Sympathetic and cardiovascular actions of orexins in conscious rats

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Shirasaka, Tetsuro, Masamitsu Nakazato, Shigeru Matsuoka, Mayumi Takasaki, and Hiroshi Kannan. Sympathetic and cardiovascular actions of orexins in conscious rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1780–R1785, 1999.—The novel hypothalamic peptides orexin-A and orexin-B are known to induce feeding behavior when administered intracerebroventricularly, but little is known about other physiological functions. The renal sympathetic nerves play important roles in the homeostasis of body fluids and the circulatory system. We examined the effects of intracerebroventricularly administered orexins on mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), and plasma catecholamine in conscious rats. Orexin-A (0.3, 3.0 nmol) provoked an increase in MAP (94.3 ± 0.7 to 101.9 ± 0.7 mmHg and 93.1 ± 1.1 to 108.3 ± 0.8 mmHg, respectively) and RSNA (28.0 ± 7.0 and 57.9 ± 12.3%, respectively). Similarly, orexin-B (0.3, 3.0 nmol) increased MAP (93.9 ± 0.9 to 97.9 ± 0.9 mmHg and 94.5 ± 1.1 to 105.3 ± 1.7 mmHg, respectively). Orexin-A and -B at 3.0 nmol also increased HR. In other conscious rats, a high dose of orexin-A and -B increased plasma norepinephrine. Plasma epinephrine only increased with a high dose of orexin-A. These results indicate that central orexins regulate sympathetic nerve activity and affect cardiovascular functions.

MATERIALS AND METHODS
Animal preparation and data collection. Male Wistar rats weighing 350–450 g each were implanted with a lateral cerebroventricular cannula while under anesthesia by intraperitoneal injection of pentobarbital sodium (50 mg/kg). A 24-gauge stainless steel guide cannula (length 19 mm) was positioned 2.5 mm from the cortex surface and 1 mm above the left lateral cerebroventricle through a burr hole located stereotaxically 0.8 mm posterior and 1.5 mm lateral to the bregma. The guide cannula was fixed to the skull with four screws and dental cement. Acute elevation (>20 mmHg) in MAP and persistent (at least 10 min) water-drinking response to intracerebroventricular administration of 10 pmol ANG II were both considered to be indicators of cannula patency and proper placement in the ventricular system. Approximately 10 days later, the cannulated rats were given pentobarbital sodium anesthesia (50 mg/kg ip). SP-31 tubing heat coupled to an SP-50 and a PE-50 catheter was inserted into the abdominal aorta and the inferior vena cava for measurement of blood pressure (BP) and for intravenous administration of drugs, respectively. The arterial cather,
were divided into five groups (Kyoto, Japan).

Immediately heparinized and centrifuged at 3,000 rpm and age-matched rats was anesthetized with pentobarbital and cardiovascular and sympathetic responses, the area under the curve (AUC) was calculated (1). Maximum changes from control values and the AUC were analyzed using a Student’s t-test. The correlation coefficient (r) was analyzed using a Pearson’s correlation coefficient. P < 0.05 was considered statistically significant.

