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The distribution of a dopamine D2 receptor mRNA in rat brain

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Based on the recently reported sequence of a dopamine D2 receptor cloned from rat brain, we prepared a series of cDNA probes to determine the distribution of mRNA encoding this receptor. Within the forebrain, D2 receptor mRNA is abundant in the caudate-putamen, accumbens nucleus and olfactory tubercle. Moderate to low levels of mRNA are observed in the medial habenular nucleus, diagonal band, lateral septal nucleus, claustrum, dorsal endopiriform nucleus, and entorhinal cortex. In the mesencephalon, D2 receptor mRNA is abundant within the substantia nigra, pars compacta, and the ventral tegmental area. Comparison of the distributions of the mRNA and ligand binding indicates that both presynaptic and postsynaptic D2 receptors of the nigrostriatal, mesolimbic and mesocortical pathways are derived from the same mRNA.

Dopamine receptor; Neurotransmitter receptor; mRNA

1. INTRODUCTION

On the basis of functional and pharmacological data central dopamine receptors have been divided into D1 and D2 subtypes. Dopamine D1 and D2 receptors stimulate and inhibit adenylate cyclase, respectively, by coupling to different G-proteins [1]. D2 receptors are known to be abundant within various forebrain regions innervated by dopaminergic neurons projecting from the mesencephalon. Three major ascending dopaminergic pathways are known. The nigrostriatal pathway originates in the dopamine cell group A9 of the substantia nigra, pars compacta, and densely innervates various striatal regions. The mesolimbic and mesocortical pathways originate in the dopamine cell group A10 of the ventral tegmental area and innervate various limbic and cortical structures [2–6]. D2 receptors have also been shown to be presynaptically located on dopaminergic neurons where they modulate dopamine release [7,8].

Abnormalities in central dopaminergic function have been implicated in the pathophysiology of schizophrenia and Parkinson's disease [9–11]. Schizophrenia is treated with antipsychotic drugs which block central D2 receptors, and Parkinson's disease by drugs which lead to their stimulation [12,15]. Prolonged treatment with antipsychotic agents is associated with extrapyramidal (motor) side effects such as tardive dyskinesia [12]. Since the motor side effects are believed to be mediated by receptors located within the striatum, and since antipsychotic activity is believed to be mediated by sites within limbic and cortical regions, investigation of a potential heterogeneity of D2 receptors between these brain regions has become a focus for drug development [16–18].

For many neurotransmitter receptors, cloning efforts have indicated a molecular basis for pharmacological heterogeneity. For example, several subtypes of muscarinic, serotonergic, and adrenergic receptors have now been cloned [19–26]. These receptor subtypes are expressed in

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distinct regions of the brain, and in the case of muscarinic receptors, different receptor gene products are expressed in presynaptic versus postsynaptic locations [27–29]. Recently, a cDNA sequence for a rat brain D2 receptor was reported showing significant sequence homology with receptors that mediate signal transduction by coupling with G-proteins [30]. We prepared a series of oligodeoxynucleotide probes to localize mRNA encoding this D2 receptor in order to determine if the distribution of the mRNA accounts for the presynaptic and postsynaptic, as well as regional, localizations of this D2 receptor.

2. MATERIALS AND METHODS

2.1. *Probes*

Three 48 base oligodeoxynucleotide probes were generated complementary to regions of the mRNA which encode the N-terminal, C-terminal and the third cytoplasmic loop of the D2 receptor cloned from rat brain and had sequences which were complementary to bases 4–51 (5′-GTT CTG CCT CTC CAG ATC GTC ATC GTA CCA GGA CAG GTT CAG TGG ATC-3′), 954–1001 (5′-CAG GAC CTT GTT CTG CTG CTC CAG CTC GTG CAC GCG CTC GAT GAA GCT-3′), and 1198–1245 (5′-GCA GTG CAA GAT CTT CAT GAA GGC CTT GCG GAA CTC GAT GTT GAA GGT-3′) of the D2 receptor cDNA [30]. The probes were synthesized on an Applied Biosystems automated DNA synthesizer and they were purified by preparative gel electrophoresis. For detection of mRNAs the probes were 3′-end labeled using terminal deoxynucleotidyl transferase (Bethesda Research Laboratories) and deoxyadenosine triphosphate labeled in the α-position with ^{35}S for in situ hybridizations and ^{32}P for Northern blots (^{35}S > 1000 Ci/mmol, ^{32}P > 3000 Ci/mmol, New England Nuclear). Labeled under these conditions, the most common length of the oligonucleotide 3′-tails was 10 bases.

