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COMPLEX ESTROGENIC REGULATION OF CATECHOL-*O*-METHYLTRANSFERASE (COMT) IN RATS

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Catechol-*O*-methyltransferase (COMT) activity depends on gender, age and physiological status suggesting that estrogen may regulate COMT activity. In fact, estrogens down-regulate the function of COMT promoters in cell cultures. On the other hand, COMT may play an important role in estrogen-induced cancers due to its ability to inactivate estrogen metabolites and thereby lowering the levels of these potential carcinogens. In this study, we explored the effect of estrogen on COMT activity *in vivo* in rats. Male and female Wistar rats received 14-day treatments with either estradiol (100 µg/kg/day; s.c.) or tamoxifen (500 µg/kg/day; s.c.), respectively; in addition ovariectomized rats were studied. COMT activity and COMT protein expression were measured from various brain- and peripheral tissues. Although we found a regulatory function of estrogen, its effects were sex and tissue dependent. Antagonizing the effects of estrogen *via* tamoxifen increased COMT protein expression in several central and peripheral tissues. However, amounts of COMT protein and COMT activities did not always match. Generally, COMT activities were quite resistant to the effects of tamoxifen and estradiol. Estradiol, unexpectedly, doubled the amount of COMT protein in the prostate but exhibited down-regulatory function in the prefrontal cortex and kidneys. Ovariectomy by itself, however, had only minor effects on COMT activity and expression. It is noteworthy that the estrogen down-regulation and tamoxifen up-regulation of COMT were best substantiated in the prefrontal cortex and kidneys where COMT is physiologically important for dopamine metabolism.

Key words: *Catechol-O-methyltransferase, estrogen, hormones, rodents, tamoxifen, ovariectomy, prefrontal cortex, dopamine*

INTRODUCTION

Catechol-*O*-methyltransferase (COMT) is present in practically all mammalian tissues (1). COMT activity is highest in the liver (1-3), followed by the kidneys and the gastrointestinal tract (4). In the brain, COMT is present in astroglia, microglia, and postsynaptic neurons (5) and plays a particularly important role in the prefrontal cortex (PFC) (6). Several neurotransmitters (such as dopamine and noradrenaline), catecholestrogens (such as 4-hydroxyestradiol), and drugs (such as L-dopa) (1, 7) are substrates of COMT. Because of these numerous substrates, COMT may play an important role in the pathophysiology of various human disorders including schizophrenia, kidney disease, hypertension, Parkinson's disease, and estrogen-induced cancers (1, 8). In fact, high estrogen levels have been linked to increased incidents of breast and prostate cancers in humans (for review see (9, 10)). In extrahepatic tissues, COMT is the key player in the inactivation of the catecholestrogens, 2- and 4-hydroxyestradiol. Especially 4-hydroxyestradiol has been implicated in cancer initiation as it can be oxidized to estrogen-3,4-quinones which in turn react with DNA. Thus, the potential functional importance of COMT has raised interest in its endogenous regulation.

The COMT protein is coded for by one single COMT gene located on chromosome 22, band q11.2b in humans and exists in two distinct isoforms. Two different promoters drive the expression of two mRNA transcripts: the P1 promoter drives the expression of the shorter (1.3 kb in human and 1.9 kb in rats) soluble COMT (S-COMT) while the upstream P2 promoter produces the longer membrane-bound COMT (MB-COMT) (1.5 kb in human and 1.9 kb in rats) as well as S-COMT (11-13).

Although COMT activity is not easily induced or suppressed, several studies report altered COMT activity depending on physiological status, gender or age. For instance, hepatic COMT activity increases about 10-fold from birth to adulthood (7). The kidney follows a similar trend with K_m values increasing about 5-fold during maturation from prepubertal period to adulthood (14).

Several studies indicate a role for sex hormones in the regulation of COMT activity. For instance, hepatic COMT activity decreases as a result of estrogen exposure in the rat (15, 16). In the rabbit, pregnancy significantly decreases COMT activity in the adrenal gland (17). On the other hand, subchronic estrogen treatment increases COMT immunostaining in hamster kidney (18), indicating tissue specific differences in COMT regulation. Lastly, hepatic COMT activity is about 30% higher in males than in females (19-21), evidently owing to an inhibitory activity of estrogens on both COMT promoters. In 1999, Xie *et al*. (22) demonstrated that estrogen is able to decrease COMT gene transcription *in vitro*. In a follow-up paper, Jiang *et al*. (23) were able to demonstrate that estrogen also significantly reduces COMT activity and COMT protein levels *in vitro*.

