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Atrial natriuretic factor stimulates renal dopamine uptake mediated by natriuretic peptide-type A receptor

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Abstract

To determine the effects of atrial natriuretic factor (ANF) on renal dopamine (DA) metabolism, ^{3}H-DA and ^{3}H-L-DOPA uptake by renal tubular cells was measured in experiments carried out in vitro in Sprague–Dawley rats. The receptor type involved was also analyzed. The results indicate that ANF increased at 30 min, DA uptake in a concentration–response fashion having 10 pM ANF as the threshold concentration. Conversely, the uptake of the precursor L-DOPA was not modified by the peptide. ANF effects were observed in tissues from external and juxtamedullar cortex and inner medulla. On this basis, 100 nM ANF was used to continue the studies in external cortex tissues. DA uptake was characterized as extraneuronal uptake, since 100 μM hydrocortisone blocked ANF-induced increase of DA uptake. Renal DA uptake was decreased at 0 °C and in sodium-free medium. The effects of ANF in these conditions were not present, confirming that renal DA uptake is mediated by temperature- and sodium-dependent transporters and that the peptide requires the presence of the ion to exhibit its actions on DA uptake.

The biological natriuretic peptide type A receptor (NPR-A) mediates ANF effects, since 100 nM anantin, a specific blocker, reversed ANF-dependent increase of DA uptake. The natriuretic peptide type C receptor (NPR-C) is not involved, since the specific analogous 100 nM 4–23 ANF amide has no effect on renal DA uptake and does not alter the effects of 100 nM ANF.

In conclusion, ANF stimulates DA uptake by kidney tubular cells. ANF effects are mediated by NPR-A receptors coupled to guanylate cyclase and cGMP as second messenger. The process involved was characterized as a typical extraneuronal uptake, and characterized as temperature- and sodium-dependent. This mechanism could be related to DA effects on sodium reabsorption and linked to ANF enhanced natriuresis in the kidney. The increment of endogenous DA into tubular cells, as a consequence of increased DA uptake, would permit D_1 receptor recruitment and Na^+,K^+-ATPase activity inhibition, which results in decreased sodium reabsorption and increased natriuresis.

Keywords: Atrial natriuretic factor; ANF; Natriuretic peptides; Dopamine

1. Introduction

Renal sodium metabolism, a major determinant of blood pressure and the main long-term regulator of blood pressure, is regulated by a variety of endocrine, autocrine and neuronal factors. These factors regulate sodium metabolism affecting the rate of tubular sodium reabsorption in all tubular segments. It is possible that natriuretic as well as antinatriuretic agents may achieve their effects through common pathways that involve reversible activation or deactivation of renal tubular Na^+,K^+-ATPase [1].

Atrial natriuretic factor (ANF), discovered by de Bold [2], is a 28-amino-acid peptide of the family that includes BNP and CNP [3]. The ANF is synthesized and stored in the atrial myocytes. It is released in response to stretch of the cardiac wall, endothelin and alpha-adrenergic stimulation [2,4]. Natriuretic ANF effects are exerted through enhanced glomerular filtration rate and tubular reabsorption processes. ANF inhibits angiotensin II (ANG II)-dependent sodium and water reabsorption at proximal kidney tubules and also decreases distal and collector tubules water absorption [5].

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Marin-Grez et al. [6] and Webb et al. [7] reported that part of the ANF inhibitory effects on sodium and water reabsorption is mediated by dopaminergic mechanisms, since haloperidol can block a percentage of natriuretic and diuretic ANF effects. Other authors [8–10] had observed that ANF decreased dopamine synthesis in the kidney.

These antecedents confirm that ANF and the renal dopaminergic system could interact and enhance the natriuretic and diuretic effects of the peptide.

We have reported that ANF, as well as BNP and CNP (the other components of the natriuretic peptide family), modulate noradrenergic neurotransmission at hypothalamic presynaptic nerve ending level [11,12]. The natriuretic peptides increase norepinephrine (NE) uptake and endogenous content of NE, and decreased NE release, synthesis, turnover and tyrosine hydroxylase (TH) activity [13,14]. Natriuretic peptides also regulate NE metabolism in adrenal medulla [15,16].

Based upon the foregoing statement, we may formulate the hypothesis that ANF could regulate catecholamine metabolism in the kidney as well as in the CNS and adrenal medulla.

Despite the main importance of the subject, there has been little information available about the cellular machinery that underlies the actions of ANF on diverse steps of dopamine (DA) metabolism in renal tubular cells. The mechanism implied in ANF–DA interaction—the chance that ANF affects endogenous DA or, on the other hand, whether the peptide alters extraneuronal DA availability in the kidney—is still unknown to our knowledge.

