RENALASE REGULATES PERIPHERAL AND CENTRAL DOPAMINERGIC ACTIVITIES

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ABSTRACT

Renalase is a recently identified FAD/NADH-dependent amine oxidase mainly expressed in kidney, which is secreted into blood and urine where it was suggested to metabolize catecholamines.

The present study evaluated central and peripheral dopaminergic activities in the renalase knockout (KO) mouse model and examined the changes induced by recombinant renalase (RR) administration on plasma and urine catecholamines levels. Compared to wild type (WT) mice, KO mice presented increased plasma levels of epinephrine (EPI), norepinephrine (NE) and DA which were accompanied by increases in the urinary excretion of EPI, NE, DA. In addition, the KO mice presented an increase in urinary DA/L-3,4-dihydroxyphenylalanine (L-DOPA) ratios without changes in renal tubular aromatic-L-amino acid decarboxylase activity. By contrast, the in vivo administration of RR (1.5mg/kg, s.c.) to KO mice was accompanied by significant decreases in plasma levels of EPI, DA and L-DOPA as well as in urinary excretion of EPI, DA and DA/L-DOPA ratios notwithstanding the accompanied increase in renal AADC activity. In addition, the increase in renal DA output observed in renalase KO mice was accompanied by an increase in the expression of the amino acid transporter LAT1 that is reversed by the administration of recombinant renalase in these animals. These results suggest that the overexpression of LAT1 in the renal cortex of the renalase KO mice might contribute to the enhanced L-DOPA availability/uptake and consequently to the activation of the renal dopaminergic system in the presence of renalase deficiency.

**Keywords:** renalase, knockout mouse model, renal dopamine, L-Dopa, epinephrine
INTRODUCTION

The catecholamines epinephrine (EPI), dopamine (DA) and norepinephrine (NE) play a key role in the regulation of blood pressure and sodium homeostasis through their action on central and peripheral adrenergic and dopaminergic receptors. The known pathway for the metabolism of these compounds involves uptake by neuronal and extraneuronal tissues and degradation by the intracellular enzymes monoamine oxidases A and B (MAO-A and B) and catechol-O-methyltransferase (COMT) (1, 21, 34).

In kidney, the epithelial cells of proximal tubules (PT) are endowed with a high aromatic-L-amino acid decarboxylase (AADC) activity and filtered or circulating L-3,4-dihydroxyphenylalanine (L-DOPA) can be converted to DA after being taken up into this cellular compartment (14, 20, 31). The candidate transport systems for L-DOPA in renal PT cells, include Na⁺-dependent amino acid transport systems, like the neutral amino acid exchanger ASCT2 (SLC1A5) and the broad specific neutral amino acid transporter B⁰AT1, and Na⁺-independent systems L-type amino acid transporter like LAT1 and LAT2 (24-25).

Dopamine of renal origin exerts natriuretic and diuretic effects by activating D₁-like receptors located at various regions in the nephron (18). The availability of DA to activate its specific receptors is determined by the interplay between the rate of synthesis and the degree of degradation of the amine (32) by deamination to 3,4-dihydroxyphenylacetic acid (DOPAC), O-methylation to 3-metoxytyramine (3-MT) and deamination plus O-methylation to homovanillic acid (HVA) (2, 9, 11). Previous work from our group demonstrated that both selective and combined inhibition of MAO-A and COMT did not change renal DA excretion, natriuresis or phosphaturia, notwithstanding the marked decrease in the urinary excretion of the corresponding metabolites (28). This observation suggested that other metabolic pathways besides
COMT and MAO might be involved in the regulation of renal DA output.

Renalase is a recently identified FAD/NADH-dependent amine oxidase mainly expressed in kidney, which is secreted into blood where it was suggested to metabolize catecholamines with preference for EPI as well as the catecholamine precursor L-DOPA (7-8, 37-38). When administered in vivo renalase acutely lowers blood pressure in a dose-dependent manner and this was accompanied by decreased cardiac contractility and heart rate without compensatory changes in peripheral vascular tone thus suggesting that these hemodynamic effects may be accounted for by circulating catecholamines degradation (3-4).