A recent study by Meyers *et al*. (24) in male rats revealed that gonadectomy itself has no effect on COMT activity in the

prefrontal cortex (PFC) and striatum. However, estradiol replacement increased S-COMT activity in the PFC (24). We are aware of only one earlier study (in aromatase knock-out mice) in which low estrogen levels were correlated to COMT expression (25). However, we are not aware of any *in vivo* study investigating the effects of estradiol on COMT in brain and peripheral tissues.

The aim of this study was to investigate the down-regulatory effect of estradiol on COMT protein expression and activity in rats. The actions of estradiol were either agonized or antagonized using selective treatments. Therefore, male and female Wistar rats received 14-day treatments with estradiol or tamoxifen, respectively. Although originally developed as an antiestrogen, tamoxifen is now classified as a first generation selective estrogen receptor modulator (SERM) (for review see (26, 27)) and can tissue-dependently act as either an estrogen receptor (ER) agonist, antagonist or mixed agonist/antagonist. In addition to drug treatments, we used ovariectomized rats, which produce only very little estradiol. Although the down-regulatory function of estrogen varied between different tissues it was most reproducible in the PFC and kidneys where COMT is a central regulator of dopamine metabolism.

MATERIALS AND METHODS

Animals

Female and male adult Wistar rats (body weights between 180-200 g) were obtained from Harlan, The Netherlands. Rats were housed in clear polycarbonate cages in groups of 2-4 and were habituated to the experimental procedure 10 days prior to the start of dosing. All rats were maintained under a 12:12 h light/dark cycle with lights on from 06:00 to 18:00 at an ambient temperature of 20-22°C. Standard rat chow and tap water were available *ad libitum*. The experiments were conducted according to the "European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific purposes", and reviewed and approved by the Animal Experiment Board of the Provincial Office in Southern Finland.

Ovariectomy

Rats were ovariectomized by the supplier (Harlan, The Netherlands). Rats were allowed to recover from the surgical procedures for 4 weeks before the experiments began. In order to reduce the number of animals used in this experiment we decided not to include a sham-ovariectomized group as any effects of surgery on COMT activity would have been acute and not detectable anymore 4 weeks post surgery.

Treatments

All test compounds were dissolved in a minimal amount of 96% ethanol and diluted to a final working concentration with sesame oil (Sigma-Aldrich, St. Louis, MO, USA). Female rats (n=10/group) received subcutaneous (s.c.) injections of tamoxifen 500 µg/kg (Sigma, St. Louis, MO, USA) or vehicle; male rats (n=10/group) received daily s.c. injections of estradiol 100 µg/kg (Sigma, St. Louis, MO, USA) or vehicle for 14 days. Ovariectomized rats (n=5/group) received s.c. injections of estradiol 100 µg/kg or vehicle daily for 14 days.

Clinical signs and body weights

Throughout the study period, each rat was observed at least once daily for clinical signs related to the drug treatment. In addition, the body weight of each rat was recorded daily prior to injections. There was no mortality during the treatment period.

Dissections

After the 14-day treatment period rats were sacrificed by decapitation. The PFC, striatum, hippocampus, hypothalamus, cerebellum, liver, and kidneys were dissected, weighed and directly stored at -80°C until further processed. In addition, ovaries and uterus were dissected from female rats and prostate and testes from male rats.

Plasma estradiol levels

Blood from each animal was collected and centrifuged at 500 g for 15 minutes. The supernatants were collected, and later analyzed using DELFIA® Estradiol kit (Wallac Oy, Turku, Finland; catalog number 1244-056).

Catechol-O-methyltransferase activity analysis

The COMT enzyme activity assay was performed as described earlier (28). In brief, the enzyme preparation was incubated at +37°C in 100 mM phosphate buffer (pH 7.4) containing 5 mM $MgCl_2$, 200 µM S-adenosyl-L-methionine (Sigma, St. Louis, MO, USA) and 500 µM 3,4-dihydroxybenzoic acid (Sigma, St. Louis, MO, USA). A high-performance liquid chromatographic (HPLC) system with electrochemical detection was used to analyze the reaction products, vanillic and isovanillic acid. The system consisted of a sample autoinjector (Jasco AS-2057, Tokyo, Japan), a pump (Merck Hitachi LaChrom L-7100, Tokyo, Japan), an RP-18 column (3 mm, 4.6x150 mm; Waters Spherisorb, Milford, MA, USA), a coulometric detector (ESA Coulochem model 5100A detector and a model 5014B cell; ESA Inc., Chelmsford, MA, USA; detector potential -0.30 mV) and an integrator (Shimadzu C-R5A; Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of 0.1 M Na_2HPO_4 (pH 3.3), 0.15 mM EDTA and 25% methanol; the flow-rate was 0.8 ml/min.

