Adenosine A1 receptor agonists inhibit trigeminovascular nociceptive transmission

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Summary

There is a considerable literature to suggest that adenosine A1 receptor agonists may have anti-nociceptive effects, and we sought to explore the role of adenosine A1 receptors in a model of trigeminovascular nociceptive transmission. Cats were anaesthetized (α-chloralose 60 mg/kg, intraperitoneally), and prepared for physiological monitoring. The superior sagittal sinus (SSS) was stimulated electrically, and linked units were recorded in the trigeminocervical complex. Post-stimulus histograms were constructed to analyse the responses and the effect of drug administration. Blood was sampled from the external jugular vein to determine levels of calcitonin gene-related peptide (CGRP) release before and after drug administration.

Intravenous administration of the highly selective adenosine A1 receptor agonist, GR79236 (3–100 μg/kg) had a dose-dependent inhibitory effect on SSS-evoked trigeminal activity. The maximal effect (80 ± 6% reduction in probability of firing) was seen at 100 μg/kg. The neuronal inhibitory effect of GR79236 could be inhibited by the selective adenosine A1 receptor antagonist DPCPX (300 μg/kg; P < 0.05). SSS stimulation increased cranial CGRP levels from 33 ± 2 pmol/l (n = 6) to 64 ± 3 pmol/l, an effect substantially reduced by pre-treatment with GR79236 (30 μg/kg; P < 0.01). The selective low efficacy adenosine A1 receptor agonist, GR190178 (30–1000 μg/kg i.v.), also inhibited SSS-evoked neuronal activity in a dose-dependent fashion. In this model of trigeminovascular nociception, adenosine A1 receptor activation leads to neuronal inhibition without concomitant vasoconstriction, suggesting a novel avenue for the treatment of migraine and cluster headache.

Keywords: migraine; cat; headache; pain; adenosine A1 receptor agonist; GR79236; GR190178

Abbreviations: CGRP = calcitonin gene-related peptide; SSS = superior sagittal sinus

Introduction

Recent advances in the acute treatment of migraine and cluster headache have contributed to our understanding of the pathophysiology of these disorders and provided considerable benefits to many patients. The development (Humphrey et al., 1990) and clinical use of sumatriptan (Ferrari, 1998) initiated a cascade of mechanistic and therapeutic advances that has included several other triptans that all share the property of being serotonin (5-HT1B/1D) agonists (Goadsby, 2000). The search for novel non-vasoconstrictor treatments of acute migraine continues because of the potential safety benefits (Welch et al., 2000) such a strategy would offer, and the considerable mechanistic insight that would be generated if it were clear that non-vascular medicines were effective.

The triptans, 5-HT1B/1D agonists, have three clearly delineated mechanisms of action in pre-clinical studies (Goadsby, 2000). These are vasoconstriction of cranial vessels through actions at 5-HT1B receptors, inhibition of peripheral trigeminal terminal activation via 5-HT1D or 5-HT1F receptors, and inhibition of second order neurones in the trigeminocervical complex via 5-HT1B/1D/1F receptors. The vasoconstrictor action has been confirmed anatomically, using highly selective 5-HT1B and 5-HT1D receptor antibodies, to be almost exclusively of the 5-HT1B subtype (Longmore et al., 1997; Nilsson et al., 1999). The peripheral trigeminal neuronal action of triptans is demonstrated by inhibition of neurogenic plasma protein extravasation...
(Moskowitz and Cutrer, 1993) and inhibition of release of calcitonin gene-related peptide (CGRP) into the cranial circulation (Goadsby and Edvinsson, 1993). Similarly, there is evidence for inhibition at central terminals of the trigeminal nerve within the trigeminal nucleus caudalis by serotonin (Goadsby and Hoskin, 2000). Inhibition of both cranial CGRP release and trigeminal neuronal transmission predict prospective anti-migraine actions for novel compounds or mechanisms (De Vries et al., 1999).

There is a considerable literature to suggest that the purine, adenosine, may have some role in nociception (Sawynok, 1998, 1999). It was observed originally that methylxanthine adenosine antagonists decreased nociceptive thresholds in rats (Paalzow and Paalzow, 1973). Based on studies comparing the rank order of potency of adenosine analogues (Sawynok et al., 1986), or on the use of selective adenosine agonists and antagonists (Sjolund et al., 1996), it is likely that the anti-nociceptive effects of adenosine are mediated via the A1 receptor (Sawynok et al., 1986). Adenosine may contribute to the anti-nociceptive effects of morphine (DeLander and Hopkins, 1986) and serotonin (DeLander and Hopkins, 1987). Recently, adenosine A1 receptor protein has been localized in human trigeminal ganglia (Schindler et al., 2001), which suggests potential utility of adenosine A1 receptor agonists to inhibit the trigeminal nerve.

