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Inhibition of trigeminal neurones after intravenous administration of naratriptan through an action at 5-hydroxytryptamine (5-$HT_{1B/1D}$) receptors

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1 The observation that 5-hydroxytryptamine (5-HT) is effective in treating acute attacks of migraine when administered intravenously resulted in a research effort that led to the discovery of the 5-$HT_{1B/1D}$ receptor agonist sumatriptan.

2 Clinical experience has shown sumatriptan to be an effective treatment with some limitations, such as relatively poor bioavailability, which naratriptan was developed to address. Increasing bioavailability has been achieved with greater lipophilicity and thus the potential for greater activity in the central nervous system.

3 In this study the increased access to central sites has been exploited in an attempt to characterize the pharmacology of those central receptors with the newer tools available. Trigeminovascular activation was examined in the model of superior sagittal sinus stimulation.

4 Cats were anaesthetized with α-chloralose (60 mg kg^{-1}, intraperitoneal), paralyzed (gallamine 6 mg kg^{-1}, intravenously) and ventilated. The superior sagittal sinus was accessed and isolated for electrical stimulation (250 μs pulses, 0.3 Hz, 100 V) by a mid-line circular craniotomy. The region of the dorsal surface of C_2 spinal cord was exposed by a laminectomy and an electrode placed for recording evoked activity from sinus stimulation.

5 Stimulation of the superior sagittal sinus resulted in activation of cells in the dorsal horn of C_2. Cells fired with a probability of 0.69 ± 0.1 at a latency of 9.2 ± 0.2 ms. Intravenous (i.v.) administration of naratriptan at clinically relevant doses (30 and 100 μg kg^{-1}), inhibited neuronal activity in trigeminal neurones of the C_2 dorsal horn, reducing probability of firing without affecting latency.

6 The effect of naratriptan could be reversed by administration of the selective 5-$HT_{1B/1D}$ receptor antagonist GR127935 (100 μg kg^{-1}, i.v.).

7 These data establish that naratriptan acts on central trigeminal neurones since sagittal sinus stimulation activates axons within the tentorial nerve and there are no inhibitory effects mediated within the trigeminal ganglion. Furthermore, given that this inhibition could be reversed by the relatively selective 5-$HT_{1B/1D}$ receptor antagonist GR127935, it is highly likely that the anti-migraine effects of drugs of this class with central nervous system access are mediated, at least in part, by 5-$HT_{1B/1D}$ receptors within the trigeminal nucleus.

Keywords: Headache; migraine; sumatriptan; trigeminovascular; blood-brain barrier

Introduction

Observations on the effect of intravenous infusion of 5-hydroxytryptamine (5-HT) in terminating acute attacks of migraine (Kimball *et al*., 1960; Lance *et al*., 1967) effectively commenced the research effort (Humphrey, personal communication) that resulted in the discovery of the 5-HT_1-like receptor agonist sumatriptan. Despite the widespread use of the compound and its undoubted efficacy in treating acute migraine attacks (Ferrari, 1991), its mechanism of action remains in some dispute (Humphrey & Goadsby, 1994) and clinical use has revealed some practical limitations. Recent studies comparing sumatriptan to either aspirin (Tfelt-Hansen *et al*., 1995) or dihydroergotamine (Winner *et al*., 1996) have highlighted the need for further development of compounds in this field. This development should ideally be targeted to avoid current problems with drugs used in the treatment of acute attacks of migraine, such as speed of effect for oral formulations, headache recurrence and side effects and may capitalize on advances in our understanding of the disorder which are relatively recent (Goadsby, 1997).

