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Antagonistic actions of renal dopamine and 5-hydroxytryptamine: endogenous 5-hydroxytryptamine, 5-HT_{1A} receptors and antinatriuresis during high sodium intake

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1 The present study has examined the effect of (+)-WAY 100135, a selective antagonist of 5-HT_{1A} receptors, and ketanserin, an antagonist of 5-HT_2 receptors, on the urinary excretion of Na^+, K^+, dopamine, 5-hydroxytryptamine (5-HT) and their metabolites in rats treated with the selective type A monoamine oxidase (MAO-A) inhibitor, Ro 41-1049 (15 mg kg^{-1} day^{-1}) in conditions of normal sodium (NS) and high sodium (HS; 1.0% NaCl in drinking water) intake.

2 Male Wistar rats were placed in metabolic cages and were given tap water (NS diet) in the first 4 days of the study and then challenged to a HS diet for another 7 days. Ro 41-1049 was given in drinking water only in the last 3 days of the HS diet, whereas (+)-WAY 100135 (5 and 10 mg kg^{-1} day^{-1}, s.c.) or ketanserin (2 mg kg^{-1} day^{-1}, s.c.) were administered in the last 4 days of the HS intake period.

3 Daily urinary excretion (in nmol kg^{-1} day^{-1}) of dopamine (82±2), 3,4-dihydroxyphenylacetic acid (DOPAC; 198±9), homovanillic acid (HVA; 915±47), 5-HT (586±37) and 5-hydroxyindoleacetic acid (5-HIAA; 1035±64) in the HS intake period was similar or higher than that in NS diet (dopamine = 68±2, DOPAC = 197±4, HVA = 923±42, 5-HT = 539±132, 5-HIAA = 1286±95). The administration of Ro 41-1049 on 3 consecutive days reduced the urinary excretion of dopamine, DOPAC and HVA, respectively, by 35–51% ($P<0.05$), 73–85% ($P<0.05$) and 59–66% ($P<0.05$); the urinary excretion of 5-HT increased 2 fold ($P<0.01$) and the levels of 5-HIAA were reduced by 39–77% ($P<0.05$).

4 During HS intake (7 days), daily urinary excretion of Na^+ increased 5.5 fold (from 6.7±0.2 to 36.5±0.9 mmol kg^{-1} day^{-1}), without changes in the urinary excretion of K^+ (from 11.2±0.2 to 11.9±0.5 mmol kg^{-1} day^{-1}) and urinary osmolality (from 1083.8±26.7 to 1117.7±24.1 mOsm kg^{-1} H_2O). MAO-A inhibition during HS intake was found to produce a 47–68% decrease in Na^+ excretion (from 39.1±0.7 to 15.1±2.5 mmol kg^{-1} day^{-1}, $n=4$; $P<0.02$) and urine volume (from 160.4±3.3 to 43.8±9.0 ml kg^{-1} day^{-1}, $n=4$; $P<0.02$) without changes in K^+ (from 11.1±0.5 to 9.2±0.6 mmol kg^{-1} day^{-1}, $n=4$) and creatinine (from 29.1±2.3 to 28.4±2.1 mg kg^{-1} day^{-1}) excretion; urine osmolality increased 2 fold (from 936.3±40.3 to 2210.7±157.4 mOsm kg^{-1} H_2O, $n=4$; $P<0.02$). Administration of (+)-WAY 100135 (5 and 10 mg kg^{-1} day^{-1}), but not of ketanserin (2 mg kg^{-1} day^{-1}), was found to inhibit the antinatriuretic effect induced by Ro 41-1049 during HS intake.

5 It is suggested that MAO-A inhibition during HS intake leads to an increased availability of 5-HT in renal tissues, the effect of which is a decrease in the urinary excretion of Na^+, involving the activation of tubular 5-HT_{1A} receptors.

