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ENHANCED PROTEIN KINASE C MEDIATED INHIBITION OF RENAL DOPAMINE SYNTHESIS DURING HIGH SODIUM INTAKE

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Abstract—The synthesis of dopamine, from L-β-3,4-dihydroxyphenylalanine (L-DOPA), in renal tissue of rats on either a normal sodium (NS) or high sodium (HS) diet for 1 week or 6 weeks was examined. Aromatic L-amino acid decarboxylase (AAAD) activity determined in kidney homogenates in "1 week HS" (V_{max} = 11.5 ± 1.6 nmol/mg protein/hr) and "6 weeks HS" rats (V_{max} = 10.6 ± 1.5 nmol/mg protein/hr) was greater ($P < 0.02$) than that in "NS" rats (V_{max} = 7.7 ± 0.8 nmol/mg protein/hr). K_m (μM) values in "NS", in "1 week HS" and "6 weeks HS" rats were similar. The formation of dopamine in kidney slices loaded with 100 μM L-DOPA depended exponentially on the concentration of sodium in the medium (0–160 mM). In kidney slices obtained from "1 week HS" rats the decarboxylation of added L-DOPA was significantly greater ($P < 0.01$) than that observed in kidney slices obtained from "NS" and "6 weeks HS" rats; the rate constant of formation of dopamine as a function of sodium concentration in the incubation medium was, however, similar in "NS" rats to that in "1 week HS" and "6 weeks HS" rats. Ouabain produced a concentration dependent decrease in the synthesis of dopamine in all three experimental groups; the magnitude of the inhibitory effect of 1.0 mM ouabain was greater in "1 week HS" rats (77% reduction; $P < 0.01$) than in "NS" rats (59% reduction; $P < 0.01$) and in "6 weeks HS" rats (23% reduction; $P = 0.08$). Activation of protein kinase C by phorbol 12,13-dibutyrate (PDBu) and the calcium ionophore A23187 produced a concentration-dependent reduction in the formation of dopamine in rat kidney slices, but not in kidney homogenates; the magnitude of the inhibitory effect was greater in "1 week HS" rats than in "NS" and "6 weeks HS" rats. Submaximal concentrations of PDBu (10 nM) were synergistic with the inhibitory effect of A23187 on the formation of dopamine: again, this effect was more marked in "1 week HS" rats than in "NS" and "6 weeks HS" rats. The effects of PDBu and PDBu plus A23187, but not those of A23187 alone, were antagonized in a concentration-dependent manner by *d*-sphingosine, a protein kinase C inhibitor. It is concluded that the increased activity of AAAD in renal tissues of rats submitted to HS intake is accompanied in "1 week HS" but not in "6 weeks HS" rats by enhanced inhibition of dopamine formation during protein kinase C activation.

Epithelial cells from renal tubules, of the proximal portion of the nephron have high aromatic L-amino acid decarboxylase (AAAD†) activity [1–3]. These cells synthesize dopamine from 3,4-dihydroxyphenylalanine (DOPA) present in the tubular filtrate and the amine formed has been shown to be of importance in the renal handling of water and electrolytes [4, 5]. The natriuretic effect of dopamine of renal origin is believed to occur as a result of the activation of specific receptors located in renal tubes [6], most probably as a consequence of inhibition of the Na^+–H^+ exchanger [7] and Na^+, K^+ATPase activities [8, 9], although it has been claimed that other mechanisms might be involved [10]. Early studies demonstrated that the renal tubular transport of DOPA occurs through an energy-dependent and stereoselective carrier-mediated process [11]. Initially, it was reported that sodium loading is accompanied by an increased excretion of dopamine in the urine of both humans and laboratory animals [1, 12–15] and that a low sodium diet results in a decrease in the urinary excretion of dopamine [1, 14, 15]. More recently, and in line with this view, it has been described in both human and rat kidney preparations that the production of dopamine is not only closely dependent on the extracellular sodium, but also appears to be related to the transtubular reabsorption of sodium [16, 17] and on the integrity of the tubular cytoskeleton and the functional integrity of the Na^+, K^+ATPase [18]. In good agreement with these results is the finding that the alpha-human atrial natriuretic peptide and the cyclic GMP, effective inhibitors of the tubular transport of sodium [19, 20], decrease the intracellular availability of L-DOPA in rat kidney slices and reduce the renal formation of dopamine [21, 22]. It appears, therefore, that sodium is an important stimuli for the renal production of dopamine, although tubular sodium reabsorption may also be affected by the amine.

The role of the renal proximal tubule dopamine system in the adaptation to changes in the renal delivery of sodium is, however, a matter of debate. While on a high sodium (HS) diet the increased urinary excretion of dopamine, which reflects mainly

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† Abbreviations: AAAD, aromatic L-amino acid decarboxylase; HPLC-ECD, HPLC with electrochemical detection; HS, high sodium; L-DOPA, L-β-3,4-dihydroxyphenylalanine; NS, normal sodium; PDBu, phorbol 12,13-dibutyrate; PKC, protein kinase C.

the tubular production of the amine, is thought to reflect an increased AAAD activity [1, 2, 23]; the increased urinary excretion of sodium during HS is also dependent on an increased production of dopamine, since blockade of dopamine receptors attenuates the natriuresis which accompanies a high renal delivery of sodium [13, 24–26]. Na^+, K^+-ATPase constitutes the driving force for sodium reabsorption and the activity of the enzyme is greatly inhibited by locally formed dopamine in rats given a HS diet for 10 [27] or 12–19 days [2]. This finding, contrasts with the view that sodium is an important stimuli for the renal formation of dopamine, under conditions of HS intake, and also conflicts with the results on the sodium-dependence and ouabain-sensitivity of dopamine synthesis in both human and rat kidney preparations. However, Bertorello *et al.* [27] found an increased sensitivity of Na^+, K^+-ATPase to inhibition by dopamine in kidney preparations obtained from rats on HS intake.