While 5-HT infusions effectively terminated attacks of migraine they were associated with marked side effects, such as flushing and diarrhoea (Lance *et al*., 1967). The many effects of 5-HT are mediated by the large number of receptors that are just being classified (Hoyer *et al*., 1994) and clarified (Hartig *et al*., 1996). Sumatriptan is an agonist at 5-HT_1-like receptors. It has been argued as to whether its anti-migraine effect is a result of agonist activity at 5-$HT_{1B/1D/1F}$ receptors (Humphrey *et al*., 1991; Phebus *et al*., 1996) or whether another receptor class exists (Yocca *et al*., 1997). Sumatriptan does not cross the blood-brain barrier insofar as this can be measured and has no effect on central nervous system structures in experimental settings (Humphrey *et al*., 1991) unless the blood-barrier is disrupted (Kaube *et al*., 1993a; Shepheard *et al*., 1995). Given the central nervous system effects of compounds, such as zolmitriptan (Goadsby & Edvinsson, 1994; Goadsby & Hoskin, 1996; Schoenen *et al*., 1996) and rizatriptan (Cumberbatch *et al*., 1997), and its greater lipophilicity in relationship to sumatriptan (Rance *et al*., 1997), the possible central nervous system actions of naratriptan have become potentially of great interest. In this study we examined the effect of intravenous administration of naratriptan upon evoked cell firing in the trigeminocervical complex and characterized the effect of naratriptan by use of the selective 5-$HT_{1B/1D}$ antagonist GR127935. These data have been presented in preliminary form (Knight & Goadsby, 1997).

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Methods

Fourteen cats weighing 2.6 ± 0.5 kg (mean $\pm$ s.d.) were anaesthetized with α-chloralose (60 mg kg^{-1}, i.p., with supplements of 20 mg kg^{-1}, i.v.) after induction and surgery with halothane (1–4%). They were intubated and ventilated (Harvard Pump, Ma) with paralysis with gallamine triethiodide (6 mg kg^{-1}, i.v.) commencing after completion of the surgery. End-expiratory CO_2 and fraction of inspired O_2 were continuously monitored (DATEX, Finland). Polyethylene catheters were also placed into the femoral artery for monitoring blood pressure and into the vein for fluid and drug administration.

Surgery

After mounting in a stereotactic frame, a circular midline craniotomy (2 cm in diameter) and $C_1\backslash C_2$-laminectomy were performed for access to the superior sagittal sinus (SSS) and the recording site in the C_2 spinal cord. To reduce movement artefacts bilateral pneumothoraces were created. In addition the thoracic cord was suspended by a spinal process and the C_2 lateral spinal process stabilized by clamping to the stereotaxic frame after limited exposure. The dura and falx adjacent to the SSS were dissected and the dural-sinus complex suspended over bipolar platinum hook electrodes. To prevent dehydration and for electrical insulation against the cortex, a paraffin bath was built with a dam of dental acrylic around the craniotomy and additionally, a small polyethylene sheet inserted under the SSS.

Stimulation and recording

To activate trigeminal primary afferents, the SSS was stimulated with a Grass S88 stimulator connected to a stimulus isolation unit (120 V, 250 μs, 0.3 Hz; SIU5A). Tungsten microelectrodes (Longreach Scientific, Ca) were lowered into the dorsolateral spinal cord caudal to the C_2-rootlets with an hydraulic micropositioner (Kopf, Model 650, U.S.A.). Electrical responses were amplified (NeuroLog, total system gain 30,000–40,000) and lowpass filtered (NeuroLog, high cut-off frequency 5.5 kHz) to prevent aliasing. The signal from the amplifier was passed to the analogue input of an A/D converter (LabMaster, Ohio) in an IBM-compatible microcomputer (80486-based) for simultaneous online analysis of single units and field potentials by a custom written programme (Microsoft C).

To obtain somatosensory field potentials the raw data were high pass filtered online with a digital 2nd order Butterworth filter (cut-off-frequency 250 Hz) to remove effects from superimposed action potentials on the amplitudes of the slow potentials and averaged over 50 or 100 repetitive recordings (sweep length 50 ms). Single unit activity was analysed after digital online high pass filtering (cut-off frequency 500 Hz) and passing a digital window discriminator to create a post-stimulus histogram over 50 or 100 recordings (sweep length 50 ms) to identify linked responses.