Keywords: 5-Hydroxytryptamine; dopamine; high sodium diet; kidney

Introduction

Dopamine and 5-hydroxytryptamine (5-HT) and their immediate percursors, L-DOPA and L-5-hydroxytryptophan, respectively, have been demonstrated to produce opposite changes in Na^+ and water excretion, without significant changes in glomerular filtration rate (Itskowitz *et al.*, 1988; Li Kam Wa *et al.*, 1993). The renal epithelial cells of the proximal convoluted tubules, have been demonstrated to constitute an important source of the two amines, particularly when the corresponding precursors are made available to the kidney. Most of the evidence available on this subject as indicated in the preceding paper (Soares-da-Silva & Pinto-do-Ó, 1996), suggests that depending on the renal delivery of L-DOPA and L-5-HTP, aromatic L-amino acid decarboxylase (AAAD)-rich tubular epithelial cells form dopamine and 5-HT, which have antagonistic actions. All these studies were, however, performed by loading the subjects under study – anaesthetized rats (Stier *et al.*, 1984; Itskowitz *et al.*, 1988) and healthy volunteers (Li Kam Wa *et al.*, 1993; 1995) – with exogenous L-5-HTP alone or in combination with L-DOPA. In all these studies, the levels of urinary dopamine or urinary 5-HT rose dramatically (360 to 2500 fold) during the infusion of exogenous L-5-HTP or L-DOPA. Although of crucial importance for the understanding of the renal actions of dopamine and 5-HT, these are non-physiological situations which do not allow evaluation of the possibility that under more physiological circumstances the two amines, dopamine and 5-HT, are in fact exerting functionally reciprocal effects.

In the kidney, apart from sharing a common synthetic pathway, dopamine and 5-HT also share a common metabolic pathway. Type A monoamine oxidase (MAO-A), the predominant form of MAO in rat renal tissues (Fernandes & Soares-da-Silva, 1992), converts dopamine into 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-HT into 5-hydroxyindoleacetic acid (5-HIAA). Previous studies in both kidney slices and isolated renal tubules have shown that newly-

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formed dopamine from added L-DOPA undergoes rapid deamination to DOPAC (Fernandes & Soares-da-Silva, 1990) and the selective inhibition of MAO-A enhances the availability of tubular dopamine (Pestana & Soares-da-Silva, 1994). The same was found to occur with 5-HT when the preparations were loaded with L-5-HTP, instead of L-DOPA (unpublished observations). In contrast to 5-HT, dopamine can also be metabolized by both MAO-B and catechol-*O*-methyltransferase (COMT) (Fernandes & Soares-da-Silva, 1990).

In the present work we took advantage of this different pattern of metabolism of 5-HT and dopamine and studied the possible functional antagonistic effects upon the kidney of endogenous dopamine and 5-HT by treating rats with a selective MAO-A inhibitor, it was expected that MAO-A inhibition might particularly enhance the renal availability of endogenous renal 5-HT, since other metabolic alternatives do exist for dopamine. In the course of these studies, MAO-A inhibition was, in fact, found to produce an increase in the urinary excretion of 5-HT associated with antinatriuresis. In order to validate the hypothesis that antinatriuresis was dependent on an enhanced availability of 5-HT, the effects of selective 5-HT receptor antagonists were also examined. A preliminary account of some of these findings has been published (Soares-da-Silva *et al.*, 1994).

Methods

Male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oeiras, Portugal) aged 45 days and weighing 180–250 g were used in the experiments. Animals were kept in metabolic cages under controlled environmental conditions (12 h light/dark cycle and room temperature, 24°C). Animals were allowed a 7 day period of acclimatization in the metabolic cages prior to initiation of the studies. The design of the different experimental protocols used is presented in Table 1.

Experimental protocols

Protocol 1 In the first set of experiments, rats ($n=4$) received tap water (normal sodium; NS) in the first 4 days of the study followed by the administration of Ro 41-1049 (15 mg kg^{-1} day^{-1}) in drinking water on the subsequent 3 days; the concentration of Ro 41-1049 in drinking water was adjusted daily in relation to the liquid intake in the previous day.

Protocol 2 In a second series of experiments, two groups of rats ($n=4$ each) were studied for 11 days. In the first 4 days, the rats received tap water and were then challenged with 1.0% (w/v) NaCl in the drinking water (HS) for the last 7 days of the study; 4 animals were tested with Ro 41-1049 (N-(2-aminoethyl)-5-(m-fluoro-phenyl)-4-thiazolecarboxamide hydrochloride) (15 mg kg^{-1} day^{-1}, in drinking water) in the last 3 days of the high sodium diet period (HS + Ro), whereas the remaining rats were given the vehicle (HS + V). For the sake of clarity, data concerning NS and HS periods were handled together ($n=8$), whereas data concerning the HS + Ro and HS + V treatment periods were considered individually ($n=4$).