2.2. *In situ hybridizations*

Brains were dissected from male Sprague-Dawley rats (200–250 g), frozen on powdered dry ice, and stored at −70°C until sectioned. Twelve (12) μm cryostat sections were collected on gelatin coated slides and stored at −70°C until use. In situ hybridizations were performed with a probe concentration of ~9 × 10^6 dpm/50 μl of buffer [4 × SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.2), 50% formamide, 1 × Denhardt's (0.02% ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 250 μg/ml sheared single-stranded salmon sperm DNA, 100 mM dithiothreitol, and 10% dextran sulfate] per brain slice at 37°C for 20–24 h in a humid chamber. Slides were washed four times for at least 15 min at 55°C in 1 × SSC, and twice for at least 30 min at room temperature also in 1 × SSC. The slides were dried and exposed to X-ray film for three weeks. To obtain higher resolution data, some slides were dipped into a one to one mixture of NTB3 emulsion (Kodak) and water at 42°C and exposed for three weeks.

2.3. *Northern blots*

Various brain regions were dissected at a gross anatomical level from male Sprague-Dawley rats (200–250 g), frozen on dry ice and stored at −70°C until used. Total RNA was extracted from these tissues as described in [31] and 15 μg of total RNA was loaded onto each lane of a formaldehyde containing agarose gel and then transferred to Genescreen (New England Nuclear). Hybridizations were performed according to [32] with ~9 × 10^6 dpm/ml of hybridization buffer. Hybridizations were done at 37°C for 20–24 h, and then washed with a similar protocol to that of in situ hybridizations in situ using 1 × SSPE (0.15 M NaCl, 10 mM sodium phosphate, 1 mM EDTA) instead. The blot was placed in saran wrap and set against film with intensifying screens for four days at −70°C.