The inhibitory effect of dopamine on Na^+, K^+ATPase activity requires the simultaneous activation of both type 1 (DA_1) and 2 (DA_2) dopamine receptors [28]. The DA_1 effect appears to involve an increased production of cyclic AMP, whereas the DA_2 mediated event has been demonstrated to be independent of adenylate cyclase activation [29]. However, inhibition of Na^+, K^+ATPase by dopamine has been shown to be mediated through activation of protein kinase C (PKC) [30]. PKC is a Ca^{2+}-activated, phospholipid-dependent enzyme closely linked to signal transduction as a result of the generation of 1,2-diacylglycerol, a product of the hydrolysis of inositol phospholipid [31]. On the other hand, the cellular responses to PKC activation have been reported to be independent of that and elicited by an increase in intracellular Ca^{2+} [32].

The aim of the present work was to study the formation of dopamine in renal tissues of rats on a normal sodium (NS) and HS diets for 1 or 6 weeks and to evaluate some of the mechanisms known to be involved in the regulation of the amine. The effects of PKC activation by phorbol 12,13-dibutyrate (PDBu) and of the calcium ionophore, A23187, which also leads to inhibition of Na^+, K^+ATPase [33], were also examined. The kinetic characteristics (V_{max} and K_m) of the enzyme AAAD in renal tissues obtained from these three animal groups were also determined.

MATERIALS AND METHODS

Male Wistar rats (Biotério do Instituto Gulbenkian de Ciência, Oeiras, Portugal) aged 45–60 days and weighing 200–280 g were used in the experiments. The animals receiving tap water are referred to as NS diet rats and those receiving 1.0% (w/v) sodium chloride as HS diet rats. One group of rats (N = 6) fed on a HS diet was maintained on this regimen for 1 week, whereas the other group (N = 6) started the HS intake 6 weeks before the study. All animals were fed *ad lib.* with ordinary rat chow; the sodium content of the food was approximately 0.1%. The daily sodium intake in NS and HS diet rats averaged, respectively, 0.5 and 5 mmol/100 g of body weight. Animals were kept two per cage under controlled environmental conditions (12 hr light/dark cycle and room temperature 24°). The experiments were all carried out during day time.

The rats were killed by decapitation under ether anaesthesia and both kidneys removed and rinsed free from blood with 0.9% w/v saline. The kidneys were then placed on an ice-cold glass plate, the kidney poles removed and slices of renal cortex approximately 0.5 mm thick and weighing 20–30 mg wet weight were prepared with a scalpel. In experiments performed with slices of renal cortex the tissues were preincubated for 30 min in 2 mL warm (37°) and gassed (95% O_2 and 5% CO_2) Krebs' solution. The Krebs' solution had the following composition (mM): NaCl 120, KCl 4.7, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, EDTA 0.4, ascorbic acid 0.57 and glucose 11; 1-α-methyl-*p*-tyrosine (50)μM), pargyline (0.1 mM), tropolone (50 μM) and copper sulphate (10 μM) were also added to the Krebs' solution in order to inhibit the enzymes tyrosine hydroxylase, monoamine oxidase and catechol-*O*-methyl-transferase and inhibit the endogenous inhibitors of dopamine β-hydroxylase, respectively. Renal slices were incubated for 15 min in Krebs' solution with added L-DOPA. In experiments performed to determine the specific activity of AAAD, samples of renal cortex were homogenized in a modified Krebs' solution with Duall–Kontes homogenizers and kept continuously on ice. The modified Krebs' solution was similar to that mentioned above except that the sodium concentration was reduced to 50 mM; the osmolarity of the medium was kept constant by the addition of 68 mM choline chloride. Aliquots of 1.0 mL of kidney homogenates plus 1.0 mL Krebs' solution were placed in glass test tubes incubated for 30 min; thereafter, increasing concentrations of L-DOPA (10–5000 μM) were added to the medium for a further 15 min. During incubation, homogenates were continuously shaken and gassed (95% O_2 and 5% CO_2) and maintained at 37°. The reaction was stopped by the addition of 250 μL of 2 M perchloric acid and the preparations kept at 4° for 60 min. The homogenates were then centrifuged (2000 rpm, 2 min, 4°) and 1.5 mL aliquots of the supernatant used for the assay of dopamine.

In experiments performed to test the influence of sodium on the renal production of dopamine, slices of renal cortex were incubated in Krebs' solution with increasing concentrations of sodium in the medium in the presence of 100 μM L-DOPA; the osmolarity of the incubation medium was kept constant by the addition of choline chloride. In experiments in which the effects of ouabain were tested, the compound was present during preincubation and incubation in the presence of 120 mM sodium in the medium. To test the effect of PDBu, the activator of PKC, and of A23187 on the formation of dopamine, kidney slices were loaded with L-DOPA (50 μM) in the presence of these compounds or their respective vehicles [A23187 and PDBu were dissolved in dimethyl sulphoxide (final concentration < 0.01%) and *d*-sphingosine in ethanol (final concentration < 0.1%)]. In some experiments, the PKC inhibitor *d*-sphingosine was

added to the medium and kept present during the preincubation and incubation periods. The concentration-dependent effect of A23187 on the renal production of dopamine was also tested in kidney slices pretreated with PDBu (10 nM). At the end of the incubation, the reaction was stopped by the addition of 250 μL of 0.2 M perchloric acid into the incubation medium; the samples of the acidified incubation medium containing the kidney slices were kept at 4°.

