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Atrial Natriuretic Factor Modifies the Biosynthesis and Turnover of Norepinephrine in the Rat Adrenal Medulla

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In the present work we investigate atrial natriuretic factor (ANF) effects on the endogenous content, utilization and turn over of norepinepinephrine (NE), on tyrosine hydroxilase (TH) activity, on cAMP and cGMP levels, and on phosphatidylinositol hydrolysis in rat adrenal medulla in order to assess the possible mechanisms underlying ANF effects on NE metabolism. Results showed that ANF (5 μg/kg) increased NE endogenous content (44%) and diminished the amine utilization. On the other hand, the atrial factor (10 nM) inhibited both spontaneous and evoked, by 100 mM KCl TH, activity (48% and 59%, respectively). When second messenger systems were studied results showed that 10 nM ANF increased cGMP levels in adrenal medulla (51%), while it modified neither cAMP levels nor phosphatidylinositol hydrolysis. These results suggest that ANF may play an important role in the modulation of the sympathoadrenergic system function, behaving as a putative neuromodulator.

Atrial natriuretic factor (ANF) was the first discovered peptide (1,2) of the family. It is synthesized and released by mammalian atrial cardiocytes in response to stretch, endothelin and α_1-adrenergic stimulation (2,3). However, experimental evidence has demonstrated the existance of extracardiac sources of ANF (3).

ANF is a vasodepressor peptide involved in the regulation of blood arterial pressure. It is closely related to body hemodynamic regulation because it decreases the peripheral vascular resistance through vasodilation and reduces blood circulating volume through natriuretic and diuretic mechanism, thus regulating blood arterial pressure (1-3). Moreover, the effects of ANF on blood arterial pressure may be indirectly mediated through modifications of central as well as peripheral catecholamine metabolism (4-6).

The atrial factor gene expression has been reported in the aortic arch, lung, anterior pituitary, central nervous system, adrenal glands (including adrenal medulla), the kidney and the gastrointestinal tract (3,7). ANF immunoractivity and precursors, as well as several mature forms of the atrial factor, have been identified in the chromaffin cells (8,9). Furthermore, ANF binding sites also been reported in the adrenal medulla (10).

We have previously reported that ANF modulated noradrenergic neurotransmission in the central nervous system and norepinephrine (NE) activity in the adrenal medulla of the rat. The atrial factor increases neuronal NE uptake and decreases neuronal NE release in hypothalamus, medulla oblongata and adrenal medulla (4-6,11). Alterations of neuronal NE uptake and release may be related to modifications in NE synthesis and/or turn over (12). Debinski et al. and Fernández et al. have reported that ANF inhibited synthesis in the superior cervical ganglia and in hypothalamus (13,14). On these basis, the aims of the present work were to study ANF effects on: the endogenous content, utilization and turn over of NE; tyrosine hydroxilase (TH) activity; cAMP and cGMP levels and phosphatidylinositol hydrolysis, in the rat adrenal medulla.

MATERIALS AND METHODS

NE endogenous content and utilization. Male Wistar rats (250-300 g of body weight) were randomly divided into the following groups: a) Control ; b) ANF (Peninsula Lab., Belmont, CA, USA) ; c) α-methyl-p-tyrosine (MT) (Sigma Chem. Co., St. Louis, MO, USA) ; d) MT plus ANF. The animals of groups c and d were intraperitoneally (i.p.) injected, 24 and 2 hs before the experiments with MT (200 mg/kg of body weight) to inhibit catecholamine syntesis, with the aim to performed the NE utilization experiment. On the other hand, groups a and b were injected with the same volume of the saline (NE endogenous content experiment). Rats were anesthetized with

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urethane (1.3 mg/kg i.p.) and the yugular vein was cannulated with a polyethylene catheter (PC-40, PL Rivero & Cia., Argentina). Groups b and d were injected with ANF (5μg/kg) and groups a and c with the same volume of saline. After 60 min, the animals were decapitated, the adrenal glands quickly removed and the adrenal medullae immediately dissected, cooled and weighed. NE was extracted according to the chromatographic method of von Euler and Lishajko (15), and meassured by the fluorometric technique of Cohen and Goldenberg (NE recovery was of 70%) (16). The amine endogenous content and utilization are expressed as μg of NE/g of fresh tissue ± S.E.M..

TH activity. Animals were decapitated and adrenal medullae dissected and cut in slices. The tissues were placed in a Dubnoff incubator and preincubated for 15 min at 37°C with modified Krebs bicarbonate solution (KBS), pH 7.4 and bubbled with a gas mixture (95% O_2 and 5% CO_2) under continuous shaking. Adrenal medulla slices were incubated for 60 min and the following groups were studied : a) control group ; b) 10nM ANF ; c) 100mM KCl ; and d) 100mM KCl plus 10nM ANF. The concentration of ANF used has been previously reported to modify both the uptake and release of NE (6). After the incubation period, slices were washed for 5 min with cooled KBM and then homogenized in 500μl of ice-cold water. TH activity was determined according to the method described by Hendry and Iversen and Zigmond and Clalazonitis (17,18). 3H-L-DOPA was determined by usual scintillation methods. TH activity results are expressed as a percentage of the control group ± S.E.M.

