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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.