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Reduced natriuretic response to acute sodium loading in COMT Gene deleted mice

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Abstract

Background: The intrarenal natriuretic hormone dopamine (DA) is metabolised by catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO). Inhibition of COMT, as opposed to MAO, results in a potent natriuretic response in the rat. The present study in anaesthetized homozygous and heterozygous COMT gene deleted mice attempted to further elucidate the importance of COMT in renal DA and sodium handling. After acute intravenous isotonic sodium loading, renal function was followed.

Results: COMT activity in heterozygous mice was about half of that in wild type mice and was zero in the homozygous mice. MAO activity did not differ between the genotypes. Urinary sodium excretion increased 10-fold after sodium loading in wild type mice. In heterozygous and homozygous mice, the natriuretic effects of sodium loading were only 29 % and 39 %, respectively, of that in wild type mice. Arterial pressure and glomerular filtration rate did not differ between genotypes. Baseline norepinephrine and DA excretions in urine were elevated in the homozygous, but not in heterozygous, COMT gene deleted mice. Urinary DA excretion increased after isotonic sodium loading in the wild type mice but not in the COMT gene deleted mice.

Conclusions: Mice with reduced or absent COMT activity have altered metabolism of catecholamines and are unable to increase renal DA activity and produce normal natriuresis in response to acute sodium loading. The results support the hypothesis that COMT has an important role in the DA-mediated regulation of renal sodium excretion.

Background

Besides the well-known effects of dopamine (DA) in the brain, this catecholamine also has specific effects in the kidney [1]. In the kidney, DA is produced in proximal tubular cells [2,3], and contributes to the natriuretic response that follows sodium loading [4]. Thus, DA is an

intrarenal natriuretic hormone with autocrine and paracrine effects that are exerted mainly by inhibiting tubular sodium transport [1]. The body sodium content is of crucial importance for the volume of the extracellular fluid (ECV), which, in turn, is closely related to the level of the mean arterial blood pressure (MAP). Thus, sodium handling is correlated to the setting of MAP. Defects in the renal DA system may reduce the natriuretic response to sodium loading, thereby leading to salt-sensitive hypertension [5,6].

DA is metabolised by monoamine oxidase (MAO) and catechol-O-methyl-transferase (COMT). The main metabolites are dihydroxyphenylacetic acid (DOPAC), formed by MAO and, after further methylation by COMT, homovanillic acid (HVA). Some of the DA is metabolised directly by COMT, forming 3-methoxytyramine (3-MT), which then also forms HVA by an action of MAO. All these metabolites are excreted into the urine, where the predominant final metabolite is HVA [7].

Although the detailed regulation of DA-induced natriuresis remains to be elucidated, several possibilities have been examined. We and others have shown in the rat that COMT inhibition leads to a pronounced natriuresis [8–12] and we have found that renal cortical COMT activity is reduced during isotonic sodium loading [11]. This suggests that COMT plays a role in the regulation of DA-induced natriuresis.

On the basis of these considerations we subjected COMT gene deleted mice and wild type mice to acute isotonic sodium loading with the aim of determining whether this would disclose a difference in sodium and DA handling between these genotypes.

Results

Control group (time control)

Urinary flow rate, DA, DOPAC and NE excretion and GFR were stable throughout the experiment in all genotypes (Table 1 and 2). Urine flow rate did not differ between the genotypes in this basal state. The excretion of DA in the urine tended to be higher in the HM than in the HT or WT mice, but, this difference was not statistically significant in a 2-way ANOVA. The urinary excretion of NE was higher in the HM than in the HT or WT mice. MAP decreased slightly at the end of the experiment in HT and HM mice (Table 1). The excretion of DOPAC was found to be very low in mice as compared to that in the rat (about 5 %, [11]) and showed no difference between the genotypes (Table 1). As pointed out in the discussion, the DOPAC levels were close to the detection limit.

