Pressor effects of orexins injected intracisternally and to rostral ventrolateral medulla of anesthetized rats

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Chen, Chiung-Tong, Ling-Ling Hwang, Jaw-Kang Chang, and Nae J. Dun. Pressor effects of orexins injected intracisternally and to rostral ventrolateral medulla of anesthetized rats. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R692–R697, 2000.—Orexin A and B, two recently isolated hypothalamic peptides, have been reported to increase food consumption upon intracerebroventricular injection in rats. In addition to the hypothalamus, orexin A-immunoreactive fibers have been observed in several areas of the medulla that are associated with cardiovascular functions. The present study was undertaken to evaluate the hypothesis that orexins may influence cardiovascular response by interacting with neurons in the medulla. Intracisternal injection of orexins A (0.005–7.0 nmol) or B (0.028–0.28 nmol) dose dependently increased mean arterial pressure (MAP) by 4–27 mmHg and heart rate (HR) by 26–80 beats/min in urethan-anesthetized rats, with orexin A being more effective in this regard. MAP and HR were not changed by intravenous injection of orexins at higher concentrations. Microinjection of orexin A (14 pmol/50.6 nl) to the rostral ventrolateral medulla, which was confirmed by histological examination, increased MAP and HR. Our results indicate that, in addition to a role in positive feeding behavior, orexins may enhance cardiovascular response via an action on medullary neurons.

THE 33- AND 28-AMINO ACID peptides orexin A and B, known also as hypocretin 1 and 2, are two recently isolated hypothalamic peptides that have been implicated in positive feeding behavior and energy metabolism (9, 23). For example, mRNA transcript of prepro-orexin and immunoreactivity to orexin A are detected in a discrete population of neurons in the lateral and dorsal hypothalamus of the rat (5, 9, 23), which have been postulated as the feeding center (2, 3). Second, prepro-orexin mRNA transcripts are overexpressed in fasting rats (23). Third, orexins injected intracerebroventricularly promoted food consumption in rats (11, 23).

Recent immunohistochemical studies showed that orexin-immunoreactive nerve fibers are present in areas of the medulla that are associated with central regulation of autonomic, neuroendocrine, and neurosecretory functions (7, 18, 20). The presence of orexin A-immunoreactive fibers in the nucleus tractus solitarius, dorsal motor nucleus of the vagus, and reticular formation (7, 9, 20) raises the possibility that the peptide may participate in central regulation of cardiovascular functions. The present study was undertaken to explore the potential role of orexins in medullary control of arterial pressure (AP) and heart rate (HR) by means of intracisternal administration and microinjection techniques. Intracisternal administration was used to deliver a relatively high concentration of orexins to the medulla where some of the pressor and depressor neurons are thought to be situated (14). Microinjection of orexin A was made to the rostral ventrolateral medulla (RVLM), the presumed output neurons from the medulla to sympathetic preganglionic neurons in the spinal cord (22).

METHODS

Chemicals. Orexin A and B were from Phoenix Pharmaceuticals (Mountain View, CA). Urethan, heparin, and all other chemicals were from Sigma Chemical (St. Louis, MO).

Preparation of animals. Sprague Dawley rats were purchased from Harlan Sprague Dawley (Indianapolis, IN). Animal protocols were approved by the University Animal Care and Use Committee.

Adult male rats, weighing 268 ± 58 g (mean ± SD, n = 62), were anesthetized with urethan (1.2 g/kg ip). The left femoral artery was cannulated with a PE-50 tube containing heparin (10 U/ml) physiological saline and was connected to a pressure transducer with its output to a Gould pen recorder as described (16). The pressure signal was used to trigger a Biotach amplifier (Gould ECG/Bi tac) for HR recording. Calculation of mean arterial pressure (MAP) is based on the following equation, MAP = diastolic pressure + (systolic pressure – diastolic pressure)/3. The right femoral vein was cannulated with a PE-50 tube for intravenous injection of orexins or supplemental urethan when necessary. Animals were then prepared for intracisternal administration or microinjection.

Intracisternal administration. The rat head was fixed on a stereotaxic device (David Kopf Instruments, Tujunga, CA). Surgery was carefully performed to expose the dura above the cisternal magna of the fourth ventricle. A 27-gauge needle was connected to one end of a PE-10 tube with the other end connected to a syringe. The needle tip was carefully inserted through the dura into the cisternal magna with minimal leakage of cerebrospinal fluid.

Artificial cerebrospinal fluid (aCSF) had the following composition (in mM): 125.1 NaCl, 2.6 KCl, 0.9 MgCl2, 1.3 CaCl2, 21.0 NaHCO3, 2.5 Na2HPO4, and 120 mg/ml BSA; the
solution was bubbled with 95% O₂ and 5% CO₂ to pH 7.4. Orexin A and orexin B were dissolved in aCSF and were infused into the cisternal magna in a fixed volume of 5 µl over 1 min. Control experiments with intracisternal injection of 5 µl aCSF were carried out in 10 rats.

