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Inhibition by sumatriptan of central trigeminal neurones only after blood-brain barrier disruption

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1 The 5-hydroxytryptamine (5-HT_1)-like agonist, sumatriptan, is highly efficient in the relief of migraine headache and its accompanying symptoms.

2 Experimental evidence has indicated that its site of action may be on the cranial vessels or on the trigeminal innervation of the cranium, or both, since sumatriptan does not pass the blood-brain barrier easily under normal circumstances. It is, however, not clear whether the blood-brain barrier is normal or abnormal during a migraine attack.

3 In this study, single unit activity and trigeminal somatosensory evoked potentials in central trigeminal neurones were monitored during electrical stimulation of the superior sagittal sinus.

4 Intravenous administration of sumatriptan ($100\,\mu g\,kg^{-1}$) did not alter trigeminal evoked activity unless the permeability of the blood-brain barrier had been increased by infusion of an hyperosmolar mannitol solution. After blood-brain barrier disruption, sumatriptan decreased the peak-to-peak amplitude of evoked potentials by $40 \pm 6\%$ and the probability of firing of single units by $30 \pm 9\%$. Mannitol infusions alone in control animals caused no changes in evoked potentials or single unit activity.

5 The data suggest that in normal circumstances sumatriptan does not have sufficient access to trigeminal neurones to alter their function.

Keywords: Headache; migraine; 5-HT receptor; sumatriptan, central trigeminal neurones; blood-brain barrier

Introduction

Migraine is well recognized clinically although its fundamental pathophysiology is unknown. Based on both clinical and experimental data we have proposed a unifying hypothesis integrating both the central nervous system structures, such as the trigeminal nucleus caudalis, and the trigeminal innervation of the meninges and cranial vessels (Goadsby *et al.*, 1991a). The synthesis and clinical trial of the novel 5-hydroxytryptamine (5-HT_1-like) agonist, sumatriptan, has provided both an important addition to clinical practice and a valuable research tool. The site of action of sumatriptan is of particular interest as it may provide a direction for further drug development and an understanding of at least part of the mechanism of migraine.

It has been proposed that the site of action of sumtriptan is at cranial arteries as a highly selective vasoconstrictor (Feniuk *et al.*, 1991), or in the periphery at the trigeminovascular innervation of the cranium (Markowitz *et al.*, 1988). Both hypotheses for a peripheral site of action are consistent with the fact that under normal circumstances sumatriptan cannot penetrate the blood-brain barrier in substantial amounts (Humphrey *et al.*, 1991).

In a series of well conceived experiments it has been shown that whereas intravenous administration of sumatriptan neither effects frontal cortex levels of 5-HT (Sleight *et al.*, 1990) nor pial vessel diameter (Connor *et al.*, 1992), local injection of the drug can alter both 5-HT levels in brain and pial vessel diameter. Since it has been shown that vasodilatation of cranial vessels may not necessarily be a sufficient stimulus to activate trigeminal neurones (Kaube *et al.*, 1992), vasoconstriction may, therefore, not be the only mechanism responsible for the clinical efficacy of the drug in migraine (Goadsby *et al.*, 1991b; Ferrari, 1991). Given that other drugs with similar pharmacological actions that are also useful in migraine, such as dihydroergotamine, have access to central nervous system binding sites (Goadsby & Gundlach, 1991) it is of interest that sumatriptan directly applied by iontophoresis to trigeminal neurones inhibits single unit activity linked to sagittal sinus stimulation in 20% of tested units (Boers, 1989). The prospect of a central locus of action of sumatriptan would then stand as a third alternative to explain its action.

In order to investigate the effects of systemically administered sumatriptan on the central processing of trigeminal noxious stimuli, we combined the electrophysiological model of sagittal sinus stimulation and recording from the dorsolateral cervical spinal cord, that we have previously characterized (Lambert *et al.*, 1988; Goadsby & Zagami, 1991), with hyperosmolar infusions to disrupt the blood-brain barrier. Mannitol was used as an hyperosmolar agent because of its low toxicity and the well-documented time course of its action (Cosolo *et al.*, 1989). As a marker for increased permeability of the blood-brain barrier we chose Evans Blue which can be detected in small quantities by fluorescence microscopy techniques.

