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Antagonistic actions of renal dopamine and 5-hydroxytryptamine: increase in Na^+, K^+-ATPase activity in renal proximal tubules via activation of 5-HT_{1A} receptors

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1 5-Hydroxytryptamine (5-HT) is antinatriuretic. Since this effect of 5-HT is not accomplished by changes in glomerular haemodynamics, we have examined in this study whether 5-HT may influence sodium excretion by affecting the Na^+, K^+-ATPase activity in renal cortical tubules.

2 Na^+, K^+-ATPase activity was determined as the rate of [^{32}P]-ATP hydrolysis in renal cortical tubules in suspension. Basal Na^+,K^+-ATPase activity in renal tubules was 4.8 ± 0.4 μmol Pi mg^{-1} protein h^{-1} ($n=8$). The 5-HT_{1A} receptor agonist, (±)-8-hydroxy-2-(di-n-propylamino) tetraline (8-OH-DPAT) (10 to 3000 nM) induced a concentration-dependent increase ($P<0.05$) in Na^+,K^+-ATPase activity with an EC_{50} value of 355 nM (95% confidence limits: 178, 708). Maximal stimulation elicited by 3000 nM of 8-OH-DPAT was antagonized by the selective 5-HT_{1A} receptor antagonist, (+)-WAY 100135 (10 to 1000 nM) with an IC_{50} value of 20 nM (14, 29); 0.3 μM (+)-WAY 100135 completely abolished ($P<0.01$) the stimulatory effect of 8-OH-DPAT. The stimulatory effect of 8-OH-DPAT was found to be time-dependent (15 ± 2% and 66 ± 7% increase at 2.5 and 5.0 min, respectively). The 5-HT_2 receptor agonist α-methyl-5-HT (100 to 3000 nM) did not induce any significant changes in Na^+,K^+-ATPase activity (5.0 ± 1.5 μmol Pi mg^{-1} protein h^{-1}; $n=4$).

3 The stimulatory effect 8-OH-DPAT was absent when homogenates were used. Stimulation occurred at a V_{max} concentration (70 mM) of sodium supporting the notion that stimulation occurs independently of increasing sodium permeability.

4 The inhibitory effect of dopamine ($P<0.05$) on Na^+,K^+-ATPase activity was blunted by co-incubation with 8-OH-DPAT (0.5 μM).

5 It is concluded that activation of 5-HT_{1A} receptors increases Na^+,K^+-ATPase activity in renal cortical tubules; this effect may represent an important cellular mechanism, at the tubule level, responsible for the antinatriuretic effect of 5-HT.

Keywords: Dopamine; kidney tubules; natriuresis; 5-hydroxytryptamine receptors

Introduction

In the kidney, dopamine and 5-hydroxytryptamine (5-HT) have been shown to originate in the same epithelial cells of renal proximal convoluted tubules and share a common synthetic pathway, decarboxylation of their respective amino acid precursors (Soares-da-Silva & Pinto-do-Ó, 1996 and references within). Locally produced dopamine increases the urinary excretion of sodium, which is not accompanied by changes in renal haemodynamics (Siragy *et al.*, 1989), but rather is the result of a combined inhibition of transcellular sodium transport by inhibition of Na^+,K^+-ATPase (for a review see Bertorello & Katz, 1993) and Na^+/H^+ exchanger (Felder *et al.*, 1990) activaties in the renal tubule. On the other hand, the renal delivery of sodium represents an important stimulus for the synthesis of the amine (for a review see Lee, 1993). In contrast to dopamine, 5-HT of renal origin is antinatriuretic (Itskowitz *et al.*, 1988; Li Kam Wa *et al.*, 1993), but both amines can modulate urinary sodium excretion without affecting renal haemodynamics (Itskowitz *et al.*, 1988; Siragy *et al.*, 1989; Li Kam Wa *et al.*, 1993). Antinatriuresis as induced by 5-HT of renal origin is accompanied by a slight reduction in glomerular filtration rate and is sensitive to inhibition by the 5-HT_{1A} receptor antagonist, (+)-WAY 100135, but not to ketanserin (a 5-HT_2 receptor antagonist) (Soares-da-Silva *et al.*, 1994b). In the rat kidney, the majority of 5-HT_2 receptors are located in the vasculature, whereas 5-HT_{1A} receptors have been found to be localized specifically in tubular epithelial cells of nephron segments particularly involved in the regulation of salt and water transport (Raymond *et al.*, 1993). This would indicate that the antinatriuretic effect of renal 5-HT might have originated at the tubular level. Since the energy for tubular sodium reabsorption is supplied by Na^+,K^+-ATPase (Bertorello & Katz, 1993) it might then be suggested that stimulation of tubular sodium absorption by 5-HT would involve activation of the sodium pump.