In addition to the kidney, renalase is also highly expressed in other organs, namely in the heart, intestine, skeletal muscle, liver as well as in the peripheral and central nervous system (CNS) (15-16, 38). Thus, one can hypothesize that renalase may be involved not only in circulating catecholamines degradation but also in tissue-specific functions including the regulation of DA levels in the CNS and peripheral organs (5).

In the present work, we used the renalase knockout (KO) mouse model to gain insight into the influence of renalase deficiency on central and peripheral dopaminergic activities and to evaluate the changes induced by recombinant renalase (RR) administration on plasma and urine catecholamines levels, namely DA and the correspondent metabolites.

MATERIALS AND METHODS

Metabolic studies

Renalase-deficient (KO) mice were generated as previously described (36).
Male C57BL/6J mice were used as wild-type (WT) group (Charles River, Barcelona, Spain). All *in vivo* experiments were performed in accordance to the European Directive number 86/609, transposed to the Portuguese Law by DL 129/92 and by Portaria 1005/92, and the rules of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (17). The present study was approved by the scientific committee of the Faculty of Medicine of University of Porto.

Both KO and WT groups of mice used were 7 to 8 weeks old. All animals were kept under controlled environmental conditions (12:12h light/dark cycle and room temperature 22±2 ºC) and were fed *ad libitum* throughout the study with a standard diet (SAFE A04, by Scientific Animal Food and Engineering). Fluid intake and food consumption were monitored daily throughout the study. The daily sodium intake averaged 25mmol/kg body wt.

Both groups of animals were placed in metabolic cages (Tecniplast, Buguggiate-VA, Italy) for later determination of biochemical parameters and 24h quantification of catecholamines and metabolites (29). On the day of sacrifice the animals were anaesthetized with pentobarbital sodium (50mg/kg body wt; ip) and blood was collected directly from the heart in tubes containing heparin for later determination of plasma catecholamines and biochemical parameters. Thereafter, the kidneys, heart, *gastrocnemius* muscle, jejunum and brain, were rapidly removed and weighted. Tibia length was also measured for organs weight normalization. Fragments of renal cortex, jejunum and brain were stored for later quantification of catecholamines and enzymatic studies.

In some experiments, renalase KO mice aged 7 to 8 weeks received recombinant renalase (1.5mg/kg) or the vehicle (25mM BisTris, 10mM NaCl, 10% glycerol, 0.5mM
DTT, 1mM EDTA, pH 6.5) by a single subcutaneous injection 24h before sacrifice as previously described (6).

Plasma and urine electrolytes and biochemistry

The quantification of sodium and potassium in plasma and urine samples was performed by ion-selective electrodes. Creatinine was measured by the Jaffé method whereas urea was measured by an enzymatic test. Total proteins in plasma were quantified by the Biuret reaction. All determinations were performed by Pentra 200 analyser (ABX Diagnostics, Geneva, Switzerland).

Assay of catecholamines

The quantification of catecholamines and its derivatives in urine, plasma samples, renal tissues, brain and jejunum and in samples from enzymatic studies was performed by high pressure liquid chromatography with electrochemical detection (HPLC-ECD), as previously described (35). In our laboratory, the lower limit of detection of NE, EPI, L-DOPA, DA, DOPAC, 3-MT and HVA ranged from 350 to 1000 fmol.

AADC activity

Fragments of renal cortex were homogenized at 4°C with a Thomas Teflon homogenizer (Poliscience Corp., ILL, USA) in incubation medium containing (in mM): 0.35 NaH₂PO₄, 0.15 Na₂HPO₄, 0.11 Na₂B₄O₇ and 0.2 pyridoxal-phosphate (pH 7.0). Activity of AADC was determined as previously described by Soares-da-Silva (33) using L-DOPA as substrate (0.1 to 10 mM, Sigma, USA). The assay of DA was performed by HPLC with electrochemical detection.
MAO-A and B activities

Fragments of renal cortex and brain were homogenised in 67 mM phosphate buffer (pH 7.2) at 4°C, with a Thomas Teflon homogeniser. MAO activity was determined with $[^3H]$-5-hydroxytryptamine (5-HT, 20 to 2000 μM, Sigma, USA) as a preferential substrate for MAO-A and $[^14C]$β-phenylethylamine (β-PEA, 0.25 to 250 μM, Sigma, USA) as a preferential substrate for MAO-B (12). Aliquots of 50 μl of the homogenates were incubated for 10 min with 50 μl of each substrate. At the end of incubation period the tubes were transferred to ice and the reaction was stopped by the addition of 50 μl of perchloric acid (2 M). The deaminated products were extracted with ethyl acetate and measured by liquid scintillation counting.

