Central Cardiovascular Action of Urotensin II in Spontaneously Hypertensive Rats

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We have previously reported that urotensin II acts on the central nervous system to increase blood pressure in normotensive rats. In the present study, we have determined the central cardiovascular action of urotensin II in spontaneously hypertensive rats (SHR). Intracerebroventricular (ICV) injection of urotensin II elicited a dose-dependent increase in blood pressure in both SHR and normotensive Wistar-Kyoto rats (WKY). The changes in mean arterial pressure induced by ICV urotensin II at doses of 1 and 10 nmol in the WKY were 8 ± 2 and 23 ± 3 mmHg, respectively. ICV administration of urotensin II caused significantly greater increases in blood pressure in SHR (16 ± 3 mmHg at 1 nmol and 35 ± 3 mmHg at 10 nmol, respectively) compared with those in WKY. Urotensin II (10 nmol) elicited significant and comparable increases in heart rate in SHR (107 ± 10 bpm) and WKY (101 ± 21 bpm). Plasma epinephrine concentrations after ICV administration of 10 nmol urotensin II were 203 ± 58 pmol/ml in SHR and 227 ± 47 pmol/ml in WKY, which tended to be higher than those in artificial cerebrospinal fluid-injected rats (73 ± 7 and 87 ± 28 pmol/ml, respectively, p < 0.1). The immunoreactivity of urotensin II receptor GPR 14 was expressed extensively in the glial cells within the brainstem, hypothalamus, and thalamus. These results suggest that central urotensin II may play a role in the pathogenesis of hypertension in SHR. Since GPR 14 was expressed in the glial cells of the brain, urotensin II may act as a neuromodulator to regulate blood pressure. (Hypertens Res 2003; 26: 839–845)

Key Words: urotensin II, GPR 14, blood pressure, central nervous system, immunohistochemistry
Microinjection of urotensin II into the A1 area of the brainstem has been shown to induce dose-related decreases in blood pressure and heart rate (HR). In contrast, microinjection of 10 pmol urotensin II into either the paraventricular or arcuate nucleus of the hypothalamus increased blood pressure and HR ([1]). These previous findings may support the idea that urotensin II participates in central regulation of blood pressure. We have previously reported that urotensin II acts on the central nervous system to increase blood pressure in normotensive rats ([2]). However, the role of central urotensin II in cardiovascular regulation in hypertensive animals has not been determined. In the present study, therefore, we examined the central cardiovascular action of urotensin II in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). The localization of urotensin II receptor GPR 14 in the brain was also determined to interpret the cardiovascular responses to central urotensin II in SHR.

Methods

Animal Preparation

Experiments were conducted on SHR (n = 24) and WKY (n = 24). The experiments were carried out following the guidelines of the Committee of Ethics in Animal Experimentation of the Faculty of Medicine, Kyushu University.

Intracerebroventricular Injection of Urotensin II

Twelve-week-old SHR (body weight 312 ± 3g, n = 20) and WKY (body weight 316 ± 2g, n = 20) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and indwelling arterial and venous catheters (PE-10 connected to PE-50) were advanced through the right femoral artery into the abdominal aorta. The catheters, filled with heparinized saline, were exteriorized and secured at the back of the rat’s neck. Intracerebroventricular (ICV) catheters were placed as described previously ([2], [3]). In brief, a 22-gauge stainless steel guide cannula was placed on the right lateral ventricle of the brain, 1.5 mm posterior from the bregma, 2 mm lateral from the midline, and 5 mm deep from the surface of the dura. The cannula was fixed to the skull with two screws and dental cement. After 24–48 h for recovery, the rats were placed in plastic boxes. The arterial catheter was connected to a pressure transducer (P50; Gould Statham Instruments, Hato Rey, Puerto Rico) for continuous recording of the arterial pressure and HR. SHR and WKY were respectively divided into three subgroups that received: 1) ICV injection of 1 nmol/10 µl urotensin II (n = 6 and n = 7, respectively); 2) ICV injection of 10 nmol/10 µl urotensin II (n = 7 each); and 3) artificial cerebrospinal fluid (aCSF, 10 µl, n = 7 and n = 6, respectively). After a stabilization period of at least 30 min, urotensin II (1 nmol or 10 nmol) or aCSF as a vehicle control was injected intracerebroventricularly. Blood samples (2 ml) were drawn from the arterial catheters to determine plasma catecholamine concentrations at 30 min after ICV injection of urotensin II. At the end of the experiments, dye (blue ink) was injected through the ICV cannula to verify its correct placement.