Methods

Eleven female cats weighing 2.7 ± 0.2 kg were anaesthetized initially with 1.5% halothane and then α-chloralose (60 mg kg^{-1}, i.p.) and prepared for physiological monitoring. The femoral artery and vein were cannulated in order to measure blood pressure and heart rate and provide access for drug administration, respectively. Cardiovascular parameters and pupillary reaction to noxious pinching of the forepaw were used to determine the need for supplementary anaesthesia. The animals were endotracheally intubated, ventilated with 40% oxygen and paralyzed after the surgical procedures with repeated doses of gallamine triethiodide (6 mg kg^{-1}, i.v., as required). Body temperature and end-expiratory CO_2 were monitored and maintained within physiological limits.

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Surgery

After mounting in a stereotactic frame, a circular midline craniotomy (2 cm in diameter) and C1/C2-laminectomy were performed for access to the superior sagittal sinus (SSS) and the recording site in the C2 spinal cord. Possible artefacts from arterial pulsation and respiratory movement were reduced by: bilateral pneumothoraces, suspension of the thoracic spinal processes, clamping of the C1 lateral spinal processes and covering the cervical spinal cord with a layer of agar gel. At the recording site the pia mater was carefully removed to facilitate the insertion of the electrode. The dura mater and falx adjacent to the SSS were dissected over 10 mm and the sinus 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 a small polyethylene sheet was inserted under the SSS.

Stimulation

To activate trigeminal primary afferents, the SSS was stimulated with a Grass S88 stimulator driving a stimulus isolation unit (SIU5A; 150 V, 250 μs duration, 0.3 s^{-1}). Tungsten-in-glass microelectrodes (tip length/diameter: 50/15 μm, impedance: <200 kΩ) were lowered into the dorsolateral spinal cord 4–5 mm caudal to the mid-point of the C2-rootlets between 500 and 1500 mm below the surface with a hydraulic micropositioner (Kopf, Model 650, USA) to record somatosensory evoked potentials and single unit activity.

Recording

Electrical responses were amplified and filtered (NeuroLog, total system gain: 20,000–30,000; bandwidth for field potentials: d.c.-5.5 kHz; for action potentials: 300 Hz–5.5 kHz). For the recording of single unit activity, the signal was first passed through a window discriminator for spike detection while for evoked potentials the amplified signal was directly fed to the analog input of an A/D conversion card (LabMaster, Ohio, U.S.A.) in an IBM-compatible microcomputer (80386/80387 based) and sampled at 20 kHz. Evoked potentials were averaged over 100 repetitive stimulations and linked single unit activity recorded in post-stimulus histograms (sweep length 50 ms) with a custom written programme (Microsoft C) and stored on hard disk. 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.

Study design

Animals were randomly assigned to two different experimental interventions. After baseline data had been obtained group one animals (controls, $n = 5$) were given an intravenous infusion with 35 ml mg^{-1} mannitol (12.5%) over 12 min to investigate the effect of increasing the permeability of the blood-brain barrier on electrophysiological responses to trigeminal stimulation in the dorsolateral cervical spinal cord. Responses were recorded during the infusion and after 15, 30, 45 and 60 min. In the second group ($n = 6$), after baseline recordings sumatriptan (Glaxo, Ware UK; 100 μg kg^{-1}) was administered intravenously and responses monitored again after 15, 30, 45 and 60 min. The animals were then subjected to the same hyperosmolar infusion and monitoring scheme as group one. Throughout the experiments electrophysiological data, blood pressure and heart rate were pulse code modulated (Vetter, 16 Channel) and stored on video tape to enable later review. After the animals were killed with KCl, 100 responses were elicited to obtain slow potentials that only consisted of the electrical artefact. This component was then subtracted from all previous recordings prior to determining the peak-to-peak amplitudes. Data were compared by the Wilcoxon matched pairs signed ranks test (Siegel, 1956) and assessed for significance at the $P < 0.05$ level.

