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Autoradiographic mapping of [^{3}H]sumatriptan binding in cat brain stem and spinal cord

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Abstract

In vitro autoradiography was performed on sections of cat brain stem and spinal cord using [^{3}H]sumatriptan. Localization studies using 20–25 nM [^{3}H]sumatriptan showed specific binding to cells in the trigeminal nucleus caudalis and nucleus tractus solitarius of the brain stem and the dorsal horn of the spinal cord. This binding was unaffected by 8-hydroxy-dipropylaminotetralin (20 nM), but was abolished by 5-carboxamidotryptamine (200 nM). Ketanserin displaced total specific binding in the brain stem with a pIC_{50} of 6.2 but no apparent regional specificity. These results indicate that [^{3}H]sumatriptan labels predominantly 5-$HT_{1D\alpha}$ and/or 5-$HT_{1D\beta}$ (but not 5-HT_{1A} or 5-HT_{1F}) receptor subtypes in cat brain stem and spinal cord.

Keywords: [^{3}H]Sumatriptan; Autoradiography; Trigeminal nucleus caudalis; Nucleus tractus solitarius; (Cat)

1. Introduction

Intracranial blood vessels receiving sensory innervation from the Vth cranial (trigeminal) nerve are now widely regarded as a primary source of pain in migraine and related vascular headaches (Moskowitz, 1993). The ergot alkaloids and the 5-HT_{1D} receptor agonist sumatriptan interact with this 'trigemino-vascular system', possibly by producing cerebral vessel constriction directly and/or inhibition of sensory neuropeptide release by a pre-junctional action on perivascular trigeminal afferents (Humphrey and Feniuk, 1991; Moskowitz, 1993). These effects appear to involve only a peripheral action of the drugs, since sumatriptan does not cross the blood-brain barrier (Kaube et al., 1993). However, ex vivo and in vitro autoradiographic studies with [^{3}H]dihydroergotamine show that this drug binds to a discrete population of cells in cat trigeminal nucleus caudalis, which also respond to superior sagittal sinus stimulation with an increase in metabolism and cell firing rate (Goadsby and Gundlach, 1991; Kaube et al., 1992). These cells may represent an important site of cranial nociceptive modulation, since locally applied dihydroergotamine is able to inhibit cell firing (Lambert et al., 1986). Although sumatriptan appears not to access these cells in vivo (Kaube et al., 1993), it displays greater receptor specificity than dihydroergotamine, and exhibits preferential affinity for the 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$ and 5-HT_{1F} subtypes. In this report we describe the use of [^{3}H]sumatriptan binding to identify and localize 5-HT_1 receptors in cat brain stem and spinal cord.

2. Materials and methods

2.1. Radioligand binding assays and autoradiography

Adult male cats (3–3.5 kg) were initially anaesthetized by isofluorane inhalation and then killed by lethal injection of chloralose. The upper cervical spinal cord and brain stem were removed, frozen immediately on dry ice and stored at −70° C until required. Cryostat sections (10 μm) were collected on Vectabond-treated slides and stored at −20° C for a minimum of 3 days. Sections were removed from the freezer and allowed to stand at room temperature for 30 min prior to pre-incubation in 170 mM Tris-HCl/4 mM $CaCl_2$, pH 7.5 for a further 30 min. Binding assays were carried out in the same buffer containing 0.01% (w/v) ascorbic acid, 10 μM pargyline and [^{3}H]sumatriptan

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(0.1–45 nM). Incubation was for 90 min at room temperature. Non-specific binding was determined on adjacent sections by co-incubating with 10 μM 5-hydroxytryptamine (5-HT). Slides were washed twice for 1 min in Tris/$CaCl_2$ buffer at 4° C, dipped in ice-cold distilled water for a few seconds and dried in a cold air stream. Autoradiograms were obtained by apposing the slides to [^{3}H]Hyperfilm for 3–6 weeks. Saturation binding and radioligand displacement data were generated by wiping the tissue sections off the slides using Whatman GF/B filters and measuring the radioactivity by liquid scintillation counting.

