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Distribution of D_5 dopamine receptor mRNA in rat brain

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The distribution of the messenger RNA encoding the dopamine D_5 receptor was determined in the rat brain by in situ hybridization. Using [^{35}S]-labelled riboprobes to either the rat or human D_5 receptor, this mRNA was localized to the hippocampus and the parafascicular nucleus of the thalamus. This mRNA could not be visualized in the more traditional brain regions associated with dopaminergic cell bodies or projection fields. This unusual distribution suggests a novel function in the brain for this subtype of the dopamine receptor.

The recent cloning of multiple subtypes of the dopamine receptor has revealed at least five distinct receptors, which cluster into D_1-like and D_2-like families [2, 4 , 7, 17, 21–25, 28, 29]. The D_2 family consists of D_2 [2], D_3 [7, 21] and D_4 [25] receptors. All three of these receptors have D_2-like pharmacology, with subtle between-receptor differences. In particular, the D_4 receptor has a 10-fold greater affinity for the atypical neuroleptic clozapine compared to the D_2 receptor, but both receptors have comparable affinities for spiperone [25]. The distributions in brain of the mRNAs encoding these receptors are also distinctive. D_2 receptor mRNA is localized in most of the traditional motor and limbic dopaminoceptive regions of the brain, as well in several of the dopamine-containing cell groups, reflecting autoreceptor synthesis [1, 10, 12–15, 18, 26]. D_3 receptor mRNA has a more limbic distribution, with minimal localization in the deep grey motor nuclei [1]. The D_4 receptor message appears to have a cortical, limbic and hypothalamic distribution [25].

The D_1 family of dopamine receptors at present contains only two members, the D_1 [4, 17, 23, 29] and D_5 [22, 24, 28] (also named D_{1A} and D_{1B}, resp.) subtypes. Both of these receptors have fairly similar D_1-like pharmacology, although the D_5 receptor has a higher affinity for dopamine itself than the D_1 receptor [22, 24]. The mRNA encoding the D_1 receptor is widely distributed in brain, very much in parallel with the distribution of the D_2 receptor in the dopaminoceptive regions of the central nervous system, although not in the dopamine-containing cell regions [5, 11, 14, 16, 27]. The distribution of D_5 receptor mRNA in brain is less clear.

In the original paper describing the cloning of the human D_5 receptor [22], in situ hybridization and Northern analysis using oligonucleotide probes suggested that the mRNA for this receptor is distributed in a number of motor and limbic regions of the rat brain, indistinguishable from the previously described distribution of the mRNA encoding the D_1 receptor. Subsequently, the rat homolog of the human D_5 receptor was cloned and referred to as D_{1B} [24]. In contrast to the earlier report using oligonucleotides to the human D_5 sequence, the distribution of the D_{1B} receptor mRNA in the rat brain was reported as quite restricted, occurring only in the hippocampus, lateral mammilary nuclei, and a thalamic nucleus identified as the anterior pretectal nucleus. Based on the high degree of homology between these two sequences, it is likely that they are encoding the same functional receptor in the respective species from which they were cloned. Accordingly, it would be expected that the anatomical distributions demonstrated in the rat brain in these earlier two reports should be identical. Given the marked discrepancies in the anatomical distributions reported, the purpose of the present study was to reexamine the distribution of the mRNA encoding the D_5/D_{1B}

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receptor in the rat brain in an attempt to resolve this inconsistency, using in situ hybridization with both rat and human probes.

Brains were rapidly removed from male Sprague Dawley rats (250 g) that had been sacrificed by decapitation, and were frozen in isopentane (−30°C) for 30 s. Frozen tissue samples were cryostat-sectioned (15 μm) and thaw- mounted onto polylysine-subbed microscope slides. Sections were obtained in a coronal plane from the frontal pole (rostral to the caudate-putamen), to just caudal to the substantia nigra. These sections were maintained at −80°C until the time of hybridization.

Riboprobes complementary to human D_5 receptor mRNA were synthesized from a partial D_5 receptor cDNA. A 1,560 bp insert corresponding to the region of the human D_5 receptor spanning transmembrane domains I–VII was synthesized using PCR and spliced into the *Sal*I site of pGEM-3Z. This plasmid was linearized with *Pst*I, to result in a 800 bp probe corresponding to most of the third cytoplasmic loop and transmembrane domains VI and VII. Riboprobes complementary to rat D_5 receptor mRNA were generated from a partial D_5 receptor cDNA corresponding to transmembrane domains II–VI subcloned into pGEM-3Z. This plasmid was linearized with *Sal*I to generate a 650 bp probe. To generate antisense riboprobes, 1 μg of these linearized DNAs were labelled with 250 μCi of [^{35}S]UTP using T7 RNA polymerase. The probes were synthesized and purified as previously described for other dopamine receptor riboprobes [10–14].