Drugs

Naratriptan, N-methyl-3-(1-methyl-4-piperidinyl)-1H-indole-5-ethanesulphonamide hydrochloride (GR85548; GlaxoWellcome R&D, U.K.), was administered in doses of either 30 μg kg^{-1} or 100 μg kg^{-1} by slow intravenous push over five minutes. This dose was chosen to match as far as is practical doses from a Phase II clinical study that resulted in a responder rate of more than 80% at a dose of 2.5 mg subcutaneously (Naratriptan Investigators' Brochure, unpublished data, GlaxoWellcome R&D, U.K.) and was reduced by weight assuming a 70 kg average body weight. GR127935, a potent selective 5-$HT_{1B/1D}$ receptor antagonist (Clitherow *et al.*, 1994) was administered intravenously in a dose of 100 μg kg^{-1} (GlaxoWellcome R&D).

Experimental design and analysis

Baseline recordings with 100 averages each were repeated at least three times to ensure that single unit and field potential responses in the spinal cord to SSS stimulation were reproducible over time. After baseline data were collected animals were treated with either vehicle or naratriptan. Data were then collected as epochs of 50 sweep averages at 5 min intervals for the ensuing 40 min. Data were analysed by reviewing each individual data set on the screen of a microcomputer by use of a locally written programme and, following preparation with a locally written filter, plots were made with SigmaPlot. Data were analysed by use of the Mann-Whitney U Test (Siegel, 1956) to compare firing with and without naratriptan and assessed at the $P < 0.05$ level for significance.

Results

All animals included in the analysis had arterial blood pH and gases (PCO_2 and PO_2) within normal ranges for the anaesthetized cat. Cardiovascular parameters, blood pressure and heart rate, were also normal. Stimulation of the superior sagittal sinus resulted in activation of neuronal elements within the caudal most part of the trigeminal nucleus caudalis. Units would fire with a mean probability of firing of 0.69 ± 0.1 at a latency of 9.2 ± 0.2 ms for the shortest latency units. These neurones had predominantly facial receptive fields in the cutaneous distribution of the ophthalmic division of the trigeminal nerve and were either wide dynamic range (WDR) or nociceptive specific (NS) by their response to noxious and non-noxious inputs (Hu *et al.*, 1981). In association with the firing of cells in the trigeminal nuclues a large trigeminal evoked potential could be measured at 234 ± 18 μV in the same area. Injection of vehicle had no effect upon cell firing ($n = 4$).

Effect of naratriptan

Intravenous injection of naratriptan (30 μg kg^{-1}; $n = 5$) had small (<10 mmHg), transient (<7 min) effects on mean blood pressure. The drug inhibited trigeminal evoked cell firing by reducing probability of firing to 0.44 ± 0.1 ($P < 0.05$) but without any effect on firing latency. Similarly, trigeminal evoked potentials were inhibited to 185 ± 15 μV ($P < 0.05$; Figure 1). At a larger dose of naratriptan (100 μg kg^{-1}; $n = 5$) mild (<15 mmHg), transient (<10 min) effects on mean

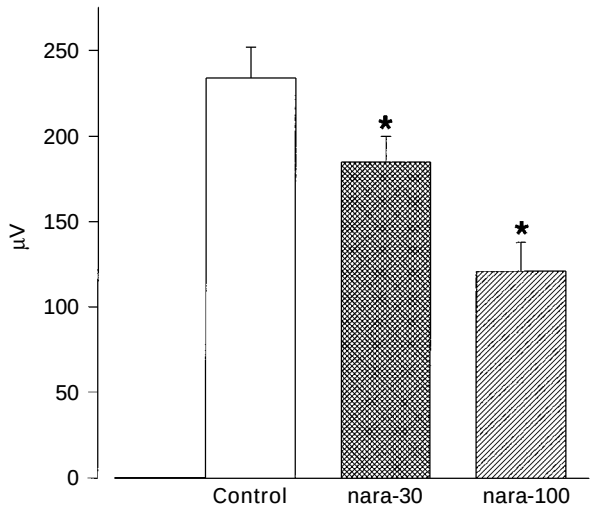

Figure 1 The effect of intravenous naratriptan on the trigeminal-evoked potential arising after electrical stimulation of the superior sagittal sinus. Naratriptan in a dose-dependent fashion at 30 μg kg^{-1} (Nara-30) and 100 μg kg^{-1} (Nara-100) inbibited the electrical activity in trigeminal neurones. The ordinate is the evoked potential in μV.

blood pressure were observed. Cell firing was markedly attenuated at this dose with a probability of firing of 0.30 ± 0.1 ($P < 0.01$; Figure 2) and again no effect on latency of firing.