In the present study, we investigated the mechanisms by which ANF could regulate DA metabolism in the renal tubular cells. We studied ANF effects on DA metabolism in the kidney and discussed the possible consequences of this interaction on renal sodium transport and urine formation. DA and L-DOPA uptakes were studied as an index of catecholamine (CA) metabolism. The receptor type involved was also analyzed.

The results obtained indicate that ANF increases DA uptake by renal tubular cells through stimulation of natriuretic peptide type A receptors (NPR-A), which in turn could induce D_1 receptor recruitment and overstimulation. By this mechanism, ANF and DA could act via a common intracellular pathway to enhance natriuresis and diuresis.

2. Materials and methods

Male Sprague–Dawley rats weighing between 250 and 300 g (from the animal room of the Pathophysiology Department, Faculty of Pharmacy and Biochemistry of Buenos Aires) were used. The animals were housed in cages, with a 12-h light/dark cycle, and temperature and humidity were controlled. All animals were given free access to water and food ad libitum (Commercial rodents Purina chow, Cooperacion, Argentina).

The following drugs were used in the experiments: ^{3}H-DA, specific activity of 28.0 Ci/mmol, and ^{3}H-L-DOPA, 37.5 specific activity of Ci/mmol (New England Nuclear, Boston, MA, USA). ANF (99–126), hydrocortisone, DL-thiorphan, nomifensine, anantin, Des (Gln 18–Ser 19–Gly 20–Leu 21–Gly 22) atrial natriuretic peptide fragment 4–23 amide (Sigma-Aldrich, Saint Louis, Missouri, USA) and EcoLite, for liquid scintillation (ICN Pharmaceutical, CA, USA).

Standard Krebs bicarbonate (SKB) solution of the following composition (mM) was used as incubation medium: 118 NaCl; 4.7 KCl; 1.2 $MgSO_4 \cdot 7\ H_2O$; 1.0 NaH_2PO_4; 2.4 $CaCl_2$; 0.004 EDTA; 11.1 glucose; 0.11 ascorbic acid; 26.0 $NaHCO_3$.

Sodium-free solution has the same composition (mM) as SKB except that NaCl was replaced by 236 D(+)-sucrose, NaH_2PO_4 by 1.0 KH_2PO_4 and $NaHCO_3$ by 26.0 $KHCO_3$.

Rats were anesthetized with 10% ethyl urethane (1.3 mg/kg, i.p.). Both kidneys were removed and slices of external and juxtamedullar cortex and inner medulla were cut and weighed. In order to determine DA uptake, experiments were carried out according to the techniques previously described by Vatta et al. [14]. The tissues were minced and then placed in 2-ml KBS incubation medium in a Dubnoff incubator and pre-incubated at 37 °C, pH 7.4, bubbled with a gaseous mixture of 95% O_2 and 5% CO_2 for 15 min. Nomifensine (50 μM) was added in the medium to avoid neuronal DA uptake. After pre-incubation, the tissues were transferred to a fresh KBS medium and incubated for 30 min, in similar conditions, with 12.5 μCi/ml of ^{3}H-DA (or 12.5 mCi/ml of ^{3}H-L-DOPA, when it corresponded), 17 μM nomifensine and the different tested drugs.

The following protocol procedures were carried out in tissues from external renal cortex, except when it is mentioned.

- Effects of ANF on ^{3}H-DA uptake:
  - Effects of ANF in different areas of the kidney: (a) control group (incubated only with KBS) and (b, c and d) incubated with 1, 10 and 100 nM ANF, respectively. This set of experiments was performed in tissues from the three renal areas mentioned above.
  - Concentration–response curve: Effects of ANF (1 pM–100 nM) on DA uptake: (a) control, (b, c, d, e, f and g) 1, 10 and 100 pM and 1, 10 and 100 nM, respectively.
  - Time course curve. Effect of ANF on ^{3}H-DA uptake at different times (1, 5, 10, 20 and 30 min): (a) control group, (b) 100 nM ANF.
- Characterization of extraneuronal uptake:
  - Effect of ANF on ^{3}H-DA uptake in the presence of the extraneuronal uptake blocker hydrocortisone: (a) control group, (b) 100 μM hydrocortisone, (c) 100 nM ANF and (d) 100 μM hydrocortisone plus 100 nM ANF.
  - Effect of temperature on renal ^{3}H-DA uptake: (a) control group (KBS 37 °C), (b) cold KBS (0 °C), both in the presence of 0–50 μM nomifensine.

- Effect of the deprivation of sodium ion in the medium on ^{3}H-DA uptake: (a) control group, (b) sodium-free medium, (c) 100 nM ANF and (d) sodium-free medium plus 100 nM ANF.