The assay of dopamine in renal tissues and kidney homogenates was performed by means of HPLC with electrochemical detectives (ECD) as described previously [16]. The lower limit for detection of dopamine was 350 fmol/50 μL.

The protein content in kidney homogenates was determine by the method of Lowry *et al.* [34], with human serum albumin as a standard.

Statistics. K_m and V_{max} values for AAAD activity were calculated by linear regression analysis.

The accumulation of dopamine in kidney slices, as a function of the concentration of sodium in the medium, was calculated from a semilog plot of the concentration of the amine versus concentration of sodium in the medium; the rate of accumulation was calculated using linear regression. The rate constant of accumulation of dopamine (k) was obtained from the expression: k = slope value/0.434 [35] and the turnover rate of amine accumulation, as a function of the concentration of sodium in the medium, calculated by multiplying the tissue levels of dopamine at 0 mM sodium by the rate constant of accumulation.

Mean values ± SEM of N experiments are given. Significance of differences between one control and several experimental groups was evaluated by the Tuckey–Kramer method [36]. $P < 0.05$ was considered significant.

Drugs. A23187 (Calcimycin), L-DOPA, dimethyl sulphoxide, dopamine hydrochloride, 1-α-methyl-*p*-tyrosine, ouabain, pargyline hydrochloride, PDBu, *d*-sphingosine and tropolone hydrochloride were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.).

RESULTS

In kidney homogenates, the decarboxylation of L-DOPA into dopamine was found to depend on the concentration of L-DOPA used and saturated at 1000 μM (Fig. 1). AAAD activity as determined in kidney homogenates was significantly ($P < 0.02$) higher in "1 week HS" rats (V_{max} = 11.5 ± 1.6 nmol/mg protein/hr) than in "NS" rats (V_{max} = 7.7 ± 0.8 nmol/mg protein/hr) and not different from that in "6 weeks HS" rats (V_{max} = 10.6 ± 1.5 nmol/mg

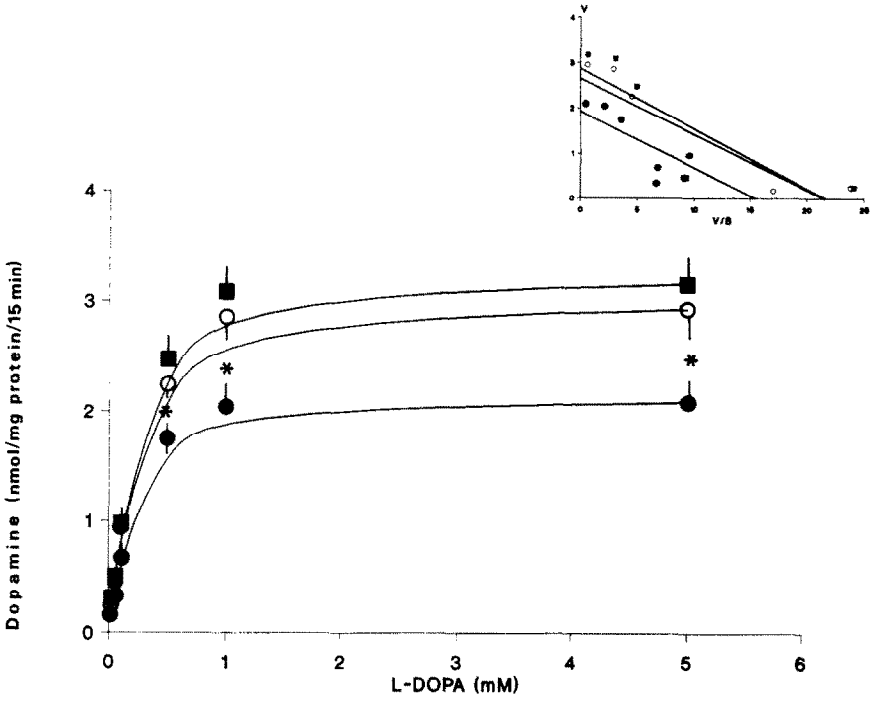

Fig. 1. Saturation curve of AAAD activity in kidney homogenates of rats in NS (closed circles), 1 week HS (open circles) and 6 weeks HS (closed squares) diets. AAAD activity is expressed as rate of decarboxylation (amount of dopamine formed in nmol/mg protein/15 min) vs increasing concentrations of L-DOPA (mM). Each point represents the mean of five experiments per group; vertical lines show SEM. Inset: Eadie–Hoffstee transformation of data ("NS" rats V_{max} = 7.7 ± 0.8 nmol/mg protein/hr, K_m = 125 ± 12 μM; "1 week HS" rats, V_{max} = 11.5 ± 1.6 nmol/mg protein/hr, K_m = 132 ± 16 μM; "6 weeks HS" rats, V_{max} = 10.6 ± 1.5 nmol/mg protein/hr, K_m = 123 ± 19 μM). *Significantly different from corresponding values for "1 week HS" and "6 weeks HS" rats ($P < 0.02$).

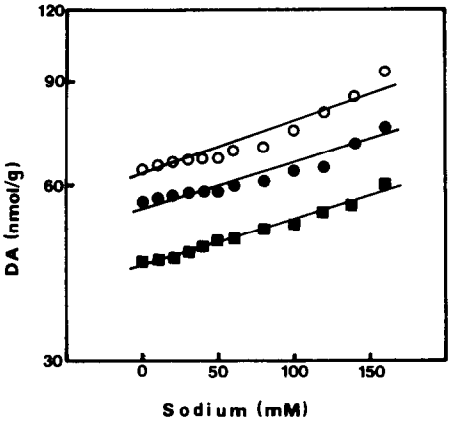

Fig. 2. Formation of dopamine (DA) in rat kidney slices incubated for 15 min with (100 μM) L-DOPA in the presence of increasing concentrations of sodium in the medium. Results are shown for observations of the formation of dopamine in kidney slices obtained from rats in NS (closed circles), 1 week HS (open circles) and 6 weeks HS (closed squares) diets. Each point represents the mean of five to six determinations; SEM values were less than 10% of the corresponding means. Linear coefficient values were as follows: NS diet rats, $r = 0.9945$, N = 78; 1 week HS diet rats, $r = 0.9763$, N = 78; 6 week HS diet rats, $r = 0.9753$, N = 60. The lower limit for detection of dopamine was 1.4 pmol/g.

protein/hr). No statistically significant differences were found between the K_m values in the three experimental groups ("NS" rats, 125 ± 12 μM; "1 week HS" rats, 132 ± 16 μM; "6 weeks HS" rats, 123 ± 19 μM).