Table 1: Mean arterial blood pressure (MAP), urinary flow rate (UV) and urinary excretion of DA ($U_{DA}V$), urinary excretion of DOPAC (UDacV) and norepinephrine (U NEV) in wild type and in heterozygous and homozygousCOMT gene deleted mice of the control groups. Kw = kidney weight. *p<0.05 vs WT (MAP) or vs WT and HT mice ($U_{NE}V$).

	C1	C2	E1	E2	E3	E4
MAP (mmHg)						
Wild type	89±2	88±2	85±2	83±2	80±2	80±7
Heterozygous	90±3	88±3	86±3	82±3*	80±2*	83±2*
Homozygous	96±2	95±2	90±2	88±2	85±2*	82±2*
UV(μl·min^{-1}·g^{-1} kw)						
Wild type	1.82±0.30	1.87±0.20	1.84±0.21	2.29±0.25	2.64±0.31	2.69±0.57
Heterozygous	2.18±0.24	1.83±0.13	2.05±0.11	2.43±0.19	2.76±0.25	2.66±0.44
Homozygous	1.76±0.29	1.77±0.21	2.01±0.18	2.73±0.21	2.58±0.24	3.09±0.26
$U_{DA}V$(ng·min^{-1}·g^{-1} kw)						
Wild type	3.17±0.67	4.31±0.80	3.62±0.81	3.91±0.86	3.77±0.78	3.80±1.67
Heterozygous	4.51±0.94	4.18±0.94	4.01±0.93	3.99±0.86	3.86±0.83	6.13±1.95
Homozygous	5.27±0.97	7.23±2.19	5.99±1.08	6.21±0.87	5.20±0.81	5.92±1.24
$U_{Dac}V$ (ng·min^{-1}·g^{-1} kw)						
Wild type	0.25±0.09	0.27±0.11	0.23±0.11	0.20±0.07	0.18±0.04	0.22±0.08
Heterozygous	0.23±0.16	0.14±0.08	0.08±0.04	0.11±0.05	0.21±0.14	0.16±0.07
Homozygous	0.30±0.19	0.33±0.19	0.35±0.24	0.31±0.21	0.29±0.21	0.54±0.41
$U_{NE}V$ (ng·min^{-1}·g^{-1} kw)						
Wild type	3.01±0.77	3.33±0.79	3.09±0.80	2.59±0.67	2.33±0.61	1.35±0.10
Heterozygous	4.50±0.89	4.82±0.82	4.55±1.05	4.24±1.04	4.44±1.02	2.92±0.45
Homozygous	7.44±1.60*	8.12±1.72*	7.25±1.28*	7.45±1.29*	7.43±0.96*	8.53±0.47*

Table 2: Glomerular filtration rate (GFR) in wild type and in heterozygous and homozygous COMT gene deleted mice in the control group and in the group subjected to isotonic sodium loading. C2 is at t = 60 min, just before the start of isotonic sodium loading, and E4 is at t = 180 min just before the end of the experiment.

	C2	E4
GFR (mlSmin^{-1}·g^{-1} kw) CONTROL GROUP		
Wild type	0.78±0.08	0.85±0.11
Heterozygous	0.87±0.13	0.70±0.06
Homozygous	0.84±0.21	0.85±0.12
GFR (ml·min^{-1}·g^{-1} kw) SODIUM LOADING		
Wild type	0.85±0.09	0.91±0.12
Heterozygous	0.84±0.07	1.16±0.05
Homozygous	0.85±0.10	0.76±0.06

Table 3: Mean arterial blood pressure (MAP), urinary flow rate (UV), urinary sodium (UNaV) and dopamine (UDA V) excretion in wild type, heterozygous and homozygous COMT gene deleted mice before (C1 and C2) and during (E1-E4) isotonic sodium loading. kw = kidney weight. *p < 0.05 vs C2 of the same genotype.