- Characterization of ANF receptor:
  - Effect of ANF on ^{3}H-DA uptake in the presence of anantin (specific NPR-A receptor blocker): (a) control, (b) 100 nM anantin, (c) 100 nM ANF and (d) 100 nM anantin plus 100 nM ANF.
  - Effect of ANF on ^{3}H-DA uptake in the presence of ANF 4–23 amide (specific agonist of type C receptors): (a) control, (b) 100 nM ANF 4–23 amide, (c) ANF 100 nM and (d) 100 nM ANF 4–23 amide 100 nM plus ANF 100 nM.
- Effects of ANF on ^{3}H-DA uptake:
  - Effect of ANF on ^{3}H-L-DOPA (precursor of DA synthesis) uptake at 10 and 30 min: (a) control group, (b) 100 nM ANF. This set of experiments was performed in tissues from the three renal areas mentioned above.

At the end of the incubation period, the tissues were washed for 5 min with cold KBS solution and then homogenized with 2.5 ml of 10% trichloroacetic acid (TCA). The homogenates were centrifuged at 2500×*g* and 4 °C for 30 min, and tritium activity in the supernatants was determined by usual scintillation counting method. Results of DA uptake are expressed as dpm/g of fresh tissue.

2.1. Statistical analysis

All values are expressed as mean±S.E.M. Student's *t*-test, one-way ANOVA and Tukey test were performed where appropriate. *P* values of 0.05 or less were considered statistically significant.

3. Results

Fig. 1 illustrates the effects of ANF (1 pM, 10 nM and 100 nM) on DA uptake in external cortex, juxtamedullar cortex and inner medulla. ANF at 10 and 100 nM increased DA uptake in the three renal regions in a concentration–response fashion.

Fig. 2 shows the effects of ANF (1 pM–100 nM) on DA uptake in external cortex. ANF at 10 and 100 nM increased DA uptake in a concentration–response fashion, 10 pM being the threshold concentration to enhance the uptake.

In Fig. 3, the time course of DA uptake between 1 and 30 min can be observed. A concentration of 100 nM ANF increased DA uptake at 30 min.

Fig. 4 demonstrates the effects of the extraneuronal amine uptake blocker, 100 μM hydrocortisone, on renal DA uptake in the presence and in the absence of 100 nM ANF. It can be observed that 100 μM hydrocortisone inhibited renal DA uptake. The stimulant effects of 100 nM ANF on renal DA uptake were blunted in the presence of 100 μM hydrocortisone.

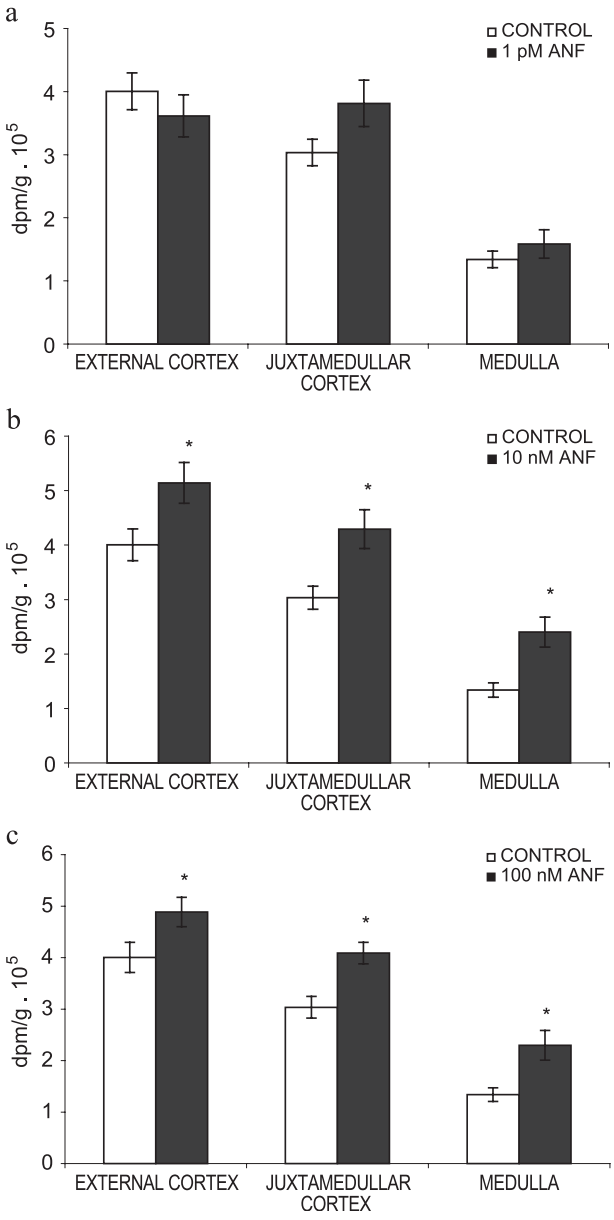

Fig. 1. (a, b and c) Effects of ANF on ^{3}H-dopamine uptake (dpm/g±S.E.M.) in renal (a) external cortex, (b) juxtamedullar cortex and (c) inner medulla. (□) Control; (■) a: 1 pM ANF, b: 10 nM ANF and c: 100 nM ANF. *p<0.05 compared with control. Number of cases: 8–13.