Blood-brain barrier integrity

In preliminary experiments carried out to determine the parameters for the hyperosmolar infusion scheme, animals were injected with 2% Evans Blue (50 mg kg^{-1}) followed in some animals by mannitol infusions and perfused with saline and 10% formaldehyde through the thoracic descending aorta. The spinal cords were removed. Spinal cord levels C1 to C3 were sectioned on a cryo-microtome at 50 μm, and inspected under a fluorescent microscope at 365 nm to evaluate the presence of Evans Blue qualitatively as an indicator for an increased permeability of the blood-brain barrier.

Results

Physiological parameters of the eleven animals included in the analysis were normal (Table 1). The studies with Evans Blue confirmed that the mannitol infusions that were used increased blood-brain barrier permeability in the upper cervical spinal cord. Systemic arterial blood pressure was increased (14 ± 5 mmHg) after the injection of sumatriptan for 5 to 15 min while mannitol infusions also caused a transient elevation of blood pressure (19 ± 3 mmHg) lasting up to 30 min.

Electrophysiological data

Electrical stimulation of the superior sagittal sinus elicited linked electrophysiological responses in the dorsolateral C2 spinal cord. Baseline peak-to-peak amplitudes for trigeminal somatosensory evoked potentials were 438 ± 161 μV in Group 1 animals and 451 ± 188 μV in Group 2. The latencies for the peak of the first component were 9.8 ± 0.4 ms and 9.3 ± 0.4 ms, respectively. The baseline probability of firing for linked single units in the dorsolateral area was 0.44 ± 0.06 with a latency of 6.9 ± 0.6 ms for the fastest component in the post stimulus histogram. Infusions with mannitol alone (group 1) had no effect on the field potentials (3 ± 10%; Figure 1). Sumatriptan alone (group 2) led only to a small transient increase in peak-to-peak amplitudes of trigeminal evoked potentials that was maximal after 30 min. After disruption of the blood-brain barrier with mannitol the evoked responses were significantly reduced by 40 ± 6% ($P = 0.03$, $n = 6$; Figure 2). The effects on single units were similar. Only the combination of intravenous sumatriptan and disruption of the blood-brain barrier led to a significant inhibition (Figure 3) in the probability of firing of single units (30 ± 9%). There was no consistent effect of sumatriptan or mannitol administration alone on the latencies of evoked potentials or single units.

Table 1 Cardiovascular and blood gas parameters†

Blood pressure (mmHg)	*Heart rate* (min^{-1})	*pH*	P_{CO_2} (mmHg)	P_{O_2} (mmHg)
114 ± 4	185 ± 5	7.33 ± 0.02	35 ± 1	212 ± 7‡

†$n = 11$
‡On 40–45% inspiratory O_2.