	C1	C2	E1	E2	E3	E4
MAP (mmHg)						
Wild type	97±3	95±3	91±3	86±3*	84±3*	90±4*
Heterozygous	93±2	90±2	86±1	81±1*	79±1*	76±3*
Homozygous	94±1	93±2	86±2	81±2*	77±2*	74±2*
UV (μl·min^{-1}·g^{-1} kw						
Wild type	1.90±0.18	1.99±0.28	2.26±0.30	3.34±0.34	5.66±0.89*	6.94±1.33*
Heterozygous	1.88±0.18	1.96±0.18	2.38±0.26	2.56±0.25	3.62±0.37	4.53±0.48
Homozygous	2.27±0.39	2.17±0.40	2.68±0.45	3.24±0.79	4.77±0.98*	6.53±1.40*
$U_{Na}V$ (μmol·min^{-1}·g^{-1} kw)						
Wild type	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.01	0.14 ± 0.05*	0.39 ± 0.16*	0.87 ± 0.28*
Heterozygous	0.07 ± 0.01	0.08 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.12 ± 0.04	0.25 ± 0.11
Homozygous	0.09 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.14 ± 0.06	0.34 ± 0.13
$U_{DA}V$ (ng·min^{-1}·g^{-1} kw						
Wild type	3.2 ± 0.5	3.4 ± 0.5	3.7 ± 0.6	4.4 ± 0.7	5.6 ± 1.1*	
Heterozygous	3.4 ± 0.6	3.6 ± 0.5	3.7 ± 0.6	3.4 ± 0.5	3.8 ± 0.5	
Homozygous	9.2 ± 2.8	8.5 ± 2.3	8.3 ± 2.5	6.7 ± 1.7	8.0 ± 2.6	

Isotonic sodium loading

Upon sodium loading the mean sodium excretion increased 10-fold in WT mice (from 0.086 ± 0.02 to 0.87 ± 0.28 μmol · min^{-1} · g^{-1} kw; p < 0.05; Table 3), whereas the mean value increased 3.5-fold in HM mice (from 0.097 ± 0.02 to 0.34 ± 0.13 μmol · min^{-1} · g^{-1} kw, ns) and 3-fold in HT mice (from 0.08 ± 0.02 to 0.25 ± 0.11 μmol · min^{-1} · g^{-1} kw, ns). Urine flow rate increased in HM and WT mice upon sodium loading. A similar trend was observed in HT mice but this response did not reach statistical significance.

The natriuretic responses in HT and HM mice in the last period of the experiment (E4) thus amounted to only 29% and 39%, respectively, of the response in the WT mice (p < 0.05, Fig. 1). GFR did not differ between the genotypes or treatments (Table 2). During acute isotonic sodium loading MAP decreased in all genotypes (Table 3). The mean urinary DA excretion during the control periods (C1-C2) was higher in the HM mice than in the wild type and HT mice (p < 0.05; Fig. 2, Table 3). The urinary DA excretion increased in the WT mice after isotonic sodium loading (from 3.17 ± 0.55 to 4.48 ± 0.80 ng · min^{-1} · g^{-1} kw; p < 0.05; Fig. 2), whereas both the HM and the HT

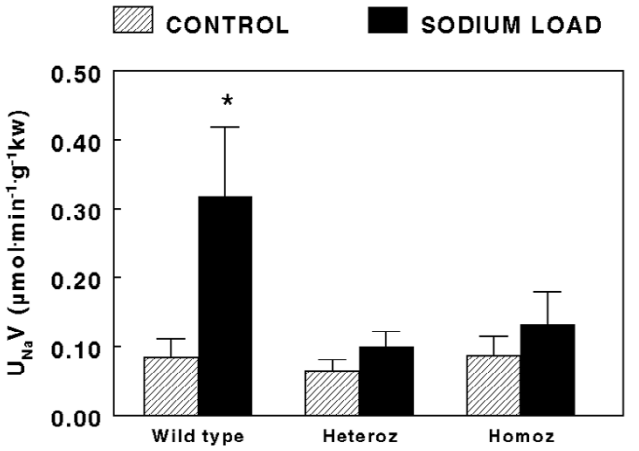

Figure 1
Mean urinary sodium excretion ($U_{Na}V$) during control treatment and in response to isotonic sodium loading in wild type mice and in heterozygous and homozygous COMT gene deleted mice. *p < 0.05 vs control treatment in the same genotype. kw = kidney weight.