COMT activity

Fragments of renal cortex and brain were homogenised in 5mM phosphate buffer (pH 7.8) at 4°C, with a Thomas Teflon homogeniser. Catechol-O-methyltransferase activity was evaluated by the methylation of EPI (0.01 to 500 μM, Sigma, USA) to metanephrine (MN), as previously described (23). The assay of MN was performed by HPLC with electrochemical detection.

Immunoblot analysis

For the determination of LAT1, LAT2 and ASCT2 expression in renal cortex extracts, equal amounts of total protein, determined using the method of Bradford (6), were separated on a 12% SDS-polyacrylamide gel and electrotransfered to a nitrocellulose membrane in Tris-glycine transfer buffer containing 20% methanol. The transblot sheets were blocked in 5% non-fat dry milk in PBS for 60 min and then incubated overnight at 4°C with specific primary antibodies (rabbit anti-LAT1 H-75 antibody, goat anti-LAT2
G-19 antibody and mouse anti-GAPDH antibody from Santa Cruz Biotechnology, and rabbit anti ASCT2 antibody from USBiological Life Sciences). The immunoblots were subsequently washed and incubated with the respective fluorescently labelled secondary antibody for 1 h at room temperature and protected from light. Membranes were washed and imaged by scanning at 800 nm with the Odyssey Infrared System (LI-COR Biosciences).

Renalase-induced catecholamines oxidation

The ability of recombinant renalase to oxidize DA to its corresponding aminochrome was examined by addition of recombinant renalase (20µg) (6) in buffer containing 25mmol/L Tris, 5mmol/L NaCl and 250mmol/L NADH (ph 7.5)1 mM, to DA (1mM), during 10 minutes. Protein precipitation was immediately performed by an acidification with perchloric acid (10%) followed by a rapid neutralization with KHCO₃ 0.76M and centrifuged for 60s at 16000g. Samples were immediately injected into a HPLC system (Waters model 2690) with a photodiode array detector (Waters model 996).

As previously described (27), the chromatograms were analysed at 279 nm which corresponds to the maximum absorption wavelength of catecholamine and at 490 nm which corresponds to the most specific wavelength of the aminochrome. Control experiments of oxidation of catecholamine to the correspondent aminochrome were carried out using NaIO₄ (2 mM).

RESULTS

Baseline characteristics

The baseline characteristics of the KO mice, namely renal function and sodium balance, did not differ from those of the corresponding WT controls (table 1). The KO mice
presented increased urine volume without differences in daily urinary excretion of both sodium and potassium (table 1).

In comparison with WT mice, the renalase KO mice presented increased renal and cardiac mass and decreased skeletal muscle mass without differences in either body weight or tibia length (table 2).

**Plasma catecholamines levels**

In comparison with WT mice, the KO mice presented increased plasma levels of both EPI and NE by ~254% and ~58%, respectively (figure 1A). In addition, the plasma levels of DA and the deaminated metabolite DOPAC were significantly increased in KO mice in comparison with WT group, by ~87% and ~22%, respectively (figure 1A). In comparison with WT mice, the plasma levels of the DA precursor L-DOPA were also increased in KO mice by ~41%, though the difference did not reach statistical significance (figure 1A).

**Renal dopamine system**

In comparison with WT mice, the daily urinary excretion of EPI, NE and DA were significantly increased in KO mice by ~42%, ~89% and ~99%, respectively (figure 1B). This was accompanied in KO mice by a ~4-fold increase in the urinary DA/L-DOPA ratios (figure 3A) in comparison to WT mice (2.41±0.54 vs 9.45±2.37, p<0.01) without changes in renal AADC activity between the two groups (table 3).