The protein concentrations of the samples were determined based on the bicinchoninic acid method using Pierce protein assay kit (Pierce Biotechnology, Rockford, IL, USA). COMT activity is expressed as picomoles vanillic acid formed in one min per mg of protein in the sample.

Catechol-O-methyltransferase protein expression

Western Blots were performed as described earlier (28). In short, the samples were diluted 1:20 with homogenization buffer (10 mM Na_2HPO_4, pH 7.4, containing 0.5 mM dithiothreitol) and subsequently homogenized using a sonicator (Rinco Ultrasonics sonicator, Arbon, Switzerland). The homogenates were centrifuged at 890xg at +4°C for 10 min and the supernatants were collected. The samples were then diluted with Laemmli buffer so that 20 µg of total protein was loaded and electrophoresis was performed in a 12% SDS-polyacrylamide gel. Subsequently, samples were blotted onto Protran® nitrocellulose transfer membrane (Schleicher&Schuell Bioscience GmbH, Dassel, Germany). After blocking nonspecific binding, the membranes were incubated with mouse anti-COMT monoclonal antibody overnight at +4°C (1:5000; BD Bioscience Pharmingen, San Diego, CA, USA). Following this, the membranes were processed with goat anti-mouse secondary antibody (1:2000; R&D Systems, Minneapolis, MN, USA) conjugated with horseradish peroxidase. Blots were visualized using chemiluminescent substrate (Thermo Scientific, Rockford, IL, USA) and detected and quantified using

GeneGnome chemiluminescent detector and corresponding software (SynGene, Synoptics LTD, UK). COMT bands were normalized to the beta actin loading control.

Statistics

All values are expressed as mean ±S.E.M. Statistical analyses for significant differences were performed with one-way ANOVA followed by Tukey's *post hoc* test (*Fig. 1A-H*) and unpaired t-test (*Fig. 2A-B*) using GraphPad Prism 5.0 program (GraphPad Software, Inc., San Diego, CA, USA). The criterion for statistical significance was $p<0.05$.

RESULTS

Ovariectomy and drug treatments

Body weights were not different between any of the respective treatment groups at the beginning of the experiments (data not shown). However, at the end of the chronic treatment period ovariectomized rats had gained more weight than their control peers ($p<0.05$), and exhibited lower uterus weights ($p<0.05$) and uterus/body weight ratios ($p<0.05$). Lower uterus weights and uterus/body weight ratios are indicative of effective removal of the ovaries. Estradiol treatment increased uterus weight ($p<0.01$) and uterus/body weight ratio ($p<0.01$) compared to ovariectomy alone and these values were also slightly, but not significantly higher than in intact rats (*Table 1*).

Plasma estradiol levels after ovariectomy were only marginally reduced compared with control rats; however, estradiol treatment of ovariectomized rats slightly, but not significantly, increased plasma estradiol levels (*Table 1*). Female rats treated with tamoxifen displayed significantly lower body weights ($p<0.05$) and weight gain ($p<0.01$) than the control rats at the end of the treatment period. Uterus weight, uterus/body weight ratio, and ovarian weight were also significantly lower in the tamoxifen group ($p<0.05$) compared to the control group, confirming the antagonistic effect of tamoxifen on the uterus of intact, vehicle-treated female rats (*Table 1*). Plasma estradiol