Figure 2 shows the semilog plots of accumulation of newly formed dopamine in kidney slices obtained from rats on a NS diet and rats on a HS diet for 1 week and 6 weeks, loaded with 100 μM L-DOPA and incubated with increasing concentrations of sodium in the medium. As has been reported before [15], the formation of dopamine in kidney slices loaded with 100 μM L-DOPA was exponential and dependent on the concentration of sodium in the medium. The synthesis of dopamine in kidney slices obtained from "1 week HS" rats was greater than that observed in kidney slices obtained from "NS" and "6 weeks HS" rats; the formation of dopamine in kidney slices of "NS" rats was also significantly higher than that in "6 weeks HS" rats. The rate constant of formation of dopamine as a function of sodium concentration in the incubation medium was, however, similar in "NS" rats to those in "1 week HS" and "6 weeks HS" rats, as shown in Table 1.

Figure 3 illustrates the results obtained in experiments in which the effects of increasing concentrations of ouabain were tested on the formation of dopamine; kidney slices obtained from "NS", "1 week HS" and "6 weeks HS" rats were loaded with L-DOPA (50 μM) in the presence of 120 mM sodium in the incubation medium. Ouabain produced a concentration-dependent decrease in the synthetics of dopamine in all three experimental groups; the magnitude of this effect was, however, not the same in the three experimental groups. The decrease in the formation of dopamine by 1.0 mM ouabain was in "1 week HS" rats (77% reduction; $P < 0.01$) higher than that in "NS" rats (59% reduction; $P < 0.01$) and in "6 weeks HS" rats (22% reduction; $P = 0.08$).

As shown in Fig. 4, incubation of kidney slices with 50 μM L-DOPA resulted in the formation of considerable amounts of dopamine. The addition of PDBu resulted in a slight decrease in the formation of dopamine in "NS" rats, which attained statistical significance at 1 μM (23% reduction); this effect was reversed in a concentration-dependent manner by

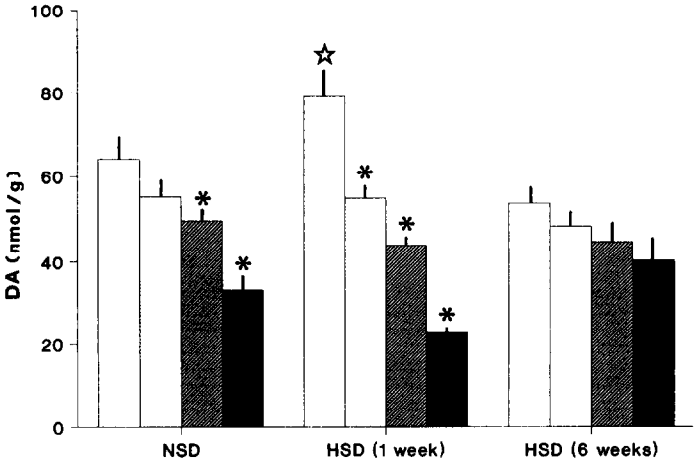

Fig. 3. Effect of ouabain (0.1, 0.5 and 1.0 mM) on the formation of dopamine (DA) in kidney slices loaded with 50 μM L-DOPA. Results are shown for observations on the formation of dopamine in kidney slices obtained from rats in NS, 1 week HS and 6 weeks HS diets. Each bar represents the mean of five experiments per group; vertical lines show SEM. Control, open columns; ouabain 0.1 mM, dotted columns; ouabain 0.5 mM hatched columns; ouabain 1.0 mM, closed columns. Significantly different from corresponding control values (*$P < 0.02$) or values for rats in NS and 6 weeks HS diets (☆$P < 0.05$) using the Tuckey–Kramer method.