NE turn over. Animals were treated with MT (200μg/kg, i.p.) 24 and 2 hs before being sacrified. Adrenal medullae were dissected and weighed as previously described. The tissues were cut in slices and divided in six fractions of about similar weight, to be studied at different times diring the incubation period with NE-HCl DL(7-^{3}H(N)) of 15 Ci/mmol of specific activity (New England Nuclear, Boston, MA, USA). Each sample was weighed and preincubated for 30 min at 37°C with KBS plus 100μM MT and 100μM hydrocortisone, extraneuronal NE uptake inhibitor (Sigma Chem. Co., St. Louis, MO, USA). Tissues were then incubated for 30 min in the presence of 1.25μCi/ml of 3H-NE plus 100μMT and 100μM hydrocortisone. After this period, the samples were incubated with KBS (control group) or 10nM ANF (experimental group) for 30, 60, 90, 120, 150 and 180 min. At the end of these periods, tissues were homogenized with 10% trichloroacetic acid and centrifuged at 27,000 G under refrigeration for 15 min. Tritium activity was determined in the supernatants by usual scintillation counting methods. Regression analysis and the comparisson of the slopes (k) of the control and experimental grops were performed.

CyclicAMP and cGMP assays. Freshly isolated adrenal medullae were incubated as previously described in the TH activity protocol. The tissues were washed with KBS and then homogenized in 10% trichloroacetic acid. The mixtures were centrifuged and supernatants extracted five times with ethyl ether, and then lyophylized. The dried samples were reconstitued in assay buffer and the concentrations of cAMP and cGMP were determined by radioimmunoassay kits (Amersham, Arlington Heights, Ill, USA). Results are expressed as pmol of cAMP or cGMP/g wet weight.

Phosphatidylinositol hydrolysis assay. Adrenal medulla slices were obtained as previously described in the TH activity protocol. The tissues were preincubated in 500μl of KBS (bubbled with 95% O_2 and 5% CO_2, adjusted to pH 7.4) at 37°C for 15 min and then prelabelled with 500μl of KBS-10mM LiCl containing 2μCi/500μl of myo-($^{2-3}$H) inositol (Amersham, Life Sci., England) for 2 hs. Thirty min before to the end of the incubation period, 10nM ANF or the same volume of KBS (experimental and control groups, respectively) were added to the medium. Phosphoinositide separation was performed as described by Berridge et al. (19). 3H phosphoinositides of the fraction eluted with 1M amonium formiate and 0.1 M formic acid that mainly represents phospholipase C activity (20) were measured by conventional scintillation methods. Results are expressed as the percentage of control values ± S.E.M..

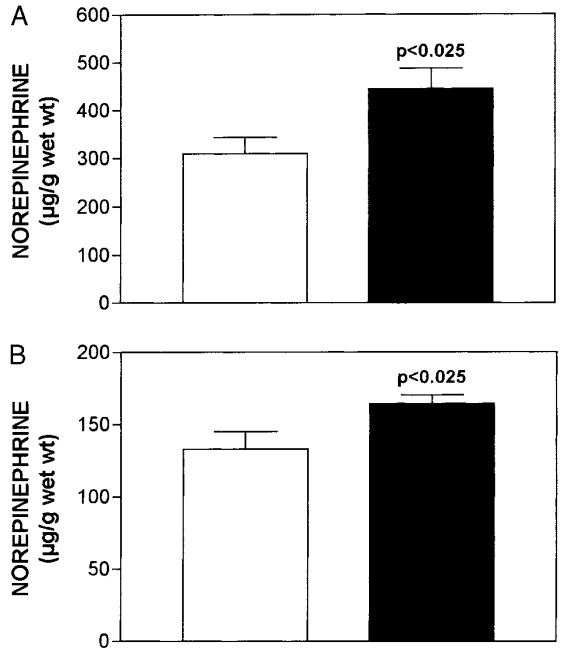

FIG. 1. (**a**) Effects of ANF on endogenous content of NE. Open column: control group injected with saline, e.v. (n=11); solid column: group injected with 5μg/kg ANF, e.v. (n=6). (**b**) Effects of ANF on NE utilization (Rats treated 24 and 2 hs before the experiments with 200mg/kg MT). Open column: control group injected with saline, e.v. (n=9); solid column: group injected with 5μg/kg ANF, e.v. (n=10). In both experimental procedures, data are shown as mean ± SEM; e.v.: endovenous; n: number of experiments.

