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Cardiovascular effects of urotensin II in different brain areas

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Abstract

It has been shown that intracerebroventricular injection of urotensin II (UII)-induced hypotensive and bradycardiac responses. Here, we tested the cardiovascular roles of UII in different brain areas by microinjection of UII into the A_1 and A_2 areas (noradrenergic cells found in the lower part of the medulla that have been designated either A_1 or A_2 areas), the paraventricular and the arcuate nucleus. In urethane-anaesthetized rats, we observed that: (1) microinjection of UII into the A_1 area induced dose-related depressor and bradycardiac responses; (2) mean arterial blood pressure (mABP) and heart rate (HR) did not change significantly after microinjection of UII into the A_2 area; and (3) significant increases in mABP and HR were induced after microinjection of 10 pmol UII into either the paraventricular or arcuate nucleus. The above results suggest that UII, in different brain areas, plays different roles in cardiovascular regulation and the A_1 area is a very important action site for UII in cardiovascular regulation.

Keywords: Urotensin II; A_1 area; A_2 area; Paraventricular nucleus; Arcuate nucleus

1. Introduction

Urotensin II (UII) is a 12-amino acid residue neuropeptide that was initially isolated from urophysical extracts of several species of fish [4,8]. Recent results indicate that UII is widely distributed in the central nervous system from molluscs to mammals [5,8,9,14], but with restricted distribution of UII mRNA to the medulla oblogata of the brain (the major integrating center for cardiovascular control) and spinal cord in humans [2]. It is also thought that UII may act as a neurotransmitter and/or neuromodulator in the central nervous system [2–5,9,10]. The expression of its specific G-protein-coupled receptor, GPR14, within the mammalian vasculature and nervous system suggests that it may regulate cardiovascular homeostasis [1,8–10,18]. Although UII acts as a strong vasoconstrictor peptide [1], it has been recently reported that intracerebroventricular injection or intraarterial injection of UII induces hypotensive and bradycardiac effects in rats [11], suggesting that central UII plays a hypotensive role in rats. However, its central acting sites in cardiovascular regulation remain unknown. As some brain areas such as the paraventricular and arcuate nuclei and the A_1 and A_2 areas play important roles in cardiovascular regulation [6,7,12,15,16], it is possible that central UII may play cardiovascular roles in these areas. Therefore, the cardiovascular effects of UII in these areas were examined by microinjection of UII into each of these areas.

2. Methods

Experiments were performed on male SD rats (180–260 g) housed 2–3 to a cage and having free access to food and water. After the rats were anesthetized with urethane (1.4 g/kg, i.p.), a catheter was inserted into the right carotid artery for measuring the mean arterial blood pressure (mABP) with a recorder (056–3002, Hitachi Ltd. Japan), and two electrodes were inserted in the forepaws for recording the heart rate (HR) with XDH-3 electrocardiography. Then, referring to the coordinate system of Jacobowitz and Palkovits [13,17], a stainless cannula was inserted into the following nuclei by means of sterotactic and positioner according to the following coordinates: the coordinate of the A_1 area was: P 6.0–8.0 mm, LR 1.8–2.0 mm, 2.3–2.5 mm under the surface of the cerebellum; the coordinate of the A_2 area was: P 7.4–8.0 mm, LR 0.1 mm, 0.2–0.3 mm under the surface of the medulla; the coordinate of the paraventriclar nucleus (NPV) was: A 5.340–5.660 mm, LR 0.3 mm, 6.7 mm under the surface of the cerebrum; and, the coordinate of the arcurate nucleus (AR) was: A 3.750–4.110 mm, LR 0.2 mm, 8.5–9.0 mm under the surface of the cerebrum. The synthetic rat UII (Phoenix Pharm Inc., USA) and bovine serum albumin (BSA, Sigma), were diluted with a 0.9% NaCl solution