The aim of the present study was, therefore, to examine whether the 5-HT effect was mediated by regulating the Na^+,K^+-ATPase in isolated renal tubules. For this purpose, Na^+,K^+-ATPase activity was examined in proximal tubules incubated with selective 5-HT_{1A} and 5-HT_2 agonists, respectively (±)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and α-methyl-5-HT. A preliminary account of some of these findings has already been published (Soares-da-Silva & Bertorello, 1994).

Methods

General

Male Sprague-Dawley rats, 40 days old and weighing 180–200 g, were used in the experiments. Animals were kept two per cage under controlled environmental conditions (12 h

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light/dark cycle and room temperature 24°C). Food and tap water were allowed *ad libitum* and the experiments were all carried out during daylight hours.

Preparation of renal tubules

The preparation of renal tubules was based on the techniques previously described and was found to contain predominantly proximal tubules (Soares-da-Silva *et al.*, 1994a; Soares-da-Silva & Pinto-do-Ó, 1996). In brief, after anaesthesia (Inactin, 50 mg kg^{-1}, i.p.), a midline incision was made, the aorta was cannulated with polyethylene catheters and both kidneys were perfused with Hanks solution (in mM): NaCl 137, KCl 5, $MgSO_4$ 0.8, Na_2PO_4 0.33, KH_2PO_4 0.44, $CaCl_2$ 0.25, $MgCl_2$ 1.0, Tris.HCl 15 and sodium butyrate 1.0, pH = 7.4. Thereafter, the kidneys were removed through a midline abdominal incision, after which they were decapsulated, cut in half and placed in ice-cold Hanks solution. The outer cortex was cut out with fine scissors and minced with a scalpel into a fine paste. The cortical paste was filtered sequentially through a series of Nybolt nylon sieves, first 180 μm and then 75 μm. Unseparated cortex remained in the upper (180 μm) sieve, while the lower one (75 μm) retained predominantly proximal nephron segments. The sieves were continuously rinsed with cold Hanks solution throughout. The retained tubules were then washed off with cold Hanks solution and collected into a pellet by centrifugation at 200 *g*, 5 min, 4°C; renal tubules used in incubation experiments were suspended in Hanks solution. The viability of proximal renal tubules used in this study was assessed by the trypan blue (0.2% w/v) exclusion method; nephron segments were pipetted on to a glass slide and observed 90 s after exposure to the dye, with a Leica microscope. Under these conditions, more than 90% of the renal tubules excluded the dye.