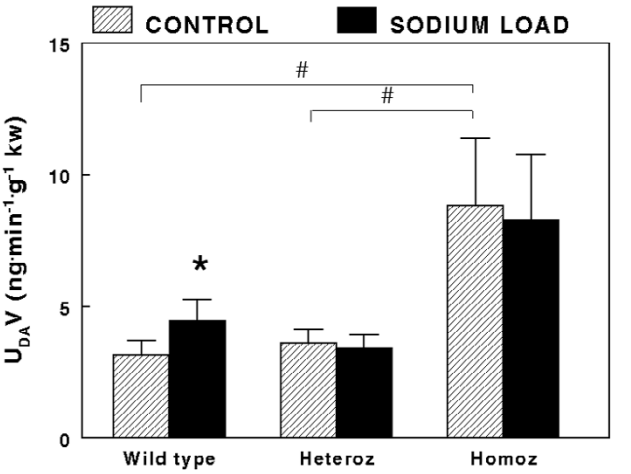

Figure 2
Mean urinary dopamine excretion ($U_{DA}V$) during control periods (C1-C2) and in response to isotonic sodium loading (E1-E4) in wild type, heterozygous and homozygous COMT gene deleted mice. *p < 0.05 vs the same genotype. #p < 0.05 vs wild type and heterozygous. kw = kidney weight.

mice failed to respond with increased DA excretion (change from 8.85 ± 2.55 to 8.32 ± 2.45 ng · min^{-1} · g^{-1} kw in HM and from 3.60 ± 0.52 to 3.44 ± 0.48 ng · min^{-1} · g^{-1} kw in HT mice; Fig. 2). Note, however, that the baseline urinary DA excretion was elevated prior to sodium loading in HM. The urinary excretion of DOPAC and of NE was not significantly changed after sodium loading in any group (data not shown).

COMT and MAO activities

The COMT activity was measured in specimens of the renal cortex, outer medulla and papilla from all animals. The basal level of specific COMT activity in WT mice was highest in the cortex (216 ± 21 pmol · mg^{-1} · min^{-1}) and lower in the outer medulla (54 ± 17 pmol · mg^{-1} · min^{-1}) and the papilla (56 ± 9 pmol · mg^{-1} · min^{-1}).

There was a tendency (p = 0.10) towards a decrease in COMT activity in the renal cortex after isotonic sodium loading (173 ± 14 pmol · mg^{-1} · min^{-1}). The COMT activity did not change in the outer medulla (64 ± 11 pmol · mg^{-1} · min^{-1}) nor in the papilla (62 ± 6 pmol · mg^{-1} · min^{-1}). Under control conditions the COMT activity in HT mice was about half of that in WT mice namely 118 ± 12 pmol · mg^{-1} · min^{-1} in the cortex and 31 ± 1 pmol · mg^{-1} · min^{-1} in the papilla and even lower in the outer medulla (19 ± 5 pmol · mg^{-1} · min^{-1}). As expected, the COMT activity was zero in all parts of the kidney in HM mice. The MAO activities did not differ significantly between the three genotypes, nor did these activities change after sodium loading (Table 4). Thus, it seems as if no compensatory effect on MAO activity occurred in animals with reduced or no COMT activity.

Discussion

This study in COMT gene deleted mice was undertaken to further elucidate the role of COMT in the regulation of renal DA and sodium handling. In previous studies in rats we and others have demonstrated that pharmacological inhibition of COMT results in a potent D_1-like receptor-mediated natriuretic response [8–12]. Furthermore, we have found in rats that renal cortical COMT activity is reduced during isotonic sodium loading [11]. The results of the present study show that mice with reduced or absent COMT activity are unable to increase the renal DA excretion from whatever baseline level and to produce normal natriuresis in response to acute sodium loading. The results support the hypothesis that COMT plays an important role in the DA-mediated regulation of renal sodium excretion.

