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Short communication

The effects of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor agonists on trigeminal nociceptive neurotransmission in anaesthetized rats

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Abstract

Pre-clinical studies have suggested that one mechanism of antimigraine action of the 'triptan' 5-$HT_{1B/1D}$ receptor agonists may be through inhibition of central nociceptive transmission in the trigeminal dorsal horn. In anaesthetized rats, the 5-$HT_{1B/1D}$ receptor agonist, zolmitriptan (up to 3 mg kg^{-1}, i.v.), inhibited the action potential discharge of single trigeminal neurones to noxious electrical stimulation of the middle meningeal artery. In contrast, the selective 5-HT_{1B} receptor agonist, CP-93,129 (3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one), and the 5-HT_{1A} receptor selective agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) had no effect in this assay at up to 3 mg kg^{-1}, i.v.. Brain penetrant, triptan 5-$HT_{1B/1D}$ receptor agonists may therefore mediate their central trigeminal anti-nociceptive action in the rat via 5-HT_{1D}, but not 5-HT_{1B} or 5-HT_{1A}, receptors.

Keywords: Zolmitriptan; Migraine; 5-$HT_{1B/1D}$ receptor; 5-HT_{1A} receptor; CP-93,129

1. Introduction

In recent years, migraine headache has received progressively greater attention following the emergence of the novel and clinically effective anti-migraine 5-$HT_{1B/1D}$ receptor agonists collectively known as the 'triptans'. It has been postulated that the mechanism of action of the 'triptans' is within the intracranial extracerebral meningeal vasculature via direct vasoconstriction (Humphrey and Feniuk, 1991) and by inhibition of neurogenic vasodilation and/or extravasation (Moskowitz, 1992).

With the advent of brain penetrant, 5-$HT_{1B/1D}$ receptor agonists (such as rizatriptan, zolmitriptan and naratriptan), an additional central anti-migraine locus of action has been proposed. It has been shown that brain penetrant triptans can inhibit the responses of neurones in the trigeminal nucleus caudalis to electrical stimulation of the meninges (Goadsby and Hoskin, 1996; Cumberbatch et al., 1997; Goadsby and Knight, 1997b). Moreover, anatomical studies show that 5-$HT_{1B/1D}$ receptors are located in the trigeminal ganglia and in the trigeminal nucleus caudalis (Bruinvels et al., 1992, 1994; Longmore et al., 1997; Bonaventure et al., 1998) in the region of termination of dural afferent fibers. These data strongly support a central role of 5-$HT_{1B/1D}$ receptors in anti-migraine therapy, in addition to any peripheral effects.

Although sumatriptan is a selective agonist for 5-$HT_{1B/1D}$ receptors in humans, binding data suggest that sumatriptan has little selectivity between rat 5-HT_{1B} and 5-HT_{1A} receptors (van Wijngaarden et al., 1990). This may also be true for other 5-$HT_{1B/1D}$ receptor agonists and interpretation of their effects requires caution, since systemic administration in the rat may have non-specific 5-HT_{1A}-mediated effects. It has also been shown that 5-HT_{1D} receptors have a more restricted trigeminal distribution than 5-HT_{1B} receptors (Longmore et al., 1997; Bonaventure et al., 1998), suggesting that 5-HT_{1D} receptor selective agonists may have a different activity profile over less selective 5-HT_1 receptor agonists. An electrophysiological assay in the anaesthetized rat was used to determine the relative contribution of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors to the central anti-nociceptive actions of the 'triptan' anti-migraine compounds in the trigeminal nucleus caudalis. The following compounds were used as agonists for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors (see Table 1); 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), CP-93,129 (3-(1,2,5,6-tetrahydropyrid-

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Table 1

	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1D}$
8-OH-DPAT	4.9[a]	3600[a]	1900[b]
CP-93,129	3000[a]	15[a]	2200[a]
Zolmitriptan	600[c]	34[a]	1.3[a]

Binding affinities (nM) for 8-OH-DPAT, CP-93,129 and zolmitriptan at rat cortex[a], bovine caudate nucleus[b] and human recombinant receptors[c]. Data from van Wijngaarden et al. (1990), Martin (1997) and Beer (unpublished observation).

4-yl)pyrrolo[3,2-*b*]pyrid-5-one; Macor et al., 1990) and zolmitriptan (Martin, 1997).

2. Materials and methods

2.1. Animal preparation

All experiments were conducted and terminated under general anaesthesia. The surgical preparation and experimental protocol have been described in detail previously (Cumberbatch et al., 1997). Briefly, male Sprague–Dawley rats ($n = 14$, 300–450 g) were anaesthetized with halothane, the trachea, the carotid artery and the jugular veins were cannulated and the animal was immobilized in a stereotactic frame. The brainstem was exposed and the skull was thinned over the middle meningeal artery. Anaesthesia was maintained by a constant intravenous infusion of sodium pentobarbitone (30 mg kg^{-1} h^{-1}) and neuromuscular blockade was initiated (2 mg kg^{-1}) and maintained (1 mg kg^{-1} h^{-1}) with pancuronium bromide. General anaesthesia was ensured by monitoring the absence of cardiovascular and pupillary responses to noxious stimuli.