For in situ hybridization, slides at −80°C were fixed and pre-hybridized as we have previously described [10–14]. Sections were hybridized with the [^{35}S]-labelled riboprobes overnight at 55°C. Probes were diluted in hybridization buffer to a final concentration of 1–2 × 10^6 dpm/30 μl. The hybridization buffer was 75% (v/v) formamide for the rat probe, 50% formamide for the human probe; 10% (w/v) dextran sulfate; 3 × SSC; 50 mM sodium phosphate, pH 7.4; 1 × Denhardt's solution (0.02% polyvinyl pyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin); 0.1 mg/ml yeast tRNA; and 10 mM dithiothreitol. Diluted probe was applied to prehybridized tissue sections, covered with a glass cover slip, and sealed with rubber cement. Following hybridization, the cover slips were removed. The sections were first rinsed in 2 × SSC at room temperature for 5 min, then in RNAse A (200 μg/ ml in 10 mM Tris, 0.5 M NaCl, pH 8.0) for 60 min at 37°C, 2 × SSC at room temperature for 10 min, 1 × SSC at room temperature for 10 min, 0.5 × SSC at 55°C for 1 h, and a final rinse in 0.5 × SSC at room temperature. These slides were dehydrated in graded ethanols and air-dried. Slides were then apposed to X-ray film, or emulsion- dipped in NTB-2 emulsion.

Using both human and rat probes, only two structures were identified to contain D_5 receptor mRNA in a comprehensive survey through the rat brain, as shown in Fig. 1 . The hippocampus showed low levels of D_5 receptor mRNA. In addition, the parafascicular nucleus of the thalamus was visualized. No other structures could be identified to contain D_5 receptor mRNA, including the striatum, cortex, amygdala, olfactory tubercle, nucleus accumbens and septum, or any of the dopamine-containing cell groups such as the substantia nigra, ventral tegmental area, or zona incerta. Control experiments using 'sense'-strand riboprobes or pretreatment with RNase A [10, 14] resulted in no detectable signal in these positively labelled regions.

These results are relatively similar to those reported by Tiberi et al. [24] in the rat D_5 (D_{1B}) cloning report, but are discrepant from the Sunahara et al. [22] cloning study of the human D_5 receptor. In the former paper, only the hippocampus, lateral mammalary nuclei and a thalamic nucleus which was identified as the anterior pretectal nucleus were identified as D_5 positive. Review of the relevant figure in this report indicates that these authors visualized the same thalamic nucleus that is demonstrated in the present report. Our identification of this nucleus as the parafascicular nucleus rather than the anterior pretectal nucleus is based on its histological appearance, especially its envelopment of the fasciculus retroflexus. This distinction is of significance, as these two nuclei have distinct circuitry and function.

In contrast, the Sunahara et al. study reported an anatomical distribution that suggested that this mRNA was distributed more widely, corresponding to the previously reported distribution of D_1 receptor mRNA. The difference between these reports is that the Sunahara et al. study employed short oligonucleotide probes rather than longer riboprobes, which likely hybridized with authentic D_1 receptor mRNA instead of (or in addition to) D_5 receptor mRNA, hence the apparent widespread distribution.

More recently, several pseudogenes of the D_5 receptor have been reported in the human in addition to the gene encoding the functional receptor [8, 19, 28]. It appears that these pseudogenes arose late in evolution, and are not identifiable in the rat [8, 28]. Accordingly, it is unlikely that detection of the distribution of D_5 receptor mRNA in the rat brain is confounded by possible transcription of a pseudogene. In contrast, at least one pseudogene may be transcribed in the human, thus potentially complicating detection of the distribution of D_5 receptor mRNA in the human brain [8, 28].

The restricted distribution of D_5 receptor mRNA in the rat brain suggests that this receptor may have a function distinct from the other identified dopamine recep-

