REVISTA ESPAÑOLA DE FISIOLOGÍA, 48 (3), 185-190, 1992

Effects of Atrial Natriuretic Peptide, Angiotensin II and III on Norepinephrine Uptake in the Rat Adrenal Medulla

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(Received on January 13, 1992)

M. S. VATTA, L. G. BIANCIOTTI and B. E. FERNÁNDEZ. *Effects of Atrial Natriuretic Peptide, Angiotensin II and III on Norepinephrine Uptake in the Rat Adrenal Medulla.* Rev. esp. Fisiol., 48 (3), 185-190, 1992.

The effects of atrial natriuretic peptide (ANP), angiotensin II (ANG II) and angiotensin III (ANG III) on norepinephrine (NE) uptake were studied in the adrenal medulla of the rat. One μM ANG II and 10 μM ANG III decreased NE uptake while 10 nM and 100 nM ANP increased it. Subthreshold concentrations of ANP (1 nM) blunted the inhibitory effect of 1 μM ANG II but did not modify the inhibitory effect of 10 μM ANG III. The increasing effects of 100 nM ANP on NE uptake were partially reversed by subthreshold concentrations of ANG II (1 nM) and blunted by 1 nM ANG III. The interaction between ANP and the renin-angiotensin system could contribute to modulate the sympathetic function in the adrenal medulla.

Key words: Adrenal medulla, Angiotensin II, Angiotensin III, Atrial natriuretic peptide, Norepinephrine uptake.

Atrial natriuretic peptide (ANP) is produced by the mammalian cardiac atria (4) and it is involved in the control of water and electrolyte balance and blood pressure regulation (3). ANP has hypotensive effects since it decreases the peripheral vascular resistance through vasodilatation and reduces blood circulating volume through natriuretic and diuretic mechanisms (4, 20). Previous reports have demonstrated that ANP modifies the uptake and release of norepinephrine (NE) in the central nervous system (18, 19).

Circulating angiotensin II (ANG II) has been reported to modify central nervous system catecholamines (6).

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The major metabolite of ANG II, angiotensin III (ANG III), has also hypertensive effects (7, 14). It has previously been demonstrated that ANG III inhibits NE uptake in the central nervous system (19).

Several authors have shown that ANP antagonizes ANG II and ANG III peripheral and central effects such as aldosterone and vasopressin secretion (2, 8), proximal tubular sodium and water reabsorption (11), constriction of vascular smooth muscle (9) and NE uptake (6).

The aim of the present work was to investigate whether ANP antagonized ANG II and ANG III effects in the adrenal medulla.

Materials and Methods

Wistar male rats weighing between 180-250 g were used in the experiments. Animals were decapitated between 10 and 12 h a.m. to avoid circadian changes (21). Adrenal glands were surgically dissected, the medullas being separated from the cortex and weighed. Adrenal medulla slices were incubated *in vitro*. The tissues were placed into a glass tube with a mesh of nylon fitted at the botton to allow free interchange with the medium. The whole system was placed in a beaker containing 2 ml of modified Krebs solution (1). Slices were equilibrated for 15 min at 37 °C, pH 7.4 and bubbled with carbogen (95 % O_2 + 5 % CO_2) under continuous shaking. NE stores were then labelled with 2.5 µCi/ml of d/1-7 (^{3}H) NE C1H (New England Nuclear) added to the incubation medium with or without the peptides. ANP (3-33 peptide, Peninsula Lab.), ANG II and ANG III (Sigma) effects on ^{3}H-NE uptake were studied in the following groups: a) Control; b), c) and d) incubated with 1 nM, 10 nM and 100 nM ANP, respectively; e), f), g) and h) incubated with 1 nM, 10 nM, 100 nM and 1 µM ANG II, respectively; i), j), k), l) and m) incubated with 1 nM, 10 nM, 100 nM, 1 µM and 10 µM ANG III, respectively. The following groups were studied to investigate ANP, ANG II and ANG III possible interactions: a) Control; b) and c) 1 nM and 100 nM ANP; d) and e) 1 nM and 1 µM ANG II; f) and g) 1 nM and 10 µM ANG III; h) 1 nM ANP + 10 µM ANG II; i) 1 nM ANP + 10 µM ANG III; j) 100 nM ANP + 1 nM ANG II; k) 100 nM ANP + 1 nM ANG III.