Microinjection in RVLM. Male Sprague-Dawley rats of 315–410 g were prepared for measurements of AP and HR as described above. The rat head was fixed on a stereotaxic device from David Kopf Instruments. The stereocoordinates for RVLM were 2.0–2.5 mm lateral to the midline, 11.5–13.5 mm caudal to the bregma, and 8.0–8.5 mm ventral to the surface of the cerebellum. The skull of the right position was marked, and the cerebellum was exposed. A glass micropipette with an opening tip of ~30 µm was used in conjunction with a Nanoject II from Drummond Scientific (Broomall, PA) to accomplish the microinjection task. The injector was held with a stereotaxic device and was adjusted to introduce 50.6 nl/administration. Orexin A (1 µg/µl) dissolved in aCSF was kept as stock solution. Orexin A (14 pmol) or aCSF was administered in 50.6 nl to one (left) side of RVLM in urethan-anesthetized rats. At the end of experiments, the rats were overdosed with urethan, and the brains were harvested and kept in 4% paraformaldehyde in PBS (pH 7.4) overnight. The brainstem was cut into 10-µm sections and stained with cresyl violet. The injection site was confirmed by microscopic examination.

Statistical data analysis. Data in the figures were expressed as means ± SE or as indicated elsewhere. Significant differences between treatment groups and dose- and time-dependent relationships were determined using one-factor or two-factor ANOVA followed by the Student-Newman-Keuls test with SPSS 8.0 from SPSS (Chicago, IL). The intracisternal ED₅₀ doses for MAP and HR were determined by dose-response curve fitting with the sigmoidal four-parameter logistic model (8) using nonlinear regression of the SAS software (Cary, NC). The dose-response equation is expressed as

$$\text{Maximal increase of MAP or HR} = \frac{a - d}{1 + \left(\frac{d}{c}\right)^b} + d$$

where $a$ is the response of vehicle control, $b$ is the curve shape parameter, $c$ is the ED₅₀, and $d$ is the achievable maximal effect at high doses.

RESULTS

Effects of intracisternal orexins on MAP and HR. Orexin A or orexin B injected intracisternally increased MAP and HR in a dose-dependent manner. Figure 1A shows an experiment in which intracisternal orexin A (0.28 nmol) produced a prolonged rise in AP and HR. The AP gradually returned toward the baseline 1 h after the injection, at which time the HR remained elevated (Fig. 1A). At the lowest dose (0.0056 nmol) tested, orexin A caused a statistically significant increase in HR but not the MAP (Fig. 2). At a higher dose (0.028 nmol), orexin A caused a significant rise in both the MAP and HR (Fig. 2). Figures 2 and 3 show dose- and time-dependent changes of MAP and HR after intracisternal orexin A. MAP and HR recovered slowly.

Fig. 1. Effects of orexins on arterial pressure (AP) and heart rate (HR) of urethan-anesthetized rats. A: orexin A (0.28 nmol) injected intracisternally (ic) caused a slow onset but long-lasting increase in both AP and HR; the latter recovered in 2–3 h (the entire time course not shown). bpm, Beats/min. B and C: orexins A or B (11 or 11 nmol/kg) administered intravenously caused no significant changes in AP and HR. D: orexin A (14 pmol) unilaterally microinjected to rostral ventrolateral medulla (RVLM) caused a rapid onset of increase of AP and HR; both recovered in 1 h.
in 2–3 h after intracisternal orexins. As a result, the time course of full recovery was not shown. A significant increase in MAP and HR was observed 2–6 min after intracisternal administration of effective doses of orexin A. At the highest dose (7.0 nmol) of orexin A tested, both effects developed within 2 min. In general, the maximal increase of MAP and HR was attained 6 and 28 min after intracisternal injection, respectively. The ED50 dose for orexin A in relation to HR (ED50 = 0.024 nmol) was less than that for MAP (ED50 = 0.11 nmol), indicating a differential sensitivity of these two components to orexin A.

At the two midrange doses (0.028 and 0.28 nmol) tested, orexin B also caused a significant increase in MAP and HR. Again, the increase in HR produced by orexin B was longer lasting compared with that of MAP (Fig. 2B). The maximal increase in MAP and HR by orexin B was attained ~6 and 12 min after intracisternal injection, respectively. The ED50 dose for orexin A in relation to HR (ED50 = 0.024 nmol) was less than that for MAP (ED50 = 0.11 nmol), indicating a differential sensitivity of these two components to orexin A.

