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Signaling pathways involved in atrial natriuretic factor and dopamine regulation of renal Na^+, K^+-ATPase activity

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Abstract

Dopamine (DA) and atrial natriuretic factor (ANF) share a number of physiological effects. We hypothesized that ANF and the renal dopaminergic system could interact and enhance the natriuretic and diuretic effects of the peptide. We have previously reported that the ANF-stimulated DA uptake in renal tubular cells is mediated by the natriuretic peptide type-A receptor (NPR-A). Our aim was to investigate the signaling pathways that mediate ANF effects on renal ^{3}H-DA uptake. Methylene blue (10 μM), an unspecific inhibitor of guanylate cyclase (GC), blunted ANF elicited increase of DA uptake. ODQ (10 μM) a specific inhibitor of soluble GC, did not modify DA uptake and did not reverse ANF-induced increase of DA uptake; then the participation of nitric oxide-dependent pathways must be discarded. The second messenger was the cGMP since the analogous 125 μM 8-Br-cGMP mimicked ANF effects. The specific inhibitor of the protein kinase G (PKG), KT 5823 (1 μM) blocked ANF effects indicating that PKG is involved. We examined if ANF effects on DA uptake were able to modify Na^+, K^+-adenosine triphosphatase (Na^+, K^+-ATPase) activity. The experiments were designed by means of inhibition of renal DA synthesis by carbidopa and neuronal DA uptake blocked by nomifensine. In these conditions renal Na^+, K^+-ATPase activity was increased, in agreement with the decrease of DA availability. When in similar conditions, exogenous DA was added to the incubation medium, the activity of the enzyme tended to decrease, following to the restored availability of DA. The addition of ANF alone had similar effects to the addition of DA on the sodium pump, but when both were added together, the activity of Na^+, K^+-ATPase was decreased. Moreover, the extraneuronal uptake blocker, hydrocortisone, inhibited the latter effect.

In conclusion, ANF stimulates extraneuronal DA uptake in external cortex tissues by activation of NPR-A receptors coupled to GC and it signals through cGMP as second messenger and PKG. Dopamine and ANF may achieve their effects through a common pathway that involves reversible deactivation of renal tubular Na^+, K^+-ATPase activity. This mechanism demonstrates a DA–ANF relationship involved in the modulation of both decreased sodium reabsorption and increased natriuresis.

Keywords: Atrial natriuretic factor; ANF; Dopamine; cGMP; PKG; Na^+; K^+-ATPase

1. Introduction

Diverse endocrine, autocrine and neuronal factors regulate blood pressure and sodium metabolism. The rate of tubular sodium reabsorption in all renal tubular segments is one of the main long-term regulators of blood pressure. 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) is a 28-amino acid peptide, synthesized and stored in the atrial myocytes and released in response to the stretch of cardiocytes or endothelin, cytoquines and alpha-adrenergic stimulation [2–4]. Natriuretic effects of ANF are exerted through enhanced glomerular filtration rate and renal tubular reabsorption processes. The natriuretic peptide inhibits angiotensin II-dependent sodium and water reabsorption at proximal kidney tubules and also decreases water absorption in distal tubules and collecting ducts [5].

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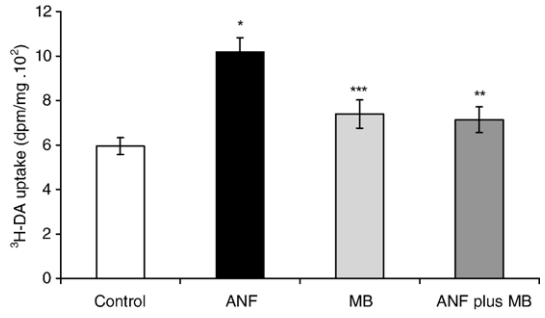

Fig. 1. Effects of methylene blue (MB) on ^{3}H-dopamine uptake (dpm/g±SEM) in renal outer cortex. □ Control; ■ 100 nM ANF; ■ 100 μM MB; ■ 100 nM ANF plus 100 μM MB. *p<0.001 compared with control; **p<0.01 and ***p<0.05 compared with 100 nM ANF. Number of cases: 6–8.

Dopamine (DA), synthesized by renal proximal tubules, plays an important autocrine/paracrine role in the regulation of renal function [6] exerted through the inhibition of Na^+, K^+-ATPase activity as well as diverse sodium influx pathways [7]. These effects are mainly mediated via the DA-1 (D_1) receptor subtype coupled to adenylyl cyclase activation and cyclic adenosine phosphate (cAMP) generation, as well as phospholipase C and protein kinase C (PKC) signaling in the renal tubular cells [8,9].

DA and ANF share a number of physiological effects. Webb et al. [10] have reported that some of the ANF inhibitory effects on sodium and water reabsorption are mediated by dopaminergic mechanisms, since haloperidol and the D_1 receptor antagonist SCH 23390 partially block the natriuretic and diuretic effects of the peptide.