2.2. Materials

[^{3}H]Sumatriptan (65–84 Ci/mmol) and [^{3}H]Hyperfilm were obtained from Amersham Life Science (Amersham, UK). Vectabond was purchased from Vector Laboratories (Peterborough, UK), 5-hydroxytryptamine (creatinine sulphate complex; 5-HT), ketanserin and 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) from Sigma (Poole, UK) and 5-carboxamidotryptamine (5-CT) from Tocris-Cookson (Bristol, UK).

3. Results

The binding of [^{3}H]sumatriptan to sections of cat spinal cord (Fig. 1) and brain stem (not shown) was saturable over the concentration range used (0.1–45 nM) with half-maximum binding being achieved at a ~ 9 nM. Scatchard analysis suggested that binding was to more than one population of sites, although the data did not permit resolution of the individual components of binding. These results are not inconsistent with binding to 5-$HT_{1D\alpha}$ and/or 5-$HT_{1D\beta}$ receptor subtypes as significant binding to 5-HT_{1F} receptors can be excluded, since reported affinities of sumatriptan are in the range 23–80 nM (Amlaiky et al., 1992; Adham et al., 1993; Lovenberg et al., 1993). In order to more fully determine the nature and distribution of the [^{3}H]sumatriptan binding site(s), autoradiographic experiments were performed using a saturating concentration of label (20–25 nM) in the absence or presence of various competing ligands with selectivity for different 5-HT receptor subtypes.

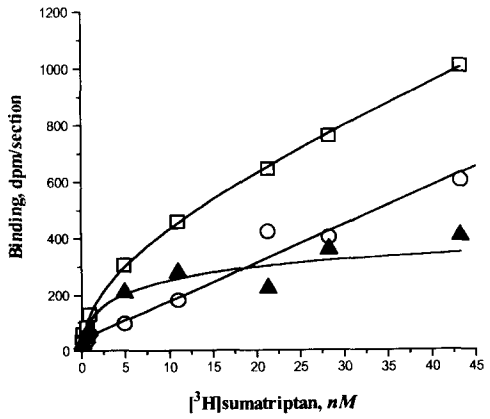

Fig. 1. Saturation binding of [^{3}H]sumatriptan to sections of cat spinal cord. Data are the mean of triplicate determinations, and S.E.M.s were less than 10% of the mean. Non-specific binding (○) was determined in the presence of 10 μM 5-HT and deducted from total binding (□) to obtain values for specific binding (▲). Similar results were obtained in two independent experiments.

[^{3}H]Sumatriptan binding to sections of cat brain stem and spinal cord revealed high densities of binding in the nucleus tractus solitarius and trigeminal nucleus caudalis of the brain stem (Fig. 2A) and the dorsal horn of the spinal cord (Fig. 2B). This binding was abolished in the presence of 10 μM 5-HT and matches that reported for ex vivo binding of [^{3}H]dihydroergotamine (Goadsby and Gundlach, 1991). Furthermore, binding was unaffected by the inclusion of 20 nM 8-OH-DPAT but absent in the presence of 200 nM 5-CT confirming that, at this concentration, [^{3}H]sumatriptan was not binding to either 5-HT_{1A} or 5-HT_{1F} receptors in these tissues.

In order to ascertain whether the binding observed was to the 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptor subtype, competition assays were carried out using ketanserin as this ligand has been reported to have a 70-fold higher affinity for 5-$HT_{1D\alpha}$ over 5-$HT_{1D\beta}$ receptors (Weinshank et al., 1991; Kaumann et al., 1993). Ketanserin caused a complete displacement of specific [^{3}H]sumatriptan binding to sections of cat brain stem (not shown) and spinal cord (Fig. 3) with a pIC_{50} of 6.2. Whilst this might imply binding predominantly to 5-$HT_{1D\alpha}$ receptors the pIC_{50} value does not concur with affinity estimates for ketanserin binding to human recombinant 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptors. Whether this discrepancy reflects species differences and/or the presence of a mixed population of sites requires further investigation. Autoradiographic analysis of [^{3}H]sumatriptan binding in the presence of 0.1, 1.0 or 10 μM ketanserin did not appear to show any differential displacement of binding from specific structures in either the brain stem or spinal cord.