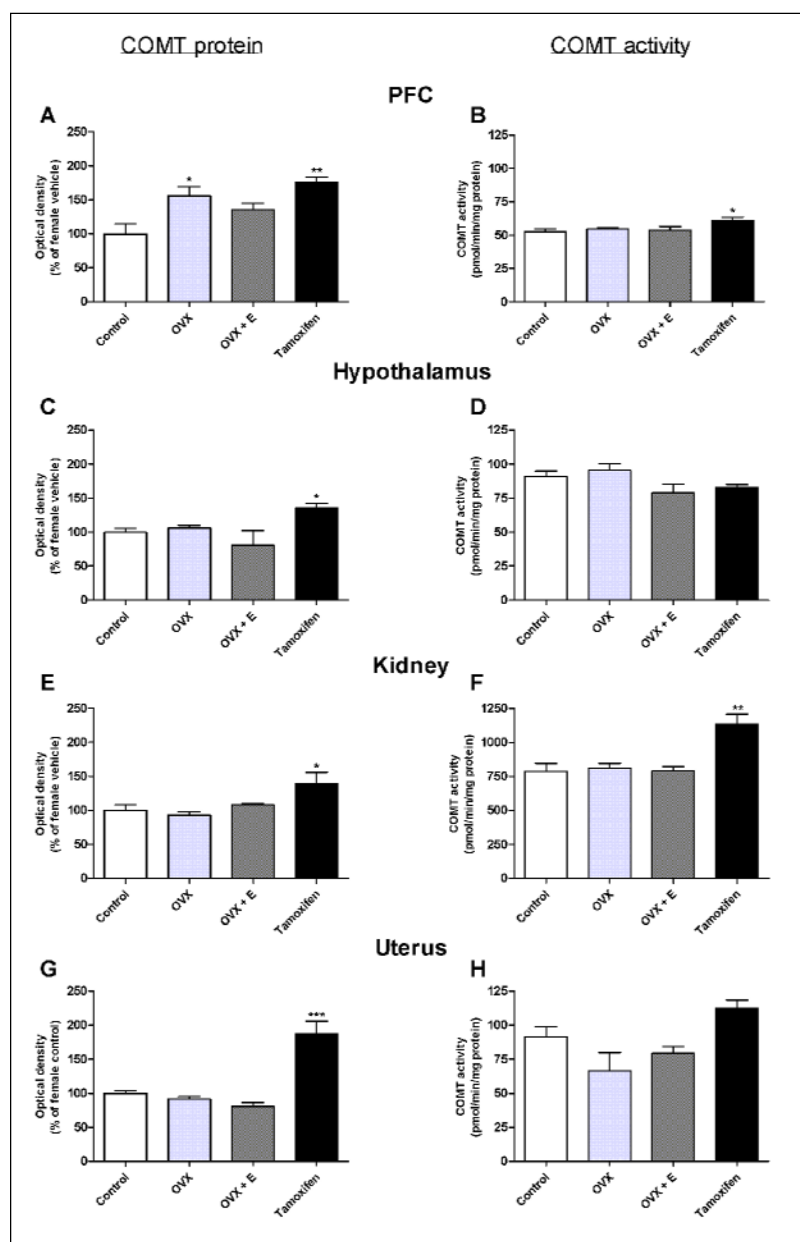

Fig. 1. COMT protein expression and enzyme activity in brain and peripheral tissues of treated and untreated female rats. Values are expressed as mean ±S.E.M. and represent total COMT values normalized to the values obtained from tissues of intact, vehicle-treated females; n=5-10. Ovariectomy itself only significantly increased S-COMT protein expression (total COMT 156%; S-COMT 168%; $p<0.05$; MB-COMT 134%) in the PFC (*Fig. 1A*) but failed to affect COMT activity (*Fig. 1B*). Reintroduction of estradiol (100 µg/kg/day) to ovariectomized rats had no effect on COMT protein expression or activity (*Fig. 1A-H*). COMT protein expression increased in the PFC (total COMT 175%; $p<0.01$; S-COMT 168% and MB-COMT 188%; $p<0.001$ and $p<0.001$ respectively), hypothalamus (total COMT 135%; $p<0.05$; S-COMT 136% and MB-COMT 132%; $p<0.05$ and $p<0.01$, respectively), kidneys (total COMT 144%; $p<0.05$; S-COMT 119% and MB-COMT 149%; $p<0.05$ and $p<0.05$ respectively) and uterus (total COMT 187%; $p<0.001$; S-COMT 195%; $p<0.01$ and MB-COMT 153%) in response to tamoxifen treatment (500 µg/kg/day) (*Fig. 1A, C, E, G*). Tamoxifen significantly increased COMT activity in the PFC (60.8 *vs*. 52.4 pmol/min/mg protein; $p<0.05$) and kidney (1119.6 *vs*. 781.5 pmol/min/mg protein; $p<0.01$) (*Fig. 1B, F*). Statistics: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ *vs*. the corresponding vehicle treated group.

Table 1. Body weight development, uterus and ovarian weights and estradiol levels of the rats.

Group	n	Weight gain	Final UW	UW/BW x 1000	Final OW	Estradiol (pg/ml)
Female rats						
Control to OVX	5	18±3	0.32±0.03	1.40±0.13	n.a.	62.5±9.8
OVX	5	24±4[a]	0.14±0.05[a]	0.58±0.21[a]	n.a.	45.9±14.1
OVX + E	5	2±5[†]	0.36±0.03[†]	1.57±0.11[†]	n.a.	163.1±78.0
Control	10	11±3	0.52±0.08	1.96±0.31	0.14 ± 0.01	65.0±32.6
Tamoxifen	10	-7±2[b]	0.26±0.02[a]	1.08±0.09[a]	0.10 ± 0.01[a]	35.7±5.0
Male rats						
Control	10	49±3	n.a.	n.a.	n.a.	34.4±2.4
Estradiol	10	2±2[c]	n.a.	n.a.	n.a.	174.5±25.4[b]