We sought to explore the role of adenosine A1 receptors in an animal model of nociceptive activation of the trigemino-vascular system (Goadsby, 1999). The effects of selective adenosine A1 receptor agonists on trigeminal neuronal activity have been determined. Their effects on a peripheral marker of trigeminovascular activation, CGRP levels in the cranial venous blood, which are elevated in acute migraine (Edvinsson and Goadsby, 1995), have also been investigated. Our data suggest that an adenosine A1 receptor agonist that has sufficient access to the trigeminal nucleus could provide a novel, non-vasoconstrictor acute treatment for migraine.

**Material and methods**

All studies reported were carried out under a project licence issued by the UK Home Office within the Animals (Scientific Procedures) Act, 1986. Adult cats were anaesthetized with α-chloralose (60 mg/kg, i.p. with supplements of 15 mg/kg i.v.). During surgical procedures, 2–4% halothane was also used (Storer et al., 1997). Before surgery, they were intubated, ventilated with 40% oxygen (Ugo Basille Varese Italy), and for the electrophysiological experiments paralysed with gallamine triethiodide (6 mg/kg, i.v.). End-expiratory CO2 and fraction of inspired O2 were monitored continuously (Datex, Helsinki, Finland). Polyethylene catheters were placed into the femoral artery for monitoring blood pressure and into the femoral vein for fluid and drug administration. Core temperature was monitored and maintained using a rectal thermistor probe and a homeothermic heater blanket system (Harvard Apparatus, Holliston, Mass., USA).

**Surgery and stimulation**

After mounting in a stereotactic frame (David Kopf, Tujunga, Calif., USA), a circular midline craniotomy (20–25 mm diameter) and C1/C2 laminectomy were performed for access to the superior sagittal sinus (SSS) and the recording site in the C2 spinal cord, respectively. The dura and falx adjacent to the SSS were dissected over 15 mm and the sinus suspended over bipolar platinum hook electrodes. To prevent dehydration of the brain, and for electrical insulation for the cortex, a paraffin bath was built with a dam of dental acrylic around the craniotomy and a small polyethylene sheet inserted under the SSS. The SSS was stimulated supramaximally with an electrical pulse (150 V, 250 μs duration; Grass S88 stimulator and Stimulus Isolation Unit SIU5A; Quincy, Mass., USA) at 0.3 Hz for electrophysiological and at 5 Hz for neuropeptide studies. From previous electrophysiological studies (Kaube et al., 1993a; Hoskin et al., 1996) and studies of fos protein expression (Kaube et al., 1993b), we had determined that trigeminal neurones would follow stimuli at a rate of 0.3 Hz for many hours. The rate of 5 Hz for the peptide study was chosen from blood flow studies of the trigeminal nucleus caudalis that demonstrated near maximal activation at this frequency (Goadsby and Classey, 2000).

**Electrophysiological studies**

**Recording**

Recordings were made using glass-coated tungsten microelectrodes with a typical tip length of 15 μm and impedance of 400–500 kΩ. After removal of the dura and pia mater from the dorsal surface of the spinal cord, the electrode was lowered into the cord substance caudal to the C2 roots aiming for dorsal horn lamina II neurones. The microelectrode was advanced or retracted in the cord substance using a manual hydraulic microdrive (Kopf Instruments, Tujunga, Calif., USA). Bacteriological agar [3% (w/v) in saline; Difco, Detroit, Mich., USA] was poured over the exposed cord after electrode insertion to reduce cardiovascularly related movements and to prevent dehydration of the cord. Signal from the electrode was fed to a Neurolog amplifier (Digitimer, Herts, UK) and then via a window discriminator to an A/D converter (DT2839, Data Translation, UK) in a microcomputer. Amplifier bandwidth was usually 300 Hz to 10 kHz. When discriminating between somatic and axonal recordings, an amplifier bandwidth of DC to 30 kHz was used.

In order to record the response of single units to stimulation, post-stimulus histograms were constructed online and saved on disk. A delay line and averaging routine were used to construct averaged action potentials. Selected single unit sites were marked for subsequent identification by an electrolytic lesion made by passing a 20–25 μA DC cathodal current through the recording electrode for 20–30 s. At the conclusion of the experiment, the animal was given a lethal dose of a barbiturate, and the brain removed. The spinal
cord was sectioned (50 μm) and recording sites identified by the lesions.