Fig. 5 shows the incidence of temperature on renal DA uptake in the presence of 0–50 μM nomifensine. DA uptake is reduced at 0 °C compared with DA uptake at 37 °C. In the presence of 50 μM nomifensine (concentration used during pre-incubation), DA uptake at 0 °C decreases by 66.8% as compared with DA uptake at 37 °C. In the presence of 17 μM nomifensine (concentration used during incubation), DA uptake at 0 °C decreases by 46.0% as compared with DA uptake at 37 °C. The slope of the linear regression curve represents the inhibition of neuronal DA uptake by nomifensine.

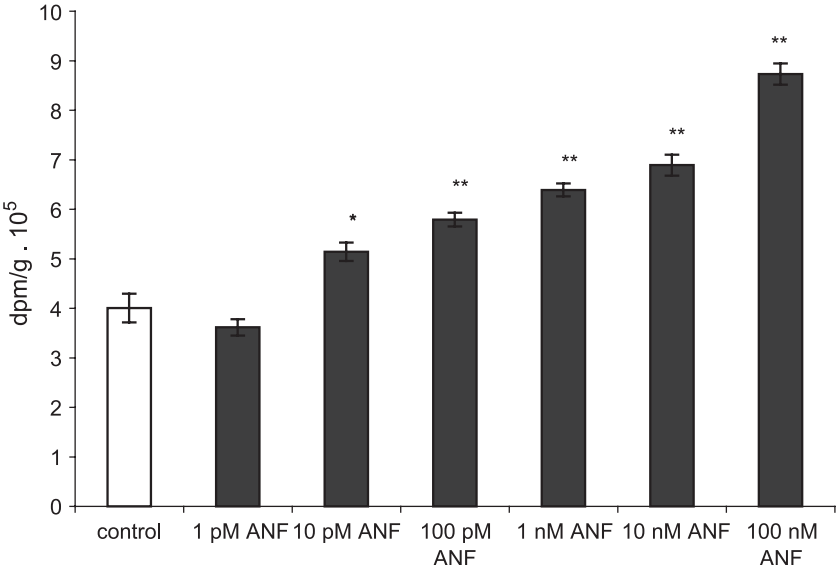

Fig. 2. Effects of ANF (1 pM–100 nM) on ^{3}H-dopamine uptake (dpm/g±S.E.M.) in renal external cortex. (□) Control; (■) ANF (1 pM–100 nM). *p<0.05 compared with control. **p<0.001 compared with control. Number of cases: 8–14.

Fig. 6 shows DA uptake in a sodium-free medium. DA uptake diminished in a medium without sodium and, under this condition, the effect of 100 nM ANF on DA uptake is inhibited.

Fig. 7 shows the effects of the specific NPR-A receptor blocker, 100 nM anantin, on DA uptake. Anantin did not alter DA uptake, but diminished ANF effects on DA uptake by 30.9%.

In Fig. 8, the effects of the specific NPR-C receptor agonist, 100 nM ANF 4–23 amide fragment, on DA uptake can be observed. The agonist NPR-C did not alter DA uptake and did not modify the increasing effects of ANF on DA uptake.

In Fig. 9, the effects of 100 nM ANF on ^{3}H-L-DOPA uptake in external cortex, juxtamedullar cortex and inner medulla can be observed. ANF at 100 nM did not modify L-DOPA uptake, at 10 or 30 min, in the mentioned renal regions.

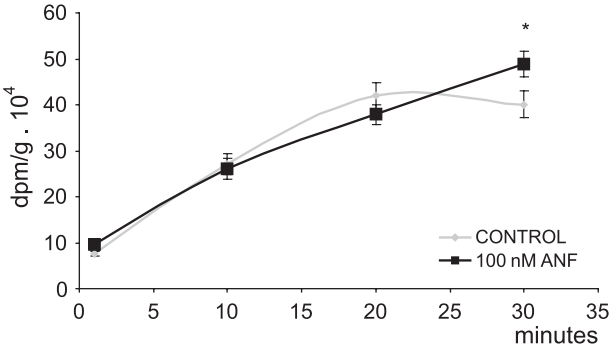

Fig. 3. Effects of 100 nM ANF on the time-course curve of ^{3}H-dopamine uptake (dpm/g±S.E.M.) in renal external cortex, between 0 and 30 min. (♦) Control; (■) 100 nM ANF. *p<0.05 compared with control. Number of cases: 5–15.