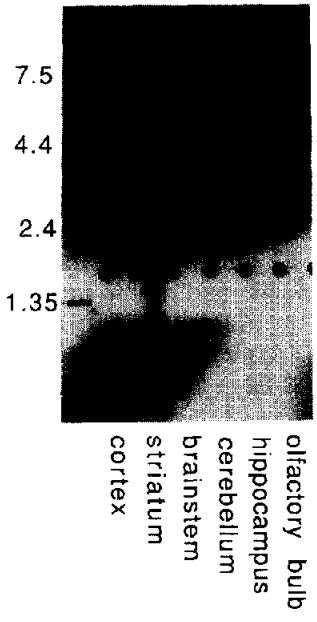
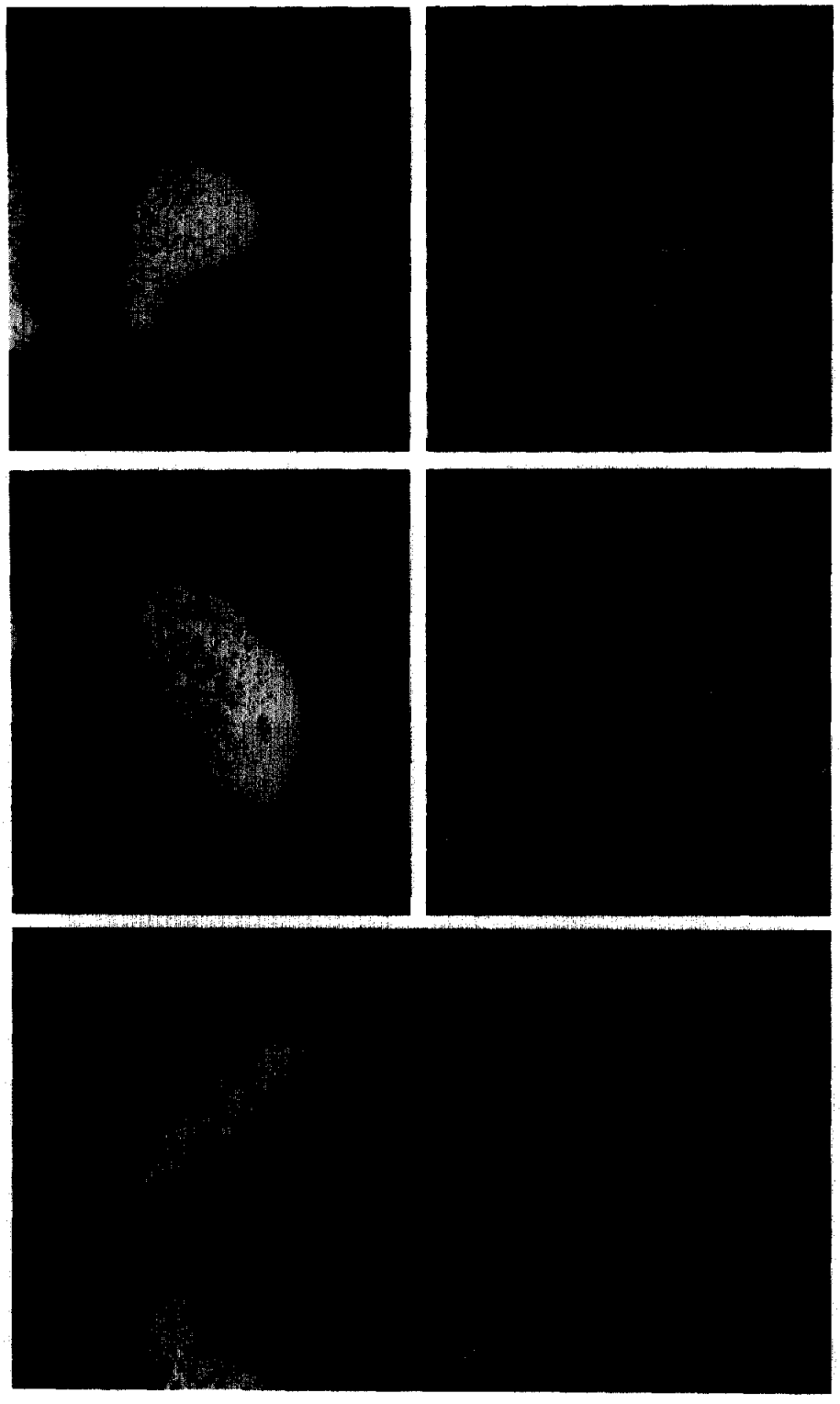
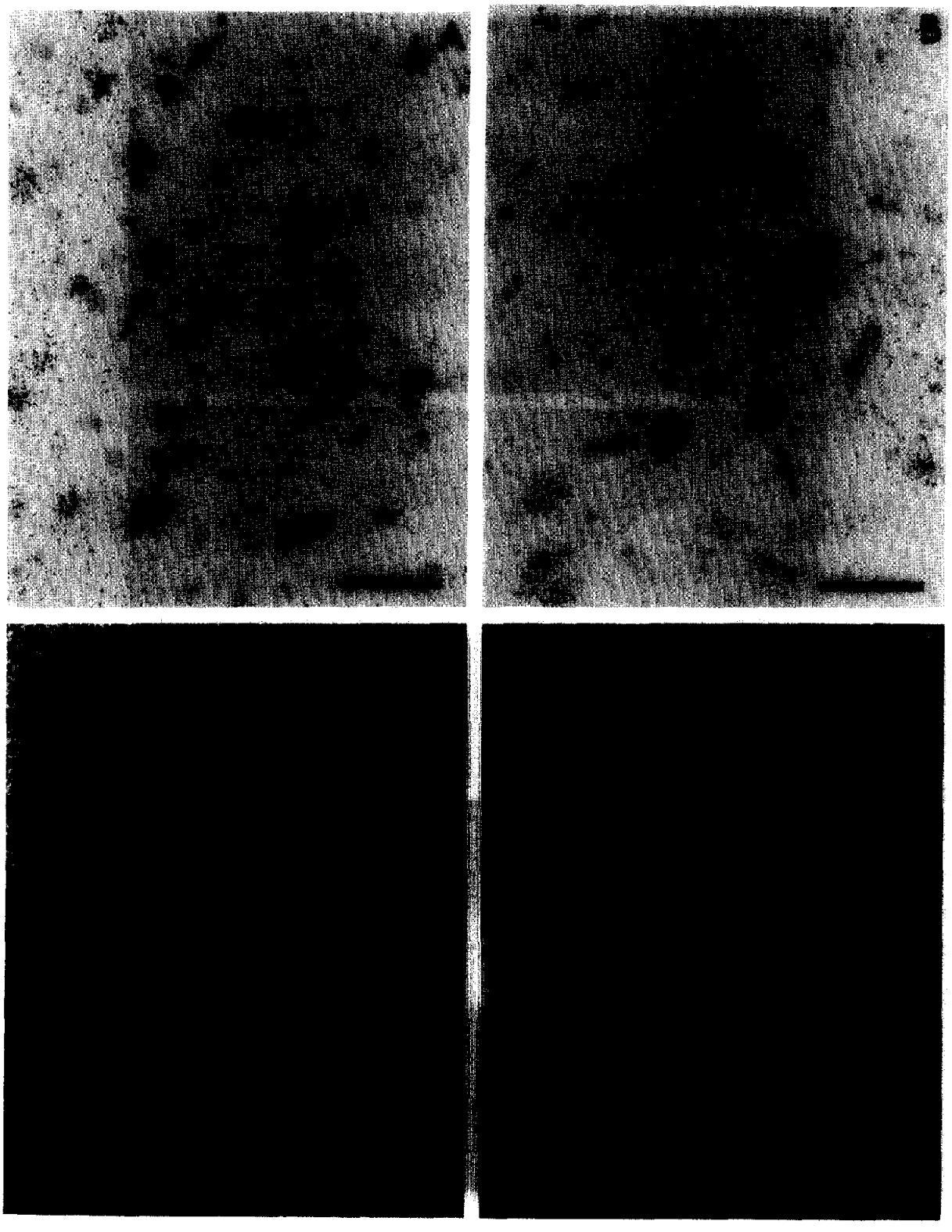