Protocol 3 The third series of experiments used 5 groups of rats: the control group ($n=4$), the Ro 41-1049 group ($n=4$), the Ro 41-1049 plus ketanserin group and two groups receiving (+)-WAY 100135 (N-tert-butyl-3-(4-(2-methoxyphenyl) piperazin-1-yl)-2-phenylpropionamide dihydrochloride) plus Ro 41-1049 ($n=4$ each). All groups were given the HS diet throughout the study (7 days); with the exception of the control group, all other groups received Ro 41-1049 (15 mg kg^{-1} day^{-1}, in drinking water) in the last 3 days of the study (HS + Ro). Ketanserin (2 mg kg^{-1} day^{-1}, s.c.) and (+)-WAY 100135 (5 or 10 mg kg^{-1} day^{-1}, s.c.) were given to rats on the last 4 days of the study, i.e. the administration of either drug started the day before the administration of Ro 41-1049; the drugs were injected between 18 h 00 min and 19 h 00 min in order to obtain the highest concentrations in plasma during the period in which the animals show the most active ingestive behaviour. The doses of ketanserin and (+)-WAY 100135 used were found not to change systolic and diastolic blood pressure and were demonstrated to produce effective antagonism at 5-HT_2 and 5-HT_{1A} receptors, respectively (Wright & Angus, 1987; Fletcher *et al.*, 1993). For the sake of clarity, data concerning the first 3 days of the study were handled together ($n=20$); from day 4 onwards, data from the five animal study groups were considered individually ($n=4$ each).

General

All animals were fed throughout the study *ad libitum* with ordinary rat chow (Letica, Barcelona, Spain; Na^+, K^+ and protein contents, respectively, 0.1%, 0.75% and 17%). The daily Na^+ intake in NS and HS diets averaged 0.5 and 5 mmol 100 g^{-1} of body weight, respectively. The vials collecting 24 h urine contained 1 ml 6 M HCl to prevent spontaneous decomposition of monoamines and amine metabolites. Blood pressure and heart rate measurements were made daily throughout the study by the tail-cuff method and a programmed electro-sphygmomanometer (Letica model LE 5000, Barcelona, Spain). Three determinations were made each time and the means used for further calculations.

The assay of monoamines (dopamine and 5-HT) and amine metabolites (DOPAC, 3-MT, homovanillic acid [HVA] and 5-HIAA) in urine samples was performed by means of high performance liquid chromatography (h.p.l.c.) with electrochemical detection, as previously described (Soares-da-Silva *et*

Table 1 Design of experimental protocols

Protocol 1	*Days 1–4*	*Days 5–8*	*Days 9–11*
	NS ($n=4$)	NS + Ro ($n=4$)	
Protocol 2	*Days 1–4*	*Days 5–8*	*Days 9–11*
	NS ($n=8$)	HS ($n=8$)	HS + V ($n=4$)
			HS + Ro ($n=4$)
Protocol 3	*Days 1–3*	*Day 4*	*Days 5–7*
	HS ($n=20$)	HS + V ($n=4$)	HS + V ($n=4$)
		HS + V ($n=4$)	HS = Ro ($n=4$)
		HS + W5 ($n=4$)	HS + Ro + W5 ($n=4$)
		HS + W10 ($n=4$)	HS + Ro + W10 ($n=4$)
		HS + Ket ($n=4$)	HS + Ro + Ket ($n=4$)

Animals on a normal sodium (NS) diet (receiving tap water) and those on a high sodium (HS) diet (receiving 1.0% NaCl in drinking water) were given Ro 41-1049 (15 mg mg^{-1} day^{-1}; NS + Ro and HS + Ro) or the vehicle (HS + V). In some experiments, rats were also given (+)-WAY 100135 (5 or 10 mg kg^{-1} day^{-1}; W5 and W10) or ketanserin (2 mg kg^{-1} day^{-1}; Ket). As mentioned in the methods section, the administration of (+)-WAY 100135 or ketanserin started the day before treatment with Ro 41-1049. Number of animals used is indicated in parentheses.

al., 1995). The h.p.l.c. system consisted of a pump (Gilson model 302; Gilson Medical Electronics, Villiers le Bel, France) connected to a manometric module (Gilson model 802 C) and a stainless-steel 5 μm ODS column (Biophase; Bioanalytical Systems, West Lafayette, IN) of 25 cm length; samples were injected by means of an automatic sample injector (Gilson model 231) connected to a Gilson dilutor (model 401). The mobile phase was a degassed solution of citric acid (0.1 mM), sodium octylsulphate (0.5 mM), sodium acetate (0.1 M), EDTA (0.17 mM), dibutylamine (1 mM) and methanol (8% v/v), adjusted to pH 3.5 with perchloric acid (2 M) and pumped at a rate of 1.0 ml min^{-1}. The detection was carried out electrochemically with a glassy carbon electrode, an Ag/AgCl reference electrode and an amperometric detector (Gilson model 141); the detector cell was operated at 0.75 V. The current produced was monitored using the Gilson 712 h.p.l.c. software. The lower limits for detection of monoamines and monoamine metabolites ranged from 350 to 1000 fmol.