Groups: Control and control to OVX=intact Wistar rat; Tamoxifen=tamoxifen (500 µg/kg/day); Estradiol=estradiol (100 µg/kg/day); OVX=ovariectomized; OVX+E=ovariectomized+estradiol (100 µg/kg/day).
Weight gain=Final BW-Initial BW; Final UW=uterus weight (g) at time of dissection; UW/BWx1000=Final uterus weight divided by final body weight x1000; Final OW=ovarian weight (g) at time of dissection; n.a.=not applicable. Statistics: ap<0.05 compared to respective control rats; b p<0.01 compared to respective control rats; c p<0.001 compared to respective control rats; † p<0.01 compared to OVX rats.

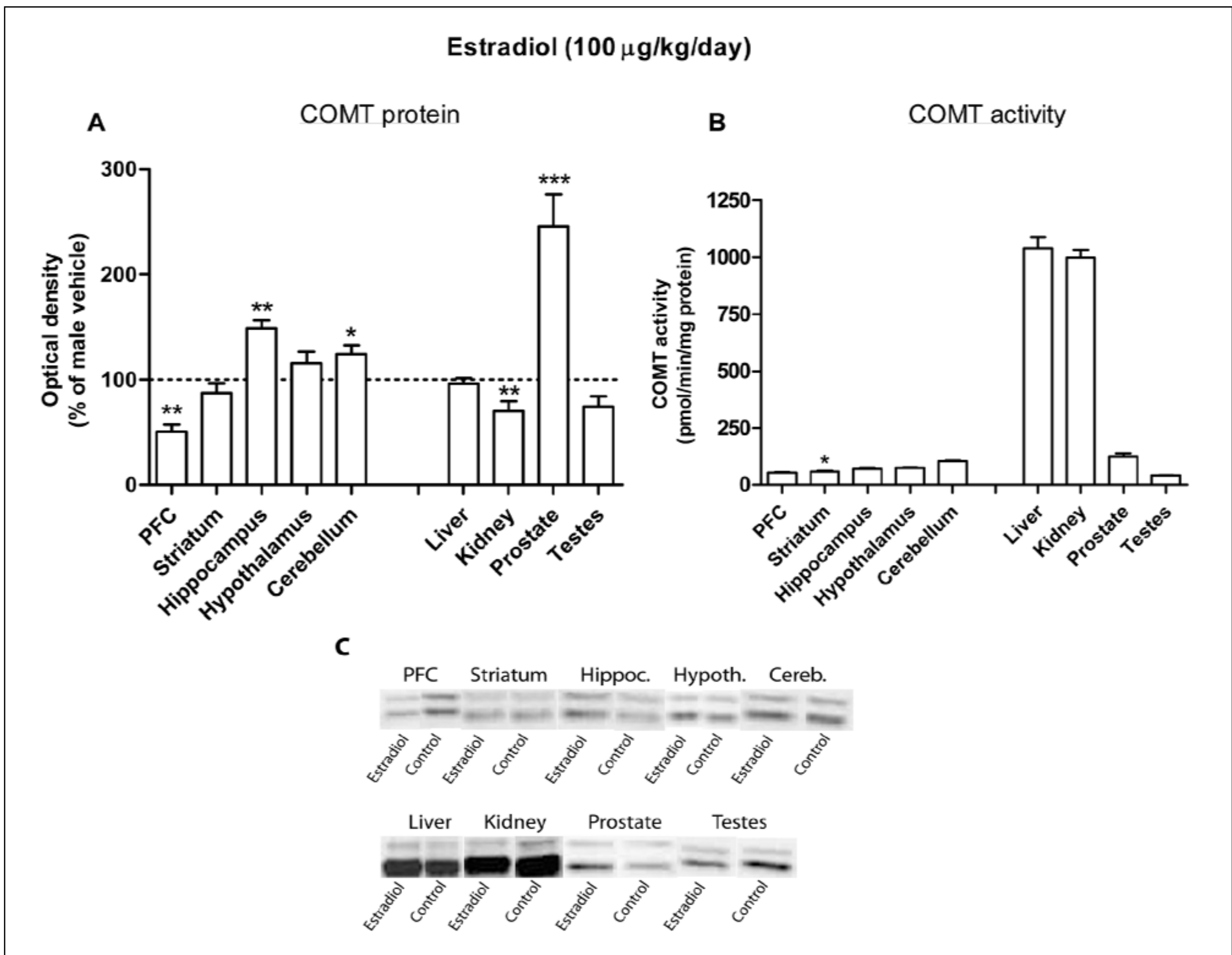

Fig. 2. COMT protein expression and enzyme activity in brain and peripheral tissues of treated and untreated male rats. Values are expressed as mean ±S.E.M. and represent total COMT values normalized to the values obtained from tissues of intact, vehicle-treated males; n=10. Estradiol treatment significantly reduced COMT protein expression in the PFC (total COMT 51%; p<0.01; S-COMT 47% and MB-COMT 58%; p<0.01 and p<0.05, respectively) and kidney (total COMT 70%; p<0.01; S-COMT 70%; p<0.05; MB-COMT 73%) (*Fig. 2A*) while increasing COMT expression in the hippocampus (total COMT 149%; p<0.01; S-COMT 153% and MB-COMT 138%; p<0.001 and p<0.01, respectively), cerebellum (total COMT 124%; p<0.05; S-COMT 122%; MB-COMT 131%; p<0.05;), and prostate (total COMT 245%; p<0.001; S-COMT 275% and MB-COMT 173%; p<0.001 and p<0.01, respectively) (*Fig. 2A*). Estradiol (100 µg/kg/day) increased COMT activity in the striatum of male rats (59.4 *vs*. 47.5 pmol/min/mg protein; p<0.05) but failed to alter COMT activity in other tissues (*Fig. 2B*). *Fig. 2C* depicts representative western blot bands. Statistics: * p<0.05, ** p<0.01 and *** p<0.001 *vs*. the corresponding vehicle treated group.

levels were somewhat decreased in rats receiving tamoxifen compared with vehicle-treated control rats; however, this difference did not reach statistical significance (*Table 1*).