4. Discussion

ANF exhibits natriuretic and diuretic properties [2,17,18] and its effects were observed on the renal glomeruli and tubuli. At proximal tubule level, ANF counteracts ANG II-sodium and water-dependent reabsorption [5,19,20]. The inhibitory effects of ANF are mediated by a cGMP-protein kinase G-dependent mechanism [21].

Intrarenal DA can act by autocrine and paracrine mechanisms on proximal and distal tubular cells inhibiting sodium transport [22,23]. The synthesis of DA in the kidney has neuronal and extraneuronal sources. The neuronal sources are noradrenergic and dopaminergic neurons [24]. The extraneuronal sources are L-DOPA decarboxylation after L-DOPA has been taken up from the tubular filtrate and DA taken up by the tubular cells from the blood [25,26]. DA

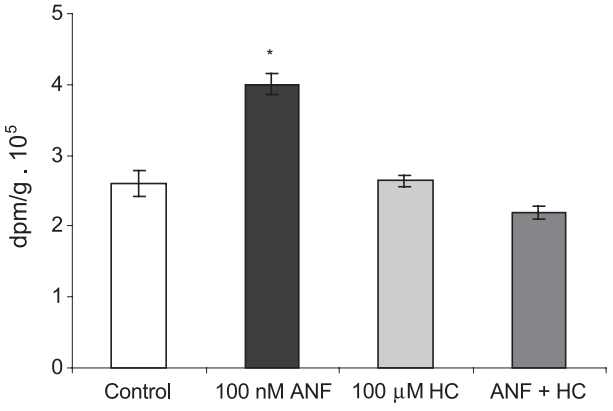

Fig. 4. Effects of 100 nM ANF on ^{3}H-dopamine uptake (dpm/g±S.E.M.) in renal external cortex in the presence and in the absence of 100 µM hydrocortisone (HC). *p<0.005 compared with 100 nM ANF. (□) Control; (■) 100 nM ANF; (▒) 100 µM HC; (▓) 100 nM ANF plus 100 µM HC. Number of cases: 6–9.

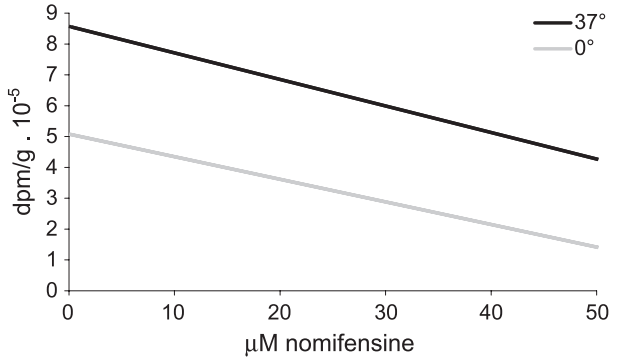

Fig. 5. Effects of temperature (0–37 °C) in the presence of nomifensine (1–50 µM) on ^{3}H-dopamine uptake (dpm/g±S.E.M.) in renal external cortex. Slope (k) of the linear regression curve represents the inhibition of neuronal DA uptake by nomifensine. r^2=0 °C: 0.60; 37 °C: 0.90; k=0 °C: −0.073±0.03; 37 °C: −0.354±0.083. Number of cases: 4–9.

produced in proximal tubular cells inhibits Na^+,K^+-ATPase activity in several tubular segments [27,28].

D_1 receptors, identified in all tubular segments, belong to the family of G-protein coupled receptors, known to cycle between plasma membrane and intracellular compartments and linked to the production of cAMP [1,29]. Receptor internalization results in receptor desensitization and is regulated by PKA and PKC activators [29,30]. Receptor sensitization involving receptor recruitment to the plasma membrane is a constitutive process partially regulated by ANF and cGMP [29,31]. The activation of DA receptors in tubular cells increases sodium and water excretion [30,32]. The inhibition of Na^+,K^+-ATPase–protein kinase A and the activation of phospholipase A2 [18] mediate DA-dependent decrease of sodium reabsorption. DA also inhibits Na^+-H^+ transport via cAMP and via phospholipase C [19,30,33].

DA and ANF exert several similar physiological effects. ANF effects on the kidney such as vasodilatation and inhibition of sodium and water reabsorption are also shared

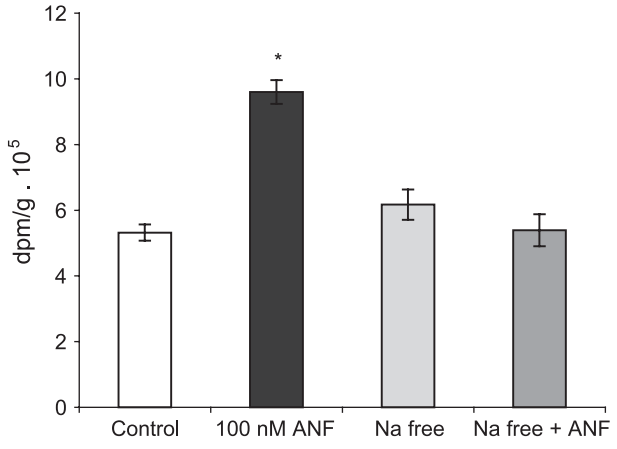

Fig. 6. Effects of ANF (in a sodium-free medium) on ^{3}H-dopamine uptake (dpm/g±S.E.M.) in renal external cortex. (□) Control; (■) 100 nM ANF; (▒) sodium-free medium; (▓) 100 nM ANF in sodium-free medium. *p<0.01 compared with control, sodium-free or 100 nM ANF (in sodium-free medium). Number of cases: 4–7.

