Studies on the Nature of the Antagonistic Actions of Dopamine and 5-Hydroxytryptamine in Renal Tissues

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The present work examines the possibility of whether the reciprocal effects of dopamine (DA) and 5-hydroxytryptamine (5-HT) are only dependent on the antagonistic nature of the signal resulting from the activation of their specific receptors or may also result from a competitive type of inhibition at different levels of the synthetic and metabolic pathways shared by DA and 5-HT. Studies performed in isolated proximal convoluted tubules (PCT) have shown that L-5-HTP and L-DOPA use the same transporter in order to be taken up into the cell and both L-DOPA and L-5-HTP exert a competitive type of inhibition upon their cellular uptake. The decrease in the formation of 5-HT in isolated PCT induced by L-DOPA reflects most probably a reduction in the intracellular availability of L-5-HTP. However, in experiments conducted in homogenates of PCT L-DOPA was found to be a better substrate for AAAD than L-5-HTP. Apart from sharing a common synthetic pathway, DA and 5-HT also share a common metabolic pathway; type A monoamine oxidase (MAO-A), the predominant form of MAO in rat renal tissues, converts DA into 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-HT into 5-hydroxyindolacetic acid (5-HIAA). However, in contrast to 5-HT, DA can be metabolized by MAO-B and catechol-O-methyltransferase. Inhibition of MAO-A was found to produce a 2-fold increase in the urinary excretion of 5-HT; this increase in the urinary excretion of 5-HT was accompanied by an unexpected reduction in the urinary excretion of DA. It is possible that the increased availability of 5-HT might have compromised the ability of the DA outward transfer to extrude the amine into the urine. Depending on the degree of sodium loading, the increased urinary excretion of 5-HT during MAO-A inhibition is accompanied by antinatriuresis and increased urine osmolality. This antinatriuretic effect does not appear to be due to a reduction on the availability of DA, but to result from an increased availability of 5-HT. In fact, this antinatriuretic effect can be antagonized in a concentration dependent manner by the selective 5-HT1A receptor antagonist (+) WAY 100135, but not by ketanserin. It is possible that stimulation of Na+–K+–ATPase might be relevant for the antinatriuretic effect of endogenous renal 5-HT, in contrast to the inhibitory effects of DA on the enzyme. In fact, we have recently found that the 5-HT1A receptor agonist 8-OH-DPAT increases Na+–K+–ATPase activity with an EC50 value of 355 nM; this effect can be antagonized by (+) WAY 100135 with a IC50 value of 20 nM. It is possible, therefore, to conclude that the nature of the antagonistic effects of DA and 5-HT in renal tissues does not only depend on the reciprocal effects related to the activation of their specific receptors, but has also to do with the availability of their amino acid precursors and the ability of the amines to leave the cellular compartment. (Hypertens Res 1995; 18 Suppl. I: 547-551)

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In kidney, dopamine (DA) has been suggested to be of physiological importance in the regulation of tubular reabsorption of sodium (1). The synthesis of DA in renal tissues has been demonstrated to result from the decarboxylation of circulating or filtered L-DOPA in epithelial cells of proximal convoluted tubules. The fact that in the kidney dopamine is produced in close proximity to renal cells which contain dopamine receptors introduces the possibility that the amine may act in a cell-to-cell manner, as a paracrine or autocrine substance. The AAAD rich epithelial cells of proximal convoluted tubules also decarboxylate L-5-hydroxytryptophan (L-5-HTP) to 5-hydroxytryptamine (5-HT) (2) and in conditions of increased renal delivery of L-5-HTP the enhanced urinary excretion of 5-HT is accompanied by significant reductions in sodium and water excretion and slight reductions in the clearance of p-aminohippurate and glomerular filtration rate (3, 4). When L-DOPA is infused together with L-5-HTP into the renal artery there is a lessening of these antinatriuretic and antidiuretic effects; the effects of L-DOPA occurred without changes in the urinary excretion of 5-HT, leading to the suggestion of a functional reciprocal effect of the two amines (3). It is possible, therefore, that depending on the availability of L-DOPA and 5-HTP, this type of cells will synthesize dopamine and 5-HT, the action

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of which will be antagonistic in nature. On the other hand, it remains to be determined whether in the absence of an increased renal delivery of L-5-HTP the amount of 5-HT formed in tubular epithelial cells exerts antinatriuretic effects.