At the end of the treatment period, male rats receiving estradiol displayed significantly lower body weights ($p<0.001$) and body weight gain ($p<0.001$) than their control peers (*Table 1*). Male rats receiving estradiol exhibited significantly increased plasma estradiol levels ($p<0.01$) than their vehicle-treated peers (*Table 1*).

Effect of hormonal treatments on COMT protein expression and activity in female rats

Ovariectomy led to markedly increased COMT protein expression (total COMT 156%; $p<0.05$; S-COMT 168% and MB-COMT 134%; $p<0.05$) (*Fig. 1A*). However, ovariectomy did not cause an increase in prefrontal COMT activity (*Fig. 1B*). COMT protein expression and activity were unaltered after ovariectomy in the hypothalamus (*Fig. 1C, D*), kidney (*Fig. 1E, F*), and uterus (*Fig. 1G, H*). Reintroduction of estradiol to ovariectomized rats failed to alter COMT protein expression (*Fig. 1A, C, E, G*) or COMT activity (*Fig. 1B, D, F, H*) compared with intact, vehicle-treated controls.

In female rats, a 14-day treatment with tamoxifen significantly increased COMT protein expression in the PFC (total COMT 175%; $p<0.01$; S-COMT 168% and MB-COMT 188%; $p<0.001$and $p<0.001$ respectively; *Fig. 1A*), hypothalamus (total COMT 135%; $p<0.05$; S-COMT 136% and MB-COMT 132%; $p<0.05$ and $p<0.01$, respectively; *Fig. 1C*), kidneys (total COMT 144%; $p<0.05$; S-COMT 119% and MB-COMT 149%; $p<0.05$and $p<0.05$ respectively; *Fig. 1E*), ovaries (S-COMT 161% and MB-COMT 159%; $p<0.01$ and $p<0.05$, respectively; data not shown) and uterus (total COMT 187%; $p<0.001$; S-COMT 195%; $p<0.01$; MB-COMT 153%; *Fig. 1G*). COMT activity in the PFC (60.8 *vs*. 52.4 pmol/min/mg protein; $p<0.05$; *Fig. 1B*), and kidney (1119.6 *vs*. 781.5 pmol/min/mg protein; $p<0.01$; *Fig. 1F*) increased in response to tamoxifen.

In addition to the above mentioned tissues, striatal, hippocampal, cerebellar and hepatic samples were analyzed. However, none of the above treatments had any effect on COMT protein expression or activity in these tissues (data not shown).