In comparison with WT mice, the KO mice presented a marked decrease in the daily urinary levels of both DOPAC and HVA, by ~85% and ~229%, respectively (figure 1B). This was accompanied in KO mice by a significant decrease in renal MAO-B activity without changes in renal MAO-A activity (table 3). In addition, the KO mice
presented a marked increase in the daily urinary levels of 3-MT by ~123% (figure 1B) which was associated with a significant increase in renal COMT activity (table 3).

**Jejunal dopamine system**

The jejunal tissue levels of both L-DOPA and DA were similar between KO mice and WT group (table 4A). The jejunal tissue levels of DOPAC in KO mice were decreased in comparison with WT group (table 4A) and this was associated with significant decreases in both MAO-A and MAO-B activities in the jejunum from KO mice (table 4B).

**Central dopamine system**

The brain tissue levels of both L-DOPA and DA were similar between KO mice and WT animals (table 5A). The brain tissue levels of DOPAC were significantly increased in KO mice in comparison with WT mice and this was associated with a significant increase in brain MAO-A activity (tables 5A and B). By contrast, the brain tissue levels of both HVA and 3-MT were significantly decreased in KO mice in comparison with WT controls and this was associated with significant decreases in both MAO-B and COMT activities (tables 5A and B).

**Effects of recombinant renalase on plasma and urine catecholamines levels**

The administration of RR did not change the baseline characteristics of KO mice, namely renal function, sodium balance as well as body and organs weight. The administration of RR to KO mice significantly reduced the plasma levels of EPI by ~61%, and also decreased the plasma levels of NE by ~39%, though the difference did not reach statistical significance (figure 2A). In addition, the administration of RR to
KO mice significantly reduced the plasma levels of both DA and L-DOPA by ~38% and ~57%, respectively (figure 2A). Moreover, the administration of RR to KO mice decreased the plasma levels of DOPAC by ~27%, though the difference did not reach statistical significance (figure 2A).

The administration of RR to KO mice did not change the daily urinary excretion of either EPI or NE (figure 2B) but significantly decreased the daily urinary excretion of DA by ~36% (figure 2B). This was accompanied in KO mice receiving the RR by a 60% reduction in the urinary DA/L-DOPA ratios (16.34±1.12 vs 6.43±0.79, p<0.001) (figure 3B). By contrast, the KO mice receiving the RR presented a marked increase in renal AADC activity (Vmax=106.6 ±11.3 vs. 66.1±7.7 nmol/mg prot/h, p<0.05). In addition, the administration of RR to KO mice was accompanied by decreases in the daily urinary excretion of DOPAC, HVA or 3-MT by ~33% ~47% and ~29%, respectively, though the differences did not reach statistical significance (figure 2B).

**Immunoblot analysis**

The changes in the amino acid transporters that are potentially involved in the uptake of L-DOPA were evaluated in the renal cortex of both WT and KO animals. As depicted in figure 4A LAT1 expression were significantly increased in KO when compared with WT animals (p<0.04). On the other hand, the administration of RR induced a significant reduction in the expression of LAT1 (p<0.04) in the renal cortex of the KO animals (figure 4B).

LAT 2 and ASCT2 expression did not change in the renal cortex of both WT and KO animals. The administration of RR did not change the expression in either LAT2 or ASCT2 (data not shown).
Oxidation of catecholamines by recombinant renalase

Figures 5 illustrate the chromatogram showing the oxidation profile of DA obtained in the presence of RR. As can be observed, DA was slightly oxidized to the correspondent aminochrome, dopaminochrome (figure 5 A and B). No oxidation of DA was observed in the absence of the RR, negative control in figure 5C and D. This catecholamine was also completely oxidized with NaIO₄ to the respective aminochrome (data not shown).

DISCUSSION

The results of the present study show that the increase in sympathetic activity in renalase KO mice is accompanied by enhanced renal DA activity, as was previously reported by Sizova et al. (30). The increased renal DA activity in KO mice was observed notwithstanding an enhanced O-methylation of renal DA and can be explained on the basis of increased renal DA synthesis going along with decreased deamination of the newly-formed amine. The finding that the administration of RR to KO mice was accompanied by significant decreases in both plasma L-DOPA levels and in urine DA/L-DOPA ratios without changes in the urinary excretion of either DOPAC or 3-MT provides evidence favouring the view that up-regulation of the renal DA synthesis may be the main mechanism underlying the increased renal DA output observed in renalase KO mice.