2.2. Electrophysiology

Single-barreled glass microelectrodes (1.5 M NaCl; 5–10 MΩ) were inserted into the brainstem to record extracellular action potentials from single trigeminal neurones. A tungsten bi-polar electrode was positioned on the thinned surface of the skull and electrical stimuli (1–3 mA, 100–300 μs square wave pulses) were applied at 0.3 Hz as search stimuli.

2.3. Drug administration and experimental design

After determining the threshold of electrical stimulus required to evoke action potentials, trains of 20 electrical stimuli were applied at 1 Hz to the thinned skull at 0.2 mA above threshold. These trains were repeated every 200 s until the responses were stable. One of the following drugs per animal were given intravenously every 10 min (every three cycles) in a cumulative dose regimen of 0.3, 1 and 3 mg kg^{-1}; the 5-HT$_{1B/1D}$ receptor agonist, zolmitriptan, the 5-HT$_{1B}$ receptor agonist, CP-93,129, or the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT (Sigma). Drug effects were calculated as a percentage of the mean of the three responses immediately prior to dosing and data are expressed as the mean ± S.E.M.

3. Results

3.1. Cell classification

A total of 14 neurones from 14 rats were used for pharmacological tests. These neurones were located between 180–910 μm (mean of 564 μm) below the pial surface in the trigeminal nucleus caudalis. All cells received single or multiple single unit action potentials following electrical stimulation of the dura mater, with a mean latency to the first action potential of 4.2 ms (range of 2–13 ms).

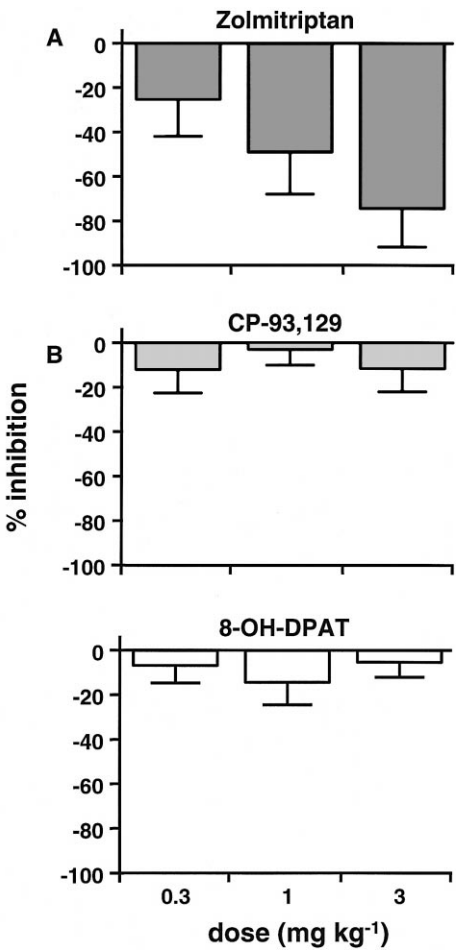

Fig. 1. The mean data for the effects of the 5-HT$_{1B/1D}$ receptor agonist, zolmitriptan (A; $n = 5$), the 5-HT$_{1B}$ receptor agonist, CP-93,129 (B; $n = 5$) and the 5-HT$_{1A}$ receptor agonist δ-OH-DPAT (C; $n = 4$) on responses of single trigeminal neurones to noxious electrical stimulation of the middle meningeal artery. Drugs were dosed i.v. in a cumulative dose regimen. Data are displayed as the percentage inhibition compared to the mean of three pre-drug control values (mean ± S.E.M.).

3.2. Effects of zolmitriptan, CP-93,129 and 8-OH-DPAT

The 5-$HT_{1B/1D}$ receptor agonist, zolmitriptan, at 0.3, 1 and 3 mg kg^{-1} (i.v.), dose-dependently inhibited durally evoked responses by a maximum of $70 \pm 15\%$ at 3 mg kg^{-1} ($n = 5$, Fig. 1a). In contrast, neither the 5-HT_{1B} receptor selective agonist, CP-93,129 ($n = 5$, Fig. 1b), or the 5-HT_{1A} receptor agonist, 8-OH-DPAT ($n = 4$, Fig. 1c), had any significant dose-related effects on durally evoked responses.

4. Discussion

The results of this study support the hypothesis that brain penetrant 5-$HT_{1B/1D}$ receptor agonists have central anti-nociceptive effects in the trigeminal nucleus caudalis (Goadsby and Hoskin, 1996; Cumberbatch et al., 1997; Goadsby and Knight, 1997b) and for the first time provide evidence that 5-HT_{1B} and 5-HT_{1A} receptors are not involved in the central processing of dural nociceptive information in the rat.