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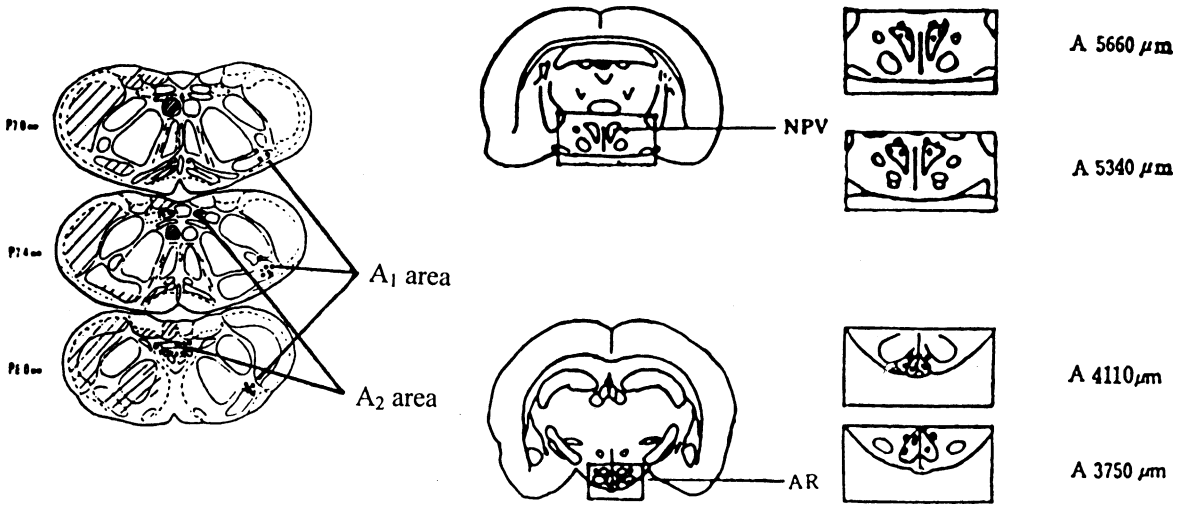

Fig. 1. Microinjection sites in paraventricular nucleus (NPV), arcuate nucleus (AR), A_1 area and A_2 area.

to 0.5, 5 or 50 nmol/ml and 8 μg/ml. Similar to our previous studies [20], after the UII or BSA was inspired into a 1 μl microsyringe, they were administered at a volume of 0.1 or 0.2 μl over a 10 s period via the stainless cannula. After each experiment, the rat was euthanized, the coronary brain slices which were parallel to the cannula were cut and the brain was taken out and fixed with 10% formalin. Then, the position of the cannula tip was histologically verified using 100 μm sections stained with Nissl stain (Fig. 1). Finally, all data were expressed as mean ± S.E. and analyzed using ANOVA, followed by Fisher LSD tests.

3. Results

In the following results, the basal mABP ranged from 83.0 ± 2.38 to 103.6 ± 7.12 mmHg and HR ranged from 357.4 ± 11.1 to 443.0 ± 24.4 beats/min before microinjection.

3.1. Effect of microinjection of UII into the A_1 area on mABP and HR

Significant dose-related depressor and bradycardiac responses were induced by microinjection of UII into the A_1 area. Significant decreases of mABP lasted more than 80 min after microinjection of 0.1, 1 or 10 pmol UII into this area, the maximal changes of mABP after microinjection of 0.1, 1 or 10 pmol UII being −20.83 ± 4.36.69, −38.83 ± 3.61 and −35.70 ± 3.61 mmHg, respectively. Furthermore, in the microinjection dose of 1 pmol, UII induced significant changes of HR in 5–30 min after microinjection of this drug and the maximal change was −25.5 ± 1.7 beats/min. In the microinjection of 10 pmol UII group, significant reductions of HR were observed in 5–75 min after the treatment and reached the maximal change value of −48.8 ± 3.2 beats/min (Fig. 2).