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At the two midrange doses (0.028 and 0.28 nmol) tested, orexin B also caused a significant increase in MAP and HR. Again, the increase in HR produced by orexin B was longer lasting compared with that of MAP (Fig. 2B). The maximal increase in MAP and HR by orexin B was attained ~6 and 12 min after intracisternal injection (Fig. 2B). In comparison with the same doses of orexin A, orexin B produced a smaller change in MAP and HR (Fig. 3). Injection of aCSF of equal volume caused no significant changes in MAP and HR in the control group. The basal MAP and HR of different intracisternal treatment groups were summarized in Table 1 and show no significant differences (P > 0.1, ANOVA).

**DISCUSSION**

The principal observation made in this study is that the orexigenic peptides, orexin A and B, cause pressor responses when administered intracisternally or microinjected into the RVLM in urethan-anesthetized rats.

Immunohistochemical studies have revealed the presence of a moderate number of orexin A-immunoreactive...
fibers in the nucleus of the solitary tract, dorsal motor nucleus of the vagus, and reticular formation (7, 9, 20). The presence of orexin-immunoreactive fibers in areas of the medulla containing pressor and/or depressor neurons provides an anatomical substrate for a role of orexins in modulating medullary output to the cardiovascular system. In view of the diffuse nature of orexin A fibers in the medulla, we first elected to administer the peptide intracisternally, and the changes in MAP and HR were taken as an approximation of the global response of medullary neurons to the peptide.

Orexin A or B administered into the cisternal magna consistently and dose dependently increased MAP and HR. Figure 3 illustrates the maximal increases of mean arterial pressure (MAP; A) and HR (B) by intracisternal orexins (Or). Maximal increases in MAP and HR after intracisternal injection of orexins at different doses were expressed as means ± SE. Dose-dependent relationship was determined by 1-factor ANOVA (P < 0.001), and the significant difference between groups was evaluated by the Student-Newman-Keuls test as indicated. *P < 0.05 and **P < 0.01.

Table 1. Basal MAP and HR in different groups of rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Basal MAP, mmHg</th>
<th>Basal HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF</td>
<td>10</td>
<td>104 ± 15</td>
<td>367 ± 16</td>
</tr>
<tr>
<td>OrA, nmol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0056</td>
<td>6</td>
<td>101 ± 7</td>
<td>370 ± 17</td>
</tr>
<tr>
<td>0.028</td>
<td>8</td>
<td>104 ± 15</td>
<td>356 ± 27</td>
</tr>
<tr>
<td>0.28</td>
<td>7</td>
<td>95 ± 10</td>
<td>373 ± 42</td>
</tr>
<tr>
<td>1.4</td>
<td>3</td>
<td>87 ± 14</td>
<td>337 ± 28</td>
</tr>
<tr>
<td>7.0</td>
<td>3</td>
<td>105 ± 11</td>
<td>352 ± 10</td>
</tr>
<tr>
<td>OrB, nmol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.028</td>
<td>6</td>
<td>86 ± 19</td>
<td>361 ± 37</td>
</tr>
<tr>
<td>0.28</td>
<td>6</td>
<td>89 ± 18</td>
<td>371 ± 29</td>
</tr>
</tbody>
</table>

Basal mean arterial pressure (MAP) and heart rate (HR) of each treatment group were expressed as means ± SD. All agents were intracisternally administered in fixed volume of 5 µl. aCSF, artificial cerebrospinal fluid was used as the vehicle control; OrA, orexin A; OrB, orexin B. P > 0.1 (ANOVA) among groups.

Figure 4 shows changes in MAP and HR with time after unilateral microinjection of orexin A to the RVLM in urethan-anesthetized rats. Orexin A (14 pmol) injected unilaterally into the RVLM caused increases in both MAP and HR (P < 0.001, ANOVA). Data in A and B were expressed as means ± SE for orexin A (●) and the vehicle aCSF control (○). *Significant difference between orexin A-treated and aCSF control groups (P < 0.05). Injection sites of orexin A (●) and aCSF control (○) can be seen in C. 10, Dorsal motor nucleus of the vagus; 12, hypoglossal nucleus; Amb, ambiguous nucleus; Cu, cuneate nucleus; ECu, external cuneate nucleus; Gi, gigantocellular reticular nucleus; GiV, ventral gigantocellular reticular nucleus; LPGi, lateral paragigantocellular nucleus; MVe, medial vestibular nucleus; py, pyramidal tract; Sol, nucleus of the solitary tract; Sp5, spinal trigeminal tract; Sp5I, interpolar spinal trigeminal nucleus.