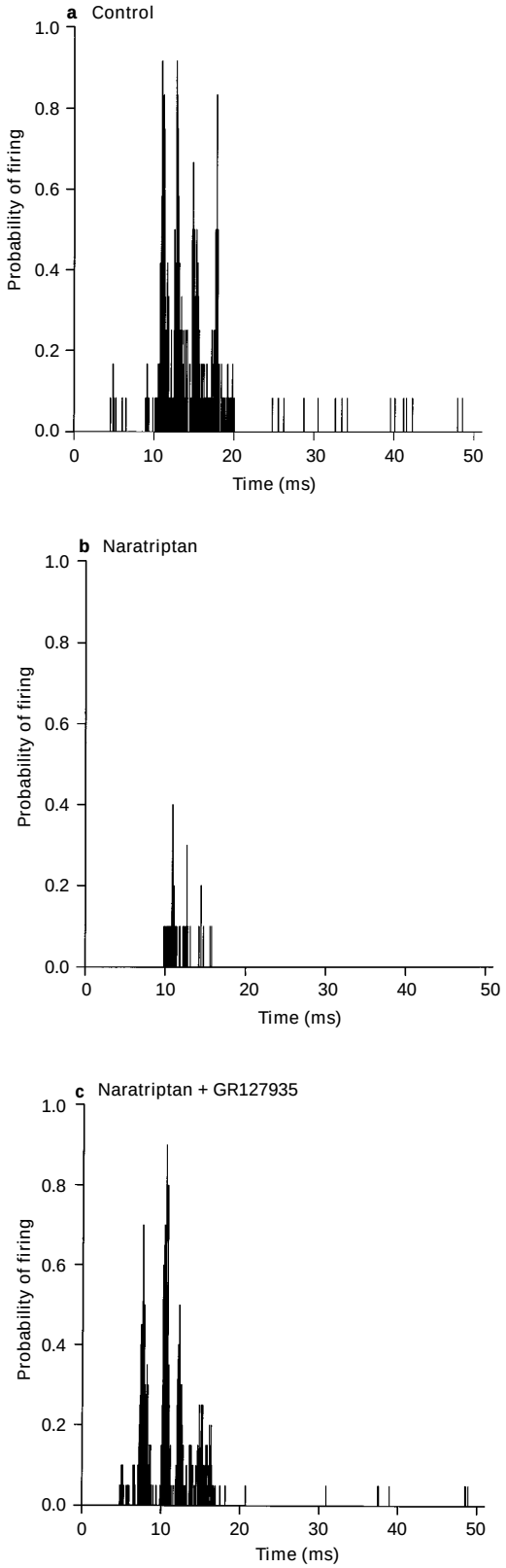

Figure 2 Original data captured by the A/D system, manipulated by a data analysis programme (see Methods) and plotted showing the probability of firing (ordinate scales) of units in the trigeminal nucleus after stimulation of the superior sagittal sinus. (a) control, (b) effect of naratriptan (100 μg kg^{-1}) on cell firing and (c) its reversal by GR127935 (100 μg kg^{-1}) illustrate that the mechanism of the effect of naratriptan is likely to be upon 5-HT$_{1B/1D}$ receptors.

Effect of a 5-HT$_{1B/1D}$ receptor antagonist

In the group of animals treated with naratriptan at 100 μg kg^{-1} GR127935, a potent selective 5-HT$_{1B/1D}$ antagonist, was administered intravenously (100 μg kg^{-1}) before the injection of naratriptan. Injection of GR127935 blocked the effects of naratriptan (Figure 3) and certainly had no added inhibitory effects, suggesting that no partial agonist activity was seen at this dose. In the group of animals receiving the smaller dose of naratriptan, vehicle administration was performed and the animals were followed for an additional 30 min after the maximal effect of the compound which had developed over 5 min. The effect of naratriptan did not reduce during the period of observation corresponding with the period over which GR127935 reduced the effect of the higher dose.