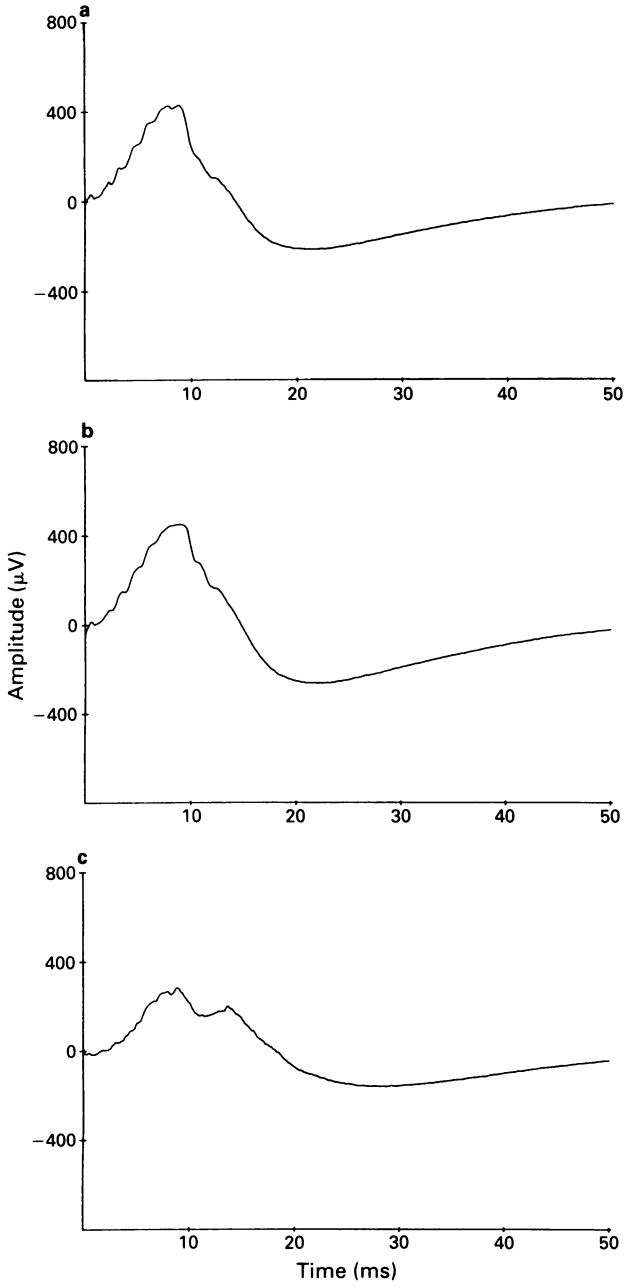

Figure 1 Trigeminal somatosensory evoked potentials (average of 100 repetitive recordings) after electrical stimulation of the superior sagittal sinus recorded from the dorsolateral C2 spinal cord. (a) Control; (b) recording 30 min after injection of sumatriptan (100 $\mu g\ kg^{-1}$); (c) evoked potentials 30 min after infusion of 12.5% mannitol (90 min after sumatriptan).

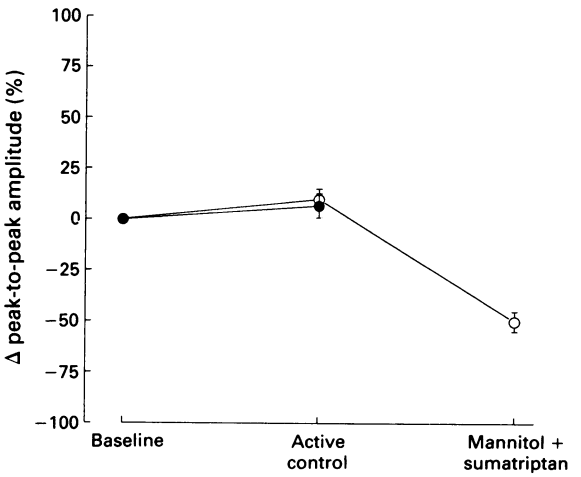

Figure 2 Group differences of means and standard errors of peak-to-peak amplitudes of trigeminal somato-sensory evoked potentials after superior sagittal sinus stimulation (100 averages) recorded from the C2 spinal cord. Experimental animals (group 2, O) received sumatriptan and mannitol; controls (group 1, ●) received only mannitol infusions.

Discussion

These data demonstrate that sumatriptan can interact with the transmission of nociceptive input in central trigeminal neurones suggesting inhibitory modulation of synapses at the second order neurone if entry of the drug into the central nervous system has been facilitated. The time interval between the injection of sumatriptan and the hyperosmolar infusion was 60 min and was long enough to allow the temporary changes in blood pressure and field potential amplitudes to recover before the next intervention. Since sumatriptan has a plasma half-life of 2–4 h, enough active compound was still available after 1 h when the mannitol infusion was given.