Receptive fields
Cells responding to SSS stimulation were characterized as receiving low threshold mechanoreceptor input if they responded to non-noxious input such as brush or stroke on cutaneous receptive fields on the face or forepaws. They were characterized as nociceptive specific if they responded to noxious mechanical stimuli, such as pinch or pricking with a needle, or wide dynamic range if they responded to both (Hu et al., 1981). These cells usually had increased firing in response to noxious stimuli.

Neuropeptide studies
In each animal, three 5 ml jugular vein blood samples were taken for peptide detection: (i) control sample, no stimulation; (ii) stimulated sample, taken within 60 s of SSS stimulation at 5 Hz; and (iii) stimulated + drug treatment sample, taken within 60 s of SSS stimulation at 5 Hz.

After removal of the 5 ml of jugular blood, the same volume of saline was administered intravenously for volume replacement. Administration of drug was carried out after the second jugular blood sample during fluid replacement. Thirty minutes later, the SSS was stimulated and the third jugular blood sample was taken.

Peptide detection
The 5 ml jugular vein blood samples were aliquoted into a centrifuge tube containing 0.5 ml of sodium heparin (Mucous) 1000 IU/ml and 0.5 ml of aprotinin 70 mg in 0.9% NaCl. The sample was then centrifuged at 2500 r.p.m. for 20 min. The plasma supernatant was withdrawn and freeze-dried. A 2 ml aliquot of freeze-dried plasma was reconstituted, and immunoreactive CGRP was quantitated using a rabbit antiserum (R-8429, Eurodiagnostics, Malmö, Sweden), raised against synthetic rat CGRP. The detection limit was 10 pmol/l, the inter-assay coefficient of variation was <10% in the 20–100 pmol/l range and the intra-assay coefficient of variation was <5% (Grunditz et al., 1986).

General study design
Animals were prepared for both the electrophysiological recordings and blood sampling in order to maximize data collection from a single study, while minimizing animal use. Blood was collected for the baseline peptide determination prior to stimulation of the SSS. Thereafter, the sinus was stimulated and neurones sought that were linked to SSS stimulation. Having identified a linked unit and recorded its properties, the test compound was administered. Separate groups of animals were used in the studies of the full agonist, GR79236, and the partial agonist, GR190178.

Drugs
The adenosine A1 receptor agonist, GR79236 (GlaxoWellcome, Stevenage, UK), was administered at cumulative doses of 3, 10, 30 and 100 μg/kg (intravenously) with intervals of ~60 min between doses. The adenosine A1 receptor low efficacy (partial) agonist GR190178 (GlaxoWellcome, Stevenage, UK) was administered at cumulative doses of 30, 100, 300 and 1000 μg/kg (intravenously) at intervals of 60 min. The adenosine A1 antagonist DPCPX (Sigma, St Louis, Mo., USA) was administered in a single dose of 300 μg/kg intravenously ~30 min after the highest dose of GR79236.

Analysis
Group data are expressed as mean ± SEM, unless otherwise indicated. Cell suppression was determined from the post-stimulus histograms using the criteria of at least a 30% shift from baseline (Nagler et al., 1973). This criterion is based on the use of the window discriminator method to generate data that consist of firing rates as a function of time. Since the collection methods allow small time units to be defined, the data may be considered Poisson (Armitage and Berry, 1994). The overall degree of inhibition for the four doses across the cohort of animals was assessed using an ANOVA (analysis of variance) with repeated measures. The ID50 was calculated (Goadsby and Lambert, 1986) using a log/logit transformation of the data (Finney, 1964). The effect of DPCPX was based on reversal of the GR79236 100 μg/kg dose alone and tested with a Wilcoxon signed ranks test. For the neuropeptide data, an ANCOVA (analysis of covariance) with repeated measures was used for the entire cohort (SPSS version 9.0), with the pre-stimulation level as the covariate; SSS-stimulated and post-drug CGRP levels were compared pair-wise with a Bonferroni correction. Significance was assessed at the P < 0.05 level.