4. Discussion

[^{3}H]Sumatriptan selectively binds to discrete areas of cat brain stem corresponding to the trigeminal nucleus caudalis and the nucleus tractus solitarius while in the spinal cord the majority of binding is to the dorsal horn. In previous studies with [^{3}H]dihydroergotamine (Goadsby and Gundlach, 1991) the binding in these areas was thought to be predominantly to

5-HT_{1A} receptors. However, this possibility has been ruled out in the present experiments and the pharmacology of the [^{3}H]sumatriptan binding sites is consistent with binding to 5-$HT_{1D\alpha}$ and/or 5-$HT_{1D\beta}$ receptors. Although competition assays with ketanserin suggest binding is predominantly to the 5-$HT_{1D\alpha}$ receptor subtype more selective ligand or subtype-specific antibodies are required to confirm this hypothesis. The presence of 5-$HT_{1D\alpha}$ receptors in these areas of the CNS would be in agreement with the result of Rebeck et al. (1994) who reported that mRNA for 5-$HT_{1D\alpha}$, but not 5-$HT_{1D\beta}$, receptors is present in ganglia of the Vth cranial (trigeminal) nerve, which projects to the trigeminal nucleus caudalis. However, Bruinvels et al.

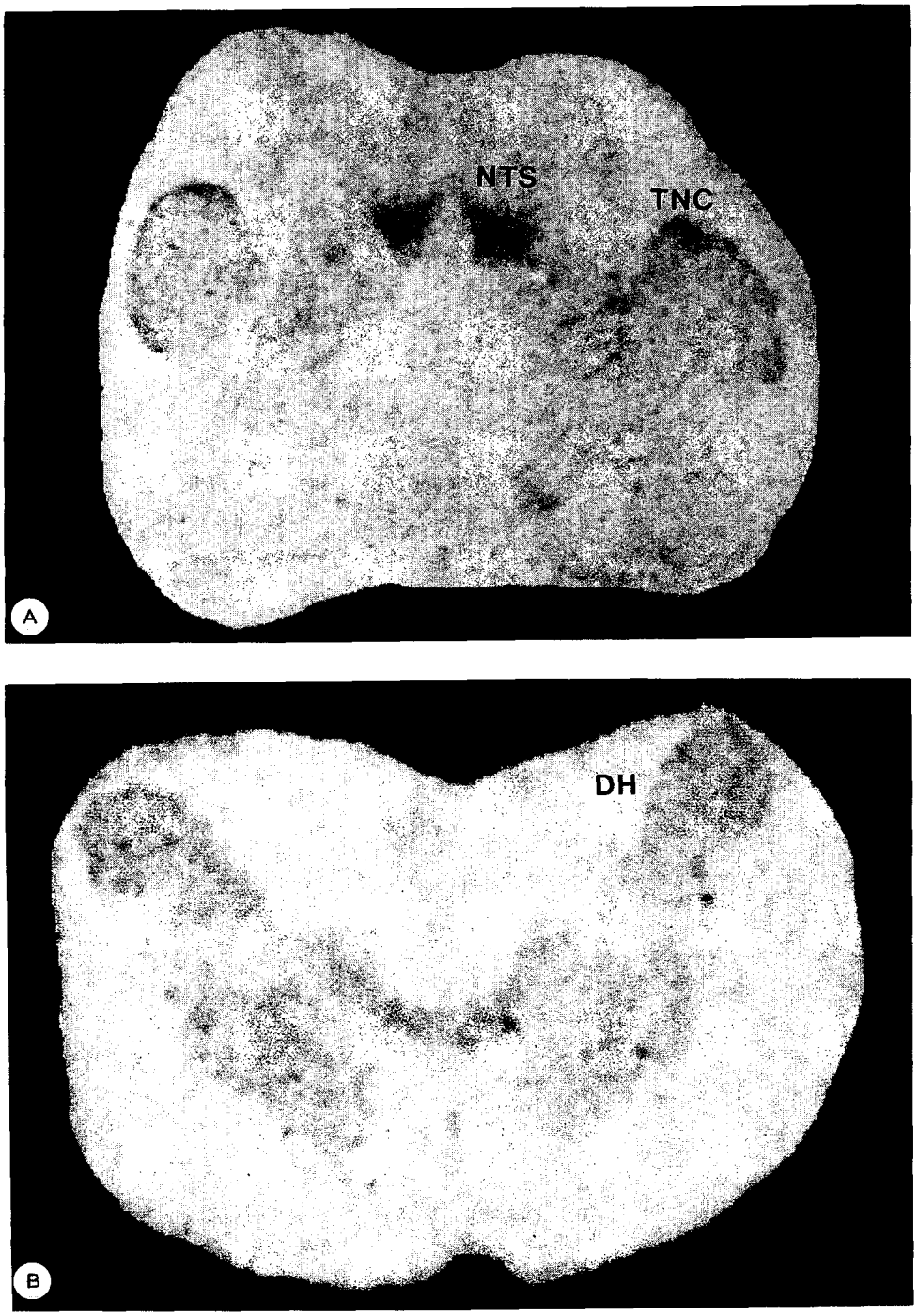