Essential to the interpretation of these data is that the blood-brain barrier was sufficiently disrupted by the intravenous mannitol infusion. Usually hyperosmolar agents are infused intra-arterially through the carotid arteries to achieve high concentrations in the cerebral circulation (Cosolo *et al.*, 1989). Because of the small calibre and complexity of the arterial supply to the cervical spinal cord and medulla in the cat selective cannulation of spinal arteries to guarantee high arterial mannitol concentrations is impractical. In addition we wished to avoid manipulations of the spinal blood supply to ensure spinal cord viability. In preliminary experiments increasing arterial perfusion pressure or cranial venous pressure rendered the recording site more unstable and led to loss of the recorded cell. Although the total mannitol concentrations in the vascular bed of the target tissue obtained by intravenous infusion are lower than can be expected from arterial infusions, the increased uptake of Evans Blue in the spinal tissue indicates an increased permeability of the blood-brain barrier in the experimental animals as opposed to the controls. It is also possible that the high fluid load during the infusions of 30 ml kg^{-1} over a short period of time may have contributed to the impairment of the blood-brain barrier while a further facilitating factor may have been some superficial neural injury to spinal cord resulting from the removal of the pia at the recording site. All of these considerations apply equally to the control and treated groups and are, therefore, unlikely to be the reason for the results as described.

As the inhibition of the electrophysiological responses only occurred after the administration of both sumatriptan and mannitol, it is most likely that the disruption of the blood-brain barrier is pivotal in facilitating the inhibitory effect of sumatriptan. The decrease in firing after mannitol infusion could have been caused by physico-mechanical changes in the neural tissue induced by the blood pressure changes or the increased intravascular volume resulting in the loss of the monitored single units. This could not explain why this effect would only occur in animals that had been pretreated with sumatriptan. The recording with long tipped, low impedance tungsten-in-glass microelectrodes enabled us to record field potentials and single unit activity together. However, the low spatial discrimination of such electrodes often leads to the recruitment of more than one single unit action potential. In the case of firing clusters our analysis software did not allow us to distinguish between cell body and axonal action potentials. It was also not possible to perform a cluster analysis of

