Orexin 1 Receptor Activation Attenuates Neurogenic Dural Vasodilation in an Animal Model of Trigeminovascular Nociception

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ABSTRACT
The pathophysiology underlying the pulsating quality of the pain of a migraine attack is not fully understood, although trigeminal vascular afferents containing the sensory neuropeptide calcitonin gene-related peptide (CGRP) must have a role. Antimigraine drugs, such as triptans, serotonin 5-hydroxytryptamine 1B/1D receptor agonists, reproducibly block neurogenic vasodilation associated with CGRP release. We examined the effects of the hypothalamic neuropeptides orexin A and orexin B on neurogenic dural vasodilation, dissecting out the receptor pharmacology with the novel orexin 1 (OX1) receptor antagonist N-(2-methyl-6-benzoxazolyl)-N'-1,5-naphthyridin-4-yl urea (SB-334867). Electrical stimulation of dural afferents (50–300 μA) resulted in reproducible dural vasodilation of 136 ± 9%. Orexin A 30 μg kg⁻¹, but not 3 and 10 μg kg⁻¹, inhibited the dilation brought about by electrical stimulation over 60 min and maximally after 15 min by 60% (t₁/₂ = 7.138; P < 0.001; n = 8). This response was reversed by pretreatment with the OX1 receptor antagonist SB-334867. Addition of CGRP₈₋₃₇ at the point of maximal effect of orexin A produced a further significant decrease in neurogenic dural vasodilation compared with orexin A only. CGRP administration (1 μg kg⁻¹) produced a reproducible dural blood vessel dilation of 145 ± 7% that was not inhibited by intravenous administration of orexin A (30 μg kg⁻¹). Orexin B had no significant effect even at the highest dose. The current study demonstrates that orexin A is able to inhibit neurogenic dural vasodilation via activation of the OX1 receptor, resulting in inhibition of prejunctional release of CGRP from trigeminal neurons.

Migraine is a common, chronic, and incapacitating neurovascular disorder resulting in severe, normally unilateral, pulsating headaches associated with sensory sensitivity (Headache Classification Committee of the International Headache Society, 2004). The pathophysiology underlying the pulsating quality of the migraine pain is not yet fully understood; however, activation of the dense plexus of trigeminal afferents that innervate the cranial blood vessels may have some role (Goadsby et al., 2002). It has been recognized for some time that most of the intracranial structures, including the dura mater and blood vessels, are pain-producing, especially when electrical stimulation is used (Penfield, 1932, 1934; Penfield and McNaughton, 1940; Wolff, 1948; Feindel et al., 1960).

Trigeminal afferents innervating the cranial blood vessels contain the sensory neuropeptides calcitonin gene-related peptide (CGRP), substance P, and neurokinin A (Edvinsson and Goadsby, 1998). Williamson et al. (1997a) demonstrated that electrical stimulation of dura mater causes CGRP release from the prejunctional nerve fibers innervating the dural blood vessels, resulting in reproducible vasodilation. Blocking this vasodilation has proved to be an effective indicator of acute antimigraine efficacy, with many compounds, including the triptans (Williamson et al., 1997b,c) and CGRP antagonists (Williamson et al., 1997a), able to inhibit the vasodilation as well as provide effective antimigraine therapy (Ferrari et al., 2002; Olesen et al., 2004).

The orexins are two hypothalamic neuropeptides cleaved from the same 130 amino acid precursor peptide prepro-orexin, which is encoded by a single gene located on chromosome 17q21 in humans. After proteolytic cleavage, orexins A and B are post-translationally modified to form a 33- and 28-residue peptide, respectively, that share 46% homology (Sakurai et al., 1998; Sakurai, 2005). The orexins bind to two G protein-coupled receptors, termed OX1 and OX2. The two receptors are 64% homologous, and the rat and human receptors for OX1 and OX2 demonstrate 94 and 95% homology, respectively, suggesting a high level of conservation across species.
mammalian species (Sakurai et al., 1998). Orexin A has equal affinity for the OX₁ and OX₂ receptors, with orexin B demonstrating a 10-fold higher affinity for the OX₂ receptor (Sakurai et al., 1998). Activation of either receptor results in elevated levels of intracellular Ca²⁺ concentrations, which results in the enhancement of the Gₛ-mediated stimulation of phospholipase C (Smart and Jerman, 2002).

