-driven regeneration of NADPH. Similar redox shift was detected upon the addition of metyrapone (Fig. 6) or cortisone (not shown) in accordance with several studies reported earlier [9,29]. When NADPH was added to a protein free EGCG solution (100 μM) in the same

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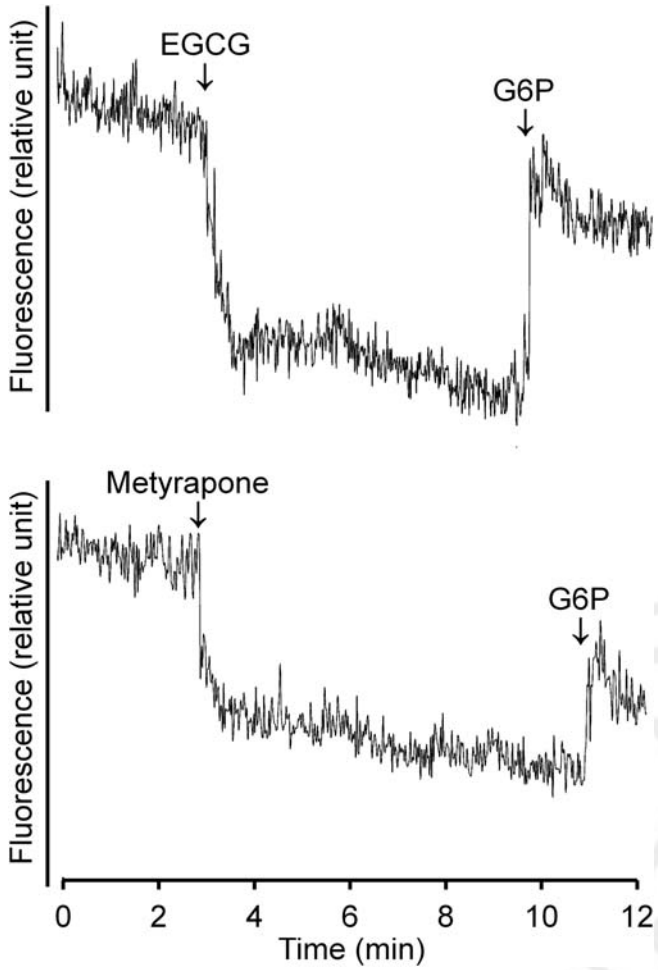

FIG 6

Modulation of NADPH level in the microsomal lumen. Endogenous NADPH content of intact microsomal vesicles was monitored at 1 mg/mL protein concentration in MOPS-KCl buffer by detecting fluorescence at 350-nm excitation and 500-nm emission wavelengths. Oxidation of the luminal pyridine nucleotide pool was found after addition of 100 μM EGCG (top panel) or 5 μM metyrapone (bottom panel). The redox shift could be, at least partly, counteracted by the administration of 100 μM G6P in both cases. Typical traces of three independent experiments are shown.

experimental condition, a sustained increase of the fluorescent signal was recorded (not shown), which rules out both nonenzymatic oxidation of NADPH and fluorescence quenching by EGCG.

The observed oxidative shift in the luminal $NADP^+$-NADPH pool could be just part of a general oxidative stress involving peroxidation. Therefore, the possible effect of EGCG on lipid peroxidation was also tested and compared to ascorbate/Fe^{2+} combination by measuring the amount of TBARS in rat liver microsomes. According to the basically antioxidant features of tea flavanols, EGCG slightly (although not significantly) decreased rather than increased peroxidation of membrane

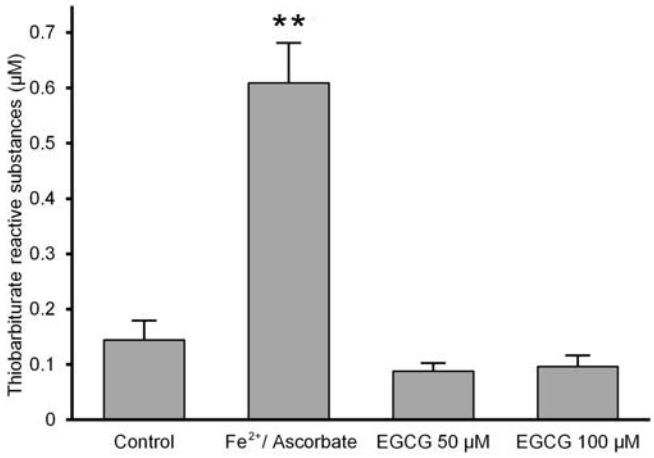

FIG 7

*Lipid peroxidation in the microsomes. Microsomal vesicles (1 mg protein/mL) were incubated in MOPS-KCl buffer for 5 min at 37 °C, in the absence or presence of EGCG (50 or 100 μM). Lipid peroxidation caused by the combination of 100 μM Fe^{2+} and 1 mM ascorbate was used as a positive control. Thiobarbituric acid reactive substances (TBARS) were detected in samples prepared by the addition of trichloroacetic acid (5% final concentration). TBARS contents of the microsomes did not change significantly upon EGCG treatment. Data are shown as mean + SEM, n = 3, **P < 0.01 versus control.*

lipids (Fig. 7), which strongly contradicts the role of a massive oxidative challenge in our system. F7

4. Discussion

Obesity epidemic is a global and growing medical problem. The condition is often associated with a complex metabolic disorder called the metabolic syndrome, which can lead to type 2 diabetes. Many of the features of the common metabolic syndrome resemble those of the rare Cushing zczc syndrome, which indicates the role of glucocorticoid action despite normal plasma cortisol levels [10]. This finding drew attention to the prereceptor mechanisms modulating the hormone actions.