The present work examines the possibility of whether the reciprocal effects of DA and 5-HT are only dependent on the antagonistic nature of the signal resulting from the activation of their specific receptors or may also result from a competitive type of inhibition at different levels of the synthetic and metabolic pathways shared by DA and 5-HT, namely at the cellular transport of their respective amino acid precursors, the decarboxylation by AAAD, the metabolic degradation by monoamine oxidase (MAO) and the cell outward amine transfer. The intrinsic mechanism responsible for the antinatriuretic effect of 5-HT and the type of receptor involved has also been evaluated.

**Uptake of L-DOPA and L-5-HTP**

Incubation of isolated renal tubules in the presence of increasing concentrations of L-5-HTP and L-DOPA results in a concentration-dependent formation of 5-HT and DA, respectively. The maximal rate of DA formation was found to be 2.5-fold that for 5-HT and occurred at similar concentrations of the substrate. The $V_{\text{max}}$ and $K_m$ values for the formation of 5-HT and DA in renal proximal convoluted tubules (PCT) were as follows: 5-HT, $V_{\text{max}} = 24.9 \pm 4.5$ nmol·mg protein$^{-1} \cdot$h$^{-1}$ and $K_m = 121 \pm 30$ pM (95% confidence limits: 75, 193) pM ($n=5$); DA, $V_{\text{max}} = 58.0 \pm 4.3$ nmol·mg protein$^{-1} \cdot$h$^{-1}$ and $K_m = 135 (97, 188)$ pM ($n=5$). When the saturation curve of 5-HT formation was performed in the presence of a constant amount of L-DOPA (250 $\mu$M), the maximal rate of decarboxylation of L-5-HTP in PCT was found to be markedly reduced ($V_{\text{max}} = 10.5 \pm 1.7$ nmol·mg protein$^{-1} \cdot$h$^{-1}$, $n=4$); this was accompanied by a significant increase in $K_m$ values (325 [199, 531] pM, $n=4$). The data obtained in experiments performed in isolated PCT reflects the activity of two different systems; the first one is involved in the cellular uptake of the substrate, whereas the other one is responsible for its decarboxylation. The $V_{\text{max}}$ and $K_m$ values obtained in isolated PCT are, therefore, apparent kinetic parameters considering the rate of decarboxylation, but reflect the kinetics of the tubular transporter.

The decrease in the formation of 5-HT in isolated PCT induced by L-DOPA reflects most probably a reduction in the intracellular availability of L-5-HTP. This view is reinforced by the result that the addition of increasing concentrations (50 to 2,000 $\mu$M) of L-DOPA produces a concentration dependent decrease in the tubular formation of 5-HT. The lowest concentration of L-DOPA (50 $\mu$M) resulting in some inhibition of L-5-HTP uptake was found to reduce by 38% the formation of 5-HT; the greatest inhibitory effect on the tubular formation of 5-HT (91% reduction) was obtained with 2,000 $\mu$M of L-DOPA. The $K_i$ value (in $\mu$M) of L-DOPA for inhibition of L-5-HTP uptake was found to be 29 (14, 61) ($n=6$). This $K_i$ value for L-DOPA is significantly lower than the $K_m$ value of 5-HT or L-DOPA formation obtained in saturation experiments.

Assuming that the kinetic parameters for the formation of both 5-HT and DA in isolated PCT reflect the kinetics of the tubular transport system for the corresponding substrates, it is interesting to underline the similarity of $K_m$ values between L-5-HTP (121 [75, 193] pM) and L-DOPA (135 [97, 188] pM). This suggest that both substrates might share the same transporter for uptake into renal tubules. This is not unique since at the level of the blood-brain barrier a similar situation has been recognized long time ago (5). The result that the concentration-dependent uptake and decarboxylation of L-5-HTP in PCT is markedly reduced by 250 $\mu$M L-DOPA, a concentration which is twice the $K_m$ value for its uptake and decarboxylation, suggests the latter is exerting a competitive type of inhibition upon the tubular uptake of the former. The finding that $K_i$ values of L-DOPA for inhibition of 5-HT formation were lower than the $K_m$ values of 5-HT or DA formation in PCT also agrees with the view that L-DOPA exerts a competitive type of inhibition upon the tubular uptake of L-5-HTP and further suggests that L-DOPA may have a higher affinity for the transporter than L-5-HTP.