Urinary Na^+ and K^+ were measured by flame photometry (model FML3) connected to a diluter (model A 6241) (Radiometer, Copenhagen, Denmark) and urine osmolality by means of an osmometer (Advanced Instruments, Inc, MA, U.S.A., model 3MO). Urinary creatinine was measured by a wave-length photometer (Hitashi Automatic Analyzer, model 717, or a Beckman Analyzer II).

Statistics

Results are means ± s.e.mean of values for the indicated number of determinations. Within-group analysis of data was performed by Friedman's test comparing all values to the baseline state or values of interest to the corresponding controls. Differences between one control and several experimental groups were estimated by the Tukey-Kramer method (Sokal & Rohlf, 1981). A P value less than 0.05 was assumed to denote a significant difference.

Results

Protocol 1

As shown in Figure 1, the administration of Ro 41-1049 (15 mg kg^{-1} day^{-1}) in drinking water to rats fed a NS diet was found to produce a significant ($P<0.05$) decrease in the urinary excretion of DOPAC, HVA and 5-HIAA. The urinary excretion of 5-hydroxytryptamine increased significantly ($P<0.05$; from 834 ± 108 to 1580 ± 84.9 and 1599 ± 77 nmol kg^{-1} day^{-1}) during the second and third days of Ro 41-1049 administration, whereas that of dopamine was found to be decreased by 32% ($P<0.05$). The urinary excretion of Na^+ was not affected by the administration of Ro 41-1049 (from 3.8 ± 0.3 to 3.9 ± 0.1 mmol kg^{-1} day^{-1}).

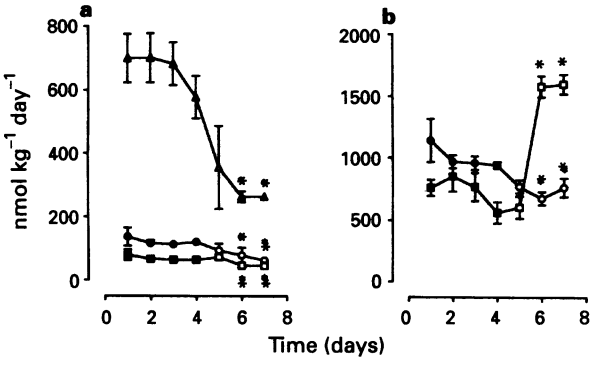

Figure 1 Urinary excretion of (a) dopamine (□, ■), DOPAC (○, ●), HVA (△, ▲) and (b) 5-HT (□, ■) and 5-HIAA (○, ●) in control conditions (closed symbols) and during treatment with Ro 41-1049 (15 mg kg^{-1} day^{-1}; open symbols) in rats subjected to a normal sodium diet. Each point represents the mean with s.e.mean of four determinations per group. Significantly different from corresponding baseline values determined by the Friedman test (*$P<0.01$).

Protocol 2

Daily liquid intake (in ml kg^{-1} day^{-1}) during HS period (198 ± 4; $n=8$) was found to be higher ($P<0.01$) than that during the NS period (138 ± 9; $n=8$). Baseline urine volume, which averaged 86 ± 4 ml kg^{-1} day^{-1} ($n=8$), increased ($P<0.02$) up to 133 ± 4 ml kg^{-1} day^{-1} ($n=8$) during the HS period. Average daily food consumption (105 ± 2 g kg^{-1} day^{-1}; $n=8$) during the NS diet period was found to be higher ($P<0.02$) than that occuring during the HS period (87 ± 2 g kg^{-1} day^{-1}; $n=8$). However, body weight during the NS diet period (259 ± 3 g; $n=8$) was found to be lower ($P<0.05$) than during the HS period (278 ± 3 g; $n=8$).

As shown in Figure 2, daily urinary excretion (in nmol kg^{-1} day^{-1}; $n=8$) of dopamine (82 ± 2), DOPAC (198 ± 9), HVA (915 ± 47), 5-HT (586 ± 37) and 5-HIAA (1035 ± 64) in the HS period was similar or higher than that in the NS diet (dopamine = 68 ± 2, DOPAC = 197 ± 4, HVA = 923 ± 42, 5-HT = 539 ± 132, 5-HIAA = 1286 ± 95). The administration of Ro 41-1049 on 3 consecutive days reduced the urinary excretion of dopamine, DOPAC and HVA, respectively, by 35–51% ($P<0.05$), 73–85% ($P<0.05$) and 59–66% ($P<0.05$); the urinary excretion of 5-HT increased 2 fold ($P<0.01$) and the levels of 5-HIAA were reduced by 39–77% ($P<0.05$).