In view of the diffuse nature of orexin A fibers in the medulla, we first elected to administer the peptide intracisternally, and the changes in MAP and HR were taken as an approximation of the global response of medullary neurons to the peptide. Orexin A or B administered into the cisternal magna consistently and dose dependently increased MAP and HR.
HR in anesthetized rats. Injection of an equal volume of aCSF resulted in no significant changes in MAP and HR. Insofar as the potency is concerned, orexin A appears to be more effective than orexin B in producing a pressor response. Furthermore, orexins caused a larger increase in HR compared with MAP in the same animals. Orexins injected intravenously in concentrations manyfold higher than those that elicited a significant pressor response by intracisternal injection produced no significant changes in MAP and HR. This observation, in conjunction with the effectiveness of orexins by intracisternal injection, indicates that the two peptides exert their pressor effect principally by acting on neurons in the medulla. Furthermore, orexin B has also been reported to increase AP after intracerebroventricular injection in a preliminary report (24).

Medullary sites such as the nucleus of the solitary tract, caudal ventrolateral medulla, and RVLM are the primary components of the baroreceptor reflex circuitry underlying central cardiovascular regulation (14), which could potentially be the targets of orexins. Area postrema has also been shown to be associated with cardiovascular control (10, 12). Recently, area postrema has been demonstrated to mediate a pressor effect of excitatory amino acid receptor agonists in the rats anesthetized with the same anesthetics used in the present study (4). However, the finding that intravenous administration of orexins caused no significant change of blood pressure implies that the area postrema, a circumventricular organ, may not be a potential site of action for orexins in eliciting a pressor response. The RVLM is thought to be the pressor area where some of the neurons project directly to sympathetetic preganglionic neurons in the spinal cord (22). For this reason, we evaluated the RVLM as the target site upon which orexins may produce a pressor response. Similar to the effects of intracisternal injection, microinjection of orexin A to the RVLM area, which was later confirmed by histological examination, elevated blood pressure and HR in all of the rats examined. Microinjection of saline to the same area produced no significant change of blood pressure and HR in any of the rats tested. These observations indicate that RVLM is one of the sites in the medulla upon which orexins may produce a pressor response. Our results do not exclude the possibility that the peptide may act on neurons in other areas of the medulla, which may or may not result in a pressor response.

Two G-protein-coupled receptors, orexin 1 and orexin 2, have been identified in the brain (23). Orexin A appears to have a higher binding affinity than orexin B to orexin 1 receptors, although they exhibit a similar binding affinity to orexin 2 receptors (23). When stably expressed in the Chinese hamster ovary cells, the two receptors mediated a transient increase in intracellular Ca$^{2+}$ upon activation by orexins in a dose-dependent manner (23).

It is of interest to note that a single intracerebroventricular injection of orexin A (3 or 30 nmol) stimulated food consumption 1 h postinjection, and the stimulating effect lasted for 4 h or longer (23). This finding, together with the prolonged increase in MAP and HR observed here, suggests that activation of orexin receptors is followed by a sustained expression of one or more intracellular signaling mechanisms. Whether or not the differential sensitivity of MAP and HR to intracisternal injection of orexins may be related to the activation of subtypes of receptors is not known. The question whether or not the intracellular signaling mechanism is triggered by Ca$^{2+}$ influx, as shown in cultured hypothalamic neurons (27), also remains to be studied.

What might be the physiological significance of a putative orexigenic peptide involved in central regulation of AP and HR? Hypertension has long been a major public health problem in the United States. It is estimated that 50 million Americans were diagnosed with some form of high blood pressure, accounting for 86% of cardiovascular disease cases (1). Obesity is one of several risk factors underlying hypertension (1). Extensive epidemiological studies from the United States and other countries have shown that the prevalence of hypertension is clearly increased among overweight persons (6, 13, 17, 19, 21, 25, 26). Viewed in this context, the present observation that the orexigenic peptides orexin A and B acting in the medulla may enhance medullary sympathetic outflow raises an interesting possibility that the peptide may be one of the common denominators contributing to high blood pressure and obesity.

Earlier studies show that orexins acted centrally to prompt food consumption in the rat (23). More recent studies show that orexins, in addition to their orexigenic property, may affect other behaviors. For example, intracerebroventricular injection of orexin A and/or B caused an immediate increase in face washing, grooming, and burrowing activities, which were followed by increased feeding behavior in rats (15). The present observation adds to the growing list of potential functions mediated by orexins.

Perspectives

Obesity, a major health problem in industrial countries, is associated with an increased risk of high blood pressure (1). In the past few years, advances in obesity research, including the discovery of leptin and novel neuropeptides, have markedly increased our understanding of the genetic and neurobiological mechanisms underlying certain forms of obesity. The present study shows that the putative obese peptide orexin A or B increases blood pressure and HR via an action on neurons in the medulla, including the pressor area of the RVLM. The demonstration of a pressor response by an obese peptide may provide new insights into the regulation of blood pressure in overweight persons and may lead to the development of novel therapeutic agents. More importantly, our result raises an interesting possibility that neuropeptides may be the common link between obesity and high blood pressure.

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REFERENCES