Results
Cardiorespiratory data
Fourteen animals (3.17 ± 0.38 kg, mean ± SD) were studied. Their cardiorespiratory parameters were kept within normal limits for the anaesthetized cat: pH 7.4 ± 0.04, pCO2 2.86 ± 0.35 kPa, pO2 74 ± 10 kPa. We report the peak cardiovascular effects of the high efficacy adenosine A1 receptor agonist, GR79236, in addition to the steady-state effect, since there were small, transient effects after immediate administration of higher doses of the compound. Cardiovascular parameters returned to basal levels within 15 min, except for the mild hypotensive effect of the 100 μg/kg dose that persisted (Table 1). The partial agonist, GR190178 (Sheehan et al., 2000), had no significant effects on cardiovascular parameters. Given these effects, we employed the doses of 30 and 300 μg/kg, for GR79236 and GR190178, respectively, for the neuropeptide studies.
Electrophysiological data

Control
Stimulation of the SSS resulted in latencies of activation that varied between 3 and 18 ms, but were typically 8±10 ms as has been reported previously (Storer and Goadsby, 1999). Given the distances involved, their latencies suggest that the fibre population is predominantly Aδ. Where they could be characterized, the units had receptive fields on the face or forepaw. The units were largely (80%) wide dynamic range in type. The onset of action of both agonists was within 5 min of administration.

Effect of GR79236
GR79236 had a dose-dependent inhibitory effect on SSS-evoked neuronal firing. At a dose of 3 μg/kg, an inhibitory effect of 36 ± 5% was noted, while the effect seen at 100 μg/kg was an 80 ± 7% inhibition (P < 0.01; Fig. 1, n = 6). The inhibitory effect persisted over the course of the study. The ID₅₀ dose for GR79236 (dose causing 50% inhibition of neuronal firing) was calculated as 4 μg/kg i.v. (n = 5). The inhibitory effect of GR79236 (100 μg/kg) could be substantially reversed by the selective A₁ receptor antagonist DPCPX (300 μg/kg i.v.), leaving an inhibition of 14 ± 3% (P < 0.05; Fig. 1, n = 5). DPCPX itself did not affect baseline SSS-evoked firing rates.

Neuropeptide studies
For the animals receiving GR79236 (30 μg/kg), pre-stimulation CGRP levels were 33 ± 3 pmol/l (n = 6); for GR190178 (300 μg/kg), the resting CGRP levels were 36 ± 3 pmol/l (n = 5). Previous studies have shown that two repeated stimulations of the SSS using this protocol would elicit increases in CGRP levels of very similar magnitude (Knight et al., 1999, 2001).

Effect of GR79236
SSS stimulation caused a significant increase in CGRP levels (64 ± 3 pmol/l; ANCOVA, P < 0.02) compared with pre-stimulation levels. Administration of GR79236 (30 μg/kg i.v.) reduced the increase in CGRP levels evoked by the second SSS stimulation to 44 ± 3 pmol/l (P < 0.01; Fig. 4).

Table 1 Cardiovascular data from the GR79236 study

<table>
<thead>
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<th>Steady state</th>
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<th>Peak effects</th>
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<tr>
<td></td>
<td>Blood pressure (mmHg ± SD)</td>
<td>Heart rate (b.p.m. ± SD)</td>
<td>Blood pressure (mmHg ± SD)</td>
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<tr>
<td>Control</td>
<td>103 ± 7</td>
<td>202 ± 7</td>
<td>103 ± 7</td>
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<tr>
<td>GR79236 (μg/kg)</td>
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<tr>
<td>3</td>
<td>103 ± 6</td>
<td>204 ± 9</td>
<td>103 ± 6</td>
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<tr>
<td>10</td>
<td>104 ± 7</td>
<td>207 ± 15</td>
<td>100 ± 8</td>
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<td>30</td>
<td>106 ± 4</td>
<td>213 ± 11</td>
<td>99 ± 7</td>
</tr>
<tr>
<td>100</td>
<td>97 ± 4</td>
<td>204 ± 9</td>
<td>92 ± 5</td>
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Effect of GR190178

The effect of SSS stimulation and subsequent GR190178 administration was significant when compared with control CGRP levels (ANCOVA, \( P < 0.05 \)). In this group, CGRP levels in each animal rose to a mean of \( 65 \pm 4 \) pmol/l during stimulation of the SSS at 5 Hz. Administration of GR190178 (300 \( \mu g/kg \) i.v.) reduced the subsequent SSS-evoked CGRP level to \( 50 \pm 3 \) pmol/l (\( P < 0.01 \); Fig. 5).

