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Research Report

Distribution of 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{1F}$ receptor expression in rat trigeminal and dorsal root ganglia neurons: Relevance to the selective anti-migraine effect of triptans

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ABSTRACT

Triptans, acting as serotonin, 5-HT$_{1B/1D/1F}$, receptor agonists, provide an effective and established treatment option in migraine and cluster headache. Clinical observations suggest a relatively specific effect of these compounds on primary headache disorders, but not in other pain syndromes. The mechanism of this specificity, however, is not well understood. Hence, we systematically studied primary sensory ganglia in rat to determine if the peripheral distribution of 5HT$_{1B/1D/1F}$ receptors showed any anatomical difference that would account for the specificity of clinical effect. Rat primary afferent and sensory ganglia neurons—trigeminal ganglia (Vg), and dorsal root ganglia (DRG): C_2, C_5, T_5, L_5—were examined using paraffin-embedded, slide-bound tissue sections reacted with specific primary antibodies for rat 5-HT$_{1B,\ 1D}$ and $_{1F}$ receptors in a peroxidase-based immunohistochemical method. Immunoreactivity specific for all three serotonergic receptor subtypes was demonstrated in the five peripheral nervous system regions examined and quantitated. There was a good agreement for 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors to that previously demonstrated in Vg and DRG L_5, while this was the first characterisation for 5-HT$_{1F}$ receptor in any of the five regions, as well as for 5-HT$_{1B}$ and 5HT$_{1D}$ receptors in DRG C_2, C_5 and T_5. In summary, all three 5-HT receptors are equally represented in Vg and the DRGs examined. We conclude that the triptans are theoretically able to bind to receptors at each level of the peripheral neuraxis without any apparent anatomical preference for the head.

1. Introduction

Migraine is an episodic neurological disorder that includes attacks of disabling headache, with pain involving the trigeminal and upper cervical dermatomes, sensory dysmodulation and, in one-fifth of patients, an aura with neurologic symptoms (Lance and Goadsby, 2005). The introduction of serotonin, 5-HT$_{1B/1D}$ receptor agonists, the triptans (Goadsby,

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Abbreviations: 5-HT, 5-hydroxytryptamine; ABC, avidin–biotin complex; CGRP, calcitonin gene-related peptide; DAB, 3,3′-diaminobenzidine tetrahydrochloride; DRG, dorsal root ganglion/ganglia; mRNA, messenger ribonucleic acid; SP, substance P; SSS, superior sagittal sinus; TNC, trigeminal nucleus caudalis; Vg, trigeminal ganglion/ganglia

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2000), provided a new effective abortive treatment of migraine (Ferrari et al., 2001) and provided fundamental insight into the neurobiology of migraine (Goadsby et al., 2002). Understanding the anatomical locus of their action may provide considerable insight into the condition and allow predictions about new treatments (Ahn and Basbaum, 2005; Goadsby et al., 2009).

Many studies investigating the site and mode of the anti-migraine effect have focused on direct 5-$HT_{1B/1D}$ actions of the triptans on the trigeminovascular system (De Vries et al., 1999; Goadsby, 2000; Moskowitz and Cutrer, 1993). Classically three putative mechanisms of anti-migraine action were considered to explain their mechanism of action: cranial vasoconstriction (Humphrey et al., 1991; Saxena and De Boer, 1991), peripheral neuronal inhibition (Moskowitz and Cutrer, 1993), inhibition of transmission through second order neurons of the trigemino-cervical complex (Kaube et al., 1993). As further data have been acquired and clinical experience accumulated, central sites of action within the brain have increasingly become more attractive to explain the action of these medicines. Certainly triptans can act in the rostral ventromedial medulla (Edelmayer et al., 2009; Vera-Portocarrero et al., 2008), the midbrain periaqueductal grey (PAG) (Bartsch et al., 2004) and in the ventroposteromedial nucleus of the thalamus (Shields and Goadsby, 2006).

Data from clinical studies suggest a remarkable specificity of the triptans with regard to migraine and cluster headache. Triptans are not effective in other forms of head pain, notably atypical facial pain (Harrison et al., 1997), paroxysmal hemicrania (Cittadini et al., 2008) and hemicrania continua (Cittadini et al., 2009) or somatic myofacial pain (Dao et al., 1995), although trigeminal mechanisms are clearly involved in these syndromes. Experimentally, the systemic application of naratriptan results in a selective inhibitory effect for trigeminal neuronal activation over spinal dorsal horn activation (Cumberbatch et al., 1998), which might suggest some anatomical specialization of the receptors. Intravenous administration of sumatriptan (Kaube et al., 1993) or naratriptan (Goadsby and Knight, 1997) inhibits trigeminal neuronal responses to stimulation of dural-vascular afferents, but neither has effects in standard spinal antinociceptive assays (Connor et al., 1997; Skingle et al., 1990).