Cortisol and the prohormone cortisone can be interconverted by 11β-hydroxysteroid dehydrogenase types 1 and 2 (11βHSD1 and 2) in the target cells. These two isoenzymes are fundamentally different regarding tissue specificity, topology, cofactor dependence, and physiological function. The cytosolic NAD^+ dependent 11βHSD2 is expressed in mineralocorticoid target tissues, such as kidney, colon, and salivary glands. It oxidizes cortisol to cortisone and thereby confers aldosterone selectivity on inherently nonselective mineralocorticoid receptors [30]. In contrast, the NADPH dependent 11βHSD1 is localized in the ER and is expressed in the glucocorticoid target tissues including liver, adipose tissue, and skeletal muscle, which play key roles in the distribution, breakdown and storage of fuel molecules in the human body. 11βHSD1 is capable of

reducing cortisone to cortisol, which makes this enzyme a major modulating factor of the glucocorticoid action in the target cells [6], and also a key player in adipocyte differentiation [5].

Mounting evidence support the role of local cortisol production in the metabolic syndrome. All or most symptoms appear in mice overexpressing 11βHSD1 [12,13], while 11βHSD1 knockout mice [16,17] seem to be protected from the disease. Selective inhibitors of 11βHSD1 are, therefore, considered as promising novel agents for the treatment of metabolic syndrome and diabetes [18]. However, reductase and dehydrogenase activities of 11βHSD1 vary in different tissues [6]. Some studies indicate cortisol oxidation by 11βHSD1 in subcutaneous adipose tissue, and this seems to contribute to a recycling between cortisol and cortisone in various human tissues *in vivo* [31]. Inhibition of both reductase and dehydrogenase activities of 11βHSD1 provides a limited effectiveness in decreasing the overall glucocorticoid activity, which might explain their limited success in phase 2 clinical trials. The selectivity of such drug molecules should not only be focused on the difference between 11βHSD1 and 2. The best therapeutic efficacy could be expected from chemicals that specifically inhibit reductase activity of 11βHSD1 and, ideally, also increase its dehydrogenase activity [18].

Localization of 11βHSD1 in a separate metabolic compartment of the cell provides a special strategy to modulate the balance between its dehydrogenase and reductase activities. The ER luminal 11βHSD1 utilizes the separate NADPH-NADP$^+$ pool of the organelle. Whether the enzyme acts as a reductase or dehydrogenase depends on the luminal redox conditions (*i.e.*, NADPH:NADP$^+$ ratio), which underlines the importance of compartmentation [7]. Reductase activity dominates in the liver, which is considered as the major source of extra-adrenal cortisol production [32]. Cortisone reducing capacity of hepatic microsomes has been shown to decrease progressively during starvation in rats [29], which strongly supports the theory that the luminal redox conditions play a central role in the nutrient sensor function of the ER [33]. In addition, NADPH generation and consequent cortisone reduction in the hepatic ER have been recently shown to be fuelled by fructose 6-phosphate, and this provides new perspective into the mechanism of how fructose ingestion contributes to obesity and the metabolic syndrome [34]. Lack of 11βHSD1 reductase activity and increased dehydrogenase activity was found in H6PD knockout mice, which have starving hypoglycemia, increased insulin sensitivity and are resistant to diabetogenic diet [14,15]. These observations indicate that chemicals capable of lowering the NADPH:NADP$^+$ ratio in the ER lumen of certain cells, particularly in the liver, should be considered as promising antiobesity and antidiabetic agents.

Oxidative shift in the ER luminal NADPH-NADP$^+$ pool can be achieved by the administration of metyrapone, as it has been already demonstrated in several studies (*e.g.*, 9, 5). However, metyrapone is primarily a steroid 11β-hydroxylase inhibitor and also a substrate of 11βHSD1, which nonselectively inhibits both types of 11βHSD [35]; therefore, it cannot be regarded as a promising drug molecule in the metabolic syndrome. Our results revealed that EGCG, the most abundant tea flavanol causes the desirable redox shift in hepatic ER vesicles. Efficient inhibition of cortisone reduction was found only in intact microsomes. Our search for the underlying mechanism revealed a pronounced oxidation of luminal NADPH to NADP$^+$, which explains the observed dependence on membrane integrity. EGCG treatment on its own did not damage the investigated enzymes and membrane lipids, that is, 11βHSD1 and H6PD activities were essentially unaffected and lipid peroxidation was not enhanced, which indicates that NADPH oxidation is not due to ROS generation in our experiments. Nonenzymatic direct interaction between EGCG and NADPH was also ruled out. Considering the small volume of the ER (and ER-derived vesicles), the phenomenon is likely attributable to an NADPH consuming luminal enzyme of low capacity, which remains to be identified.

The NADPH-NADP$^+$ redox shift caused by EGCG in the ER lumen might significantly contribute to the beneficial health effects of tea flavanols. Moreover, the mean by which EGCG interferes with microsomal cortisol generation highlights the importance of the separate ER luminal pyridine nucleotide pool as a potential drug target. This mechanism should be regarded as a promising strategy for the development of novel 11βHSD1 inhibitors of double selectivity as it only affects the ER luminal isoenzyme and definitely shifts cortisone–cortisol conversion toward oxidation, which is desirable for the prevention and treatment of obesity, metabolic syndrome, and diabetes.

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