At the end of the incubation period, slices were washed in Krebs solution for 15 min and homogenized with 2.5 ml of 10 % trichloroacetic acid. The homogenates were centrifuged at 27,000 g. Tritium activity was determined in the supernatants by conventional scintillation counting methods.

Results are expressed as d.p.m./g of fresh tissue ± S.E.M. and submitted to the one way analysis of variance (ANOVA) and «t» test modified (17).

Results

Figure 1 illustrates that 100 nM and 10 nM ANP, 1 µM ANG II and 10 µM ANG III were the threshold concentrations required to modify total NE uptake, and 100 nM and 10 nM ANP increased total NE uptake while no modifications were observed with 1 nM ANP. On the other hand, 1 µM ANG II and 10 µM ANG III inhibited local ^{3}H-NE uptake, while 1 nM, 10 nM and 100 nM ANG II and 1 nM, 10 nM, 100 nM and 1 µM ANG III did not alter the amine uptake.

Subthreshold concentrations of ANP (1 nM) blunted 1 µM ANG II inhibitory effects whereas it did modify 10 µM ANG III inhibitory effects (fig. 2). Conversely 100 nM ANP increasing effects on NE uptake were only partially reversed by non-effective concentration of 1 nM ANG II and completely blunted by non-effective concentration of 1 nM ANG III.

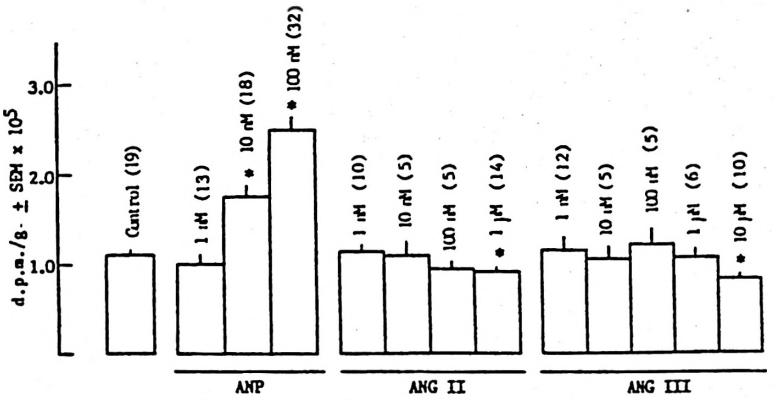

Fig. 1. *Effects of ANP, ANG II and ANG III on ^{3}H-NE uptake*
In parenthesis, number of experiments. * $p < 0.05$ vs control.

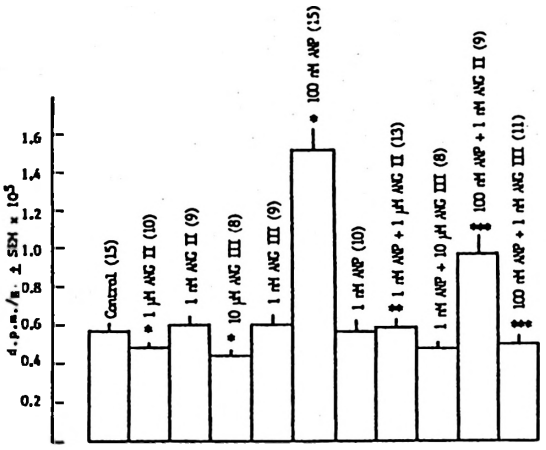

Fig. 2. *ANP, ANG II and ANG III interaction on ^{3}H-NE uptake*
In parenthesis number of experiments. * $p < 0.05$ compared with control; ** $p < 0.05$ compared with 1 μM ANG II and *** $p < 0.05$ compared with 100 nM ANP.