As was previously reported (36), we found significant increases in circulating levels of EPI, NE and DA in renalase KO mice thus providing evidence for increased sympathetic activity in the renalase deficiency status. Because EPI was found to be the preferred substrate for renalase (7), it was suggested that the mechanism underlying the increased sympathetic activity in KO mice might be related to EPI induced release of NE in sympathetic neurons (13, 26). The same mechanism may also account, at least in
part, for the increase in circulating DA levels in KO mice because enhanced sympathetic nervous system activity has been shown to increase the secretion of NE as well as DA (19). The finding that the administration of RR to KO mice induced significant decreases in circulating levels of both EPI and DA is also in agreement with this suggestion.

The increased urine DA output in KO mice was observed in the presence of significant changes in the renal activities of DA metabolizing enzymes, namely an increase in renal COMT activity and a decrease in renal MAO-B activity, without changes in renal MAO-A activity. Because the urinary excretion of DOPAC and 3-MT are used as good parameters for the assessment of deamination and O-methylation of renal DA respectively (10-11), the observation that KO mice presented a significant decrease in urinary excretion of DOPAC going along with a significant increase in urinary excretion of 3-MT, further reinforce the view that renal DA is less deaminated and more O-methylated in KO mice in comparison with WT controls. On the other hand, the finding that the urinary excretion of both 3-MT and DOPAC did not increase after RR administration to KO mice suggests that increases in neither deamination nor O-methylation of renal DA can account for the RR induced decrease in urine DA output. However, one cannot exclude that RR administration may have contributed to decreased renal DA output through oxidation of DA into the corresponding aminochrome as was evidenced in vitro.

In addition to the kidney, renalase is also highly expressed in other organs, namely in the intestine and CNS where it was suggested to contribute to tissue-specific modulation of catecholamine levels (15-16). In the present study, renalase deficiency in KO mice was associated with significant changes in brain and jejunal activities of catecholamine metabolizing enzymes, namely MAO-A and B and COMT, which were accompanied by
the expected changes in the tissue levels of the corresponding metabolites. These findings, when viewed collectively with the observation that both jejunal and brain tissue levels of DA did not differ between renalase KO mice and WT controls suggest that in the presence of renalase deficiency both central and peripheral monoaminergic systems may undergo adjustments involving changes in the local activities of enzymes that ultimately regulate local catecholamine levels.

In the present study, renalase deficiency in KO mice was accompanied by a 2-fold increase in urinary DA levels. The regulation of renal DA output is well recognized to depend mainly on the renal availability/uptake of L-DOPA by renal PT cells, its fast decarboxylation to DA by AADC as well as on both deamination and methylation of the newly-formed amine (2, 9, 11, 22). Because urinary DA/L-DOPA ratios are a rough measure of renal uptake/decarboxylation of L-DOPA in PT cells, the finding that renalase KO mice presented a 4-fold increase in the urinary DA/L-DOPA ratios in comparison to WT controls provides evidence for enhanced renal DA synthesis in KO mice. Furthermore, the finding that renal AADC activity was similar between KO and WT mice suggests that increased availability/uptake of L-DOPA in renal PT cells might be the main mechanism underlying the enhanced renal DA synthesis in KO mice. The effects of RR administration to KO mice further reinforce this view. Actually, the administration of RR to KO mice significantly decreased both urine DA output and urinary DA/L-DOPA ratios notwithstanding the accompanied increase in renal AADC activity. Interestingly, this was accompanied by a RR induced decrease in plasma levels of L-DOPA which was recently described as a good substrate for the enzyme (6).