Orexin fibers project throughout the nervous system, including dense projections to the locus coeruleus, dorsal raphe nucleus of the brainstem as well as less dense projections to the spinal cord dorsal and ventral horns, brainstem motor nuclei, periaqueductal gray, thalamus, and hypothalamus (Peyron et al., 1998; Cutler et al., 1999; van den Pol, 1999). Peripherally, orexin fibers and receptors have been localized to the pituitary, adrenal glands, gut, and testis (Blanco et al., 2001; Johren et al., 2001). The broad projections of the orexinergic system have led to its implication in a variety of functions, including feeding, sleep wake cycle, cardiovascular function, hormone secretion (Ferguson and Samson, 2003; Siegel, 2004; Sakurai, 2005; Samson et al., 2005) and more recently the modulation of nociceptive processing (Bingham et al., 2001; Cheng et al., 2003; Yamamoto et al., 2003; Kajiyama et al., 2005).

The hypothalamus is known to play an important role in the pathophysiology of cluster headache and the same may be true for migraine (Peres et al., 2001). The presence of premonitory symptoms up to 48 h preceding an attack (Giffin et al., 2001; Cheng et al., 2003; Yamamoto et al., 2003; Kajiyama et al., 2005) have the potential to inhibit trigeminovascular nociceptive transmission.

Materials and Methods

Surgical Preparation

All experiments were conducted under the UK Home Office Animals (Scientific Procedures) Act (1986). Male Sprague-Dawley rats (280–350 g) were anesthetized throughout the experiments with 60 mg kg⁻¹ pentobarbital sodium salt intraperitoneally and then 10 mg kg⁻¹ intravenously as required (Sigma Chemical, Poole, Dorset, UK). The left femoral artery and vein were cannulated for blood pressure recording and intravenous infusion of anesthetic and test compounds, respectively. Temperature was maintained throughout by using a homoeothermic blanket system. The rats were placed in a stereotaxic frame and ventilated with oxygen-enriched air, 3 to 5 ml, 60 to 80 strokes per min (Small Rodent Ventilator model 683; Harvard Apparatus, Ltd., Edenbridge, Kent, UK). End-tidal CO₂ was monitored (Capstar-100; CWE Inc., Ardmore, PA) and kept between 3.5 and 4.5%, and blood pressure was monitored continually, allowing monitoring for changes in respiration and blood pressure due to anesthesia. The skull was exposed, and the right or left parietal bone was thinned by drilling with a saline-cooled drill until the blood vessels of the dura mater were clearly visible through the intact skull.

Intravitral Microscopy

The cranial window was covered with mineral oil (37°C), and a branch of the middle meningeal artery was viewed using an intravital microscope (Microvision MV2100; Finlay Microvision, Warwickshire, UK), and the image was displayed on a television monitor. Dural blood vessel diameter was continuously measured using a video dimension analyzer (Living Systems Instrumentation, Burlington, VT) and displayed with blood pressure on an online data analysis system (CED spike2v5 software; CED, Cambridge, UK).

Experimental Protocols

Defining Electrical Stimulation Parameters. Electrical stimulation was used to evoke dilation of the dural blood vessels with a bipolar stimulating electrode (NE 200X; Clarke Electromedical Instruments, Pangbourne, UK) placed on the surface of the cranial window approximately 200 μm from the vessel of interest. The surface of the cranial window was stimulated at 5 Hz, 1 ms for 10 s (Grass Stimulator S88; Grass Instruments, Quincy, MA), with increasing voltage until maximal dilation was observed. Subsequent electrically induced responses in the same animal were then evoked using that voltage (Williamson et al., 1997a; Akerman et al., 2002). The reproducibility of this vasodilator response to electrical stimulation has been demonstrated previously using consecutive saline-controlled stimuli (Akerman et al., 2002).

Effect of Orexin on Evoked Dural Vessel Dilation. Two control responses to dural electrical stimulation were performed, and at least 10 min later, orexin A (n = 9) or B (n = 7), at doses of 30, 10, and 30 μg kg⁻¹ was administered intravenously. Electrical stimulation was then repeated 5, 15, 30, 45, and 60 min after the orexin treatment. For the antagonist studies, two control responses to dural electrical stimulation were performed and, at least 10 min later, SB-334867 was administered intravenously. This was followed by further electrical stimulation after 5 min and then followed by orexin A or B (n = 6) treatment, after which stimulation was repeated 5, 15, 30, 45, and 60 min later.

Effect of Orexin A and CGRP₈₋₃₇ on Evoked Dural Vessel Dilation. Two control responses to dural electrical stimulation were performed, and at least 10 min later, 30 μg kg⁻¹ orexin A was administered intravenously and then the electrical stimulation was repeated 5, 15, 30, 45, and 60 min after the orexin treatment. To investigate the effect of orexin A and CGRP₈₋₃₇, the CGRP₈₋₃₇ was administered intravenously immediately before the 15 min stimulation postorexin A (n = 6).