Determination of Na^+,K^+-ATPase activity

Na^+,K^+-ATPase activity was determined as the rate of [γ-^{32}P]-ATP hydrolysis essentially as described before (Bertorello *et al.*, 1990). Briefly, aliquots of tubules in suspension were pre-incubated for different time intervals at room temperature with the desired agonists and antagonists. The pre-incubation period was terminated by placing the tubules on ice. Thereafter, aliquots of 10 μl containing approximately 10–20 μg protein were transferred to the Na^+,K^+-ATPase assay medium. The assay medium contained the following (in mM): NaCl 70, KCl 5, $MgCl_2$ 10, EGTA 1, Tris.HCl 100, Na_2ATP 10 and 2–5 Ci $mmol^{-1}$ in tracer amounts (5 nCi μl^{-1}) of [γ-^{32}P]-ATP. For determination of ouabain-insensitive ATPase (Mg-dependent), NaCl and KCl were omitted and Tris.HCl (150 mM) and ouabain (2 mM) were added. The pH of both solutions was adjusted to 7.4. In each experiment, total ATPase and ouabain-insensitive ATPase were determined; the difference between total ATPase and ouabain-insensitive ATPase was noted as Na^+,K^+-ATPase. In each assay we also determined the non-specific ^{32}P release from [γ-^{32}P]-ATP. Na^+,K^+-ATPase-mediated hydrolytic activity was determined in the tubule suspension for 15 min at 37°C at V_{max} conditions for Na^+,K^+ and ATP. Drugs to be tested were present during preincubation (15 min) and incubation (15 min) periods. In some experiments, the exposure of renal tubules to 8-OHDPAT during preincubation varied from 1 to 15 min. The reaction was terminated by placing the test tubes (100 μl) on ice and addition of 700 μl TCA/charcoal (10% w/v). After centrifugation (12.000 *g* for 5 min) an aliquot from the supernatant was placed in the scintillation counter and the liberated ^{32}P was counted.

The protein content of the suspensions of renal tubules was determined by the method of Bradford (1976), with human serum albumin as a standard.

Statistics

Arithmetic means are given with s.e.mean and geometric means are given with 95% confidence limits; *n* is the number of experiments (= number of suspensions of renal tubules). For the calculation of the IC_{50}s the parameters of the Hill-equation for multisite inhibition were fitted to the experimental data (Segel, 1975). Statistical analysis was performed by one-way analysis of variance (ANOVA) using Student's *t* test to compare values. The Newman-Keuls' test was employed in multiple comparison of values. A *P* value less than 0.05 was considered to denote a significant difference.

Drugs

Drugs used were: α-methyl-5-hydroxytryptamine maleate (Research Biochemicals Inc. Natick, MA, U.S.A.), dopamine hydrochloride (Sigma Chemical Company, St. Louis, Mo, U.S.A.), (+)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (Research Biochemicals Inc.), (+)-WAY 100135 (N-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropionamide dihydrochloride (Wyeth Research U.K. Ltd, Berkshire, U.K.).

Results

Basal Na^+,K^+-ATPase activity in suspensions of isolated renal tubules was 4.8 ± 0.4 μmol Pi mg^{-1} protein h^{-1}. As shown in Figure 1, the 5-HT_{1A} receptor agonist, 8-OH-DPAT (10 to 3000 nM) induced a concentration-dependent increase in Na^+,K^+-ATPase activity with an EC_{50} value of 355 nM (95% confidence limits: 178, 708). By contrast, the selective 5-HT_2 receptor agonist, α-methyl-5-HT (100 to 3000 nM) induced no changes in Na^+,K^+-ATPase activity.

The maximal stimulation by 8-OH-DPAT (3000 nM) on renal Na^+,K^+-ATPase activity (12.0 ± 1.8 μmol Pi mg^{-1} protein h^{-1}) in suspensions of isolated renal tubules was antagonized by the selective 5-HT_{1A} receptor antagonist (+)-WAY 100135 (10 to 1000 nM) in a concentration-dependent manner with an IC_{50} value of 20 nM (14, 29) (Figure 2).

The stimulatory effect of 8-OH-DPAT (1 μM) on Na^+,K^+-ATPase activity was found to be time-dependent (Figure 3). In this set of experiments, renal tubules were exposed during preincubation for 1, 2.5, 5 and 15 min to 8-OH-DPAT. As shown in this figure, 1 min exposure to 8-OH-DPAT was found to