The semiquantitative evaluation of the renal cortical expression of Na+-independent amino acid transporter LAT1, involved in renal tubular uptake of L-DOPA also
reinforce the view that the increased renal DA activity in renalse KO mice appears to result mainly from an enhanced availability/uptake of L-DOPA in renal PT cells. Although, LAT1 has been previously described to have a very limited tissue distribution in the kidney, in the present study we found that the increase in renal DA output observed in renalse KO mice was accompanied by an increase in LAT1 expression that is reversed by the administration of recombinant renalse in these animals. These results suggest that the overexpression of LAT1 in the renal cortex of the renalse KO mice might contribute to the enhanced L-DOPA uptake and consequently to the activation of the renal dopaminergic system in the presence of renalse deficiency. This conclusion is supported further by the findings that recombinant renalse administration induced a marked decrease of LAT1 expression in the renal cortex of renalse KO mice, which would account for decreased L-DOPA uptake, and diminished renal dopamine output. In summary, the up-regulation of renal DA activity in renalse KO mice appears to result mainly from an enhanced availability/uptake of L-DOPA in renal PT cells that is reverted by the administration of RR.

ACKNOWLEDGEMENTS

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We thank the Department of Biochemistry and the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto for the facilities. We also thank Dr. Luisa Guardão for veterinarian assistance and Liliana Leite for technical support.

CONFLICTS OF INTEREST/DISCLOSURES

GVD is a named inventor on issued patents for the discovery and use of renalase.
REFERENCES


Table 1: Metabolic balance in WT and renalase KO mice.

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.36 ± 0.01</td>
<td>0.38 ± 0.003</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>43.84 ± 3.37</td>
<td>41.41 ± 2.52</td>
</tr>
<tr>
<td>Protein, g/dl</td>
<td>37.98 ± 1.43</td>
<td>36.87 ± 1.90</td>
</tr>
<tr>
<td>Na⁺, mmol/l</td>
<td>143.75 ± 1.06</td>
<td>143.27 ± 0.66</td>
</tr>
<tr>
<td>K⁺, mmol/l</td>
<td>6.17 ± 0.28</td>
<td>5.67 ± 0.34</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
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</tr>
<tr>
<td>Volume, µl/24h</td>
<td>640.32 ± 95.36</td>
<td>1009.26 ± 105.88*</td>
</tr>
<tr>
<td>Na⁺, mmol/24h</td>
<td>1.31 ± 0.11</td>
<td>1.42 ± 0.20</td>
</tr>
<tr>
<td>K⁺, mmol/24h</td>
<td>3.26 ± 0.44</td>
<td>3.46 ± 0.24</td>
</tr>
</tbody>
</table>

*Footnote:* Symbols represent mean values ± SEM (n = 9-11). Significantly different from corresponding values in WT mice (*p<0.05).
Table 2: Body and organs weight in WT and renalase KO mice.

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
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<tr>
<td>Body weight, g</td>
<td>20.2 ± 0.3</td>
<td>19.6 ± 0.7</td>
</tr>
<tr>
<td>Tibia length, cm</td>
<td>1.7 ± 0.02</td>
<td>1.6 ± 0.02</td>
</tr>
<tr>
<td>Right kidney, mg</td>
<td>130.5 ± 3.1</td>
<td>147.8 ± 4.9*</td>
</tr>
<tr>
<td>Left kidney, mg</td>
<td>120.5 ± 4.0</td>
<td>141.3 ± 5.1*</td>
</tr>
<tr>
<td>Heart, mg</td>
<td>101.0 ± 1.8</td>
<td>108.4 ± 1.5*</td>
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<tr>
<td>Left ventricle, mg</td>
<td>69.5 ± 1.2</td>
<td>74.8 ± 1.8*</td>
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<tr>
<td>Skeletal Muscle, mg</td>
<td>117.6 ± 3.1</td>
<td>102.4 ± 6.5*</td>
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<tr>
<td>Brain, mg</td>
<td>305.1 ± 3.1</td>
<td>287.0 ± 12.6</td>
</tr>
</tbody>
</table>

*Footnote: Symbols represent mean values ± SEM (n = 9-11). Significantly different from corresponding values in WT mice (*p<0.05).
Table 3: Kinetic parameters ($V_{\text{max}}$ and $K_{\text{m}}$) of enzymatic activities of AADC, MAO-A, MAO-B and COMT in homogenates of renal cortex from WT and renalase KO mice.