During moderate isotonic sodium loading in rats, up to 60 % of sodium excretion is mediated by D_1-like receptors [6,11,13,14]. The contribution of the dopaminergic system to natriuresis in mice has been reported in one study previously [14]. The study found that disruption of the D_3 receptor gene attenuated the ability of homozygous mice to respond with natriuresis upon a similar saline load as given in the present study. Since we in the present study have not tested the dependency of the natriuresis on DA receptors, we cannot categorically state that the blunted natriuretic response in COMT gene deleted mice after

Table 4: Renal cortical monoamine oxidase (MAO) activity in wild type (WT) and in heterozygous (HT) and homozygous (HM) COMT gene deleted mice during control treatment and after isotonic sodium loading.

	WT	HT	HM
MAO activity (pmol·mg^{-1}·min^{-1})			
CONTROL GROUP	16.9±4.3	15.1±3.9	9.6±2.3
SODIUM LOADING	16.9±4.8	13.1±2.9	15.4±5.4

acute sodium loading is due to the defective DA system. The wild type mice, however, responded with an increased DA activity after sodium loading, as has previously been demonstrated in rats [11–13,16]. The fact that the homozygous and heterozygous mice did not respond with an increased DA activity after sodium loading strongly indicates a renal dopaminergic defect in these animals. COMT metabolises other substances involved in sodium excretion besides DA, such as NE, and urinary NE excretion was indeed increased in the homozygous but not in the heterozygous mice compared to their wild type littermates. NE is considered to act antinatriuretically via adrenoceptors [17,18] and its urinary excretion is decreased after sodium loading in normal rats [19]. In the present study urinary NE excretion did not change in any of the genotypes after sodium loading. Furthermore, the renal α_1-adrenoceptor expression is similar between the genotypes (data not shown). This would suggest that NE plays only a minor role, if any, in the regulation of the natriuresis provoked in this experimental setting.

On the basis of our previous results, we hypothesise that COMT is necessary for upregulating DA activity in response to sodium loading, thereby affecting renal sodium handling. In the wild type mice, as in normal rats [9,16,20], urinary DA excretion increased in response to isotonic sodium loading. The cortical COMT activity showed a tendency to decrease, an effect which we have previously observed in rats during sodium loading [11]. This may well contribute to an increase in DA activity. These changes in renal DA regulation in wild type mice were accompanied by a 10-fold increase in urinary sodium excretion.

In the heterozygous COMT gene deleted mice the cortical COMT activity was about half of that in the wild type mice. The baseline urinary DA and NE excretions were similar to those in the wild type mice, suggesting that the reduced COMT activity is still sufficient to metabolise DA and NE. However, during isotonic sodium loading, the heterozygous mice, as opposed to the wild type mice, were unable to increase the DA excretion and the natriuretic response was only 29% of that in the wild type mice. This would again suggest an important regulatory defect in these animals due to reduced COMT function.

In the homozygous COMT gene deleted mice the baseline urinary DA and NE excretions were elevated as a result of the reduced ability to metabolise these catecholamines. During isotonic sodium loading these animals failed to respond with a further elevation in DA activity (beyond basal values) and the natriuretic response was only 39% of that in the wild type mice. These results together strengthen the hypothesis that COMT has an important role in regulating DA activity in response to sodium loading and thereby affecting renal sodium handling.

The urinary excretion of DOPAC in mice was found to be very low and very close to the detection limit of the HPLC system thereby restricting the conclusions that can be drawn regarding genotype differences and the response to sodium loading. The low levels are in contrast to our previous observations in rats that urinary DOPAC excretion was larger than DA excretion [4,10,11]. This implies a difference in the handling of DA metabolism between mice and rats. A comparison of the renal enzyme activities between mice (present study) and rats [11] has shown that the COMT activity is similar, while the MAO activity is significantly weaker in mice than in rats. MAO forms the main metabolite DOPAC from DA. It is therefore obvious that low MAO activity will result in low DOPAC generation, analogously to pharmacological MAO inhibition [11]. The renal MAO activity in mice is about 10 % of that in the rat and the corresponding urinary DOPAC excretion is about 5 % [11].