Urinary excretion of Na^+, K^+ and creatinine during the NS period ($n=8$) averaged 6.7 ± 0.2, 11.2 ± 0.2 mmol kg^{-1} day^{-1} and 26.9 ± 2.3 mg kg^{-1} day^{-1} respectively, and remained stable throughout this period. In the HS period ($n=8$), the urinary excretion of Na^+, K^+ and creatinine averaged 36.5 ± 0.9, 11.9 ± 0.5 mmol kg^{-1} day^{-1} and 28.6 ± 2.7 mg kg^{-1} day^{-1}, respectively. Baseline urine osmolality, which averaged 1083.8 ± 26.7 mOsm kg^{-1} H_2O during the NS period was similar to that observed during the HS period (1117.7 ± 24.1 mOsm kg^{-1} H_2O). MAO-A inhibition during HS intake (HS + Ro period) was found to produce a 47–68% decrease in Na^+ excretion (from 39.1 ± 0.7 to 15.1 mmol kg^{-1} day^{-1}, $n=4$; $P<0.02$) and urine volume (from 160.4 ± 3.3 to 43.8 ± 9.0 ml kg^{-1} day^{-1}, $n=4$; $P<0.02$) without changes in K^+ (from 11.1 ± 0.5 to 9.2 ± 0.6 mmol kg^{-1} day^{-1}, $n=4$) and creatinine (from 29.1 ± 2.3 to 28.4 ± 2.1 mg kg^{-1} day^{-1}) excretion; urine osmolality increased 2 fold (from 936.3 ± 40.3 to 2210.7 ± 157.4 mOsm kg^{-1} H_2O, $n=4$; $P<0.02$).

Protocol 3

The results shown in Figure 3 were obtained in a third set of experiments in which five groups of rats ($n=4$) submitted to a seven day HS intake were then challenged with the MAO-A inhibitor in the last three days of the study; some of the animals were pretreated with the 5-HT_{1A} antagonist, (+)-WAY 100135 or the 5-HT_2 antagonist, ketanserin. Animals in the HS diet and receiving the MAO-A inhibitor presented the typical reduction in urinary Na^+ excretion and increase in urine osmolality, without changes in the urinary excretion of K^+ and creatinine (data not shown). Pretreatment with (+)-WAY 100135 partially or completely prevented the decrease in urinary Na^+ excretion and increase in urine osmolality during MAO-A inhibition when, respectively, 5 or 10 mg kg^{-1} day^{-1} were administered; these effects were not accompanied by changes in the urinary excretion of K^+ and creatinine (data not shown). Pretreatment with ketanserin (2 mg kg^{-1} day^{-1}) was found not to reverse the decrease in the urinary Na^+ excretion and the increase in urine osmolality observed during MAO-A inhibition.

Blood pressure was not affected either during HS intake or during the administration of Ro 41-1049 (Table 2).

Discussion

The data presented here indicate that depending on the degree of Na^+ loading, the increased urinary excretion of 5-HT, which occurred during MAO-A inhibition with Ro 41-1049, is accompanied by antinatriuresis and increased urine osmolality. Since these effects are accompanied by a decrease in the urinary excretion of dopamine (an intrarenal natriuretic hormone), it is suggested that antinatriuresis might result from an imbalance in the renal availability of these two monoamines. The finding that pretreatment with the selective 5-HT_{1A} receptor antagonist, (+)-WAY 100135, abolishes the antinatriuretic effect induced by Ro 41-1049 indicates the involvement of endogenous 5-HT and the activation of this type of receptor.

The first point which deserves some discussion concerns the origin of this endogenous 5-HT which appears to be responsible for the antinatriuresis in conditions of HS intake. As for urinary dopamine, for which there is a considerable amount of information suggesting that the amine reflects mainly the tubular decarboxylation of filtered or circulating L-DOPA (for review see Lee, 1993), the origin of urinary 5-HT is also believed to be mainly the kidney. Studies involving the whole kidney (Stier *et al.*, 1984; Itskowitz *et al.*, 1988; Li Kam Wa *et al.*, 1993) and isolated renal tubules (Sole *et al.*, 1986; Pinto-do-Ó & Soares-da-Silva, 1994; Soares-da-Silva & Pinto-do-Ó, 1996) have demonstrated that L-5-HTP is decarboxylated to 5-HT quite efficiently. On the other hand, the extrarenal production of 5-HT cannot account completely for the high urinary excretion of the amine. In fact, 5-HT pro-