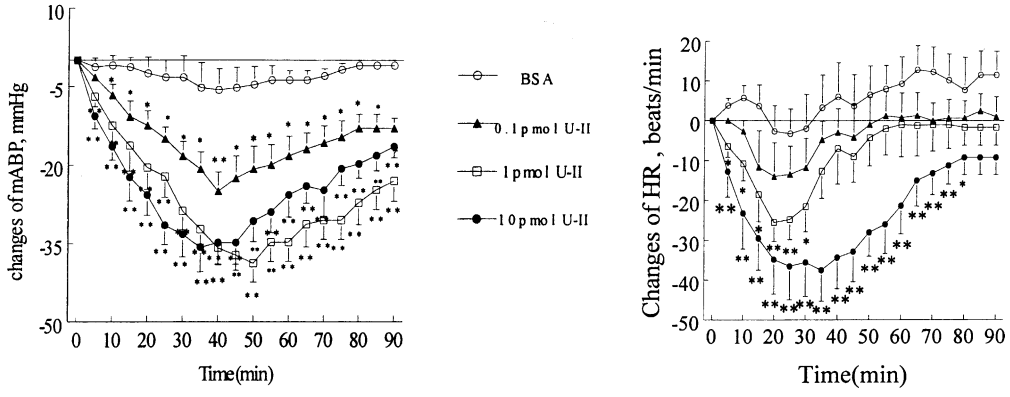

Fig. 2. Effects of microinjection of UII into A_1 area on mABP and HR. BSA: microinjection of BSA into A_1 area ($n = 6$); 0.1 pmol UII: microinjection of 0.1 pmol UII into A_1 area ($n = 6$); 1 pmol UII: microinjection of 1 pmol UII into A_1 area ($n = 6$); 10 pmol UII: microinjection of 10 pmol UII into A_1 area ($n = 6$). The symbols (∗) $0.01 < P < 0.05$, (∗∗) $P < 0.01$ compared with microinjection of BSA.

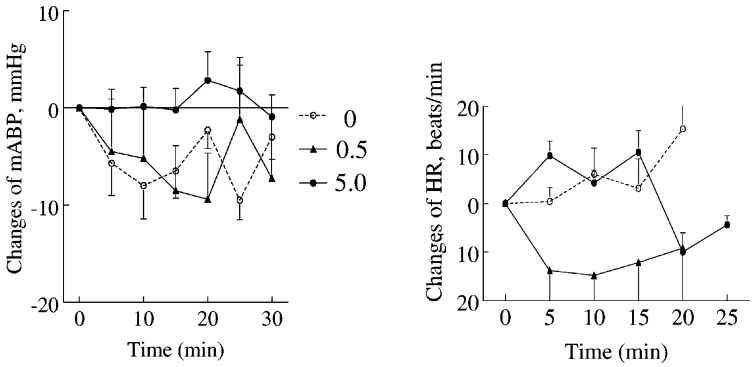

Fig. 3. Effects of microinjection of UII into A_2 area on mABP and HR. BSA: microinjection of BSA into A_2 area ($n = 6$); 0: microinjection of BSA into A_2 area ($n = 6$); 0.5: microinjection of 0.5 pmol UII into A_2 area ($n = 6$); 5.0: microinjection of 5 pmol UII into A_2 area ($n = 6$).