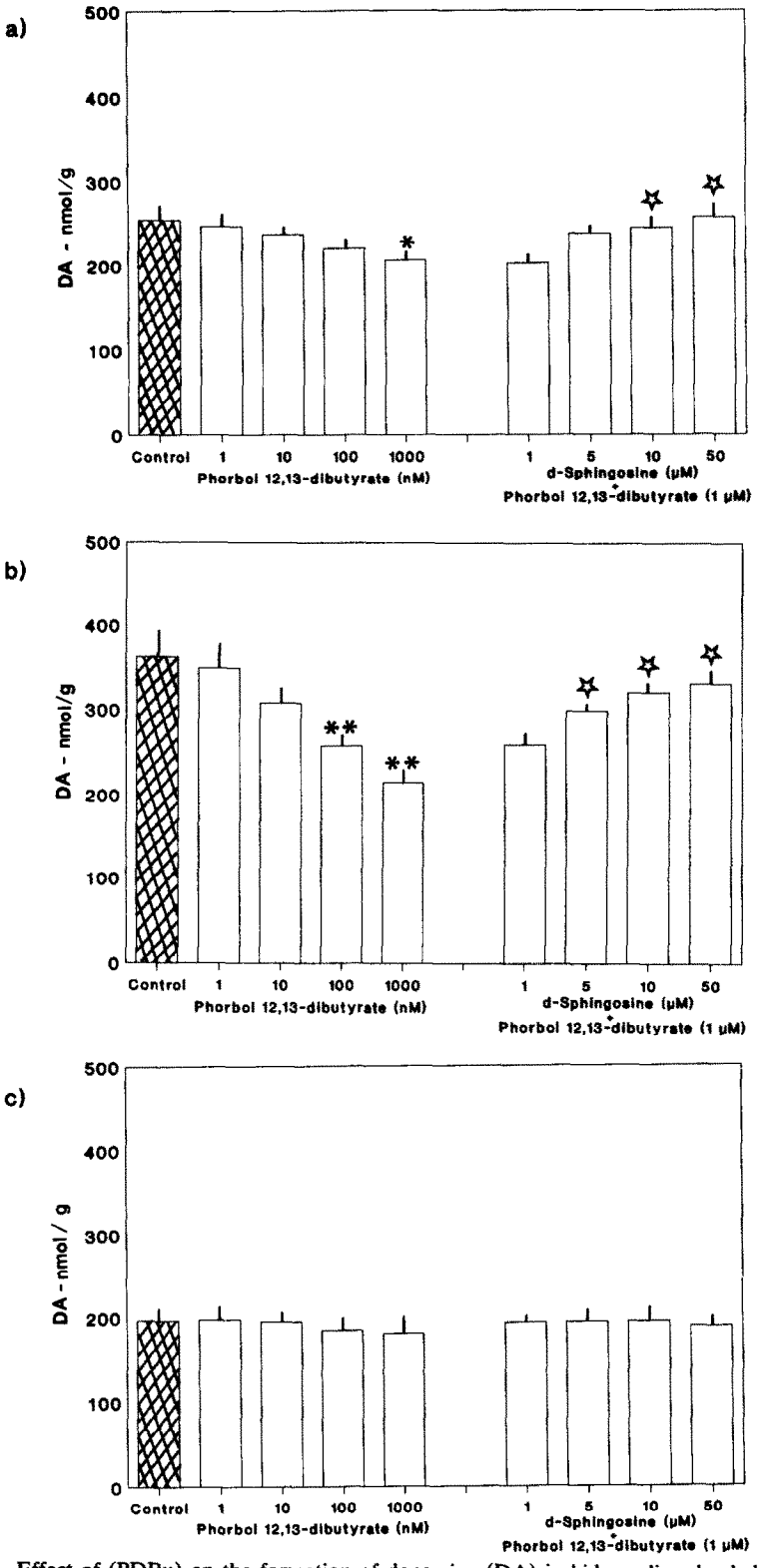

Fig. 4. Effect of (PDBu) on the formation of dopamine (DA) in kidney slices loaded with 50 μM L-DOPA. Tissues were incubated with PDBu alone or with PDBu (1 μM) in the presence of *d*-sphingosine for 15 min. PDBu and *d*-sphingosine were dissolved in, respectively, dimethyl sulphoxide and ethanol; the final concentrations of dimethyl sulphoxide and ethanol in the incubation medium were, respectively, less than 0.01% and 0.1% and appropriate controls were used. Each bar represents the mean of five to six experiments per group; vertical lines show SEM. Significantly different from corresponding control values using the Tuckey–Kramer method (*$P < 0.05$, **$P < 0.02$). These results are from kidney slices obtained from rats in NS (a), 1 week HS (b) and 6 weeks HS (c) diets.

Table 1. Rate constant of formation (k) and turnover times of dopamine (in nmol/g/15 min/mM sodium) in kidney slices obtained from NS rats and 1 week HS rats or 6 weeks HS rats

	Rate constant of formation k (min^{-1})	Turnover rate (nmol/g/15 min/mM Na^+)
NS rats	0.00385 ± 0.00034	0.2363 ± 0.0219
1 week HS rats	0.00395 ± 0.00035	0.2518 ± 0.0154
6 weeks HS rats	0.00447 ± 0.00039	0.1989 ± 0.0175

Results are shown for observations of the formation of dopamine in slices of rat renal cortex incubated with 100 μM L-DOPA for 15 min in the presence of increasing concentrations (0–160 mM) of sodium in the medium. Values are means ± SEM of five experiments per group.

the PKC inhibitor, *d*-sphingosine (Fig. 4a). Apart from having a higher capacity to synthetize dopamine, renal tissues obtained from rats fed a HS diet for 1 week also showed an increased sensitivity to the inhibitory effect of PDBu (Fig. 4b). Again, the effect of 1 μM PDBu was completely reversed by *d*-sphingosine. In contrast to the results obtained from renal tissues taken from "1 week HS" rats, neither PDBu nor *d*-sphingosine affected the renal formation of dopamine in tissues obtained from rats fed a HS diet for 6 weeks (Fig. 4c).

Incubation of renal tissues obtained from "NS" rats with the calcium ionophore A23187 resulted in a slight decrease in the formation of dopamine; this effect attained statistical significance at 1.0 μM A23187, corresponding to a 22% reduction in the formation of dopamine, and was insensitive to *d*-sphingosine (Fig. 5a). The inhibitory effect of A23187 on the renal formation of dopamine was more marked in renal tissues obtained from rats fed a HS diet for 1 week; 0.5 and 1.0 μM A23187 significantly decreased the formation of dopamine, respectively, (Fig. 5b). Again, the effect of 1.0 μM A23187 was completely insensitive to *d*-sphingosine. As in experiments with PDBu, A23187 was devoid of any inhibitory effect on the formation of dopamine in renal tissues obtained from "6 weeks HS" rats (Fig. 5c).