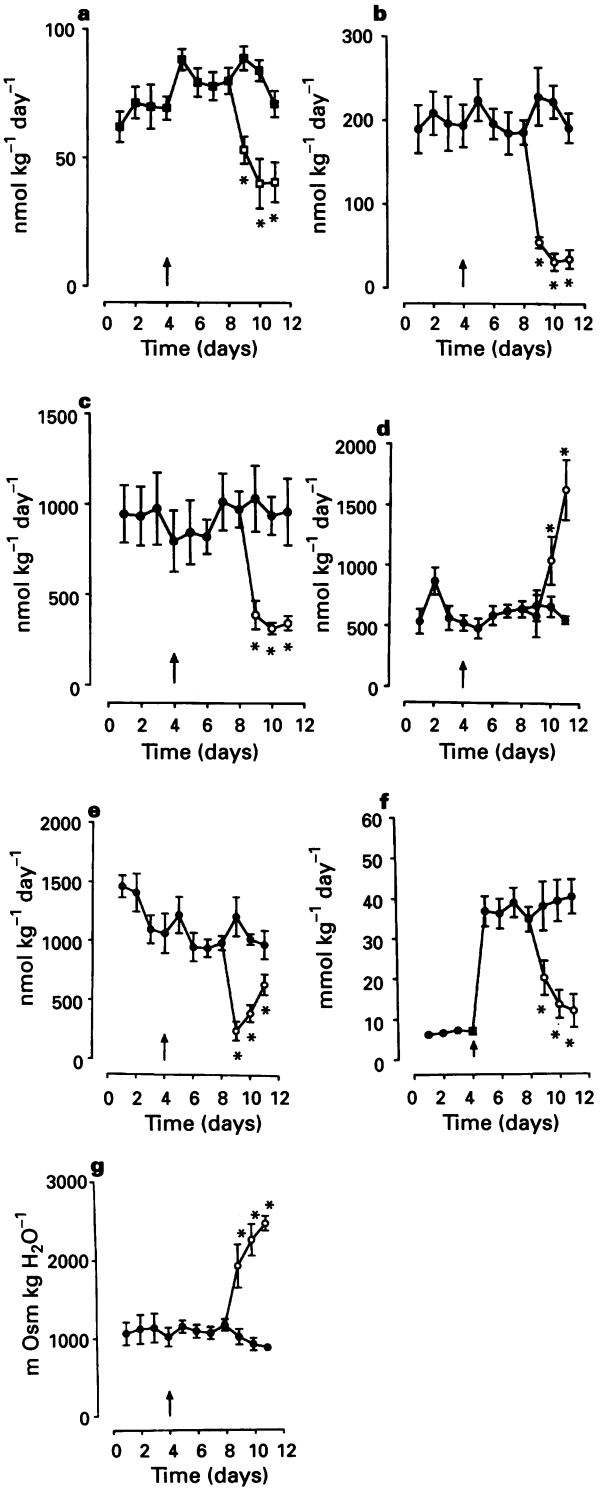

Figure 2 Urinary excretion of (a) dopamine, (b) DOPAC, (c) HVA, (d) 5-HT (e) 5-HIAA, (f) Na^+ and (g) osmolality in control conditions (closed symbols) and during treatment with Ro 41-1049 (15 mg kg^{-1} day^{-1}; open symbols) in rats subjected to a high sodium (HS) diet (1% NaCl in drinking water). In the first four days, animals were given a normal sodium diet and the beginning of HS diet is indicated by the arrow. Each point represents the mean with s.e.mean of four determinations per group. Significantly different from corresponding control values using Student's *t* test (*$P<0.01$).