The effects of zolmitriptan were unlikely to be via 5-HT_{1A} or 5-HT_{1B} receptors because the 5-HT_{1A} and the 5-HT_{1B} receptor agonists (8-OH-DPAT and CP-93,129) produced only small and non-significant changes in the evoked activity. These small, spontaneous changes in neuronal activity were similar to those observed following administration of saline vehicle control (Cumberbatch et al., 1997).

We have considered the issue of relative brain penetration for the compounds that were used in these experiments. Zolmitriptan has been shown to enter the brain and to occupy 5-$HT_{1B/1D}$ receptors (Goadsby and Knight, 1997a) and the inhibitory effects of zolmitriptan in the current study are consistent with this. These data are also consistent with the inhibitory effects of micro-iontophoretically applied zolmitriptan, which support the hypothesis that activation of central 5-HT_{1D} receptors is anti-nociceptive (Storer and Goadsby, 1997).

The lack of effect of 8-OH-DPAT is unlikely to be due to inadequate brain penetration since previous studies have shown this agonist to inhibit central neuronal firing at 30 $\mu g\ kg^{-1}$ i.v.; (Ceci et al., 1994). In addition, it was noticed that 8-OH-DPAT produced a small decrease in blood pressure in the present study ($\approx 10\%$ decrease; data not shown). This hypotension is thought to be mediated via activation of central 5-HT_{1A} receptors (Fozard et al., 1987). These observations suggest that 8-OH-DPAT entered the brain and that 5-HT_{1A} receptors are not involved in trigeminal nociceptive processing.

Previous studies with CP-93,129 have involved direct injections into the central nervous system (CNS; Macor et al., 1990) and have therefore avoided the issue of brain penetration. CP-93,129 has high affinity for the rat 5-HT_{1B} receptor (Table 1) and has been shown to inhibit dural neurogenic vasodilation in the rat at a dose of 10 $\mu g\ kg^{-1}$ (Shepheard et al., 1997). The dose used in the current experiments was considerably higher than this and would have been expected to achieve effective CNS concentrations. Using in situ brain perfusion techniques, it was found that CP-93,129 and zolmitriptan have similar uni-directional rates of entry into the brain (1.9 and 1.4 $\mu l\ min^{-1}\ g^{-1}$, respectively; Tattersall, unpublished observations; see Tattersall et al., 1996 for methods) and that these compounds also have similar plasma decay kinetics following a 3 mg kg^{-1} i.v. dose in the rat (area under the curve for the first 15 min was 88 $\mu M \cdot min$ for zolmitriptan and 87 $\mu M \cdot min$ for CP-93,129; Williamson, unpublished observation). These data suggest that the inward driving force for brain entry of CP-93,129 and zolmitriptan, and therefore, the extent of brain penetration, would be similar. Since CP-93,129 and zolmitriptan have similar affinities for the 5-HT_{1B} and 5-HT_{1D} receptors, respectively (Table 1), the lack of effect of CP-93,129 under the conditions of these experiments was probably not due to ineffective brain penetration.

It is widely recognized that 5-$HT_{1B/1D}$ receptor agonists directly constrict the meningeal and cerebral vascular beds and that normalization of painfully distended blood vessels may represent a significant 5-HT_{1B} receptor mediated component of the anti-migraine action of this class of drugs (Humphrey and Feniuk, 1991). However, it is unlikely that the effects of zolmitriptan in the current study were due to a meningeal vasoconstriction, and hence a reduced tonic mechanoreceptor activation, because CP-93,129 and other 5-$HT_{1B/1D}$ receptor agonists have no direct vasoconstrictor effects on dural blood vessels in the rat (Shepheard et al., 1997; Williamson et al., 1997). In addition, the electrical stimuli used in the present study would have depolarized the perivascular afferent neurones and thus any direct vasoconstriction by zolmitriptan would have been irrelevant.

Recent evidence suggests that in man there are no 5-HT_{1D} receptors on the meningeal vasculature and that these receptors are found specifically on trigeminal perivascular afferent fibers (Longmore et al., 1997). Additionally, 5-HT_{1D} receptors are located in the trigeminal ganglion and on pre-synaptic elements within the nucleus caudalis but have a restricted distribution elsewhere in the brain compared to 5-HT_{1B} receptors (Longmore et al., 1997; Bonaventure et al., 1998). This suggests that brain penetrant 5-HT_{1D} selective receptor agonists may be centrally antinociceptive without vasoconstrictor activity and with reduced central side effects.

5. Conclusion

These data show that 5-HT_{1A} and 5-HT_{1B} receptors do not appear to be involved in dural nociceptive processing in rats and that the central effects of 'triptan' 5-$HT_{1B/1D}$ receptor agonists in this assay may be mediated by 5-HT_{1D}

receptors. Whilst it is sometimes difficult to predict clinical efficacy, these results suggest that the rat may be a suitable species for the identification of centrally acting 5-HT_{1D} selective agonists that could have therapeutic utility in migraine headache.

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