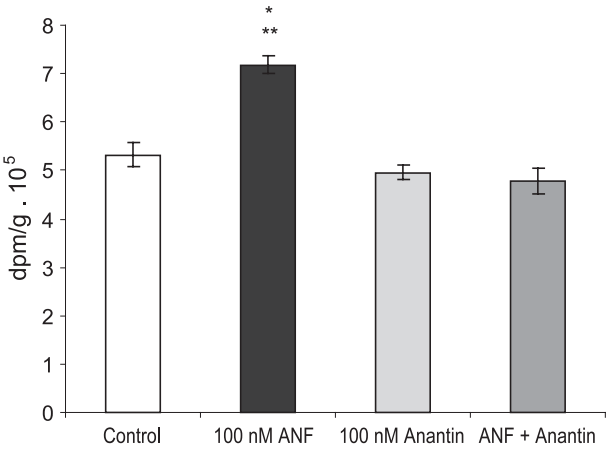

Fig. 7. Effects of 100 nM anantin on ^{3}H-dopamine uptake (dpm/g±S.E.M.) in renal external cortex. (□) Control; (■) 100 nM ANF; (▒) 100 nM anantin; (▓) 100 nM ANF plus 100 nM anantin. *p<0.01 compared with control, **p<0.001 compared with 100 nM anantin or 100 nM ANF plus 100 nM anantin. Number of cases: 6–8.

by dopamine. Both lower blood pressure, increase renal flow and filtration rate, enhance diuresis and natriuresis, and suppress aldosterone release [5,34]. They differ in their receptors that are linked to different second messengers: ANF receptors are coupled to the particulate form of guanilate cyclase, the activation of which results in an increase in the generation of cGMP [3,5]. Conversely, stimulation of D_1 receptors is coupled to the enzyme adenylate cyclase and the consequent increase of cAMP formation [30].

Renal DA availability can be regulated on different levels: synthesis, storage or metabolism [30]. It was hypothesized that DA generation or uptake is essential to the expression of ANF renal effects [19]. Krayacich et al. [35] demonstrated that DA nerves are unnecessary for the action of ANF and that the response to ANF was identical in rats with innervated or denervated kidneys. However, the mechanisms that regulate the synthesis, storage and metabolism of DA in the renal tubular cells are little known.

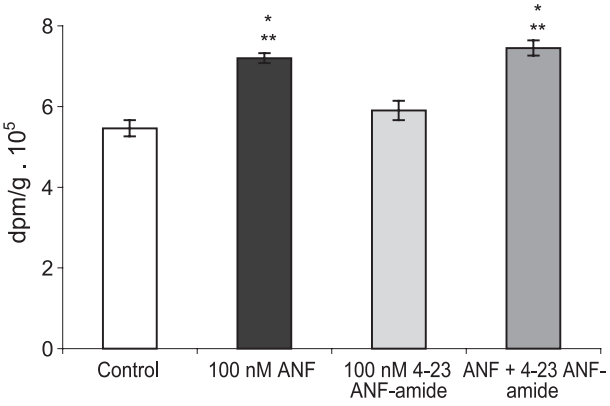

Fig. 8. Effects of 4–23 ANF-amide on ^{3}H-dopamine uptake (dpm/g±S.E.M.) in renal external cortex. (□) Control; (■) 100 nM ANF; (▒) 100 nM 4–23 ANF-amide; (▓) 100 nM ANF plus 100 nM 4–23 ANF-amide. *p<0.05 compared with 100 nM 4–23 ANF-amide; **p<0.001 compared with control. Number of cases: 6–8.