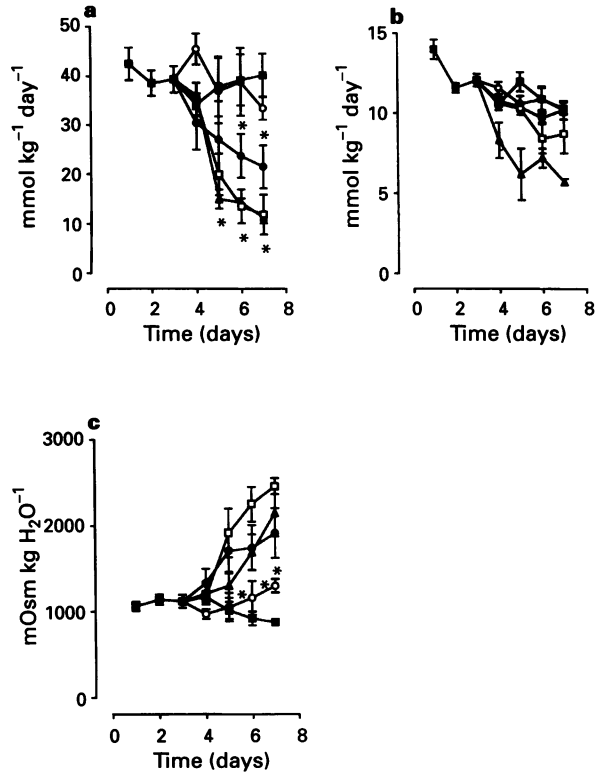

Figure 3 Effect (+)-WAY 100135 (5 and 10 mg kg^{-1} day^{-1}) and ketanserin (2 mg kg^{-1} day^{-1}) on the urinary excretion of (a) sodium, (b) potassium and (c) osmolality in rats subjected to a high sodium diet (1% NaCl in drinking water) and given the MAO-A inhibitor Ro 41-1049 (15 mg kg^{-1} day^{-1}) in the last three days of the study; (+)-WAY 100135 and ketanserin were given to rats in the last 4 days of the study, i.e. the administration of either drug started the day before beginning the administration of Ro 41-1049. Each point represents the mean with s.e.mean of four determinations per group. Significantly different from corresponding control values using the Student's *t* test (*$P<0.01$). Control (■); Ro 41-1049 alone (□); Ro 41-1049 plus (+)-WAY 100135 (5 mg kg^{-1} day^{-1}) (●); Ro 41-1049 plus (+)-WAY 100135 (10 mg kg^{-1} day^{-1}) (○); Ro 41-1049 plus ketanserin (2 mg kg^{-1} day^{-1}) (▲).

Table 2 Systolic (SBP) and diastolic (DBP) blood pressure (mmHg) and heart rate (beats min^{-1}) during normal sodium (NS) and high sodium (HS) diets and during HS diet in the presence of Ro 41-1049 (15 mg kg^{-1} day^{-1}) alone or in combination with (+)-WAY 100135 (10 mg kg^{-1} day^{-1}) or ketanserin (2 mg kg^{-1} day^{-1})

	NS diet	*HS diet*	*HS diet plus Ro 41-149*	*HS diet plus Ro 41-149 plus (+)-WAY 100135*	*HS Diet plus Ro 41-149 plus ketanserin*
SBP	125±8	142±11	147±11	148±11	147±10
DBP	82±11	107±10	110±12	110±11	112±12
Heart rate	416±26	386±14	377±19	378±19	391±15

Results are means±s.e.mean of four determinations per group; data concern blood pressure and heart rate for the last day of corresponding drug treatment.

duced outside the kidney and entering the circulation is expected to be rapidly deaminated by MAO in the liver and lung or sequestered into platelets, leaving little available for filtration at the glomerulus. This is also supported by the finding that intravenous administration of 5-HT results in small increases in plasma 5-HT and has no effect on the urinary excretion of the amine (Davidson *et al.*, 1957). More recently, the administration of γ-glutamyl-L-5-HTP, a renal 5-HT prodrug which is sequentially converted to L-5-HTP by γ-glutamyltransferase and then decarboxylated to 5-HT, has been shown in healthy volunteers to result in significant increments in urinary 5-HT (Li Kam Wa *et al.*, 1993) without changes in plasma 5-HT (Li Kam Wa *et al.*, 1995). It is possible, therefore, to conclude that most urinary 5-HT has its origin within the kidney and both dopamine and 5-HT are probably synthesized in the same AAAD-rich epithelial cells of the renal tubules. However, this does not mean that the increased amount of 5-HT in the urine during MAO-A inhibition is derived from a pool of renal 5-HT which has not been deaminated to 5-HIAA. Although the kidney is endowed with one of the highest MAO-A activities in the body, it is possible that inhibition of this enzyme in other organs might have enhanced the availability of circulating 5-HT to be delivered to the kidney. In fact, most of the body 5-HT is located in the enterochromaffin cells of the gastrointestinal tract (Resnick & Seimmour, 1961) and the evidence available suggests that the 5-HIAA appearing in urine derives largely from extrarenal sources and reflects the turnover of gastrointestinal 5-HT (Alfieri & Cubeddu, 1995). The type of study reported here did not allow collection of plasma samples simultaneously with the urine sampling; therefore, it is impossible to ascertain the extent to which the increases in urine 5-HT during inhibition of MAO-A are related to increases in the availability of extrarenal 5-HT to the kidney. However, the finding that no change in the urinary excretion of creatinine was observed during this period of increased urinary 5-HT excretion strongly suggests that most of the amine appearing in the urine may be of renal origin. The two main arguments which give support to this suggestion are the following: (1) urinary creatinine can be used as a rough estimation of glomerular filtration rate and (2) increased plasma levels of 5-HT, at the level of the renal circulation, have been demonstrated to produce marked vasoconstrictor effects (Page & Glendening, 1955; Colliss & Vanhoutte, 1977; Blackshear *et al.*, 1991).