Discussion

These data demonstrate that two highly selective adenosine \( A_1 \) receptor agonists, GR79236 (Gurden et al., 1993) and GR190178 (Sheehan et al., 2000), can inhibit trigeminovascular activation, both in the trigeminal nucleus and by inhibition of release of CGRP in the cranial circulation. The effect within the trigeminal nucleus reflects a central action, whilst inhibition of CGRP release is likely to be attributable to an action at adenosine \( A_1 \) receptors on peripheral terminals of the trigeminal nerve. Both effects are in keeping with the concept of inhibitory adenosine \( A_1 \) receptors being located pre-junctionally on primary afferent neurones and causing inhibition of transmitter release, as has been described in other systems (Santicioli et al., 1993). Importantly, the adenosine \( A_1 \) receptor agonists caused inhibition of nociceptive trigeminovascular activation without any concomitant vasoconstrictor effects. Indeed, only modest bradycardia and
Adenosine agonists and migraine

Fig. 5 Inhibition of cranial release of calcitonin gene-related peptide (CGRP) by GR190178 (300 µg/kg, i.v.). There is a significant inhibition of CGRP release after GR190178 administration. *P < 0.05 with controls; #P < 0.05 with SSS.

hypotension were observed after the highest dose of GR79236, as has been reported previously for adenosine A1 receptor agonists (Gardner et al., 1994). Furthermore, GR79236 (10 µg/kg i.v.) has no effect on resting meningeal artery diameter in rats (Honey et al., 2000). This is in contrast to the effects of triptans that cause cranial vasoconstriction in a range of species, including cat and humans, in addition to inhibiting trigeminal nerve firing (Goadsby, 2000). The ability of the selective A1 receptor antagonist, DPCPX, to reverse the neuronal inhibitory effects of GR79236 in the present study provides further confirmation that these effects are mediated via activation of the adenosine A1 receptor subtype. The data establish a role for adenosine A1 receptor modulation of the trigeminovascular system and suggest a novel candidate target for therapeutic intervention in acute migraine and cluster headache.

SSS-evoked trigeminal neuronal activation has proved to be a robust method for studying trigeminovascular nociception. The trigeminocervical complex has receptors that bind a number of compounds that are specific acute migraine treatments; notably [3H]dihydroergotamine in cat (Goadsby and Gundlach, 1991), [3H]sumatriptan in cat (Mills and Martin, 1995), guinea pig (Waerber and Moskowitz, 1995) and humans (Pascual et al., 1996), and [3H]zolmitriptan in the cat (Goadsby and Knight, 1997). These same neuronal cells can be functionally inhibited by anti-migraine drugs, such as dihydroergotamine (Hoskin et al., 1996), eletriptan (Goadsby and Hoskin, 1999), naratriptan (Knight and Goadsby, 1997; Cumberbatch et al., 1998), rizatriptan (Cumberbatch et al., 1997) and zolmitriptan (Goadsby and Hoskin, 1996), using clinically relevant doses. For both serotonin (Goadsby and Hoskin, 1998), which itself can inhibit trigeminal neurones, and the 5-HT1B/1D agonist naratriptan (Knight and Goadsby, 1997), the inhibition of trigeminal activity is blocked by the specific 5-HT1B/1D antagonist GR127935 (Clitheroe et al., 1994). It is clear from microiontophoretic studies that the action of the triptans includes a locus within the trigeminal nucleus itself (Storer and Goadsby, 1997; Storer et al., 2001). In this study, the adenosine A1 receptor agonists elicit a dose-dependent block SSS-evoked trigeminal firing. The inhibitory effects seen are comparable in magnitude and time course with those observed for the triptans in this model, with the caveat that such comparisons should be done cautiously. The potential clinical relevance of the present findings is highlighted by the reported localization of adenosine A1 receptor protein in human trigeminal ganglia. Adenosine A1 receptor immunoreactivity was present in a large number of trigeminal neuronal cells and showed both membrane-bound and cytoplasmic localization (Schindler et al., 2001).

Adenosine receptor agonists are effective in preclinical anti-nociceptive tests when administered systemically, intrathecally or centrally. Use of selective adenosine A1 receptor agonists and antagonists suggests that the adenosine A1 receptor is the predominant receptor subtype mediating these effects. There is little evidence to suggest that other adenosine receptor subtypes (A2A, A2B or A3) can mediate an anti-nociceptive effect (Segerdahl and Sollevi, 1998; Sawynok, 1999; Sheehan and Bountra, 2002). Adenosine A1 receptor agonists appear to be most effective against experimental inflammatory and neuropathic pain. For example, the selective adenosine A1 receptor agonist GR79236 caused a rapid reversal of sciatic ligature-induced allodynia (Collins et al., 2000) and inhibited carrageenin-induced inflammatory hyperalgesia (Clayton et al., 2000) in rats. Adenosine itself has been reported to be effective following intravenous infusion in patients with peripheral neuropathic pain (Belfrage et al., 1995) and, following intrathecal administration to neuropathic pain patients, adenosine had a long duration of analgesic effect (Sawynok et al., 1998). Moreover, consistent with our new data, protein kinase A-induced CGRP release from rat trigeminal neurones can be inhibited by adenosine A1 receptor agonists (Carruthers et al., 2001).