Effect of hormonal treatments on COMT activity and protein expression in male rats

In male rats, estradiol treatment caused a significant decrease of COMT protein expression in the PFC (total COMT 51%; $p<0.05$; S-COMT 47% and MB-COMT 58%; $p<0.01$ and $p<0.05$, respectively) and kidney (total COMT 70%; $p<0.01$; S-COMT 70%; $p<0.05$; MB-COMT 73%) (*Fig. 2A*). On the other hand, a significant increase in COMT expression occurred in the hippocampus (total COMT 149%; $p<0.01$; S-COMT 153% and MB-COMT 138%; $p<0.001$ and $p<0.01$, respectively), cerebellum (total COMT 124%; $p<0.05$; S-COMT 122%; MB-COMT 131%; $p<0.05$), and prostate (total COMT 245%; $p<0.001$; S-COMT 275% and MB-COMT 173%; $p<0.001$ and $p<0.01$, respectively) in response to the 14-day estradiol treatment (*Fig. 2B*). COMT activity in the striatum increased (59.4 *vs*. 47.5 pmol/min/mg protein; $p<0.05$) in response to estradiol (*Fig. 2B*).

DISCUSSION

The aim of this study was to elucidate the effect of manipulation of estrogen balance on COMT activity and COMT protein expression in an *in vivo* rat model. Female and male Wistar rats received different 14-day drug treatments and the effects on COMT activity and protein expression in different tissues were subsequently measured. Although the outcome of these studies was less than anticipated, the most consistent results were observed in the PFC and kidney.

Lowering estradiol levels by means of ovariectomy increased COMT protein expression in the PFC. Accordingly, tamoxifen treatment increased COMT protein expression and activity in the PFC, pointing to an antiestrogenic effect of tamoxifen in the PFC. Supportingly, increasing estrogen levels in male rats resulted in markedly lower COMT activity and protein expression. COMT is particularly important in the PFC which contains significantly less dopamine transporter (DAT) protein than other brain regions such as the striatum. COMT accounts for less than 15% of dopamine degradation in the striatum (6, 27), but for about 60% of dopamine degradation in the PFC (6, 29, 30). Therefore, the PFC is more likely to be affected by variations in COMT activity than other brain areas as changes in the elimination of synaptic dopamine *via* COMT is much more important in the PFC than in other brain areas. In line with our findings, a recent study by Aydin and colleagues reports that dopamine levels in the PFC significantly decrease after ovariectomy (31). The unexpected absence of significant changes in plasma estradiol levels after ovariectomy may be explained by compensatory mechanisms. Ovariectomy results in significant weight gain and non-reproductive tissues constitute a considerable source of estrogen (32-34). Furthermore, a study by Zhao and colleagues (35) has shown that estradiol levels increase with time after ovariectomy. As we obtained the plasma samples 6 weeks after ovariectomy, it is possible that some of the initial down-regulatory effects of ovariectomy were lost. However, it needs to be kept in mind that our data are only suggestive as they reflect the systemic plasma estradiol concentrations in a limited number of rats. Furthermore, it is possible that specific tissues (*e.g.* the PFC) experienced a more robust change in estradiol levels after ovariectomy. Supporting this hypothesis, Franczak and colleagues report that local intra-uterine concentrations of steroids, rather than blood plasma concentrations, determine endometrial activity and its response to stimuli in pigs (36). Notably, unchanged estradiol levels correlate with likewise unchanged COMT protein and activity levels. Curiously, administration of estrogen to male rats increased COMT protein expression in several central and peripheral tissues. However, estrogen lowered COMT activity and/or protein expression in the PFC and kidney, obeying a previously introduced general rule of down-regulation of the COMT gene function by estrogens (22, 23).

Male (but not female) aromatase knock-out mice, with low estrogen levels, develop compulsive behaviours that are reversed by a 3-week estradiol replacement therapy (25). Interestingly, the presentation of compulsive behaviours is accompanied by concomitant decreases in hypothalamic COMT protein levels. In contrast, hepatic and frontal cortical COMT levels were not affected by the estrogen status, indicating region- and tissue-specific up-regulation of COMT levels by estrogen. A similar trend occured in our studies but tamoxifen increased hypothalamic COMT proteins more strongly than estradiol. In our hands, estradiol significantly increased hippocampal and cerebellar COMT proteins, supporting a site-specific up-regulating action of estrogens in male rats.

In the periphery, manipulation of COMT *via* hormonal treatments yielded most consistent results in the kidney. COMT is able to modulate the rate of dopamine metabolism and consequently the dopaminergic tone of the kidneys. Kidney dopamine exerts natriuretic and diuretic effects by activating D_1-like dopamine receptors in the nephron (37). Activation of D_1-like receptors in turn results in an inhibition of the main sodium

transport mechanisms (namely Na^+, K^+ -ATPase and Na^+/H^+ exchanger), located in the proximal tubulus of the kidney (38-41). A previous study by Odlind *et al*. (42) revealed that mice with reduced or absent COMT activity are unable to increase renal dopaminergic activity and produce normal natriuresis in response to sodium loading. Furthermore, COMT is more important than monoamine oxidase in regulating dopamine-mediated natriuresis in the rat kidney (43). In our study, tamoxifen treatment resulted in an increase in COMT protein expression and activity whereas increasing estrogen levels led to a decrease in kidney COMT, stressing the physiological importance of hormonal regulation of renal COMT activity. As increasing estradiol levels led to a downregulation of COMT in the kidney, the observed upregulation of kidney COMT following tamoxifen treatment is most likely due to an antiestrogenic action of tamoxifen.