**Decarboxylation of L-DOPA and L-5-HTP**

Incubation of homogenates of PCT with L-5-HTP or L-DOPA (50 to 10,000 $\mu$M) resulted in a concentration-dependent formation of 5-HT and DA, respectively. The $V_{\text{max}}$ and $K_m$ values for AAAD using L-DOPA as the substrate ($V_{\text{max}} = 479.9 \pm 74.0$ nmol·mg protein$^{-1} \cdot$h$^{-1}$; $K_m = 2380 [1630, 3476]$ pM; $n=4$) were both found to be significantly higher than those observed when using L-5-HTP ($V_{\text{max}} = 81.4 \pm 5.2$ nmol·mg protein$^{-1} \cdot$h$^{-1}$; $K_m = 97 [87, 107]$ pM, $n=10$). The addition of 10 mM L-DOPA to the incubation medium reduced by 30% ($p<0.02$) the maximal rate of decarboxylation of L-5-HTP ($V_{\text{max}} = 56.7 \pm 3.1$ nmol·mg protein$^{-1} \cdot$h$^{-1}$, $n=10$) and resulted in a significant increase of $K_m$ values (249 [228, 270] pM, $n=10$).

The results obtained in homogenates of PCT show that the maximal rate of decarboxylation of L-DOPA was found to be 6.1-fold that for L-5-HTP. This suggest that L-DOPA is a better substrate for AAAD than 5-HTP, though the affinity of 5-HTP for the enzyme, as indicated by the $K_m$ values, would favour the decarboxylation of the latter. This discrepancy has been described in several tissues, both in the central nervous system and the periphery, by several groups of researchers in the past 30 years (6-11). The different kinetic parameters for decarboxylation of L-DOPA and L-5-HTP have been even considered as evidence for the presence of similar but different enzymes (7, 9, 12). Recently, however, it has been demonstrated that the purified rat renal AAAD decarboxylates preferentially L-DOPA in comparison to L-5-HTP (10). Similar results have been obtained by Sumi et
al. (11) using a recombinant human AAAD expressed in COS cells. The expressed enzyme decarboxylates both L-DOPA and L-5-HTP; however, differences concerning the kinetic properties for decarboxylation of L-DOPA and L-5-HTP were found to be similar to those described in crude homogenates or preparations with different degrees of enzyme purification (6–9). The mutual competitive inhibition between L-DOPA and L-5-HTP described in several studies and present one in the kidney is consistent with the finding of a single catalytic unit. According to Bender and Coulson (8), it is possible for L-DOPA and L-5-HTP to be arranged in such a manner that the α-amino and the α-carboxyl groups and the 3-hydroxyl group of L-DOPA and the 5-hydroxyl group of L-5-HTP occupy corresponding sites. With such an arrangement the aromatic rings of both substances would no longer occupy corresponding planes or sites. It is possible that this different arrangement might explain differences in kinetic properties for the two substrates.

**Intracellular Availability of DA and 5-HT**

Apart from sharing a common synthetic pathway, DA and 5-HT also share a common metabolic pathway; type A monoamine oxidase (MAO-A), the predominant form of MAO in rat renal tissues (13, 14), converts DA into 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-HT into 5-hydroxyindolacetic acid (5-HIAA). However, in contrast to 5-HT, DA can be metabolized by MAO-B and catechol-O-methyltransferase.

In studies carried out under in vivo conditions (15), rats given a selective MAO-A inhibitor (Ro 41-1049 50 μmol·kg⁻¹·day⁻¹) in three consecutive days were found to excrete slightly less DA (15.9±0.8 vs. 12.9±1.6 nmol·24 h⁻¹) and 3-MT (439.1±15.8 vs. 395.6±26.7 nmol·24 h⁻¹) in the urine; the urinary excretion of DOPAC (29.6±0.6 nmol·24 h⁻¹) and HVA (154.8±6.2 nmol·24 h⁻¹) were found to be reduced by 37-54% and 15-53%, respectively. The urinary excretion of 5-HT was found to be increased by 3-fold (135.5±19.0 vs. 410.7±30.9 nmol·24 h⁻¹) during the administration of the MAO-A inhibitor Ro 41-1049, whereas the levels of 5-HIAA in the urine (228.4±8.7 nmol·24 h⁻¹) were reduced by 14-25%. The administration of the MAO-B inhibitor Ro 19-6327 did not change the urinary excretion of DA, DOPAC, 5-HT, and 5-HIAA, but significantly decreased the urinary excretion of HVA by 42-62%. The urinary excretion of sodium and potassium were not affected by the administration of both MAO inhibitors. It is possible that the increased availability of 5-HT might have compromised the ability of the DA outward transfer to extrude the amine into the urine, but this did not compromised renal sodium homeostasis.

**Antinatriuretic Effect of 5-HT**

Depending on the degree of sodium loading, the increased urinary excretion of 5-HT during MAO-A inhibition is accompanied by antinatriuresis and increased urine osmolality. This antinatriuretic effect does not appear to be due to a reduction on the availability of DA, but to result from an increased availability of 5-HT (16). In that study, rats were given tap water (normal salt diet) in the first 7 days of the study and then challenged to a high sodium diet (HSD, 1% NaCl in drinking water). The decrease in urinary sodium during the 3-day period of MAO-A inhibition can be antagonized by (+)-WAY 100135 (10 mg·kg⁻¹·day⁻¹) but not by ketanserin (2 mg·kg⁻¹·day⁻¹).