Urotensin II was dissolved in aCSF (in mmol/l: NaCl 133.3, KCl 3.4, CaCl2 1.3, MgCl2 1.2, NaH2PO4 0.6, NaHCO3 32.0, and glucose 3.4, pH 7.4).

Immunohistochemistry

Tissue Preparation

Separate groups of SHR (n = 4) and WKY (n = 4) were used for the experiments of immunohistochemical assays. Rats were anesthetized with sodium pentobarbital (100 mg/kg, i.p.), and were perfused transcardially with 100 ml of 0.1 mol/l phosphate-buffered saline (PBS, pH 7.4), followed by 200 ml of 10% formaldehyde solution. The brains were removed and kept in 10% formaldehyde solution for 24–36 h. The fixed brains were embedded in paraffin and processed for immunohistochemistry.

Single Immunohistochemistry

Serial sections were cut at 3 µm thickness, then were deparaffinized and rehydrated in graded alcohols. The slides were incubated in a 500-W microwave oven for 10 min in 10 mmol/l citrate solution buffer (pH 6.0) for antigen retrieval. The antibodies specific to rat GPR 14 (L-16, 1:400 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, USA) and glial filament protein (GFP, clone GF 12.24, 1:50 dilution; Progen Biotektronik Gmbh, Heidelberg, Germany) were applied at 4 ºC overnight (18 h). The primary antibodies were visualized by using appropriate biotinylated secondary antibodies and the streptavidine peroxidase staining method (Histofine SAB-PO Kit and DAB Kit; Nichirei Co., Osaka, Japan). Control slides were incubated with appropriate control immunoglobulin or PBS. The numbers of GPR 14-positive glial cells in the brain in each of 3 views in the high power field (40 × 1,200) of a microscope were counted and averaged.

Double Immunohistochemistry with Anti-GPR 14 and Anti-GFP Antibodies

After GPR 14 (L-16, 1:400 dilution) immunoreactivity was visualized with 3,3′-diaminobenzidine solution (DAB), and the slides were incubated at 4ºC overnight (18 h) in solution containing anti-GFP antibody (clone GF 12.24, 1:50 dilution). Then they were incubated for 30 min in the appropriate biotinylated secondary antibody. Brain regions positive for GFP immunoreactivity were visualized by the streptavidine alkaline phosphatase staining method (Histofine SAB-AP Kit and New Fuchsin Substrate Kit; Nichirei Co.).

Biochemical Measurements

Blood samples were centrifuged at 4ºC. Plasma samples for
the measurement of catecholamines were stored at -80°C. Plasma catecholamine concentrations were determined by high performance liquid chromatography.

**Statistical Analysis**

Values are expressed as the means ± SEM. In order to determine the cardiovascular effects of ICV urotensin II in SHR and WKY, one-way ANOVA with repeated measurements was performed in each strain, followed by Scheffe’s multiple range test to determine which means differed from the control means. Student’s t-test was used to compare the cardiovascular and plasma catecholamine responses to ICV urotensin II between SHR and WKY. P values less than 0.05 were considered statistically significant.

**Results**

**Intracerebroventricular Injection of Urotensin II**

ICV administration of urotensin II increased arterial pressure and HR in SHR and WKY, which returned to the baseline levels at 30 min after injection (Fig. 1). Figure 2 shows the time courses of mean arterial pressure (MAP) and HR before and after ICV injection of urotensin II in both strains. In SHR, ICV injection of 10 nmol of urotensin II resulted in significant (p < 0.01) increases in MAP and HR at 3 and 5 min after the injection. Urotensin II (10 nmol) also produced a significant (p < 0.05) increase in MAP at 3 to 10 min after the injection in WKY, whereas the change in HR was not significant. As shown in Fig. 3, the maximal increases in MAP were 8 ± 2 mmHg at 1 nmol (n = 7) and 23 ± 3 mmHg at 10 nmol (n = 7) of urotensin II in WKY. In contrast, the pressor responses in SHR were 16 ± 3 mmHg (n = 6) and 35 ± 3 mmHg (n = 7), respectively, which were significantly greater than those in WKY (p < 0.05). Since the baseline MAP in SHR was significantly higher than that in WKY (23.5 ± 2.0% vs. 19.4 ± 3.5%, at 10 nmol urotensin II).