Effect of Orexin A on CGRP-Evoked Dural Vessel Dilation. Two control responses to CGRP-induced dilation were performed, and at least 10 min later 30 μg kg⁻¹, orexin A (n = 6) was administered intravenously and then the CGRP induced dilation was repeated 5, 15, 30, 45, and 60 min after the orexin treatment.

Data Analysis

The effects of electrical stimulation and CGRP administration on dural vessel diameter were calculated as a percentage increase from the prestimulation baseline diameters. The nature of the experimental setup, where the magnification of the dural vessel visualized was
different in each animal by virtue of selecting an appropriate target vessel, made it impossible to standardize the dural vessel measurement; therefore, the change in dural vessel diameter is reported as a percentage change from baseline. The typical vessel diameter measured in the study ranged from 120 to 150 μm. All data are expressed as mean ± S.E.M. Statistical analysis was performed using an analysis of variance for repeated measures with Bonferroni post hoc correction for multiple comparisons followed by Student’s paired t test. To compare the effect of orexin A only and orexin A and CGRP8-37, an independent samples t test was used (SPSS version 12.0; SPSS Inc., Chicago, IL). Significance was assessed at the P < 0.05 level.

Drugs

The delivery of anesthetic and experimental drugs was via the same femoral catheter; however, the line was always flushed with saline first, several minutes before administering a different compound. Orexins A and B (Tocris Cookson Inc., Bristol, UK) were dissolved in water for injection at a maximum dose of 30 μg kg⁻¹. SB-334867, a kind donation from GlaxoSmithKline (Uxbridge, Middlesex, UK) was dissolved in 2-hydroxy-β-cycloexetrin (Sigma Chemical) with a few drops of dimethyl sulfoxide to aid solubility and injected at a dose of 10 mg kg⁻¹. CGRP (rat; Tocris Cookson Inc.) was initially dissolved in distilled water, aliquoted, and frozen. Subsequent dilutions were made in 0.9% saline before injection at a dose of 1 μg kg⁻¹. CGRP8-37 (rat; Tocris Cookson Inc.) was dissolved in 0.9% saline for injection at a dose of 300 μg kg⁻¹. All drugs were made fresh on the morning of an experiment and administered in volumes ranging from 0.1 to 0.3 ml.

Results

Effects of Orexin A on Neurogenic Dural Vasodilation. Electrical stimulation (50–300 μA) produced reproducible dural blood vessel dilation of 136 ± 9% (n = 35). Orexin A given intravenously at 3 and 10 μg kg⁻¹ did not significantly attenuate the response to electrical stimulation of the cranial window, but it had a significant effect at 30 μg kg⁻¹ (F(2,4,16.6) = 16.4; P < 0.001; Figs. 1 and 2; Table 1). Orexin A produced its maximum effect after 15 min, reducing the neurogenic dural vasodilation by 60% (t₇ = 2.4,16.6 P < 0.001; Figs. 1 and 2; Table 1). Control vehicle injections demonstrated no significant changes, and SB-334867 demonstrated no effect on neurogenic vasodilation when given alone. The addition of CGRP8-37, 13 min postorexin A, produced a significant decrease in neurogenic dural vasodilation at the 15-min time point compared with orexin A alone (t₅ = 3.5; P < 0.005; n = 6; Fig. 2) and baseline (F(13,225) = 142.4; P < 0.001; Fig. 2; Table 1). Control vehicle injections demonstrated no significant effect.

Effects of Orexin B on Neurogenic Dural Vasodilation. Orexin B given intravenously at 3 and 10 μg kg⁻¹ had no effect on neurogenic dural vasodilation (Fig. 3). Orexin B given intravenously at 30 μg kg⁻¹ elicited a nonsignificant decrease in neurogenic dural vasodilation (F(2,1,18.8) = 0.86; P = 0.48; Fig. 3). Pretreatment with SB-334867 followed 5 min later by 30 μg kg⁻¹ orexin B elicited a slight increase in neurogenic dural vasodilation; however, this was not significantly different from baseline (F(2,25) = 1.63; P = 0.26; Fig. 3). Grouped comparisons of orexin B and orexin B post-SB-334867 showed no significant difference between the two treatments. Orexin B administration did not elicit any sig-
significant blood pressure changes, and control vehicle injections demonstrated no significant effect.