Previous studies in the rat have examined the quantitative distribution of 5-$HT_{1B,\ 1D\ and\ 1F}$ receptors in Vg or in L_5 dorsal root ganglion, or both (Ma, 2001; Ma et al., 2001; Pierce et al., 1997; Wotherspoon and Priestley, 2000). In particular, Potrebic and colleagues did not find evidence of a differential peripheral distribution when comparing the distribution of 5-HT_{1D} receptor-immunoreactivity within the trigeminal ganglion (TRG) and lumbar dorsal root ganglion of the rat (Potrebic et al., 2003). Another study on 5-HT_{1B} receptors did not find evidence for a selective distribution of the 5-HT_{1B} receptor in cranial targets, such as arterial blood vessels and primary afferents (Longmore et al., 1997; Wotherspoon and Priestley, 2000). Previous work offers good evidence for a peripheral component to the action of triptans (Levy et al., 2004), including providing an explanation for one of the side effects of triptans in migraine: initial headache worsening (Burstein et al., 2005).

In view of these experimental findings we have considered a sole peripheral action for triptans as an unlikely explanation for the specific efficacy in particular headache syndromes. Hence, we systematically studied primary sensory ganglia in all levels of the neuraxis in rat (Vg, C_2, C_5, T_5, and L_5) to determine if the peripheral distribution of 5$HT_{1B/1D/1F}$ receptors showed some degree of a cranio–caudal gradient that may explain the apparent trigeminovascular selectivity of triptans. In essence, we found the receptors equally distributed in the periphery, by virtue of the fact they were found consistently in large numbers in a set of randomly selected sections for all three receptors.

2. Results

2.1. *Distribution of 5-HT_{1B} receptor-IR neurons*

Within all of the five types of ganglia (Vg, DRG C_2, C_5, T_5 and L_5), 5-HT_{1B} receptor-immunoreactive (IR) cells were clearly observed (Vg: Figs. 1A; DRG C_2: D; DRG C_5: G; DRG T_5: J and DRG L_5: Fig. 2A) and represented the full range of neuronal sizes from small through to medium and large diameter neurons (Vg: Figs. 1B, C; DRG C_2: E, F; DRG C_5: H, I; DRG T_5: K, L and DRG L_5: Figs. 2B, C). In addition, it was possible to discern a broadly defined subdivision of cells into two sub-groupings (Table 1), often regardless of their size for some nuclei, based upon the texture/quality of their resulting immunoreaction product. So for exclusively larger cells in the Vg there was typically a "punctuate" appearance. By contrast all DRG C_5 cells were also largely represented by a "punctuate" staining pattern. DRG C_2 and L_5 both wholly exhibited "dense" staining irrespective of cell size and finally DRG T_5 demonstrated uniformly "punctuate" labelling of all its cells across the size range.

2.2. *Distribution of 5-HT_{1D} receptor-IR neurons*

Again, each of the five peripheral nuclei (Vg, DRG C_2, C_5, T_5 and L_5) contained numerous 5-HT_{1D} receptor-IR cells (Vg: Figs. 2D; DRG C_2: G; DRG C_5: J; DRG T_5: Figs. 3A and DRG L_5: D) and represented the entire range of neuronal phenotypes from small through to medium and large diameter neurons (Vg: Figs. 2E, F; DRG C_2: H, I; DRG C_5: K, L; DRG T_5 Figs. 3B, C and DRG L_5: E, F). As was the case for the 5-HT_{1B} receptor, 5-HT_{1D} receptor-IR neurons were similarly classifiable into two subtypes based upon their immunoreactive staining properties (Table 1). In this instance, DRG C_2 neurons like those staining for 5-HT_{1B} receptor-IR were generally distinguished by a "dense" staining outline for all cell sizes. In all other cases, the cells in each of the nuclei fell into different subdivisions from those of their counterparts observed for 5-HT_{1B} receptor-IR. Like DRG C_2, the immunostaining for DRG C_5 neurons was determined as "dense" across all cell sizes. The staining seen in the Vg was unique, mainly "punctuate", whatever the neuron class. Lastly, only the larger cells were selectively "punctuate" in their 5-HT_{1D} receptor staining quality for both DRG T_5 and L_5, while small and medium neurons were characterised by the "dense" labelling property.

2.3. *Distribution of 5-HT_{1F} receptor-IR neurons*

Those cells immunostaining for 5-HT_{1F} receptors (Vg: Figs. 3G; DRG C_2: J; DRG C_5: Figs. 4A; DRG T_5: D; DRG L_5: G) represented