Urotensin II (10 nmol) elicited significant and comparable increases in HR in SHR (107 ± 10 bpm) and WKY (101 ± 21 bpm). Plasma epinephrine concentrations after ICV administration of 10 nmol urotensin II were 203 ± 58 pmol/ml in SHR and 227 ± 47 pmol/ml in WKY, which tended to be higher than those in aCSF-injected rats (73 ± 7 and 87 ± 28 pmol/ml, respectively, p < 0.1, Fig. 4). The responses in plasma catecholamine concentrations did not differ between SHR and WKY.

**Immunohistochemistry**

The GPR 14 receptor was expressed exclusively in the glial cells of the rat brainstem (Fig. 5A) and hypothalamus (Fig. 5B). The results of double immunohistochemical staining with anti-GPR 14 and anti-GFP antibodies are shown in Fig. 5C. GPR 14-positive glial cells were also stained for anti-GFP antibody. The negative controls for normal goat serum did not exhibit any specific staining patterns (Fig. 5D). Numbers of GPR 14-positive glial cells in the hypothalamus and
in the brainstem did not differ between SHR and WKY (hypothalamus: 17.1 ± 3.1% vs. 16.8 ± 1.5%). GPR 14 immunoreactivity was also expressed in glial cells of the hippocampus and thalamus.

**Discussion**

In the present study, ICV injection of urotensin II caused significant increases in blood pressure and HR in both SHR and WKY. However, the pressor response of SHR was significantly greater than that of WKY. To the best of our knowledge, this is the first study to show that the central cardiovascular action of urotensin II was exaggerated in hypertensive animals.

Urotensin II was originally characterized as a vasoconstrictor peptide (2, 14). However, intravenous injection of urotensin II has been shown to decrease blood pressure in conscious rats, suggesting that urotensin II has a vasodilatory action (12, 15). These divergent cardiovascular effects of urotensin II may be attributable to the differences of the species or tissues used in the experiments (16, 17). It has been reported that urotensin II acts not only on the peripheral vascular bed but also on the central nervous system (7, 12).

In the present study, ICV injection of urotensin II caused an insignificant increase in plasma epinephrine concentrations determined at 30 min after the injection. We have previously reported that pretreatment with intravenous injection of pentolinium, a ganglion-blocking agent, prevented the pressor response to ICV urotensin II in conscious rats (12). Therefore, it is likely that the sympathetic nervous system contributed to the central pressor action of urotensin II in the present study, even though the plasma norepinephrine concentrations did not change.

Fig. 2. *Time courses of mean arterial pressure and heart rate in SHR and WKY given artificial cerebrospinal fluid (aCSF) or 1 nmol or 10 nmol of urotensin II (UTII). Values are the means ± SEM. ** p < 0.01, * p < 0.05 vs. SHR-aCSF; † p < 0.05 vs. WKY-aCSF.*

Fig. 3. *Bar graphs showing the central effects of 1 nmol and 10 nmol of urotensin II or artificial cerebrospinal fluid (aCSF) on maximal changes in mean arterial pressure (MAP) and heart rate (HR) in SHR and WKY. Values are the means ± SEM. * p < 0.01 vs. SHR-aCSF; ** p < 0.01 vs. WKY-aCSF.*
In the present study, ICV injection of urotensin II caused some dissociated responses of plasma epinephrine and norepinephrine concentrations—i.e., the plasma epinephrine concentration increased more than the plasma norepinephrine concentration. This dissociation is somewhat difficult to interpret; however, it may be due, at least in part, to some stimulatory effect of urotensin II on the adrenal gland. ICV injection of urotensin II has also been shown to increase the plasma concentrations of prolactin and thyroid stimulating hormone in rats (7). The present and the previous findings

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**Fig. 4.** Bar graphs showing the central effects of 1 nmol and 10 nmol of urotensin II or artificial cerebrospinal fluid (aCSF) on plasma epinephrine and norepinephrine concentrations in SHR and WKY. Values are the means \( \pm \) SEM. *p < 0.1 vs. aCSF.