Hepatic COMT activity and protein expression were unaltered by the different treatments in male and female rats. This may be explained by the particularly high capacity of COMT in the liver. Only a minor fraction of the protein may be needed for metabolism and therefore small changes in COMT activity and/or protein expression may not be noticeable (1).

The uterus and ovaries were particularly responsive to the effects of tamoxifen. In line with previous findings, tamoxifen caused a significant decrease in uterine (44-47) and ovarian weight (46), suggestive of antiestrogenic activity. Accordingly, COMT protein amounts were significantly increased in both tissues, confirming the down-regulatory effect of estrogen on COMT. Unexpectedly, the amount of COMT protein in the prostate doubled in response to estradiol treatment. Estrogens may initiate prostate cancer *via* formation of reactive catechol estrogen-3,4-quinones which can react with DNA and cause mutations (48). Catechol estrogens are substrates of COMT and the observed upregulation of COMT upon estradiol treatment may be a compensatory mechanism to protect the prostate from developing cancer.

Estrogen exerts its function by binding to estrogen receptors (ERs). Two ERs have been identified: ER alpha (ERα) and ER beta (ERβ). Both ERα and ERβ are capable of activating transcription in response to estradiol. However, *in vitro* studies suggest that ERα is substantially more efficient in activating transcription than ERβ. Different tissues contain distinct amounts of these receptors and may therefore respond differently to the same stimulus owing to their individual ER subtype composition. In *in vitro* studies with cells expressing both ER subtypes, ERβ dampens ERα activity and decreases overall sensitivity to agonists. Both ERs are present in the brain (49-51). However, there are tissue-dependent differences in distribution. Most peripheral tissues such as the liver, kidneys, uterus and testes mainly express ERα (52), whereas the prostate and ovaries are more abundant in ERβ (52). Still, in most tissues the distribution patterns of ERα and ERβ are usually distinct from each other, *i.e.* that they do not overlap and certain subregions may only contain one type of ER. Unfortunately, one of our drugs, tamoxifen, is a SERM. Tamoxifen can tissue-dependently act as a partial ER agonist or as an ER antagonist (53). Several studies suggest that the differential pharmacology of tamoxifen is due to differences in coactivator/corepressor availability in different tissues (for review see (26, 54). Coactivators interact with agonist-activated ER and facilitate transcription while corepressors oppose this effect. Therefore, tamoxifen's seemingly inconsistent actions may be due to differences in coactivator/corepressor availability between tissues. Furthermore, as ERβ dampens the effects of ERα, the magnitude of tamoxifen's partial agonist activity on ERα seems to be depedent on the expression levels of ERβ. Additionally, tamoxifen acts as a full agonist on the G-protein-coupled estrogen receptor (GPR30) which is postulated to mediate rapid non-genomic signalling events (55). GPR30 is widely expressed in estrogen target tissues, but its physiological roles remain to be elucidated. A recent study by Filice and colleagues reports that in addition to ERα and ERβ, GPR30 is also involved in cardiac activity (56). Therefore, the differences in response to our treatments may be, at least partially, due to distinct tissue distribution and function of GPR30.

The diverse distribution of ER throughout the brain and periphery may explain some of our results but by no means all of them. Whereas in female rats COMT expression was either upregulated or unaffected by tamoxifen treatment, we observed a variety of different responses in male rats. Male COMT tissue expression was either downregulated, unaffected or in some cases even upregulated in response to estradiol treatment. Male and female brains develop under the influence of very different hormonal environments (for review see (57)). Brain regions which have been implied as being sexually dimorphic are the PFC, the nigro-striatal pathway, the hippocampus and the hypothalamus. Therefore, some of our results may be explained by sexual dimorphisms and consequent differences in tissue responses to hormonal interventions in male and female rats.

In conclusion, our results revealed a complex regulatory effect of estradiol on COMT expression in male and female rats. However, the effects of estradiol were sex and tissue specific. Furthermore, COMT protein expressions and COMT activities did not always correlate (*e.g.* in the reproductive organs). Therefore, the question of how exactly estrogen regulates COMT expression in specific tissues warrants further investigation. Nevertheless, estrogen down-regulation was most prominent in the PFC and kidneys, two tissues in which COMT is highly important for dopamine metabolism.

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