**Effects of Orexin A on CGRP-Induced Dural Vasodilation.** CGRP administration (1 μg kg⁻¹) produced a reproducible dural blood vessel dilation of 145 ± 7% (n = 6) and a characteristic decrease in blood pressure. Intravenous administration of orexin A at 30 μg kg⁻¹, which inhibited neurogenic dural vasodilation, had no effect on dural vasodilation in response to CGRP administration (F₅,₂₋₅ = 0.52; P = 0.75; Fig. 4; Table 3).

**TABLE 1**

<table>
<thead>
<tr>
<th>Time</th>
<th>Increase in Dural Vessel Diameter</th>
<th>Orexin A</th>
<th>Orexin A and CGRP₈⁻³⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control response</td>
<td>143.5 ± 10</td>
<td>126.2 ± 5</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>71.9 ± 11* (tₛ = 4.27; n = 8)</td>
<td>83.4 ± 6* (tₛ = 8.42; n = 6)</td>
<td></td>
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<tr>
<td>15 min</td>
<td>54.6 ± 10* (tₛ = 7.14; n = 8)</td>
<td>114.7 ± 1.5* (tₛ = 20.8; n = 6)</td>
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</tr>
<tr>
<td>30 min</td>
<td>95.2 ± 14* (tₛ = 3.43; n = 8)</td>
<td>83.8 ± 2* (tₛ = 8.88; n = 6)</td>
<td></td>
</tr>
<tr>
<td>45 min</td>
<td>120.9 ± 10* (tₛ = 2.66; n = 8)</td>
<td>101.5 ± 5* (tₛ = 6.46; n = 6)</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>146.5 ± 9</td>
<td>130.3 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 significance compared with control response; *P < 0.05 significance compared with orexin response only.

**TABLE 2**

<table>
<thead>
<tr>
<th>Neurogenic Dural Vasodilation</th>
<th>Increase in Dural Vessel Diameter</th>
<th>% age change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control response</td>
<td>131.1 ± 7</td>
<td>1</td>
</tr>
<tr>
<td>5 min post-SB-334867</td>
<td>132.6 ± 6</td>
<td>2</td>
</tr>
<tr>
<td>5 min postorexin A</td>
<td>115.5 ± 7</td>
<td>3</td>
</tr>
<tr>
<td>15 min</td>
<td>128.3 ± 9</td>
<td>4</td>
</tr>
<tr>
<td>30 min</td>
<td>127.2 ± 7</td>
<td>5</td>
</tr>
<tr>
<td>45 min</td>
<td>134.1 ± 10</td>
<td>6</td>
</tr>
<tr>
<td>60 min</td>
<td>131.8 ± 6</td>
<td>7</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Neurogenic Dural Vasodilation</th>
<th>Increase in Dural Vessel Diameter</th>
<th>% age change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control response</td>
<td>143.5 ± 6</td>
<td>1</td>
</tr>
<tr>
<td>5 min</td>
<td>138.2 ± 7</td>
<td>2</td>
</tr>
<tr>
<td>15 min</td>
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<td>4</td>
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<tr>
<td>45 min</td>
<td>139.5 ± 5</td>
<td>5</td>
</tr>
<tr>
<td>60 min</td>
<td>135.7 ± 3</td>
<td>6</td>
</tr>
</tbody>
</table>

![Fig. 4. Effects of intravenous orexin A (30 μg kg⁻¹) on CGRP (1 μg kg⁻¹)-induced vasodilation. Following control responses to CGRP administration, rats were injected with orexin A at 30 μg kg⁻¹ and CGRP injection was repeated after 5, 15, 30, 45, and 60 min (n = 6). Overall, no significant effect of orexin A on CGRP-induced vasodilation was seen.](image)

**Discussion**

Orexin A was able to significantly inhibit neurogenic dural vasodilation at a dose of 30 μg kg⁻¹ by a maximum of 60%, and this inhibition was blocked by pretreatment with the selective OX₁ receptor antagonist SB-334867. Together, the data indicate that activation of the OX₁ receptor inhibits neurogenic vasodilation in the dura mater. This effect of orexin A may involve either pre- or postsynaptic receptors, or both, to inhibit dural vasodilation. To examine this further, we have reasoned that exogenous orexins are acting directly on postsynaptic CGRP receptors in the smooth muscle of dural arteries to induce dural vasodilation. Orexin A is unable to block this effect at concentrations that were able to signifi-
stantly inhibit neurogenic dural vasodilation, suggesting that the effect of orexin A is presynaptic. The absence of evidence for orexin receptor localization on blood vessels and the absence of both OX₁ and OX₂ receptor mRNA from the heart and aorta (Johren et al., 2001) are in agreement with the current effect being neural. The CGRP receptor antagonist CGRP₉₋₃₇, which has been shown to inhibit neurogenic dural vasodilation to 19% when given alone (Williamson et al., 1997a), was able to further inhibit neurogenic dural vasodilation (11%) when administered at the point of maximal inhibition (60%) by orexin A, suggesting that orexin A does not completely block the presynaptic release of CGRP.