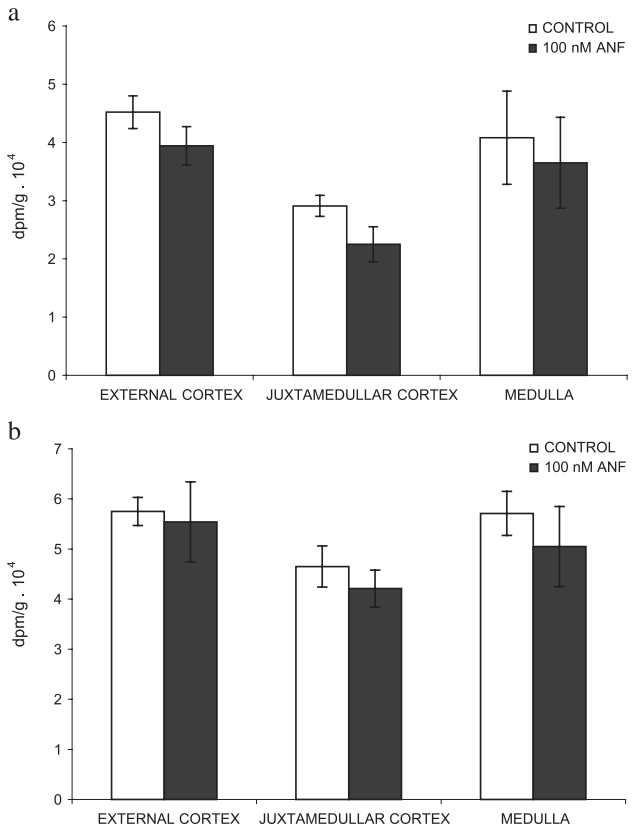

Fig. 9. (a and b) Effects of ANF on ^{3}H-L-DOPA uptake (dpm/g±S.E.M.) at (a) 10 min and (b) 30 min in renal external cortex, juxtamedullar cortex and inner medulla. (□) Control; (■) 100 nM ANF. Number of cases: 4–7.

Several observations suggested that D_1 receptors contribute to the actions of ANF in the kidney and it was observed that the natriuretic response to ANF was completely blocked by pretreatment with D_1 receptor antagonists. Marin-Grez et al. [6] and Webb et al. [7] reported that part of the ANF inhibitory effects on sodium and water reabsorption is mediated by dopaminergic mechanisms, since haloperidol blocked a percentage of natriuretic and diuretic ANF effects. After the administration of DA_1 receptor antagonists, haloperidol, Sch 23390, or in the presence of carbidopa, ANF-induced increase of phosphate and sodium in urine was blunted [36–38]. When DA was administrated, ANF effects were restored. The results of other studies suggest that D_2 receptors are unlikely to be involved since metaclopramide and sulpiride were reported not to influence the response to ANF [39,40]. ANF decreases NA^+,K^+-ATPase activity in rat proximal tubule segments. This effect was abolished in the presence of SCH 23390 and was enhanced in the presence of DA [29]. Furthermore, a synergistic effect between DA and ANF was observed on the Na^+,H^+ exchanger activity [38]. Other authors [8,9,41] had observed that ANF decreased DA synthesis in the kidney.

Na^+,K^+-ATPase is present in the basolateral membrane of all tubular cells, generally in a very high concentration. The electrochemical gradient generated by this enzyme allows sodium to enter the apical side of the cells via channels and is mediated by symport and antiport systems [1,30]. ANG II by itself stimulates Na^+,K^+-ATPase activity [19,42]. ANF inhibits tubular fluid reabsorption only in the presence of stimulatory factors such as angiotensin II and NE [1,43]. Catecholamines and peptide hormones exert short-term regulation that includes alterations of tubular sodium transporters, carried out through reversible protein phosphorylation by kinases on specific phosphorylation sites and dephosphorylation by phosphatases. These reactions result in conformational changes and thereby alterations in activity of the modified proteins. Second messengers such as cGMP, cAMP and Ca^{2+} could regulate these processes, which in turn, are under hormonal control by ANF, DA and angiotensin II [1]. Natriuretic factors (DA, ANF, parathyroid hormone, endothelin and PGE_2) inhibit Na^+,K^+-ATPase by different pathways [42,44–46]. Conversely, antinatriuretic agents (norepinephrine, neuropeptide Y, insulin and angiotensin II) stimulate enzyme activity [42,47–49]. The intrinsic balance between the mentioned systems modulates this bidirectional regulation of Na^+,K^+-ATPase [30]. Activation of the enzyme is blocked by DA and ANF and by their respective second messengers, cAMP and cGMP, as well as by the specific protein phosphatase 2B inhibitor, FK 506 [1,30]. DA promotes sodium excretion by raising intracellular cAMP, thereby activating PKA (cAMP activating protein kinase), which in turn, phosphorylates and inactivates Na^+,K^+-ATPase. ANF promotes sodium excretion by raising cGMP, thereby activating PKG (cGMP activating protein kinase) [50,51].