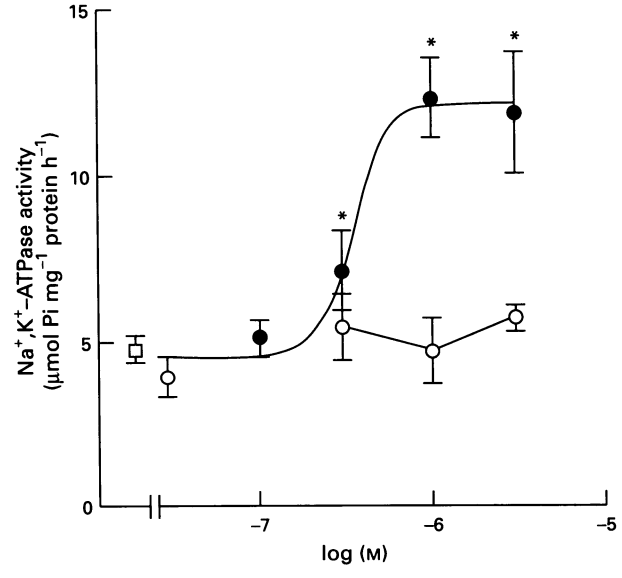

Figure 1 Effect of increasing concentrations of 8-OH-DPAT (●) and α-methyl-5-HT (○) on Na^+, K^+-ATPase activity in renal proximal convoluted tubules; basal Na^+, K^+-ATPase activity is indicated by (□). Results are means ± s.e.man of 5 experiments per group. *Significantly different from control baseline values ($P < 0.05$) using Student's *t* test.

produce no significant change in Na^+,K^+-ATPase activity, whereas a 2.5 or 5 min exposure to 8-OH-DPAT already produce a significant ($P<0.05$) increase in Na^+,K^+-ATPase activity.

In another set of experiments, homogenates of renal tubules instead of intact tubules were used. The preparations were exposed to 1 μM 8-OH-DPAT (15 min pre-incubation plus 15 min incubation) and no change in Na^+,K^+-ATPase activity was found to occur (data not shown).

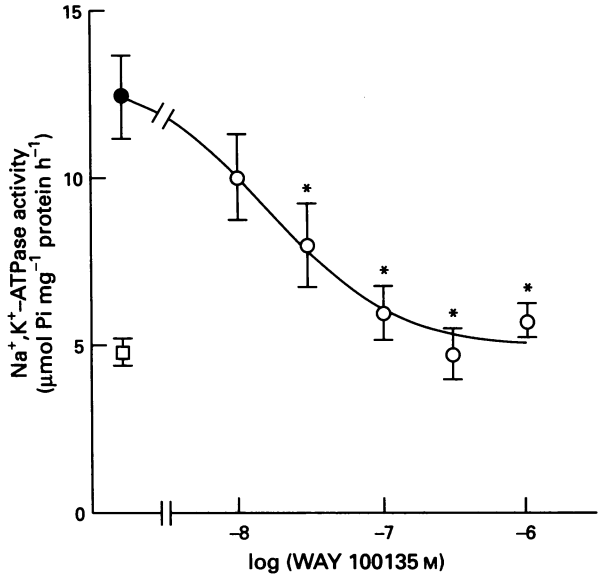

Figure 2 Na^+, K^+-ATPase activity in renal proximal convoluted tubules incubated in the presence of 8-OH-DPAT (1 μM) in the absence (●) and the presence of increasing concentrations of (+)-WAY 100135 (○); basal Na^+, K^+-ATPase activity is indicated by (□). Results are means ± s.e.mean of 5 to 6 experiments per group. *Significantly different from corresponding control values ($P<0.05$) using Student's t test.