A potential issue for clinical use of adenosine A1 receptor agonists is the therapeutic window between the dose required for clinical efficacy and the dose producing mechanistic side effects. Higher doses of adenosine A1 receptor agonists have been reported to cause bradycardia and CNS effects (Sosnowski et al., 1989; Gardner et al., 1994). Preclinical studies measuring anti-nociceptive effects and locomotor activity, as an index of CNS effects or bradycardia, or both, in rats showed that the low efficacy adenosine A1 receptor agonist, GR190178, had a greater therapeutic index than the high efficacy adenosine A1 receptor agonist, GR79236 (Clayton et al., 2002). This difference presumably reflects differences in receptor coupling for these two responses and suggests that low efficacy adenosine A1 receptor agonists may offer benefit over full adenosine A1 receptor agonists in the clinic, including efficacy in migraine. The lower potency of GR190178, compared with GR79236, in this model is in keeping with their rank order of potency in adenosine A1 receptor in vitro assays (M. Sheehan, GlaxoWellcome,
unpublished observations) and in in vivo pain models (Clayton et al., 2000, 2002).

The role played by CGRP in the trigeminovascular system has been postulated to be that of cerebrovascular regulation, or sensory transmission, or both (McCulloch et al., 1986; Edvinsson et al., 1987; Biella et al., 1997; Edvinsson and Goadsby, 1995). Trigeminal ganglion stimulation in cat and humans increases cranial levels of CGRP (Goadsby et al., 1988). CGRP is elevated in the cranial circulation of humans during acute attacks of migraine (Goadsby and Edvinsson, 1993). CGRP is released by stimulation of the trigeminal nerve, either by direct electrical stimulation of its peripheral terminations in the SSS in the cat (Zagami et al., 1990), or by electrical stimulation of the trigeminal ganglion in the rat (Buzzi et al., 1991), cat and in humans (Goadsby et al., 1988). Triptans, 5-HT1B/1D agonists, can attenuate both peripheral (Williamson et al., 1997a, b) and central (Goadsby and Hoskin, 1996, 1999; Cumberbatch et al., 1997) effects that may be mediated by CGRP. We report here that both GR79236 and GR190178 can inhibit SSS-evoked CGRP release in the cranial circulation. These data are consistent with the electrophysiological data that demonstrated inhibition of trigeminal neuronal activation with GR79236 and GR190178.

In conclusion, we have shown a potent, robust, dose-dependent inhibition of nociceptive trigeminovascular activation by selective adenosine A1 receptor agonists. The high efficacy agonist, GR79236, and the low efficacy agonist, GR190178, had a dose-dependent inhibitory effect on SSS-evoked trigeminal neuronal activity. This effect could be reversed with the selective A1 antagonist DPCPX. Similarly, both agonists reduced the level of CGRP in the cranial circulation evoked by SSS stimulation. These findings are broadly consistent with the reported anti-nociceptive effects of adenosine A1 receptor activation. The present data support the clinical testing of adenosine A1 receptor agonists in migraine and cluster headache and, indeed, in an initial proof-of-concept study in migraine GR79236 did abort the acute attack (Humphrey et al., 2001). Our data suggest that such compounds need to gain access to the crucial site of action, which we speculate is the trigeminal nucleus caudalis. A clinically effective non-vasoconstrictor acute anti-migraine drug would answer a fundamental issue in the pathophysiology of migraine as to whether a neural inhibitory mechanism was sufficient to abort an acute migraine, and provide significant benefit to many patients.

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References


Knight YE, Goadsby PJ. Naratriptan inhibits central trigeminal neurones after systemic administration by a 5HT1B/1D receptor in cat [abstract]. Cephalalgia 1997; 17: 403.


Storer RJ, Butler P, Hoskin KL, Goadsby PJ. A simple method, using 2-hydroxypropyl-beta-cyclodextrin, of administering alpha-