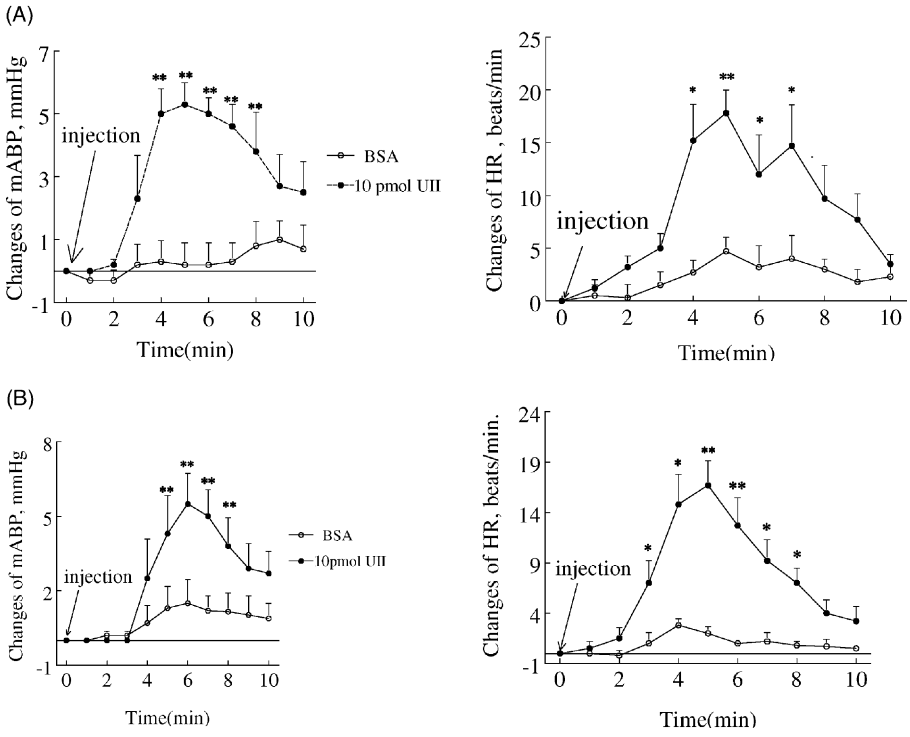

Fig. 4. Effects of microinjection of UII into NPV, AR on mABP and HR. BSA: microinjection of BSA into NPV ($n = 5$) or AR ($n = 6$); 10 pmol UII: microinjection of UII into NPV ($n = 6$); AR ($n = 5$). The symbols (∗) $0.01 < P < 0.05$, (∗∗) $P < 0.01$ compared with microinjection of BSA.

3.2. Effect of microinjection of UII into the A_2 area on mABP and HR

As depicted in Fig. 3, no significant changes in mABP or HR were observed after microinjection of UII into the A_2 area.

3.3. Effects of microinjection of UII into the NPV or AR on mABP and HR

Significant pressor and tachycardiac responses were induced in 3–8 min after microinjection of UII into either the NPV or AR, the maximal hypertensive effect of microinjection of UII into the NPV or AR being 6.5 ± 0.52 and 9.5 ± 0.64 mmHg, respectively, and the maximal increase in HR after microinjection of UII into the NPV or AR, respectively were 16.7 ± 3.0 and 17.8 ± 2.7 beats/min (Fig. 4).

4. Discussion

As mentioned above, intracerebroventricular injection of UII induces dose-related depressor effects. But, central

acting areas of UII on cardiovascular regulation remain unclear. The A_1 and A_2 areas are main noradrenergic neurons in the medulla [7]; noradrenergic and/or adreneric transmission are very important for cardiovascular control and restricted distribution of UII mRNA to the medulla oblogata of the brain (the major integrating center for cardiovascular control) and spinal cord in humans has been reported [2,7]. Thus, we tested the roles of UII in the A_1 and A_2 areas. In the present results, dose-related and long-lasting hypotensive and bradycardiac responses were induced by microinjection of UII into the A_1 area. Further, the A_1 area plays a very important role in cardiovascular regulation [7,19]. Thus, our results suggest that the A_1 area is an important hypotensive site for central UII. However, although the A_2 area also plays a very important role in cardiovascular regulation and significant depressor responses can be induced by chemical excitation of this area [6], no significant changes in mABP and HR were observed after microinjection of UII into this area. Since UII is widely distributed in the central nervous system from molluscs to mammals [5,8,9,14], this possibly suggests that UII selectively acts upon some areas of the brain in cardiovascular regulation. In rats, a hypertensive response is induced by microinjection of sodium glutamate into the paraventriclar nucleus but a hypotensive response was also observed by injection of this drug into the arcurate nucleus [6,12,15,16]. Interestingly, transient, slight but significant hypertensive and tachycardiac responses were observed by microinjection of UII into either the paraventriclar or arcurate nucleus. Thus, these results not only suggest that central UII plays different cardiovascular roles in different brain areas, but also supports an inference that it plays different roles in the neurons of different brain areas. That is, UII is likely to mainly play an inhibitory role in some cardiovascular regulating neurons in AR but an excitatory role in those neurons in the NPV. On the other hand, GPR14 is identical to the rat sensory epiththelium neuropeptide-like receptor that is expressed in neural tissue [18]. Furthermore, as an agonist for GPR14 and a potent stimulator of Ca^{2+} responses, UII activates this kind of receptor [1,2,18]. Thus, the inhibitory role of UII in some cardiovascular neurons is unlikely to result from the inhibition of the GPR14 receptors but likely to result from the excitation of inhibitory interneurons (such as GABAergic neurons in the AR etc.) in these areas.

In our present experiment, transient, smaller hypertensive and tachycardiac effects or no significant changes in mABP and HR were induced by microinjection of UII into the paraventricular nucleus, arcuate nucleus or A_2 area, while long-lasting, obviously hypotensive (over -30 mmHg in maximal change) and bradycardiac (over -40 beats/min in maximal change) effects were induced by microinjection of UII into the A_1 area. Since the i.c.v. injection of UII induces depressor responses, it seems possible that the A_1 area is a very important action site for UII in cardiovascular regulation.

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