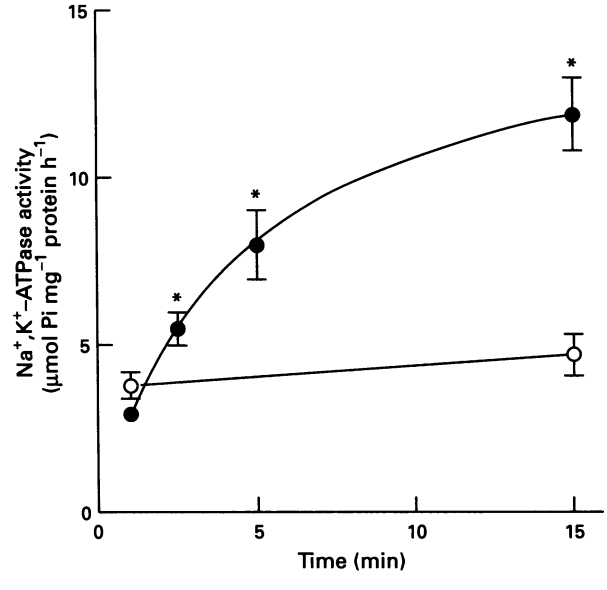

Figure 3 Time-course of Na^+, K^+-ATPase activity in renal proximal convoluted tubules incubated in the absence (○) and the presence (●) of 1 μM 8-OH-DPAT. Renal tubules were exposed during pre-incubation for 1, 2.5, 5 and 15 min to 8-OH-DPAT. Results are means ± s.e.mean of 3 experiments per group. *Significantly different from control baseline values ($P<0.05$) using Student's t test.

Incubation of renal tubules with dopamine (1 μM) produced a significant ($P<0.05$) inhibition (27% reduction) of Na^+,K^+-ATPase activity (basal levels 4.0 ± 0.8 μmol Pi mg^{-1} protein h^{-1}). Co-incubation of 8-OH-DPAT (0.5 or 1 μM) with dopamine (1 μM) was found to reverse the inhibitory effect on Na^+,K^+-ATPase activity observed with dopamine alone and the stimulatory effect of 8-OH-DPAT (0.5 or 1 μM) alone (Figure 4).

Discussion

The results presented here show that the selective 5-HT_{1A} receptor agonist 8-OH-DPAT, but not the selective 5-HT_2 receptor agonist, α-methyl-5-HT, stimulates Na^+,K^+-ATPase in suspensions of isolated proximal convoluted renal tubules. This stimulatory effect was found to be time-dependent and was absent when homogenates of renal tubules were used, indicating the requirement of an intracellular signalling system. Stimulation occurred at V_{max} for sodium supporting the concept of a direct effect on the Na^+,K^+-ATPase molecule rather than secondary to changes in sodium permeability and this might be a relevant mechanism behind the antinatriuretic effect of endogenous renal 5-HT.

Several subtypes of 5-HT receptors have been identified and their characterization described quite extensively in the brain (Schmidt & Peroutka, 1989). Much less is known regarding the properties and functions of 5-HT receptors in peripheral tissues. There is evidence, however, that 5-HT, in the kidney, can exert marked effects, namely at the level of vasculature and glomerular and tubular structures. The subtypes of 5-HT receptors identified in the kidney and renal cell lines include the 5-HT_2 subtype in renal blood vessels (Janssens & Van Nueten, 1986; Wright & Angus, 1987; Shoji *et al.*, 1989; Blackshear *et al.*, 1991) and the glomerulus (Hamamori *et al.*, 1988; Takuwa *et al.*, 1989), the 5-HT_{1A} subtype in the medullary and cortical tick ascending limb of Henle and distal convoluted tubules (Raymond *et al.*, 1993), the 5-HT_{1B} subtype in OK cells from the opossum kidney (Murphy & Bylund, 1990) and the 5-HT_{1D} in MDCK cells from the canine kidney (Frederich *et al.*, 1988). In a recent study in the rat kidney, Ramond *et al.* (1993), employing an immunohistochemical technique, failed to identify the 5-HT_{1A} receptor subtype in the proximal nephron. This contrasts with the evidence presented here, since the preparation used in the present study contains mainly proximal tubules (Soares-da-Silva *et al* 1994a; Soares-da-Silva & Pinto-do-Ó, 1996). However, our results agree with those of