Incubation of renal tissues obtained from "NS" rats in medium containing 10 nM PDBu plus increasing amounts of A23187 (0.1, 0.5 and 1.0 μM) resulted in a marked decrease in the formation of dopamine (17–52% reduction): this inhibitory effect was greater than that obtained during the exposure of renal tissues with A23187 alone (results in Fig. 6a) and was reversed, in a concentration-dependent manner, by *d*-sphingosine (Fig. 6a). A similar pattern occurred in renal tissues obtained from "1 week HS" rats; in the presence of 10 nM PDBu, the inhibitory effect of A23187 ranged from 23% to 63% (Fig. 6b). As found in "NS" rats, the inhibitory effect obtained by the simultaneous addition of PDBu and A23187 was reversed by the PKC inhibitor. In contrast to the results obtained with A23187 alone, the formation of dopamine in renal tissues obtained from the "6 weeks HS" rats in the presence of A23187 plus PDBu (10 nM) was significantly decreased at 0.5 and 1.0 μM A23187: *d*-sphingosine completely abolished this inhibitory effect (Fig. 6c).

The addition of PDBu (1000 nM) or A23187 (1.0 μM) did not affect AAAD activity, as determined in homogenates of renal tissues obtained from animals in the three experimental groups (data not shown).

DISCUSSION

A considerable body of evidence favours the view that dopamine of intrarenal origin plays a physiological role in the regulation of tubular sodium reabsorption [4, 5, 8, 9, 13, 24–26]. One of the mechanisms through which the amine is thought to produce such an effect concerns the inhibition of Na^+, K^+ATPase [2, 8, 9, 27], the enzyme governing the net transtubular flux of sodium. Dopamine has also been demonstrated to inhibit the activity of the Na^+–H^+ exchanger, the main apical tubular transporter of sodium [7, 37]. Other types of evidence show, however, that sodium may be an important stimuli for the renal production of dopamine [16–18, 21, 22, 38].

The results presented here show that the renal production of dopamine is increased in rats submitted for 1 week to a HS intake. This is in line with evidence presented by several authors both under *in vivo* and *in vitro* experimental conditions, that this increased formation of dopamine is related to a high activity of the enzyme AAAD, as indicated by the higher V_{max} values (Ref. 2 and the present study). The synthesis of dopamine from L-DOPA under *in vitro* conditions has also been demonstrated to be sensitive to inhibition of Na^+, K^+ATPase by ouabain and also to depend on the concentration of sodium in the medium [16, 38]. Both phenomena have been found to occur while using kidney slices, but not in kidney homogenates [16, 38], suggesting the involvement of a membrane-operated mechanism, most probably responsible for the cellular uptake of L-DOPA. The increased formation of dopamine found to occur in "1 week HS" rats appears to be also related to an increased sensitivity to sodium, since the inhibitory effect of ouabain was almost twice that observed in renal tissues obtained from rats fed a NS diet. The finding that the rate constant of dopamine synthesis as a function of the concentration of sodium in the incubation medium did not differ between "1 week HS" and "NS" rats, strongly suggests that the number of membrane