Discussion

These data demonstrate that naratriptan when administered intravenously in the experimental animal can, unlike sumatriptan, inhibit activity in the trigeminal nucleus. The effect of naratriptan is robust and reproducible, tightly linked to its administration and reversible by the 5-HT$_{1B/1D}$ antagonist GR127935. These data are compatible with its increased lipophilicity when compared to sumatriptan (Rance *et al*., 1997) and, in some measure, are reflected in its good clinical efficacy. The data support a direct inhibitory effect for this class of compounds, when they access the central nervous system, by an action upon 5-HT$_{1B/1D}$ receptors in the trigeminal nucleus by virtue of the specific agonist/antagonist combination employed.

The model of sagittal sinus stimulation has been used because it offers several distinct advantages in trying to understand the basic pathophysiology of primary vascular headaches and its impact upon the analysis of the data. First, it is clear that the large venous sinuses and large arteries at the base of the brain are pain-producing in man (Ray & Wolff, 1940; Wolff, 1963; McNaughton & Feindel, 1977) while the trigeminal ganglion contains all sensory modalities. Consistent with this human data is the fact that the innervation of the sinus is by small myelinated and unmyelinated fibres (Penfield, 1934; Penfield & McNaughton, 1940; Keller *et al*., 1985). The cells recorded from were either wide dynamic range or nociceptive-specific. When mapped with either 2-deoxyglucose autoradiography (Goadsby & Zagami, 1991) or Fos immunohistochemistry (Kaube *et al*., 1993b; Hoskin *et al*., 1996a,b; Goadsby & Hoskin, 1997) sinus stimulation results in activation of cells in the superficial laminae of the trigeminal nucleus caudalis and dorsal horns of the $C_{1/2}$ spinal cord, which is recognized to be a site receiving nociceptive information. Again consistent with the predominantly first (ophthalmic) division of trigeminal innervation by the tentorial nerve (Feindel *et al*., 1960), the cutaneous receptive fields in this study were largely in the first division of the trigeminal nerve. Similarly, sinus stimulation results in increased jugular vein levels of calcitonin gene-related peptide (CGRP) (Zagami *et al*., 1990), a feature of the acute attack of both migraine (Goadsby *et al*., 1990; Gallai *et al*., 1995) and cluster headache (Goadsby, 1994; Fanciullacci *et al*., 1995).

Since the aim of the study was to examine trigeminal cells within the central nervous system, the sinus offers the opportunity to stimulate effectively the end-organ and the tentorial nerve as a unit. By electrically stimulating the entire structure the model effectively bypasses the nerve-vessel synapse (Kaube *et al*., 1992). The site of stimulation is important since the 5-HT$_{1B}$ receptors are found on vessels (Hamel *et al*., 1993; Bouchelet *et al*., 1996) and in the trigeminal ganglion (Rebeck *et al*., 1994). A consequence of this expression is that electrical stimulation of the sinus, which bypasses the peripheral targets, results in activation of trigeminal neurones that is unaffected

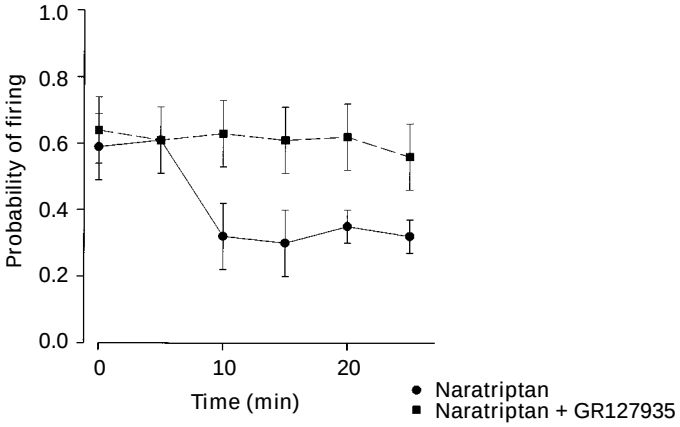

Figure 3 Time course data (abcissae in minutes) for the effect of naratriptan upon probability of firing of trigeminal units and its inhibition by GR127935. The ordinate scale shows probability of firing after stimulation of the superior sagittal sinus.