The conclusions that can be drawn from experiments with genetically modified animals are limited. Compensatory mechanisms are likely to develop. Changes in gene expression might have effects on other systems during early development, and phenotypic differences may not always be obvious. However, the expressions of D_1-like and D_2-like receptors are not changed in the brain or in renal tissue in COMT gene deleted mice as compared to the wild type mice (Garcia and Männistö, unpublished results, 2001). Furthermore, we have found that the density of α_1-

adrenoceptors (α_{1A} and α_{1B}) are also similar between all three genotypes (data not shown). Thus, it is not likely that the difference in natriuretic response is due to changes in the DA or adrenoceptor density. However, the functionality of these receptors has not been studied. Furthermore, in a recent study by Houtari et al. [21] using the COMT gene deleted mice it was found that no compensatory changes in protein levels of catecholamine-synthesizing (tyrosine hydroxylase, dopa decarboxylase, dopamine-beta-hydroxylase) and catecholamine-metabolising enzymes (MAO-A/B, phenylsulfotransferase) had occurred.

Acute pharmacological COMT inhibition in rats results in an acute elevation of dopamine levels which results in an acute elevation of sodium excretion [8–10]. In the genetically COMT inactivated mice the chronically elevated basal dopamine levels does not result in a chronically elevated basal sodium excretion as compared with the wildtype mice. This may seem as inconsistent regarding the relationship between basal urinary dopamine and sodium excretion. It is, however, important to acknowledge that longterm sodium excretion will always depend and be in balance with that which has been ingested. It seems primarily to be the changes in dopamine levels from whatever baseline level which will cause a change in the excretion of sodium and not the baseline level per se.

A defect in the renal DA system resulting in a reduced ability to respond with natriuresis after sodium loading may lead to salt-sensitive hypertension [5,6]. Defects in the DA system have been found in association with some forms of hypertension in rats [6,22,23] and mice [15,24]. In the present study we found no differences in MAP between the genotypes, either under control conditions or after sodium loading. It is difficult, however, to compare MAP in gas anaesthetised mice, since one of the determinants of the level of isoflurane used is MAP. Thus, to correctly elucidate this issue, measurements of MAP in non-anaesthetised mice using the telemetric method are preferred.

The tonic control over renal vascular resistance exerted by the renin-angiotensin system has been shown to be greater in mice than in rats [25]. The reduced MAP after sodium loading seen in all genotypes in the present study may be secondary to reduced levels of angiotensin II, resulting in vasodilation. Such a suggestion would be in line with the results of Traynor & Schnermann [26] showing a major contribution of the renin-angiotensin system to the vasodilation caused by isotonic sodium loading. The activity of the renin-angiotensin system in the different genotypes used in the present study is unknown and renders further investigations.

We have observed in an earlier study in rats that after acute isotonic sodium loading, cortical COMT activity was reduced. A similar trend ($p = 0.10$) was seen in the sodium-loaded wild type mice in this study. It is therefore possible that at least part of the increased DA activity noted after isotonic sodium loading is due to reduced degradation of DA. The MAO activity was not altered after isotonic sodium loading in wild type mice which is in line with our previous study in rats [11]. It is also worthy of mention that in the rat it has been demonstrated that both COMT inhibitor and the atrial natriuretic peptide (ANP) result in translocation of the D_1-like receptor to the cell membrane [26,27]. This may represent another way of regulating DA-mediated natriuresis, especially since it is known that ANP is released in response to isotonic sodium loading [4].

Conclusions

The present study shows that mice with reduced or absent COMT activity are unable to increase the DA activity and to produce normal natriuresis in response to sodium loading. The results support the hypothesis that COMT plays an important role in the DA-mediated regulation of renal sodium excretion.