RESULTS

Effects of orexins on MAP, HR, and RSNA. Intracerebroventricularly administered orexin-A resulted in a rapid and progressive increase in MAP, with a 15.3 ± 1.1 mmHg increase at a high dose (3.0 nmol; Fig. 1). HR also increased rapidly and reached peak value 15 min after orexin-A (3.0 nmol) administration. This high dose of orexin-A additionally resulted in an ~60% increase in RSNA 10 min after injection, which persisted for ~15 min. RSNA also increased transiently at a low dose (0.3 nmol) of orexin-A. Increases in all these parameters were significantly larger at 3.0 nmol than at 0.3 nmol. Vehicle (saline; n = 8) did not produce any effects on MAP, HR, or RSNA (Fig. 1). To further clarify the relationship between sympathetic nerve activity and cardiovascular responses, the correlation coefficient (r) between RSNA and MAP or HR was examined. There was a statistically significant correlation between RSNA and MAP (r = 0.69 and r = 0.83, respectively; both P values < 0.001) or HR (r = 0.76 and r = 0.89, respectively; both P values < 0.001) at 0.3- and 3.0-nmol doses in the orexin-A-injected group. Central orexin-B also produced a significant increase in MAP, with a 10.8 ± 0.2 mmHg increase at the high dose, this response pattern being similar to what was observed for orexin-A administration (Fig. 2). HR also rapidly increased and returned to the control level within 30 min at 3.0 nmol. In contrast to the results with orexin-A, RSNA did not increase significantly at any dose of orexin-B (Fig. 2). Increases in MAP and HR were larger at 3.0 nmol than at 0.3 nmol. For each dose, the maximum changes from control values during recording time (60 min) were compared for orexin-A and -B (Fig. 3A). An increase in MAP induced by central orexin-A was 1.5-fold larger than that of orexin-B for both doses, but significant differences were not observed in HR. The increase in RSNA produced by intracerebroventricularly administered orexin-A was larger than that of orexin-B at 3.0 nmol (Fig. 3A). The AUC was calculated for the 60-min period immediately after peptide injection for each animal within a group to provide a description of both the duration and magnitude of the cardiovascular and sympathetic responses.
(Fig. 3B). The AUC in MAP and HR was significantly larger in orexin-A than -B at only 3.0 nmol. On the other hand, the AUC in RSNA was 10-fold larger in orexin-A (2,027.3 ± 383.4 %·m; P < 0.05) than -B (186.0 ± 68.1 %·m) at 3.0 nmol (Fig. 3B). At a low dose (0.3 nmol) of orexin-A, the AUC in RSNA (607.2 ± 118.9 %·m; P < 0.05) was approximately sixfold larger than at the same dose of orexin-B (99.1 ± 51.2 %·m).

Effects of orexins on plasma catecholamine. A high dose of orexin-A (3.0 nmol) increased plasma epinephrine (Epi) from 136.4 ± 9.1 to 433.7 ± 59 pg/ml 10 min after injection (Fig. 4A). A tendency toward an increase in Epi was observed with a high dose of orexin-B, but it was not significant (Fig. 4B). Changes in plasma norepinephrine (NE) concentration did not occur at low doses of orexin-A and -B (Fig. 4, A and B). However, with the high dose of orexin-A, plasma NE increased from 104.5 ± 4.2 to 351.1 ± 34.6 pg/ml 10 min after injection, and the increase continued to 284.7 ± 23.8 pg/ml after 60 min. The magnitude of increase in plasma CA
induced by central orexin-A was significantly larger at 3.0 nmol than at 0.3 nmol (Fig. 4A). The high dose of orexin-B produced an increase in plasma NE from 104.1 ± 5.1 to 253.6 ± 54.1 pg/ml 10 min after injection, but the level decreased after 60 min (Fig. 4B). Vehicle (saline; n = 7) did not produce any effects on plasma CA (Fig. 4A).

**DISCUSSION**

This is the first study showing the effects of central orexins on cardiovascular parameters and sympathetic nerve activity recorded directly in conscious, unrestrained rats. Intracerebroventricular administration of orexin-A (0.3 and 3.0 nmol) resulted in a progressive increase in MAP; this MAP increase is also associated with increases in HR and RSNA. Significant correlations were observed between RSNA and MAP or HR for both doses in orexin-A. Although RSNA does not always reflect general sympathetic nerve activity, it is likely that orexin-A induced increases in MAP and HR due to an increase in sympathetic outflow. Similarly, centrally administered orexin-B increased MAP and HR dose dependently, but there were no significant changes in RSNA at any dose. In the regulation of food intake, no significant differences between the effects of orexin-A and -B have been observed (15). Our study indicated that the responses of cardiovascular and sympathetic nerve activity induced by central orexin-A were larger than those of orexin-B. In almost all orexin intracerebroventricularly administered rats, increases in locomotor activities, such as chewing and grooming, were observed. Muscle exercise and postural change are well known to induce the activation of sympathetic outflow (12). To exclude the effects of locomotion on these parameters, we also injected orexin-A and -B (3.0 nmol; n = 6) centrally in rats anesthetized with pentobarbital sodium (50 mg/kg ip). Intracerebroventricularly admin-
istered orexin-A increased MAP (control value: 95.2 ± 0.9 mmHg; maximum value: 105.9 ± 3.2 mmHg; P < 0.05), HR (control value: 359.2 ± 2.6 beats/min; maximum value: 394.3 ± 12.2 beats/min; P < 0.05), and RSNA (control value: 100%; maximum value: 131.1 ± 4.5%; P < 0.05), and orexin-B increased MAP (control value: 93.4 ± 1.5 mmHg; maximum value: 101.8 ± 2.3 mmHg; P < 0.05) and HR (control value: 361.5 ± 4.1 beats/min; maximum value: 388.2 ± 8.7 beats/min; P < 0.05), indicating that the increases in these parameters were not due to the rats' activated locomotion.