by sumatriptan (Kaube *et al*., 1993a) just as direct trigeminal ganglion stimulation bypasses these peripheral elements and again results in activation of trigeminal neurones that is unaffected by sumatriptan (Shepheard *et al*., 1995). However, if the sinus is mechanically stimulated on its luminal surface by stretching, trigeminal neurones are activated (Kaube *et al*., 1992) and this activation may be inhibited by sumatriptan (Hoskin *et al*., 1996b). Lastly, it is important to note that 5-$HT_{1B/1D}$ receptor agonists as a class do not inhibit trigeminal ganglion cells directly (O'Shaughnessy *et al*., 1993).

Functional anatomical evidence from autoradiographic studies is consistent with the data of this study. By use of [^{3}H]-sumatriptan, binding has been found in the dorsal horn of the upper cervical spinal cord and caudal trigeminal nucleus caudalis in cat (Mills & Martin, 1995), guinea-pig (Waeber & Moskowitz, 1995) and man (Pascual *et al*., 1996). A similar distribution can be seen for [^{3}H]-dihydroergotamine (Goadsby & Gundlach, 1991), which binds 5-HT receptors that sumatriptan activates (Hamblin *et al*., 1987; Humphrey *et al*., 1991), and for another member of this drug class, zolmitriptan (Goadsby & Knight, 1997). Given that both dihydroergotamine (Hoskin *et al*., 1996a) and zolmitriptan (Goadsby & Hoskin, 1996) also inhibit sagittal sinus evoked caudal trigeminal nucleus activity, both the current anatomical and functional data are consistent with the effects of naratriptan presented here. However, the pharmacological nature of this site is as yet not clearly defined.

Is the effect observed pharmacologically relevant? Given that the inhibition of trigeminal firing is dose-dependent at doses well within clinically used levels the inhibition is potentially relevant *in vivo*. Similarly, the fact that the effect could be antagonized by a dose of GR127935 which is relatively specific for the 5-$HT_{1B/1D}$ receptor (Clitherow *et al*., 1994; Starkey & Skingle, 1994) supports the possibility that the effect seen is indeed related to activation of functionally relevant receptors on cells in the trigeminal nucleus. The reversal of the effect eliminates the possibility that the cells being observed were lost by either subtle movements in the electrode during recording or that local changes around the electorde resulted in loss of signal. The data are consistent with results obtained for zolmitriptan (Goadsby & Hoskin, 1996), rizatriptan (Cumberbatch *et al*., 1997) and dihydroergotamine (Hoskin *et al*., 1996a), and offer the very important addition of the effect of the 5-$HT_{1B/1D}$ antagonist GR127935. Taken together the data convincingly demonstrate the existence of a functional receptor at this site which is inhibitory. Given that the same cells that have been observed in the cat have now been demonstrated in non-human primates (Goadsby & Hoskin, 1997) it is highly likely that they have a functional role in man.

In summary, the data demonstrate a dose-dependent time-locked potent inhibition of sagittal sinus evoked trigeminal nucleus activity by the highly specific 5-$HT_{1B/1D}$ receptor agonist, naratriptan. Further, it has been shown that this inhibition can be reversed by the 5-$HT_{1B/1D}$ receptor antagonist GR127935 at doses without intrinsic agonist activity or other known receptor activity. The second order neurones of the trigeminovascular pain pathway are an ideal candidate as a site for controlling pain in migraine, since specific inhibitors would bypass the vascular effects that are widely seen as a weakness of many of the current treatments. Moreover, the characterization of the pathways and receptor systems involved in migraine must precede any understanding of the pathophysiology of the condition.

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