Fig. 2. Autoradiographic localization of [^{3}H]sumatriptan binding to sections of cat brain stem (A) and spinal cord (B). Highest densities of binding are seen in the nucleus tractus solitarius (NTS) and trigeminal nucleus caudalis (TNC) of the brain stem and the dorsal horn (DH) of the spinal cord.

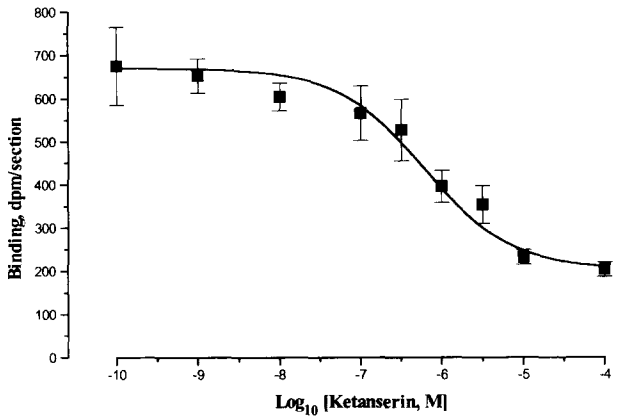

Fig. 3. [^{3}H]Sumatriptan (20 nM) binding to sections of cat spinal cord in the presence of ketanserin. Results shown are the means of triplicate determinations ± S.E.M.s. Non-specific binding was determined in the presence of 10 μM 5-HT and was equal to that observed at 1×10^{-4} M ketanserin. Similar results were obtained in two independent experiments.

(1994) have found mRNA for both 5-HT_{1D} receptor subtypes in the trigeminal nucleus of rodent brain.

The selective labelling by [^{3}H]sumatriptan of regions involved in the central processing of cranial pain emphasizes the potential importance of these sites as a target for anti-migraine 5-HT_{1D} receptor agonists. Furthermore, the presence of [^{3}H]sumatriptan-sensitive binding sites in the nucleus tractus solitarius of the cat raises the possibility that a central action of 5-HT_{1D} receptor agonists may contribute directly to the ability of this drug class to reduce the nausea and vomiting associated with migraine headache.

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