<table>
<thead>
<tr>
<th>Renal cortex</th>
<th>WT</th>
<th>KO</th>
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<tbody>
<tr>
<td></td>
<td>$V_{\text{max}}, \text{nmol.mg prot}^{-1}.\text{h}^{-1}$</td>
<td>$V_{\text{max}}, \text{nmol.mg prot}^{-1}.\text{h}^{-1}$</td>
</tr>
<tr>
<td>AADC</td>
<td>106.4 ± 5.2</td>
<td>103.5 ± 5.9</td>
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<tr>
<td></td>
<td>$K_{\text{m}}, \text{mM}$</td>
<td>$K_{\text{m}}, \text{mM}$</td>
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<tr>
<td></td>
<td>1.4 ± 0.23</td>
<td>1.5 ± 0.28</td>
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<tr>
<td>MAO-A</td>
<td>12.09 ± 0.87</td>
<td>10.45 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>$K_{\text{m}}, \text{mM}$</td>
<td>$K_{\text{m}}, \text{mM}$</td>
</tr>
<tr>
<td></td>
<td>1001.0 ± 145.0</td>
<td>878.3 ± 99.6</td>
</tr>
<tr>
<td>MAO-B</td>
<td>1.77 ± 0.07</td>
<td>1.22 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>$K_{\text{m}}, \text{µM}$</td>
<td>$K_{\text{m}}, \text{µM}$</td>
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<tr>
<td></td>
<td>48.2 ± 5.3</td>
<td>50.60 ± 5.6</td>
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<tr>
<td>COMT</td>
<td>3.68 ± 0.07</td>
<td>3.99 ± 0.13*</td>
</tr>
<tr>
<td></td>
<td>$K_{\text{m}}, \text{µM}$</td>
<td>$K_{\text{m}}, \text{µM}$</td>
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<tr>
<td></td>
<td>2.5 ± 0.22</td>
<td>2.2 ± 0.32</td>
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</table>

Footnote: Symbols represent mean values ± SEM (n = 9-11). Significantly different from corresponding values in WT mice (*p<0.05).
Table 4:

A) Catecholamines in jejunum from WT and renalase KO mice.

<table>
<thead>
<tr>
<th></th>
<th>pmol/mg</th>
<th>WT</th>
<th>KO</th>
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<tr>
<td>L-DOPA</td>
<td></td>
<td>0.005 ± 0.0004</td>
<td>0.005 ± 0.001</td>
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<tr>
<td>DA</td>
<td></td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.002</td>
</tr>
<tr>
<td>DOPAC</td>
<td></td>
<td>0.006 ± 0.001</td>
<td>0.003 ± 0.003 *</td>
</tr>
</tbody>
</table>

Footnote: Symbols represent mean value ± SEM (n = 9-11). Significantly different from corresponding values in WT mice (*p<0.05).

B) Kinetic parameters (V_{max} and K_{m}) of enzymatic activities of MAO-A, MAO-B and COMT in homogenates of brain from WT and renalase KO mice.

<table>
<thead>
<tr>
<th>Jejunum</th>
<th>WT</th>
<th>KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAO-A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_{max}, nmol/mg prot/h</td>
<td>74.76 ± 1.76</td>
<td>60.53 ± 3.97 *</td>
</tr>
<tr>
<td>K_{m}, µM</td>
<td>411.1 ± 26.6</td>
<td>355.9 ± 64.3</td>
</tr>
<tr>
<td>MAO-B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_{max}, nmol/mg prot/h</td>
<td>18.32 ± 1.08</td>
<td>13.10 ± 1.30 *</td>
</tr>
<tr>
<td>K_{m}, µM</td>
<td>61.0 ± 9.1</td>
<td>57.9 ± 14.3</td>
</tr>
</tbody>
</table>

Footnote: Symbols represent mean values ± SEM (n = 9-11). Significantly different from corresponding values in WT mice (*p<0.05).
Table 5:

A) Catecholamines in brain from WT and renalase KO mice.