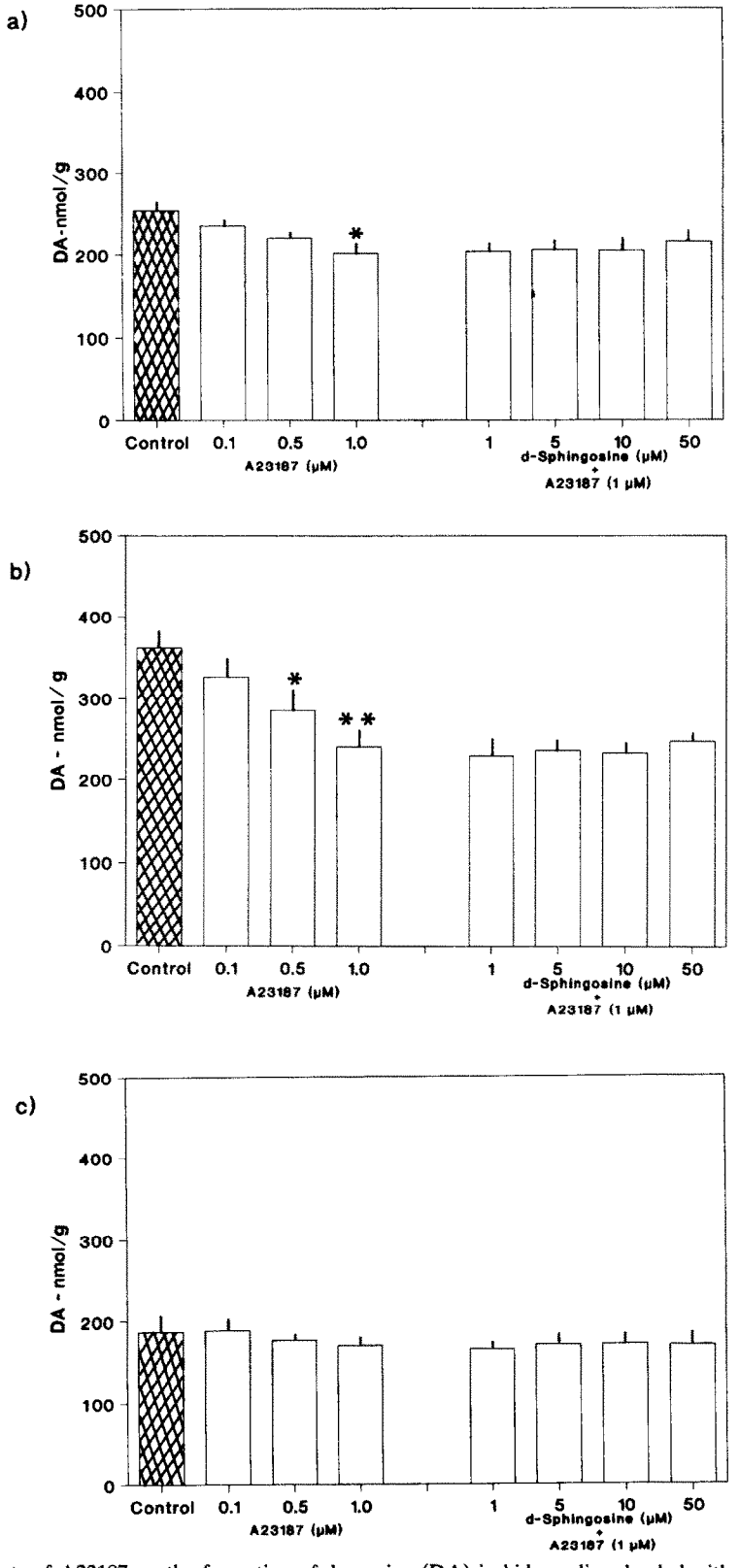

Fig. 5. Effects of A23187 on the formation of dopamine (DA) in kidney slices loaded with 50 μM L-DOPA. Tissues were incubated with A23187 alone or with A23187 (1.0 μM) in the presence of *d*-sphingosine for 15 min. Each bar represents the mean of five to six experiments per group; vertical lines show SEM. Significantly different from corresponding control values using the Tuckey–Kramer method (*$P < 0.05$, **$P < 0.02$). These results are from kidney slices obtained from rats in NS (a), 1 week HS (b) and 6 weeks HS (c) diets.

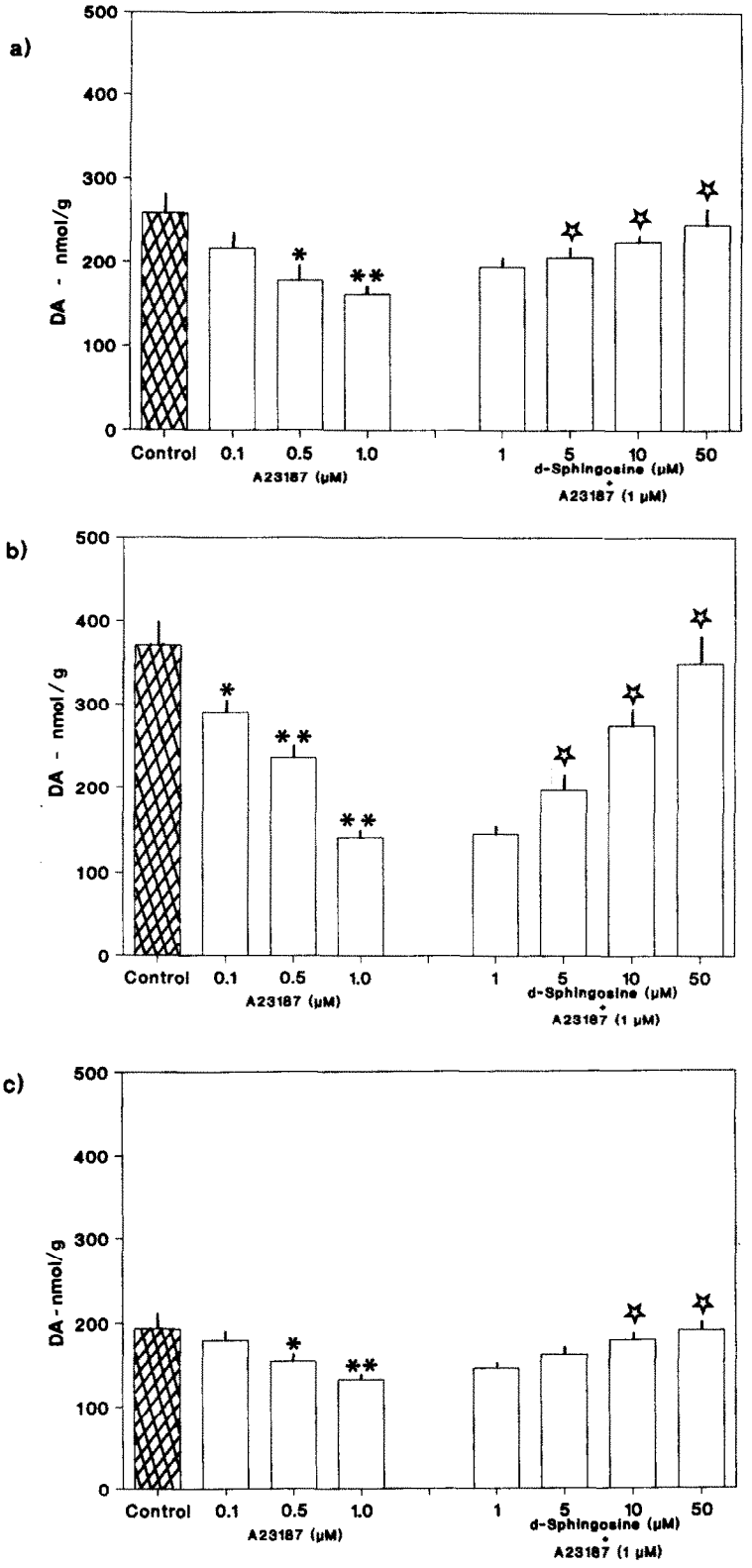

Fig. 6. Effect of A23187 in the formation of dopamine (DA) in kidney slices loaded with 50 μM L-DOPA in the presence of PDBu. Tissues were incubated with A23187 (0.1, 0.5 and 1.0 μM) plus PDBu (10 nM) or with A23187 (1.0 μM) plus PDBu (10 nM) in the presence of *d*-sphingosine for 15 min. Each bar represents the mean of five to six experiments per group; vertical lines show SEM. Significantly different from corresponding control values using the Tuckey–Kramer method (*$P < 0.05$, **$P < 0.02$). These results are from kidney slices obtained from rats in NS (a) 1 week HS (b) and 6 weeks HS (c) diets.