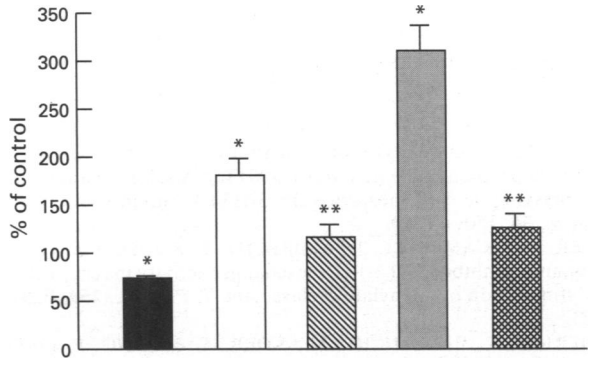

Figure 4 Effect of dopamine (1 μM) alone (solid column) and with added 8-OH-DPAT (0.5 μM, hatched column; 1 μM, cross-hatched column) on Na^+, K^+-ATPase activity in intact renal proximal convoluted tubules; the stimulatory effect of 0.5 μM and 1 μM 8-OH-DPAT is indicated by the open and stippled columns, respectively. Results are means ± s.e.mean of 5 experiments per group. Significantly different from control values (*$P<0.02$) and values for dopamine or 8-OH-DPAT alone (**$P<0.02$) by the Newman-Keuls test.

(Middleton *et al.*, 1990) while showing stimulation of Na^+,K^+-ATPase by 8-OH-DPAT in HeLa cells expressing the human 5-HT_{1A}. The notion that stimulation of Na^+,K^+-ATPase activity is accomplished by activation of the 5-HT_{1A} receptor subtype is further supported by the fact that the effect of 8-OH-DPAT is abolished in a concentration-dependent manner by the specific 5-HT_{1A} receptor antagonist, (+)-WAY 100135. This compound is the first silent 5-HT_{1A} receptor antagonist for which a considerable degree of specificity has been demonstrated (Cliffe *et al.*, 1993). Therefore, despite the failure of immunohistochemical techniques to detect the presence of 5-HT_{1A} receptors in the proximal nephron, the present data provide functional evidence for their presence in this nephron segment.

In this present study, enzyme activity was determined under V_{max} conditions for its major substrates, among them sodium. The fact that stimulation of enzyme activity is seen at concentrations of sodium above 70 mM, indicates that the stimulatory effect is not the consequence of increased sodium permeability, but rather a direct effect on Na^+,K^+-ATPase molecules, probably representing incorporation of new preformed molecules from intracellular compartments. This could be an important cellular mechanism behind the antinatriuretic action of endogenous renal 5-HT, as described in a previous work (Soares-da-Silva *et al.*, 1994b) and by other authors (Itskowitz *et al.*, 1988; Li Kam Wa *et al.*, 1993).

The role of endogenous 5-HT on Na^+,K^+-ATPase has not yet been studied *in vivo*. Nevertheless, it is likely that the rate of sodium transport, and thereby excretion, may be dependent on the balance between a 5-hydroxytryptaminergic and dopaminergic tonus on Na^+,K^+-ATPase activity. Evidence favouring this possibility has been presented before (Soares-da-Silva *et al.*, 1994a), where it is shown that when the availability of endogenous 5-HT is increased, a marked antinatriuresis is observed. Furthermore, the finding that the inhibitory-effect of dopamine on Na^+,K^+-ATPase activity is abolished by the simultaneous activation of 5-HT_{1A} receptors also agrees with this view. From a conceptual point of view, the antagonism of 5-HT on the tubular effects of dopamine represents a challenge in renal physiology and constitutes a local alternative to the sympathetic nervous system to renal sodium conservation (Beach *et al.*, 1987; Ibarra *et al.*, 1993; Gopalakrishnan *et al.*, 1995), since the formation of natriuretic dopamine and antinatriuretic 5-HT can occur in the same cell. It is possible, however, that under *in vivo* conditions locally formed dopamine or 5-HT might also have access to renal sympathetic nerve terminals and would exert a modulatory effect upon renal sympathetic tone, through activation of specific prejunctional receptors.

In conclusion, the data presented here show that selective 5-HT_{1A} receptor activation stimulates Na^+,K^+-ATPase activity in suspensions of isolated renal tubules. The stimulatory effect is receptor mediated and absent in a cell free system, indicating the requirement of an intracellular signalling system. Moreover, the results point to the existence of 5-hydroxytryptaminergic/dopaminergic tonus, antagonistic in nature, that may be responsible for controlling tubular sodium transport, and thereby its excretion.

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