Orexin B was unable to significantly alter neurogenic dural vasodilation at all three doses studied. At the 30 μg kg⁻¹ dose, a nonsignificant trend to a reduction in the dilator response was observed, possibly as a result of an effect at the OX₁ receptor. In the presence of orexin B, pretreatment with the selective OX₁ receptor antagonist SB-334867, although still having no significant effect, caused a slight increase in dural vessel dilation as a result of electrical stimulation. The experimental model used in this study results in dilation of the dural blood vessels under examination, and as such, there is an upper physiological limit to the level of dilation achievable; therefore, one is unable to rule out a possible small effect of orexin B either with or without SB-334867 pretreatment.

The data presented indicate a prejunctional effect for orexin A on the trigeminovascular system, probably from decreased CGRP release. The OX₁ receptor has been identified in the dorsal root ganglion, suggesting its possible presence in the trigeminal ganglion. The exact mechanisms of this inhibitory effect are unknown, because the orexins normally elicit an excitatory response on neurons. It is possible that orexin A is inhibiting the central branch of the trigeminal nerve, in agreement with previous studies demonstrating orexnergic inhibition via spinal orexin receptors (Kajiyama et al., 2005), thus reducing CGRP release from the peripheral end of the trigeminal nerve. This may also explain the lack of effect of orexin B in the current study, because it has been shown to be highly degraded in the blood and crosses the blood-brain barrier poorly (Kastin and Akerstrom, 1999). The possibility of an as yet unknown peripheral effect of OX₁ receptor activation cannot be ruled out (Bingham et al., 2001).

Orexin A injected into the hypothalamus has been shown previously to inhibit significantly cell firing in the trigeminocephalic complex in response to dural electrical stimulation and nociceptive thermal stimulation of the facial skin in the rat (Bartsch et al., 2004). The level of inhibition achieved by intravenous administration of orexin A at a dose of 30 μg kg⁻¹ in our model of trigeminovascular activation indicates a potent ability to influence the trigeminovascular system directly. As previously reported, the ability to inhibit neurogenic vasodilation has been used to identify potential migraine therapy targets, such as sumatriptan (Williamson et al., 1997b). OX₁ receptor activation causes an inhibition comparable with that of other acute attack treatments, and as such, it may be considered a putative target for migraine medicine development.

The trigeminal ganglion is bipolar, with its afferent fibers innervating the dural blood vessels in the periphery and centrally projecting to the trigeminal nucleus caudalis (TNC). The pharmacology of the peripheral and central branches of the trigeminal ganglion in general matches each other; thus, work demonstrating an effect on the neuronal mechanisms involving the dural blood vessels may also apply to the central neuronal mechanisms in the TNC. A number of compounds, including the triptans, 5-hydroxytryptamine₁B/₁D receptor agonists (Williamson et al., 1997c; Boers et al., 2000, 2004), and the CGRP receptor blockers (Williamson et al., 1997a; Storer et al., 2004), are able to inhibit the activation of both the central and peripheral branches of the trigeminal ganglion. Our new data suggest the possibility that OX₁ receptor agonists may be able to inhibit neurons in the TNC directly.

The current study demonstrates that orexin A is able to inhibit neurogenic dural vasodilation via activation of the OX₁ receptor. This effect is most likely prejunctional, acting on the trigeminovascular system and resulting in decreased but not complete blockade of CGRP release. The data complement electrophysiological work in the rat demonstrating that OX₁ receptor activation in the hypothalamus is able to inhibit neuronal firing in the TNC in response to electrical stimulation of the dura mater and noxious thermal stimulation of the facial receptive field (Bartsch et al., 2004). The widespread distribution of the orexins and their receptors throughout the central nervous system, including the hypothalamus, periaqueductal gray, and lamina I, II, and X of the spinal cord (Peyron et al., 1998; Trivedi et al., 1998; van den Pol, 1999), which are all areas involved in nociceptive processing, provides the potential for further research into the role of the orexins in neuronal systems potentially involved in migraine and cluster headache. Orexins may provide some part of the link between some of the remarkable triggering and behavioral manifestations of migraine, such as those involved in feeding, and the disorders of the neuroendocrine and autonomic systems that can be characteristic of the condition.

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References


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