In keeping with the suggestion that a considerable amount of 5-HT in the urine reflects the production of the amine in epithelial cells of renal tubules and assuming urinary creatinine as an index of glomerular filtration rate, it might be concluded that antinatriuresis occurring during increases in the urinary excretion of 5-HT may not be related to changes in glomerular filtration rate. In fact, antinatriuresis was accompanied by no changes in the urinary excretion of creatinine and by an increase in urine osmolality. These effects occurred only when rats were on an HS diet and not in experiments performed with rats on a NS diet. Since the basal urinary excretion of 5-HT and that occurring during the administration of Ro 41-1049 were found to be similar in both NS and HS diets, one might exclude the possibility that the occurrence of antinatriuresis observed during MAO-A inhibition is not related to differences in the availability of renal 5-HT. It is possible, however, that the increased delivery of Na^+ to the kidney might have changed the responsiveness of tubular structures to the amine. Alternatively, it may be suggested that the enhanced effects of 5-HT during HS could be related to the unexpected decrease in the urinary excretion of dopamine, leading to an imbalance between the two amines. In fact, the urinary excretion of dopamine was found to be reduced during MAO-A inhibition and this was even more marked when rats were on an HS diet. The present data do not allow any plausible explanation for these findings to be put forward. Of course, one might anticipate that inhibition of MAO-A could remove a metabolic barrier and, therefore, allow free access of tubular dopamine to the circulation. This, however, has been demonstrated to occur only when Ro 41-1049-treated rats were injected with exogenous L-DOPA (Vieira-Coelho *et al.*, 1994). Obviously, the same could apply to tubular 5-HT, but all the evidence suggests that this was not the case. The possibility that the compound Ro 41-1049 might have reduced the tubular uptake of L-DOPA or its conversion to dopamine is very unlikely, since in *in vitro* studies using kidney slices and isolated renal tubules this compound was always found to enhance the accumulation of newly-formed dopamine, an effect accompanied by a decrease in DOPAC formation (Fernandes & Soares-da-Silva, 1990; Guimarães & Soares-da-Silva, 1994), the main metabolite of dopamine of renal origin (Fernandes & Soares-da-Silva, 1990; Wolfovitz *et al.*, 1993; Vieira-Coelho *et al.*, 1994).

The finding that (+)-WAY 100135, a selective antagonist of 5-HT_{1A} receptors, but not ketanserin, a 5-HT_2 antagonist, inhibited the antinatriuretic effect induced by Ro 41-1049, suggests that MAO-A inhibition during HS intake leads to an increased availability of 5-HT in renal tissues, the effect of which is a decrease in the urinary excretion of Na^+, involving the activation of tubular 5-HT_{1A} receptors. This fits well with the evidence that in the human and rat kidney, 5-HT_{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 (Raymond *et al.*, 1993). Apparently, this conflicts with the findings that sodium restriction in human subjects results in an increase in the renal production of 5-HT (Sharma *et al.*, 1993). However, the finding that (+)-WAY 100135 also reversed the increase in urine osmolality during MAO-A inhibition constitutes evidence favouring the view that this effect of 5-HT is of tubular origin and most probably does not produce changes in the renal circulation, otherwise ketanserin would have produced some effect, since most of the 5-HT_2 receptors described within the kidney are located on the renal vasculature (Wright & Angus, 1987; Shoji *et al.*, 1989; Blackshear *et al.*, 1991). Assuming this effect of 5-HT is a tubular one, two other points which deserve some discussion concern the possible localization along the nephron where this could occur and the nature of the mechanisms involved. The recent immunohistochemical

study by Raymond *et al.* (1993) provides evidence for the presence of 5-HT_{1A} receptors mainly in the thick ascending limb of Henle and distal collecting tubules. Activation of 5-HT_{1A} receptors with the selective agonist (±)-8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) has been shown to result in marked activation of Na^+-K^+ ATPase in isolated renal cortical tubules (Soares-da-Silva & Bertorello, 1994). It is suggested that this effect may represent an important cellular mechanism, at the tubule level, responsible for the antinatriuretic effect of 5-HT, though the preparation used is expected to contain mainly proximal convoluted tubules (Soares-da-Silva *et al.*, 1994). The possible involvement of a neuronal or vascular component on this 5-HT_{1A} receptor-mediated antinatriuresis appears to be rather low, since the activation of vascular 5-HT_{1A} receptors produces an endothelium-dependent renal vasodilatation (Shoji *et al.*, 1989) and the administration of 5-HT_{1A} receptor agonists causes suppression of renal sympathetic tone leading to increased sodium and water excretion (Chamienia & Johns, 1994).

In conclusion, the data presented here suggest that MAO-A inhibition during HS intake leads to an increased availability of 5-HT in renal tissues, the effect of which is a decrease in the urinary excretion of Na^+, involving the activation of tubular 5-HT_{1A} receptors.

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