**Fig. 1.** Effect of MAO-A inhibition by Ro 41-1049 (50 mol·kg⁻¹·day⁻¹) on changes in urinary sodium excretion in rats submitted to a high sodium diet (HSD, 1% NaCl in drinking water). The decrease in urinary sodium during the 3-day period of MAO-A inhibition can be antagonized by (+)-WAY 100135 (10 mg·kg⁻¹·day⁻¹) but not by ketanserin (2 mg·kg⁻¹·day⁻¹).
that in the human and rat kidney, 5-HT\textsubscript{1A} receptors have been found to be specifically localized in tubular epithelial cells of nephron segments particularly involved in the regulation of salt and water transport (17). However, it remains to be explained the reason why this antinatriuretic effect of 5-HT evident only when rats are submitted to a HSD. Apparently, this conflicts with the findings that salt restriction in humans results in an increase in the renal production of renal 5-HT (18).

**Concept of a direct effect on the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase molecule rather than secondary to changes in sodium permeability.** The selective 5-HT\textsubscript{2} receptor agonist \(\alpha\)-methyl-5-HT (100 to 3,000 nM) induce no changes in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase. Taken together the available data agrees with the view that activation of 5-HT\textsubscript{1A} receptors increases Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in renal proximal tubules and this might be a relevant mechanism behind the antinatriuretic effect of endogenous renal 5-HT.

**Nature of Mechanism Involved in 5-HT-Mediated Antinatriuresis**

It is possible that stimulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase might be relevant for the antinatriuretic effect of endogenous renal 5-HT, in contrast to the inhibitory effects of DA on the enzyme (19, 20). In fact, we have recently found that activation of 5-HT\textsubscript{1A} receptors in isolated renal tubules increases Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity (21). In that study, Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was determined as the rate of \(32\text{P}\)-ATP hydrolysis in the presence and absence of 2 mM ouabain. The 5-HT\textsubscript{1A} receptor agonist (\(\pm\))-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) 10 to 3,000 nM induced a concentration dependendent increase in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity with an EC\textsubscript{50} value of 355 nM (95% confidence limits: 178, 708). The maximal stimulation by 8-OH-DPAT (3,000 nM) was antagonized by the selective 5-HT\textsubscript{1A} receptor antagonist (+) WAY 100135 (10 to 1,000 nM) with an IC\textsubscript{50} value of 20 nM (14, 29). The stimulatory effect of 8-OH-DPAT (1,000 nM) on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity by was found to be a time dependent: 15 \(\pm\) 2\% and 66 \(\pm\) 7\% increase activity at 2.5 and 5.0 min, respectively. The stimulatory effect was absent when homogenates and membrane preparations were used, indicating the requirement of an intracellular signalling system. Stimulation occurred at \(V\text{max}\) for sodium (70 mM) supporting the concept of a direct effect on the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase molecule rather than secondary to changes in sodium permeability. The selective 5-HT\textsubscript{2} receptor agonist \(\alpha\)-methyl-5-HT (100 to 3,000 nM) induce no changes in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase. Taken together the available data agrees with the view that activation of 5-HT\textsubscript{1A} receptors increases Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in renal proximal tubules and this might be a relevant mechanism behind the antinatriuretic effect of endogenous renal 5-HT.

**Conclusions and Future Directions**

The nature of the antagonistic effects of DA and 5-HT in renal tissues appears not only to depend on the reciprocal effects related to the activation of their specific receptors, but has also to do with the intracellular availability of their amino acid precursors, competition for decarboxylation by AAAD and the ability of the newly-formed amines to leave the cellular compartment (Fig. 2). The stimulatory effect of 5-HT on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase involves the activation of 5-HT\textsubscript{1A} receptors and requires the integrity of an intracellular signalling system. Since the formation of natriuretic DA and antinatriuretic 5-HT can occur in the same cell, it will be interesting to study the implications of this balance at a molecular level, namely at the post-receptor transducing mechanisms, on the regulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. From a conceptual point of view the antagonism of 5-HT on the tubular effects of DA represents a challenge in renal physiology and constitute a local alternative to the sympathetic nervous system to renal sodium conservation. It remains to be determined the importance and role of the renal tubular 5-hydroxytryptaminergic system in the pathophysiology of hypertension and renal disorders.
References