sodium/L-DOPA transporter units in operation was in both experimental groups probably the same. The same argument applies to the results obtained in renal tissues taken from rats fed a HS diet for 6 weeks. In fact, the rate constant of newly formed dopamine synthesis was not different from that in the two other experimental groups, suggesting that the lower ability to synthesize dopamine observed in "6 weeks HS" rats is not related to a decreased number or affinity of the membrane sodium/L-DOPA transporter units. As the V_{max} of AAAD was significantly increased in kidney homogenates in "6 weeks HS" rats, the lower ability of kidney slices to synthesize dopamine might be related to a net decrease in the cellular uptake of L--DOPA; in fact, the ouabain-sensitive component of dopamine formation was found to be almost completely absent in "6 weeks HS" rats. Decreased tubular sodium reabsorption has been reported to occur in chronically sodium-loaded animals [39] and this is thought to result from an overriding mechanism, which is believed to have its origin in an increased tubular hydrostatic pressure, decreased peritubular colloidal osmotic pressure and passive sodium backleakage [40]. In agreement with the view that the decreased formation of dopamine in kidney slices of "6 weeks HS" rats is related to a decrease in the cellular uptake of sodium and L-DOPA is also the finding that the specific activity of AAAD in homogenates is higher than that in "NS" rats (Ref. 23 and the present study).

The increased inhibitory effect of ouabain on the formation of dopamine found to occur in renal tissues from "1 week HS" rats suggests the presence of a more pronounced effect of the glycoside on Na^+, K^+ATPase. A HS intake has been reported to result in decreased activity of Na^+, K^+ATPase [2, 27], a situation reversed by previous inhibition of dopamine formation [27]. This led to the suggestion that the decreased activity of Na^+, K^+ATPase was related to an increased production of dopamine; furthermore, evidence for an increased sensitivity of Na^+, K^+ATPase to the inhibitory effect of the amine has also been produced [27]. The results presented here agree with the view of an increased sensitivity of Na^+, K^+ATPase during HS intake and the more pronounced inhibitory effect of ouabain in "1 week HS" rats agrees well with the suggestion of an increased sensitivity of Na^+, K^+ATPase.

Na^+, K^+ATPase has been reported to be an effector protein for pKC in renal proximal tubules [30]; PDBu has been found to exert a pronounced inhibitory action on the activity of enzyme, an effect antagonized by the PKC inhibitor, *d*-sphingosine [30]. The inhibitory effect of PDBu and its reversibility by *d*-sphingosine on the formation of dopamine in kidney slices, but not in kidney homogenates, suggest that this may occur as a result of inhibition of a membrane-operated mechanism, most probably related with the cellular uptake of the precursor, L-DOPA. As the cellular uptake of L-DOPA is a sodium-dependent and ouabain-sensitive mechanism [16, 38] and PDBu is an effective inhibitor of Na^+, K^+ATPase, it might be suggested that the inhibitory effect of PDBu on the formation of dopamine in kidney slices results from the inhibition of the enzyme Na^+, K^+ATPase. One argument which favours this suggestion is the simultaneously increased sensitivity of dopamine formation which is inhibited by both ouabain and PDBu in "1 week HS" rats. This interpretation agrees with the suggestion that the enhanced dopamine-induced inhibition of Na^+, K^+ATPase might involve an increased activity of PKC. Inhibition of Na^+, K^+ATPase activity has been also suggested to occur in conditions of increased intracellular Ca^{2+}, an event which would also favour activation of PKC [33]. The calcium ionophore A23187, was found in the present study to produce a slight reduction in the production of dopamine. However, as has been found with PDBu the inhibitory effect of A23187 was more marked in "1 week HS" rats than in the two other experimental groups. It is possible, again, that this enhanced sensitivity may involve facilitation of the inhibition of Na^+, K^+ATPase. Since, this effect was not sensitive to the PKC inhibitor, *d*-sphingosine, it is possible that the effect of A23187 was not related to PKC activation. In fact, this agrees with the view that the cellular responses to PKC activation are separate from those dependent on an increase in the intracellular levels of Ca^{2+} [32]. These responses may, however, be synergistic [32] and this may explain the potentiation of the effects of A23187 in the presence of a submaximal concentration of PDBu (10 nM). It should be stressed that the synergistic effect between A23187 and PDBu was greater in "1 week HS" rats, followed by that in "NS" and "6 weeks HS" rats. These findings strongly support the view of an enhanced sensitivity of PKC in renal tissues obtained from "1 week HS" rats. It might therefore be, hypothesized that the enhanced sensitivity of PKC activation constitutes a regulatory mechanism to prevent an excessive formation of dopamine. This appears to be an acute response to HS diet since it is no longer observed in "6 weeks HS" rats. In contrast, the increased ability of renal tissues to synthetize dopamine, as evidenced by an increased specific activity of AAAD, was found to be similar in "1 week HS" and "6 weeks HS" rats. The mechanism(s) responsible for the increased activity of AAAD in conditions of HS intake remain, however, to be clarified.

In conclusion, the present study demonstrates the presence of an increased activity of AAAD in renal tissues of rats subjected to HS intake; a prolonged period of HS intake appears also to involve changes in the membrane-operated mechanism responsible for the coupled transport of sodium and L-DOPA. The enhanced inhibition of the ouabain-sensitive component of dopamine formation appears to be a PKC mediated event.

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