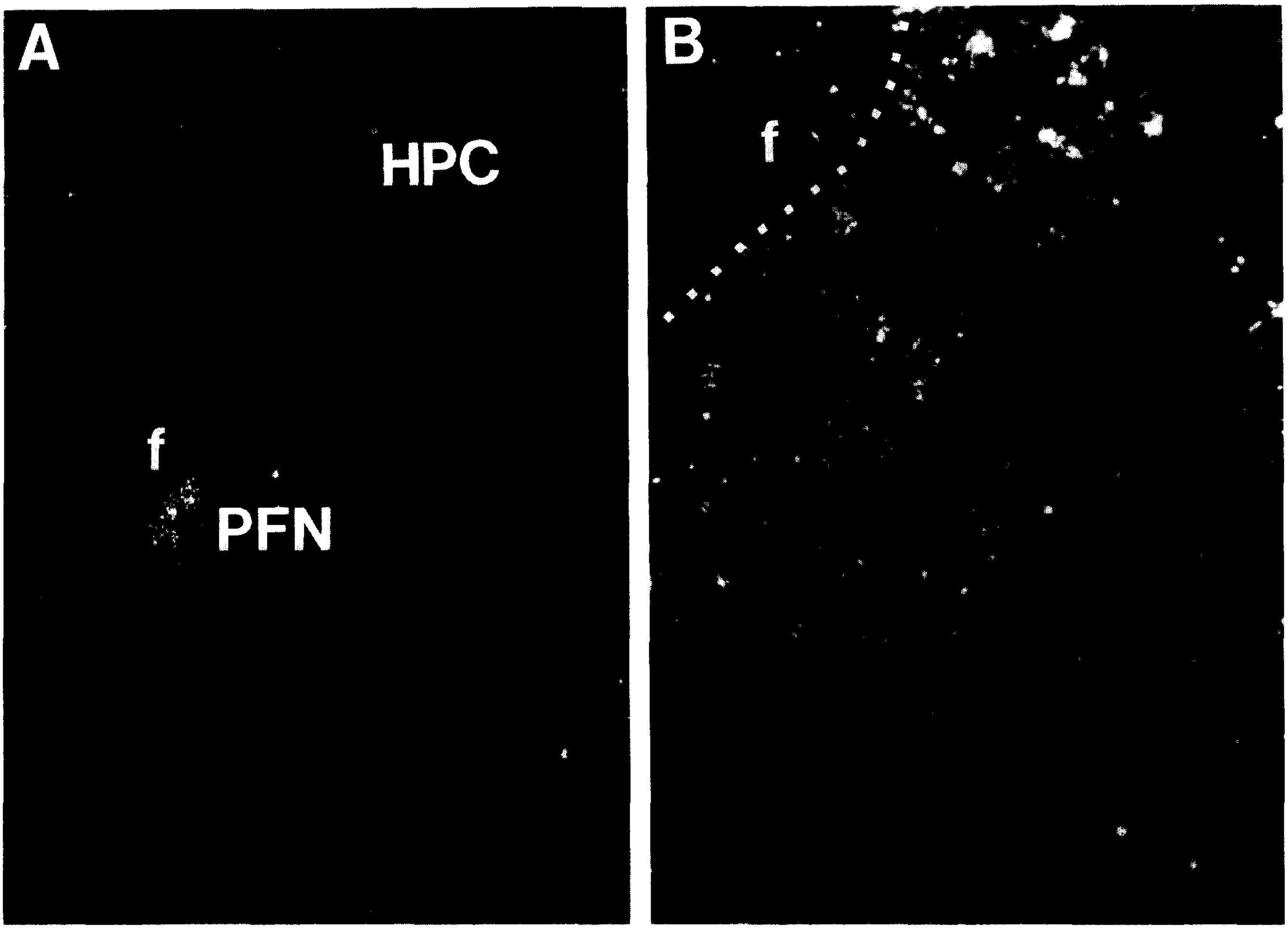

Fig. 1. Distibution of dopamine D_5 receptor mRNA in the rat brain as determined by in situ hybridization. These images were generated with a riboprobe to the rat sequence, although similar results were obtained with a human cRNA probe. A: low power view demonstrating faint labelling in the hippocampus (HPC) and parafascicular nucleus of the thalamus (PFN). The fasciculus retroflexus (f) is identified for orientation. B: high power dark field image demonstrating labelling of the parafascicular nucleus: the fasciculus (f) is dorsomedial to the PFN. Note the relatively higher density of labelling of D_5 receptor mRNA in the dorsolateral aspect of this nucleus.

tors. While all of the dopamine receptors are apparently encoded in regions of the hippocampus, this is the first dopamine receptor that has been identified as being encoded in the parafascicular nucleus. This particular nucleus receives afferents from the substantia nigra [3], making it a likely recipient of the dopamine projections arising in the midbrain. It also sends a projection to the striatum [9], thus suggesting a possible role for this structure in the integration and regulation of multiple levels of dopaminergic neurotransmission. This thalamic nucleus is involved in the perception of pain [6] , and appears to be a neuroanatomical locus associated with the antinocioceptive actions of opiates [20]. The localization of D_5 receptor mRNA in this nucleus suggests that this receptor subtype may be involved in the neurochemical regulation of the thalamic processing of painful stimuli, which would not be considered a traditional role for dopamine in the central nervous system.

In summary, using both rat and human riboprobes generated to the D_5 dopamine receptor, we have determined the distribution of the mRNA encoding this receptor in the rat brain, and could only identify this mRNA by in situ hybridization in the hippocampus and in the parafascicular nucleus of the thalamus. This restricted distribution suggests that the D_5 receptor may have a function quite dissimilar from other dopamine receptors.

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