Statistical analysis. All values are expressed as the mean ± S.E.M.. One way ANOVA and the *t* test modified by Bonferroni were used for statistical analysis. *P* values of 0.05 or less were considered statistically significant.

RESULTS

Figures 1a and b show the effects of ANF on the endogenous content and utilization of NE in the rat hypothalamic tissue. The atrial factor (5μg/kg) injected in the jugular vein increased the endogenous content of NE in the rat adrenal medulla (Fig. 1a). Furthermore, ANF (5μ/kg, e.v.) diminished NE utilization in the same tissue. Figure 1b illustrates that rats treated with both MT and ANF showed significantly higher levels of NE in the rat adrenal medulla than those injected only with MT.

Figure 2 illustrates ANF effect on basal and induced TH activity. Ten nM ANF diminished basal TH activity. As catecholamine biosynthesis was stimulated by a high potassium solution (25mM) the enzyme activity was significantly enhanced. however, in the presence of ANF, TH activity was similar to the control group. These results show that the stimulatory effect, pro-

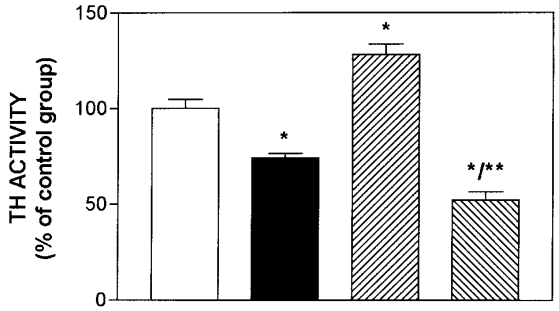

FIG. 2. Effects of ANF on basal and induced, by 100mM KCl, TH activity. Open column: control group (basal TH activity; n=8); solid column: group treated with 10nM ANF (n=7); up-hatched column: group treated with 100mM KCl (induced TH activity; n=7); down-hatched column: group treated with 100mM KCl and 10nM ANF (n=7). *: $p<0.001$ as compared with control group; **: $p<0.001$ as compared with 100mM KCl. Data are shown as mean ± SEM; n: number of experiments.

duced by high potassium solution on catecholamine synthesis, is abolished by the presence of ANF.

The effect of ANF on NE turn over is illustrated in figure 3. Results showed that the atrial factor diminished the turn over of the amine, wich is represented by the slope of the linear regression rect (control: −90.67 ± 7.95 vs ANF: −60.95 ± 8.22, $p<0.025$).

Figures 4a and b show the ANF effect on cAMP and cGMP levels in the rat adrenal medulla. The atrial factor (10nM) did not modify cAMP content (control: 1.47 ± 0.11 vs ANF: 1.77 ± 0.22 pmol/g wet wt) (Fig. 4a) but, it increased significantly adrenomedular cGMP levels as compared with the control group (control: 6.65 ± 0.93 vs ANF: 10.06 ± 0.54 pmol/g wet wt, $p<0.01$) (Fig. 4b).

On the other hand, 10nM ANF did not affect phosphatidylinositol hydrolysis in the rat adrenal medulla (control: 100 ± 20 vs ANF: 76 ± 9)(Fig. 5).

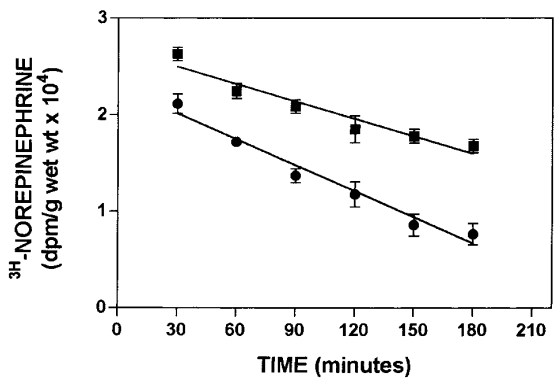

FIG. 3. Effects of ANF on NE turn over. ●: control group (n=6); ■: group incubated with 10nM ANF (n=6). Slope (k) of the linear regresion rect represents the NE turn over (r, control: 0.97 and ANF: 0.93; k, control: −90.67 ± 7.95 vs ANF: −60.95 ± 8.22, $p<0.025$). n: number of experiments.