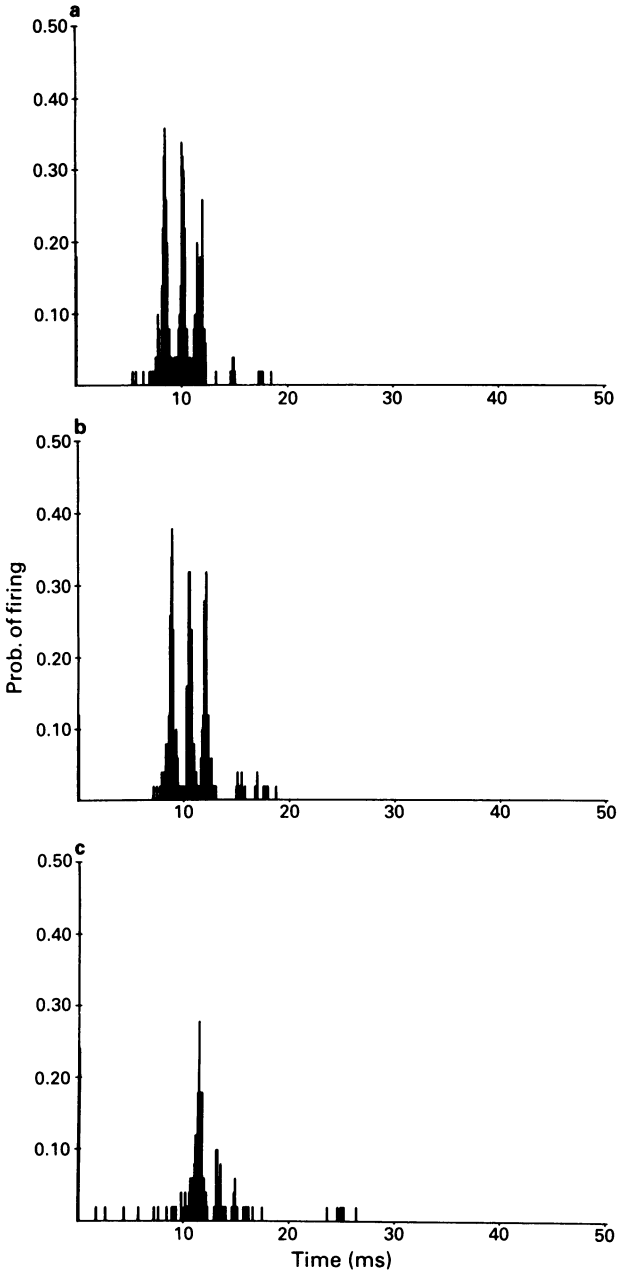

Figure 3 Poststimulus histograms of single unit activity (average of 100 repetitive recordings) after electrical stimulation of the superior sagittal sinus recorded from the dorsolateral C2 spinal cord. (a) Control recording; (b) recording 30 min after injection of sumatriptan, 100 $\mu g\,kg^{-1}$; (c) single unit activity 30 min after infusion of 12.5% mannitol (90 min after sumatriptan).

multiple single unit firing to identify which or how many units stopped firing after the pharmacological intervention. However, it was a consistent finding that the total probability of firing computed for the whole post-stimulus interval was markedly decreased only after the administration of sumatriptan and mannitol.

Data in the literature support the view that under normal circumstances there is a minimal entry of sumtriptan into the brain. Ten minutes after an intravenous dose of 1 $mg\,kg^{-1}$ of [^{14}C]-sumatriptan in the rat only some 0.05% of the dose could be located in the brain (Humphrey *et al.*, 1990). In a study in rat it has been shown that intravenous injection of sumatriptan did not alter brain production of 5-HT while a local injection, that would bypass the blood-brain barrier, reduced brain 5-HT levels (Sleight *et al.*, 1990). Furthermore, sumatriptan has no effect on isolated pial vessels unless it is injected directly around those vessels when it acts as a vasoconstrictor, again suggesting that the blood-brain barrier must be passed for a significant action to be seen (Connor *et al.*, 1992). The data reported here extend this concept more directly into the brain regions likely to be involved in migraine. Neither the frontal cortex nor pial vessels are thought to play a role in the pathogenesis of the disorder nor are they thought to be affected during resolution of an attack. Our data represent a clear demonstration of an effect of sumatriptan in the trigeminal nucleus, after blood-brain barrier disruption.

It was not possible to distinguish in these studies whether the observed modulation of the central trigeminal pathway was based on a direct effect of sumatriptan on inhibitory 5-HT_1-like receptors at second order synapses or mediated through descending 5-hydroxytryptaminergic antinociceptive pathways from supraspinal centres but it was certainly not peripheral. Since supramaximal stimulation of afferent C-fibres in the superior sagittal sinus was used in the studies, peripheral transduction mechanisms were bypassed and it is, therefore, very unlikely that the vasoconstrictive properties of sumatriptan on cranial vessels (Feniuk *et al.*, 1991), its inhibitory effect on the release of calcitonin gene-related peptide from trigeminal nerve terminals (Goadsby & Edvinsson, 1993) or its blockade of neurogenic inflammation (Moskowitz & Buzzi, 1991) could have accounted for the results observed. Such peripheral effects would also not depend on the presence of hyperosmolar infusions. It is not known whether the integrity of the blood-brain barrier is impaired during migraine attacks although it is important to note that drowsiness or sedation are recognized as albeit less common side effects of parenteral sumatriptan administration indicating a possible central site of action.

These data are certainly consistent with a peripheral action of sumatriptan on the as yet unproven assumption that the blood-brain barrier is normal in migraine and suggest a further target for future anti-migraine drugs. Future clinical research with better imaging techniques, radiolabelled drugs and tracers will reveal more about the role of supraspinal and spinal structures in the pathophysiology of migraine. These data suggest that the status of the blood-brain barrier in migraine must be studied if the action and site of action of antimigraine compounds is to be understood since these drugs have significant actions in the central nervous system if access to the brain is possible.

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