Peptide hormones can regulate sodium homeostasis indirectly via sensitization of catecholamine receptors [42,47,48]. ANF-silent recruitment of DA receptors from the interior of the cell to the plasma membrane provides a plausible explanation for this phenomenon [29]. The permissive role of DA on the natriuretic effects of ANF may be dependent on the mobilization of dopamine of renal origin or the regulation of its metabolism. Different probabilities could justify ANF–DA relationship in renal tissues: (1) ANF could stimulate the production or release of endogenous DA in the kidney. It is not probable since ANF seems to diminish DA synthesis [10,41]. (2) The endogenous DA inhibits (mediated by D1 receptors) the clearance of ANF. It is not probable since plasmatic ANF concentration is not modified after treatment with the D_1 antagonist SCH 23390 [36]. (3) The ANF binds to tubular D_1 receptors. It is not probable since D_1 receptor activates cAMP production and ANF activates cGMP formation [36]. (4) Most probably, ANF increases endogenous DA and this amine, through D_1 receptor, decreases reabsorption of sodium.

ANF required tubular cell integrity to exert its inhibitory effect on the formation of DA [41]. The inhibition of ANF-elicited natriuresis and diuresis in response to dopamine D_1 receptor blockade is not quantitatively significant [32]. Then, it can be deduced that DA mechanisms involved in ANF diuretic effects are not completely related to the amount of newly synthesized DA from all the sources

mentioned above. Since ANF only increases (in our experiments) the uptake of DA by the tubular cells but not the uptake of L-DOPA, ANF does not affect the main source of the newly synthesized ones.

Present results show that ANF increased DA uptake by renal tissue in a concentration–response fashion. The analysis of the concentration–response curve demonstrates that 10 pM ANF was the threshold concentration of ANF to elicit the enhancement of DA uptake. On this basis, 100 nM ANF was the concentration used to continue the studies. Conversely, uptake of the precursor L-DOPA was not modified by the peptide. Three areas from the kidney were studied: the external cortex, the juxtamedullar cortex and the inner medulla. The effects of ANF were observed in the three segments. In view of this result, former experiments were carried out only in the external cortex. In the CNS, the effect of ANF on NE uptake was rapid (the increase was significant at one minute) and continued increasing up to 30 min. Conversely, in the kidney, ANF increased DA uptake after 30 min of incubation. It is probable that the quantity and availability of uptake transporters for amines are smaller in the kidney than in the central nervous system (CNS) and could be the cause of the different response between the CNS and the kidney. The DA uptake measured belonged to extraneuronal tissues, since neuronal uptake was blocked by the presence of nomifensine.

In order to define the extraneuronal amine uptake, the experiments were then performed in the presence of hydrocortisone. This extraneuronal blocker blunted ANF effects on DA uptake. Also, the uptake was temperature- and sodium-dependent.

Two types of natriuretic peptide receptors have been identified: two guanylate cyclase-coupled receptors (NPR-A and NPR-B) that modulate most of the biological effects and a non-guanylate-coupled receptor (NPR-C), called the clearance receptor, that regulates the circulating levels of natriuretic peptides, also reported to be coupled to other signaling mechanisms different from cGMP [52,53]. NPR-B receptors are mainly present in the central nervous system and widely distributed NPR-A receptors have been described to be present in renal tissues. Anantin, a specific blocker of biological type A receptor (NPR-A), reversed ANF-dependent increase of DA uptake and demonstrated that NPR-A mediates ANF responses on renal DA uptake. In order to discard the participation of ANF type C (NPR-C) receptors, which are coupled to phosphatidylinositol hydrolysis and inositol triphosphate (IP3) and diacylglycerol (DAG) generation or inhibition of cAMP production [52,54], the experiments on DA uptake were performed in the presence of the truncated peptide 4–23 ANF amide. This peptide binds to NPR-C receptors as an analogue of ANF. The 4–23 amide ANF did not alter DA uptake in renal tissues. Besides, the response to ANF in the presence of the truncated peptide did not differ from the response obtained with ANF alone. These results suggest that NPR-C receptors are not implied in the induced increase of DA uptake in renal tubules.

The renal uptake of DA was decreased at 0 °C and in sodium-free medium. The effects of ANF on DA uptake in these conditions were not present. These results confirm that renal uptake of DA is mediated by a transporter that is temperature- and sodium-dependent, and that the peptide requires the presence of the ion to exhibit its actions on DA uptake. The effects of ANF on DA uptake may be mediated by different mechanisms such as the increase of transporters, an alteration in the membrane potential and/or an alteration in the carrier affinity.

In conclusion, ANF stimulates DA uptake by the tubular cells in the kidney mediated by NPR-A receptors coupled to guanylate cyclase and cGMP as second messenger. The process was characterized as a typical extraneuronal uptake, and temperature- and sodium-dependent. This mechanism could be related to DA effects on sodium reabsorption and linked to ANF enhanced natriuresis in the kidney. The increment of endogenous DA into tubular cells, as a consequence of increased DA uptake, would permit D_1 receptor recruitment and stimulation and, in turn, Na^+,K^+-ATPase activity overinhibition that results in decreased sodium reabsorption and increased natriuresis.

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