We have previously reported that ANF stimulates DA uptake by tubular cells in the kidney, an effect mediated by the natriuretic peptide type-A receptor (NPR-A), that process being characterized as a typical temperature and sodium-dependent extraneuronal uptake [11].

These previous findings lead us to hypothesize that ANF and the renal dopaminergic system could interact and enhance the natriuretic and diuretic effects of the peptide.

The aim of the present work is to study the signaling pathways that mediate the NPR-A stimulation by ANF and identify the second messenger and protein kinase involved.

The results indicate that ANF increases DA uptake in renal tubular cells through stimulation of NPR-A, cGMP production and PKG activation. Thus, the increment of endogenous DA into tubular cells would permit D_1 receptor recruitment and inhibition of Na^+, K^+-ATPase activity that results in decreased sodium reabsorption and increased natriuresis. By this mechanism, ANF and DA seem to 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 Cátedra de Fisiopatología Facultad de Farmacia y Bioquímica, Buenos Aires) were used. The animals were housed in cages, with a 12-h light/dark cycle and controlled temperature and humidity. All animals were given free access to water and food (Commercial rodents Purina chow, Cooperacion SRL, Argentina). All procedures were carried out in accordance with the guidelines edited by the Canadian Council on Animal Care.

The following drugs were used in the experiments: ^{3}H-DA, 28.0 Ci/mmol of specific activity (New England Nuclear, Boston, MA, USA), ANF (99–126, rat), hydrocortisone, methylene blue, 8-Br-cGMP, KT 5823, nomifensine, DL-Thiorphan, imidazole, ATP (adenosine 5′ triphosphate), Bovine Seroalbumin fraction V of Cohn (Sigma Chem. Co., St. Louis, MO, USA), Folin reactive (Merck Co., USA), carbidopa (gently provided by Dr. Victor Nahmod, Buenos Aires, Argentina), ODQ (1H-[1,2,4] Oxadiazolo [4,3-a]quinoxalin-1-one] (Calbiochem, San Diego, CA, USA) and EcoLite, for liquid scintillation (ICN Pharmaceutical Inc., CA, USA).

The standard Krebs bicarbonate (SKB) solution composition (mM) was: 118 NaCl; 4.7 KCl; 1.2 $MgSO_4 \cdot 7H_2O$; 1.0 NaH_2PO_4; 2.4 $CaCl_2$; 0.004 EDTA; 11.1 glucose; 0.11 ascorbic acid; 26.0 $NaHCO_3$.

Rats were anesthetized with 10% ethyl urethane (1.3 mg/kg, i.p.). Both kidneys were removed and slices of the external cortex were cut and weighed. In order to determine ^{3}H-DA uptake, experiments were carried out according to the techniques previously described [11]. The tissues were minced and then placed in a 2 ml SKB 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 to avoid neuronal DA uptake. After pre-incubation, the tissues were transferred to fresh SKB medium and incubated for 30 min, in similar conditions, with 12.5 μCi/ml of ^{3}H-DA, 17 μM of nomifensine, without (control) or with the different tested drugs (experimental groups).

The following experimental groups were studied:

◆ *Identification of second messenger*

– Effect of ANF on ^{3}H-DA uptake in the presence of methylene blue (unspecific inhibitor of GC): (a) control; (b) 100 nM ANF; (c) 10 μM methylene blue; (d) 10 μM methylene blue plus 100 nM ANF.
– Effect of ANF on ^{3}H-DA uptake in the presence of ODQ (specific inhibitor of soluble GC): (a) control; (b) 100 nM ANF; (c) 10 μM ODQ; (d) 10 μM ODQ plus 100 nM ANF.

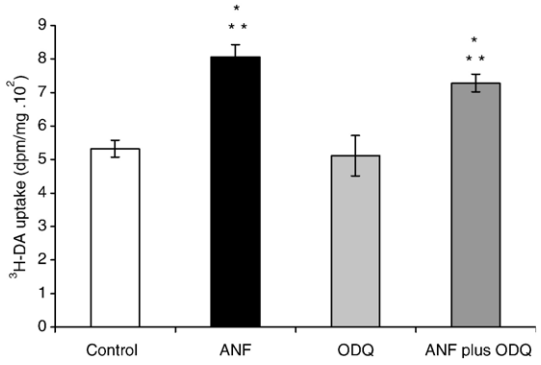

Fig. 2. Effects of ODQ on ^{3}H-dopamine uptake (dpm/g±SEM) in the renal outer cortex. □ Control; ■ 100 nM ANF; ■ 10 μM ODQ; ■ 100 nM ANF plus 10 μM ODQ. *p<0.001 compared with control; **p<0.01 compared with 10 μM ODQ. Number of cases: 6–10.