Materials and methods

Production and genotyping of COMT gene deleted mice

The COMT gene-deleted strain was produced as previously described [28]. In brief, the gene encoding COMT was disrupted by replacing part of the fragment with a cassette including the neo gene. The construct was introduced into the genome of embryonic stem (ES) cells of 129/Sv mice by means of homologous recombination. ES cells with positive homologous recombination were selected by neomycin resistance (~15 %) and were injected into C57B6 early mouse embryos in the blastocyst stage. Chimeric males (mice containing both cells from the microinjected ES cell and the host embryo) were mated with C57B6 females and in cases where the injected recombinant ES cells had contributed to the germ line of the chimeric mouse, the mating resulted in heterozygous (HT) 129/Sv/C57B6 offspring with respect to the target gene. HT animals were mated and mice of all three genotypes were obtained (F1 generation). Each mouse was then typed by southern blot analysis [28]. DNA from tail biopsies was extracted and digested with *Eco*RV, and fragments were separated on a gel. The fragments were blotted with two different radioactively marked probes and on the exposed films an 11.5 kb fragment was seen in wild type (WT) samples, a 3.5 kb signal in homozygous (HM) genotypes and both of these in HT genotypes.

Animal procedures

The experiments were approved by the Ethics Committee for Animal Experimentation at the University of Uppsala. The study was performed on a total of 66 mice: 11 WT, 13

HT and 8 HM male COMT gene deleted mice, weighing 31.0 ± 0.9, 30.8 ± 1.0 and 30.4 ± 1.3 g, respectively, and 10 WT, 15 HT and 9 HM female such mice, weighing 23.7 ± 0.5, 24.4 ± 0.5 and 23.0 ± 0.3 g, respectively. Up to the day of the experiment all animals had free access to tap water and standardised chow (R3, Ewos, Södertälje, Sweden) containing 0.3 % sodium, 0.8 % potassium and 21 % protein. Anaesthesia was induced by placing the mice in a polystyrene box with isoflurane flowing through it (Forene^{R}, Abbott Scandinavia AB, Kista, Sweden). After induction, the animals were placed in the supine position on a thermostatically controlled surgical table to maintain the body temperature at 37°C and a breathing mask was placed over the head. The inhalation gas contained ~2 % isoflurane in air with 37.5 % O_2 during surgery and the amount of isoflurane was subsequently adjusted to maintain the respiratory rate and MAP. Polyethylene catheters were inserted into the right femoral vein and artery, the former for infusion of isotonic saline (0.15 M NaCl) and the latter for continuous monitoring of mean arterial blood pressure (MAP) and for blood sampling. The urinary bladder was catheterised through a suprapubic incision for urine collection. After the experimental procedures described below and excision of the kidneys, all animals were euthanised by an intravenous injection of saturated KCl.

Protocol

After completion of the surgical procedures, the mice received an intravenous bolus infusion of 0.08 ml isotonic saline to replace fluid losses during surgery. This bolus dose contained 0.5 μCi of [^{3}H]methoxy-inulin. A maintenance infusion of 0.15 M NaCl containing 2.5 $\mu Ci \cdot ml^{-1}$ of inulin was administered at a rate of 0.5 $ml \cdot h^{-1}$. After a 45 min stabilisation period and 60 min of control sampling (2 × 30 min, C1-C2), the mice were divided into two groups. One group was given isotonic saline (0.15 M NaCl, n = 11 WT, n = 14 HT and n = 8 HM) continuously at a rate of 1.5 $ml \cdot h^{-1}$ (ECV expansion, 5% of body weight) during four 30-min observation periods (E1-E4). The other group, the control animals, (n = 10 WT, n = 12 HT and n = 9 HM) received isotonic saline at the maintenance infusion rate (0.5 $ml \cdot h^{-1}$) throughout the study (E1-E4). Because of the small blood volume in the mouse (~1.5 ml), blood samples for GFR estimation (~15 μl) were only drawn at two time points during the experiment (C2 and E4). Before the animal was killed, the kidneys were excised, placed on carbon-dioxide ice and sliced under a microscope into sections of cortex, outer medulla and papilla; the pieces were frozen in a mixture of alcohol and carbonic ice and subsequently stored at -70°C pending assay of COMT and MAO activities.