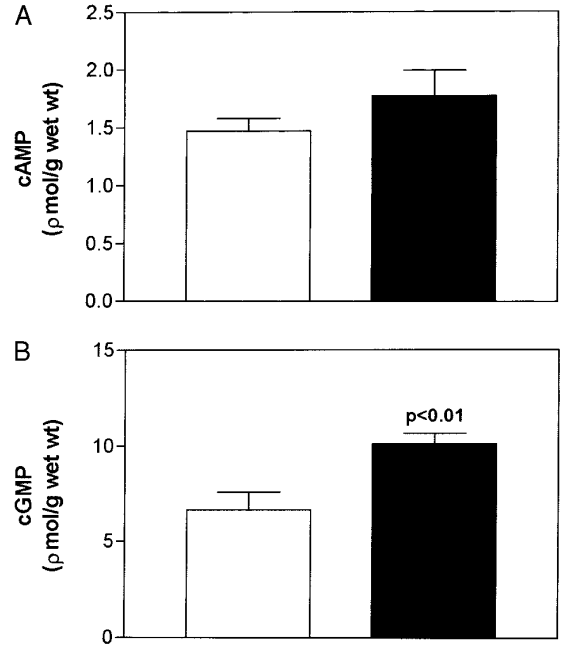

FIG. 4. Effects of ANF on cAMP (**a**) and cGMP (**b**) levels. Open column: control group; solid column: group incubated with 10nM ANF. Data are shown as mean ± SEM; number of experiments: 6-7.

DISCUSSION

In the present work, we report that ANF increases NE endogenous content. The mechanism underlaying this experimental observation could be related to several effects of ANF on NE metabolism that have been previously described. We have reported that ANF increased NE neuronal uptake and diminished the amine output without modifications on the activity of the enzymes involved in NE catabolism (6,14,21). These effects on NE metabolism result in the accumulation of the amine in the chromaffin cell.

TH is the enzime that catalyzes the rate limiting step in the biosynthesis of the catecholamines. The regula-

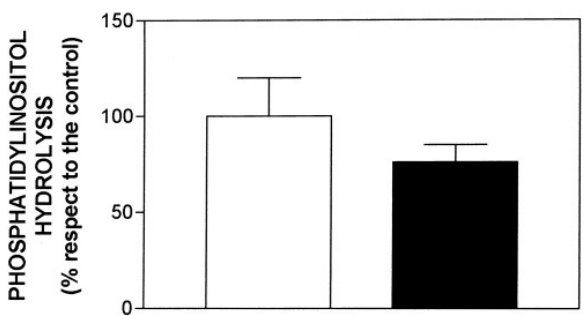

FIG. 5. Effects of ANF on phosphatidylinositol hydrolysis. Open column: control group (n=9); solid column: group incubated with 10nM ANF (n=8). Data are shown as mean ± SEM; n: number of experiments.

tion of TH enzyme number and intrinsec enzymatic activity represent the central means for controlling the biosynthetic pathway. Several mechanisms have been involved in the regulation of TH activity, such as a feedback inhibition of the enzyme by final product, allosteric modulation of the enzyme activity, and phosphorylation-dependent activation of the enzyme by different kinase systems (22,23). TH is phosphorilated in vitro by at least seven different protein kinases system, and many of these phosphorilation events can be associated with the increase or decrease in the enzyme activity (22,23). Cyclic AMP-dependent protein kinase, cGMP-dependent protein kinase, Ca^{2+} calmodulin-dependent protein kinase and Ca^{2+} phospholipid-dependent protein kinase have been identified as regulators of TH activity (22,23). Moreover, Ca^{2+} has been demonstrated to trigger phosphorylation and/or activation of TH in adrenal chromaffin cells (23). Furthermore, catecholamine biosynthesis is known to be tightly coupled to the amine secretion which depends upon the increase of Ca^{2+} in the cytoplasma (24). The effects of ANF on basal and induced, by 100mM KCl, TH activity in adrenal medulla were studied in order to assess a possible role for ANF as a putative neuromodulator of symphatoadrenergic function. Results showed that the atrial factor inhibited both, spontaneous and induced TH activity (24% and 59%, respectively). Moreover, ANF did not modify cAMP levels and phosphatidylinositol hydrolysis but, increased cGMP levels in the adrenomedular tissue, which suggest that cGMP is the signal transduction mechanism involved in the adrenal medulla for ANF. We have previouslyreported that ANF behaved as a partial blocker of calcium channels (14,25). On these basis, we can conclude that the atrial factor is likely to inhibit TH activity through a mechanism that involves a feedback inhibition induced by the increase of NE endogenous content, and/or an inhibition of TH phosphorilation through the reduction of the intracellular Ca^{2+} disposition, events that could be directly or indirectly mediated by cGMP (26). These mechanisms would finally lead to the reduction of the catecholamines biosynthesis.

On the other hand, the atrial factor reduced both, NE utilization and turn over. These results are in accordance with ANF effect on the synthesis, neuronal uptake and release of NE in the adrenal medulla. ANF increases NE uptake and diminished its biosynthesis and secretion, which strongly support the decrease in the utilization and turn over of NE.

In conclusion, with the results presented in this work and the data showed in other works (6,14,21,25) we could conclude that ANF may play a role in modulating the function of the symphatoadrenergic system, behaving as a putative neuromodulator in the adrenal medulla.

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