<table>
<thead>
<tr>
<th>pmol/mg</th>
<th>WT</th>
<th>KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-DOPA</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>DA</td>
<td>6.27 ± 0.61</td>
<td>5.40 ± 0.34</td>
</tr>
<tr>
<td>DOPAC</td>
<td>1.37 ± 0.16</td>
<td>1.87 ± 0.12*</td>
</tr>
<tr>
<td>HVA</td>
<td>1.73 ± 0.17</td>
<td>1.23 ± 0.11*</td>
</tr>
<tr>
<td>3-MT</td>
<td>1.17 ± 0.13</td>
<td>0.72 ± 0.08*</td>
</tr>
</tbody>
</table>

Footnote: Symbols represent mean value ± SEM (n = 9-11). Significantly different from corresponding values in WT mice (*p<0.05).

B) Kinetic parameters ($V_{max}$ and $K_m$) of enzymatic activities of MAO-A, MAO-B and COMT in homogenates of brain from WT and renalase KO mice.

<table>
<thead>
<tr>
<th>Brain</th>
<th>WT</th>
<th>KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAO-A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max}$, nmol/mg prot˚h</td>
<td>9.88 ± 0.53</td>
<td>13.01 ± 0.69*</td>
</tr>
<tr>
<td>$K_m$, µM</td>
<td>630.1 ± 78.76</td>
<td>952.9 ± 115.6</td>
</tr>
<tr>
<td>MAO-B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max}$, nmol/mg prot˚h</td>
<td>8.61 ± 0.31</td>
<td>7.58 ± 0.31*</td>
</tr>
<tr>
<td>$K_m$, µM</td>
<td>27.9 ± 3.4</td>
<td>27.3 ± 3.9</td>
</tr>
<tr>
<td>COMT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max}$, nmol/mg prot˚h</td>
<td>1.47 ± 0.05</td>
<td>0.94 ± 0.04*</td>
</tr>
<tr>
<td>$K_m$, µM</td>
<td>7.1 ± 0.8</td>
<td>7.5 ± 1.1</td>
</tr>
</tbody>
</table>

Footnote: Symbols represent mean values ± SEM (n = 9-11). Significantly different from corresponding values in WT mice (*p<0.05).
FIGURES CAPTIONS

Figure 1: EPI, NE, L-DOPA, DA and DA metabolites levels in plasma (A) and urine (B). Footnote: Bars represent mean values ± SEM. * KO mice significantly different from corresponding values in WT mice (n = 9-11). L-DOPA, DA and DOPAC corresponding to left Y axis; HVA and 3-MT corresponding to right Y axis.

Figure 2: Recombinant renalase mediated effects in EPI, NE, L-Dopa, DA, DOPAC, HVA and 3-MT levels in plasma (A) and urine (B). Footnote: Bars represent mean values ± SEM, expressed in % of control. *KO mice treated with recombinant renalase significantly different from corresponding values in KO mice treated with vehicle (n=4-8).

Figure 3: Urinary DA/L-DOPA ratios in WT and KO mice at baseline (A) and in KO mice treated with buffer or recombinant renalase (B). Footnote: Bars represent mean values ± SEM (*p<0.01, * KO mice significantly different from corresponding values in WT mice (n = 9-11).

Figure 4: Semiquantitative analysis and representative immunoblots of the expression of LAT1 in the renal cortex from WT and renalase KO mice (A) as well as in KO mice injected with buffer or recombinant renalase (B). Footnote: Bars represent mean values ± SEM (*p<0.05, *KO mice treated with recombinant renalase significantly different from corresponding values in KO mice treated with vehicle (n=4-8).
Figure 5: Renalase-induced DA oxidation assessed by HPLC-DAD at normal range (A) and amplified (B). Negative control in the absence of RR (C) at normal range (A) and amplified (D). **Footnote:** Pink line represents the channel at 279 nm which corresponds to the maximum absorption wavelength of catecholamine and orange line represents the channel at 490 nm which corresponds to the most specific wavelength of the respective aminochrome.
FIGURES

Figure 1:

A)

B)
Figure 3:

A) Urinary DA/L-DOPA (ratio)

B) Urinary DA/L-DOPA (ratio)
Figure 4:

A) Renal cortex
LAT-1 expression

LAT-1 / GAPDH

B) Renal cortex
LAT-1 expression

LAT-1 / GAPDH
Figure 5:

A)

B)
C) 

D)