The existence of regional differences in sympathetic outflow has been demonstrated (23). Thus, to examine systemic sympathetic outflow induced by central orexin, plasma CA was measured under similar conditions to record nerve activity. High doses of orexin-A and -B increased plasma NE, the effect being larger and lasting longer with orexin-A. Therefore, it is likely that the orexin-induced increase in sympathetic nerve outflow leads to the increase in plasma NE, which produces cardiovascular responses. The elevated circulating levels of Epi, as well as NE after injections of the high dose of orexin-A, suggest the activation of the sympathoadrenal medullary system (SA system). In contrast to orexin-A, central orexin-B did not produce an increase in plasma Epi. The large pressor response induced by central orexin-A, compared with that induced by orexin-B may be due to activation of the SA system in addition to sympathetic outflow. These results suggest that intracerebroventricularly administered orexin-A and -B produce cardiovascular responses via different central mechanisms.

Both orexin-A and -B nerve fibers projected widely into the rat brain (4). mRNA for two orexin receptors (OX1R and OX2R) distributed extensively in the rat brain (21). Within the hypothalamus, OX1R mRNA is most abundant in the ventromedial hypothalamic nucleus (21), which plays an important role in the homeostatic regulation of body metabolism mediated through the sympathetic nerves (17). In contrast, OX2R mRNA exists mainly in the PVN (21), which is involved in the integration of the autonomic nervous and neuroendocrine systems (19). Orexin-A and -B bind to both receptors, but the affinity for OX1R and OX2R is different (15). Although our study could not elucidate the action sites of both orexins, our observation that the effects of orexin-A and -B were quantitatively and qualitatively different suggests that both orexins play an important, but different, role in the central regulation of the autonomic nervous system. Although the
pathophysiological role of the activation of sympathetic outflow induced by orexin is not clear, a number of studies have clearly documented the close relationship between obesity, hypertension, and altered cardiovascular responses (10). In this regard, leptin, the peptide product of the obese gene (25), has been reported to decrease food intake (3) and to produce an increase in MAP (5, 16) and RSNA (5). Therefore, these neuropeptides, leptin and orexin, which are involved in the control of energy balance, may be chemical mediators in the brain that are responsible for the generation and maintenance of hypertension.

In conclusion, in addition to their potent effects on appetite, orexins may interact with the brain system, controlling sympathetic outflow and cardiovascular function and may, therefore, have a broader spectrum of action than previously hypothesized.

Perspectives

Orexin was initially reported as a regulator of food intake (15). More recent reports suggest its possible important roles in the multiple functions of neuronal systems (8, 14, 22). Central orexin-A induces face washing behavior and grooming (8), which is known to be related to a stress response (7). Stress has been reported to cause an increase in sympathetic nerve activity (11, 20). Our observation is that increases in RSNA and plasma CA produced by central orexin-A may be relevant to CA-induced excessive baroreceptor activation. Stress increases sympathetic nerve activity during spinal cord heating and cooling in rats. Proc. Natl. Acad. Sci. USA 96: 748–753, 1999.

Orexin is a neuropeptide that is also expressed in the lateral hypothalamus. Its expression is increased in response to stress and epinephrine in humans. Am. J. Physiol. 257 (2 Pt 2): R1785–R1790, 1989.

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