Discussion

Present results showed that both ANG II and ANG III inhibit ^{3}H-NE uptake in the adrenal medulla of the rat. ANG II threshold concentration to inhibit NE uptake was ten fold higher than that of ANG III (10 μM and 1 μM, respectively). The inhibitory effect of ANG III on NE uptake is a relevant finding since it has not been previously reported. ANP showed opposite effects to those of ANG II and III on the process of NE uptake. ANP stimulatory effects on the uptake were observed with 10 nM and 100 nM.

Previous reports have demonstrated that ANG II and ANG III bind to a single class of binding sites in the adrenal medulla. The concentrations of both peptide binding sites are similar, and unlabelled ANG II and ANG III displace (^{125}I) ANG III from its binding sites with the same potency in adrenal medulla which supports the view that ANG II and ANG III could bind to similar binding sites in adrenal medulla. PEACH *et al.* (15, 16) demonstrated that ANG III is less potent than ANG II as regards catecholamine secretion. Nevertheless the binding affinity of ANG III is slighty higher than that of ANG II, which suggests that such a difference may indicate that ANG III could modify the catecholamine releasing effects of ANG II. Present results indicate that ANG III also inhibits NE uptake, but with a 10 fold higher concentration than that of ANG II to produce the same effects. This suggests an ANG III physiological role on catecholamine metabolism modulating the adrenal medulla functionality. The presence of ANP has been observed in adrenal medulla vesicles (12).

ANP binding sites have also been described in adrenal medulla cells and correlate well with those of ANG II and ANG III in the same areas (13). Results show that ANP increased NE uptake in adrenal medulla slices and antagonized ANG II and ANG III effect on that process.

Similar ANP effects on catecholamine uptake have been reported in hypothalamus and medulla oblongata, which suggests the existence of an indirect mediated central effect of ANP on blood arterial pressure regulation (5). Present results demonstrate the existence of peripheral ANP effects which support the hypothesis that it can modulate central as well as peripheral sympathetic functions.

Subthreshold concentrations of ANP (1 nM) reversed the inhibitory effects of 1 μM ANG II on NE uptake, but failed to reverse 10 μM ANG III effects. On the other hand, non-effective concentrations of ANG II (1 nM) blunted the stimulatory effects of ANP on the amine uptake. The reversing effect of ANG II was partial whereas similar concentrations of ANG III blunted ANP effects and restored control levels of NE uptake. These results show a physiological interaction between the vasoactive peptides in the adrenal medulla. A similar interaction was reported in specific structures of the central nervous system such as hypothalamus and medulla oblongata (6, 19). NE was found to decrease 17 % by ANG II (1 μM) and 8 % by ANG III (10 μM), and to increase 158 % by 100 nM ANP. Subthreshold concentrations of ANP (1 nM) inhibited the ANG II effects on NE uptake but not the ANG III effects. In addition, 100 nM ANP stimulated uptake of the amine was inhibited 55 % and 100 % by ANG II and ANG III respectively in the adrenal medulla. Present results prove that ANG II and ANG III have differential effects on NE uptake inhibition depending on whether ANP is added; both antagonize ANP effects in the adrenal medulla.

Acknowledgements

This work was supported by grants from the «Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina» (CONICET)

Resumen

Se estudian los efectos del péptido natriurético auricular (ANP), la angiotensina II (ANG II) y la angiotensina III (ANG III) sobre el proceso de captación de noradrenalina (NA) en la médula adrenal de la rata.

La ANG II y la ANG III a 1 y 10 μM, respectivamente, disminuyen la captación de NA, mientras que concentraciones de ANP 10 nM y 100 nM la aumentan. Concentraciones subliminales de ANP (1 nM) reducen el efecto inhibidor de la ANG II (1 μM), pero no modifican la acción de la ANG III 10 μM. Concentraciones subumbrales de ANG II revierten parcialmente el aumento de la captación de NA producido por ANP 100 nM, mientras que la ANG III (1 nM) anula el incremento de la captación de NA inducido por el ANP. Los resultados obtenidos muestran que la interacción entre el ANP y el sistema renina-angiotensina podría contribuir a la regulación de la actividad simpática en la médula adrenal.

Palabras clave: Médula adrenal, Angiotensina II, Angiotensina III, Péptido natriurético atrial, Consumo de norepinefrina.

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