Urine analysis

The urine volumes were measured gravimetrically. The urinary sodium concentration (U_{Na}) was determined by flame photometry (FLM3, Radiometer, Copenhagen, Denmark). For assay of DA, DOPAC and norepinephrine (NE), urine samples were immediately transferred to polyethylene vials containing 1 ml 0.4 M perchloric acid (PCA), 0.1 ml 10 % EDTA-Na_2 and 0.05 ml 5 % $Na_2S_2O_5$ and immediately frozen to -70°C. The DA, DOPAC and NE contents were measured electrochemically following alumina adsorption and ion-pair, reverse-phase high-performance liquid chromatography (HPLC, [29]). An internal standard (3,4-dihydroxybenzylamine) was used and all values were corrected for its recovery. Recovery through the alumina extraction step was determined from aqueous mixtures of catecholamines.

The amount of [^{3}H]methoxy-inulin in samples of plasma and urine was determined in a liquid scintillation counter. The glomerular filtration rate (GFR) was estimated from the clearance of [^{3}H]methoxy-inulin (C_{in}) according to the equation:

$$C_{in} = (U_{in} \cdot V) / P_{in}$$

where U_{in} and P_{in} are the urinary and plasma concentrations, respectively, of inulin and V is the urine flow rate.

Measurements of COMT and MAO activities

COMT and MAO activities were measured in renal sections of cortex, outer medulla and papilla (prepared as described under *Protocol*).

COMT activity was measured by electrochemical detection of the reaction products formed from the substrate dihydroxybenzoic acid by COMT using HPLC, as previously described in detail [30,31]. In short, 100 μl enzyme preparation was incubated for 30 min at 37°C in the presence of 240 μM dihydroxybenzoic acid, 5 mM $MgCl_2$ and 200 μM SAM in 100 mM sodium phosphate buffer, pH 7.4. The reaction was terminated with ice-cold PCA (4 M, 25 μl) and centrifuged for 10 min at 5530 × g at 4°C. The supernatants were subjected to HPLC analysis for vanillic acid and isovanillic acid.

Determination of MAO activity was based on the detection of hydrogen peroxide in a horseradish peroxidase-coupled reaction using 10-acetyl-3,7-dihydroxy-phenoxazine, a probe for hydrogen peroxide [32,33]. An AmplexTM Red Monoamine Oxidase Assay Kit (A-12214, Molecular Probes) was used for measurement of the activity. One hundred microlitres of supernatant was mixed with 100 μl reaction buffer containing 50 mM phosphate buffer, pH 7.4, horseradish peroxidase 2 $U \cdot ml^{-1}$, 400 μM 10-acetyl-3,7-dihydroxy-phenoxazine and 2 mM p-

tyramine as a substrate of both MAO-A and B. The increase in fluorescence intensity (excitation 530/25 nm, emission 645/40 nm) was measured with a fluorescence microplate reader, with resorufin used as standard.

Statistical analysis

All data are presented as means ± 1 standard error of the mean (SEM). Differences within and between groups were tested for significance by two-way analysis of variance (ANOVA) followed by the Tukey test (STATISTICA, StatSoft, Tulsa, OK, USA) or by an unpaired t-test. A p value of less than 0.05 was adopted as statistically significant. No gender differences in excretory data or enzymatic activities were found, and results for males and females of the same genotype and treatment group were therefore pooled. Mice with an MAP below 70 mmHg were excluded from the study.

Authors' contributions

CO carried out all physiological in vivo experiments, sampled tissues and drafted the manuscript. IR performed the COMT analysis. PTM participated in, and coordinated the COMT and MAO analysis, helped in the manuscript writing and supplied the genotyped mice. RJ performed the MAO analysis. SU performed the adrenoceptor assay. JAG and MK originally supplied the COMT gene deficient mice and participated in the manuscript writing. PH conceived of the study, was responsible for its design and coordination and for production of the final manuscript. All authors read and approved the manuscript.

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