**Fig. 5.** Photographs showing immunohistochemical localization of urotensin II receptor GPR 14 in the brain of SHR. A, GPR 14 immunoreactivity in the brainstem; B, GPR 14 immunoreactivity in the hypothalamus; C, GPR 14-GFP double immunohistochemistry of the brainstem; D, Control staining for normal goat serum in the brainstem.
suggest that central urotensin II modulates the hypothalamos- pituitary-adrenal axis. Furthermore, microinjection of uroten- sin II into the A1 area has been reported to induce dose-related depressor and bradycardiac responses, while microinjection of 10 pmol urotensin II into the paraventricular or arcuate nucleus of the hypothalamus increased blood pressure and HR (11). These previous findings support the idea that urotensin II acts at the central nervous system to participate in cardiovascular and hormonal regulation.

ICV-injected urotensin II is widely distributed to many brain regions through the cerebrospinal stream; therefore, in the present study, we were not able to determine the particular brain site at which urotensin II acts. Previous studies have shown that urotensin II, its precursor propro-urotensin II, and GPR 14 are mainly localized in the brainstem and spinal cord (2, 4, 8). GPR 14 mRNA expression is found in ubiquitous brain areas, with the highest levels occurring in the amygdala, cerebellum, cerebral cortex, medulla oblongata, pons, and thalamus, and lower levels occurring in the hippocampus, hypothalamus, striatum, and spinal cord (7, 8). In the present study, immunoreactivity of GPR 14 was extensively expressed in the glial cells of the rat brainstem and hypo- thealamus, suggesting that urotensin II may act as a neuro- modulator rather than a neurotransmitter in the brain to activate the sympathetic nervous system. Enhanced sympathetic outflow from the central nervous system to the peripheral or- gans could be blocked by a ganglion-blocking agent. The hy- pothalamus and the nuclei in the medulla oblongata, such as the nucleus of the solitary tract, ventrolateral medulla, and area postrema, are candidate brain regions involved in the central cardiovascular action of urotensin II. In addition, in our preliminary experiment in the present study, immuno- reactivity of GPR 14 in the brainstem and the hypothalamus was not different between SHR and WKY. It is unlikely that differences in the number of urotensin II receptors, GPR 14, in the brain contributed to the exaggerated pressor response in SHR. Further studies focusing on the cardiovascular ac- tion of urotensin II in these regions are required.

ICV injection of urotensin II caused significantly greater increases in blood pressure in SHR than in WKY. In contrast, urotensin II elicited significant and comparable increases in HR in SHR and WKY. Thus the blood pressure and HR response to ICV urotensin II were not parallel between SHR and WKY. These dissociated responses may be explained by the differential regulation of blood pressure and HR—i.e., HR is influenced by the changes in blood pressure via the baroreceptor reflex and is dually regulated by the sympatheti- c and parasympathetic nervous systems. An enhanced in- crease in the blood pressure response to ICV urotensin II might suppress any further increase in HR via the barorecep- tor reflex in SHR.

The present study did not clarify the underlying mecha- nisms of the exaggerated pressor response to ICV urotensin II in SHR. Microinjection of excitatory neurotransmitters such as L-glutamate or N-methyl-d-aspartate into the rostral ventrolateral medulla (RVLM) has been shown to evoke the enhanced pressor response in SHR compared to WKY (18, 19). In the present study, the role of the RVLM on the cardio- vascular response to urotensin II was not determined. However, the differences of the responses within the RVLM might contribute to the augmented pressor response by ICV urotensin II in SHR.

In conclusion, ICV injection of urotensin II elicited an ex-aggerated pressor response in SHR compared with that in WKY. Urotensin II acts at the central nervous system to in- crease blood pressure, and central urotensin II may con- tribute to the pathogenesis of hypertension in SHR. Since GPR 14 was expressed in the glial cells of the brain, urotensin II may act as a neuromodulator to regulate blood pressure.

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References