Fig.1. Northern blot analysis of D2 receptor mRNA extracted from various rat brain areas as indicated. The ribosomal bands are indicated by black dots, and the molecular size markers in kilobases are shown on the left.

Fig.2. The distribution of D2 receptor mRNA within rat brain. Regions rich in mRNA are light in the photomicrograph. Panels (A–D) are coronal sections, and panel (E) is a horizontal section. cp, caudate-putamen; ep, endopiriform nucleus; ac, accumbens nucleus; ot, olfactory tubercle; ls, lateral septal nucleus, dorsal; is, lateral septal nucleus, intermediate; db, diagonal band; snc, substantia nigra pars compacta (A9); vta, ventral tegmental area (A10); cl, claustrum; ec, entorhinal cortex; pvn, paraventricular nucleus; cb, cerebellum.





3. RESULTS

The specificity of these probes was verified based on two criteria. First, all three probes labeled an identical pattern of mRNA on cryostat sections of rat forebrain, and when the three probes were combined the signals were roughly additive (data not shown). Second, as shown in fig.1, a mixture of the three probes label a single ~2.9 kb species of mRNA on Northern blots of total RNA extracted from various rat brain regions. The size of this mRNA species is the same as that reported using a long cloned probe derived from the rat cDNA [30]. The striatum expresses the largest amount of D2 receptor mRNA, while less mRNA was detected in cerebral cortex, brain stem, and cerebellum, and none was detected in peripheral tissues (data not shown).

The distribution of D2 receptor mRNA was evaluated using in situ hybridization with the oligodeoxynucleotide probes to sections of rat forebrain. Regions which are richest in D2 receptor mRNA are the caudate-putamen, the accumbens nucleus and olfactory tubercle (fig.2). D2 receptor message is expressed throughout the entire caudate-putamen, and a greater number of cells express the mRNA in the lateral, rather than the medial aspects of this structure. Within the olfactory tubercle, the highest concentration of D2 receptor mRNA is in the Islands of Calleja. Additional limbic structures express moderate levels of mRNA, including the horizontal limb of the diagonal band, and both the dorsal and intermediate aspects of the lateral septal nucleus. Amygdaloid nuclei as well as the medial habenular nucleus also express moderate levels of mRNA (data not shown). Cortical regions expressing this mRNA include the claustrum, dorsal endopiriform nucleus, and entorhinal cortex.

We also evaluated the presence of D2 receptor mRNA within dopamine containing cells in the rat mesencephalon. As illustrated in fig.2D, E and fig.3, a high density of mRNA is observed within the substantia nigra, pars compacta, and is widely distributed in the ventral tegmental area. The more lateral aspects of the pars compacta show the highest degree of expression while little is seen in the substantia nigra, pars reticulata. High levels of expression are also seen in the paraventricular nucleus. In sections which are not shown, a moderate level of mRNA is observed in the dopamine cell group A8 which is a caudal extension of A9 and innervates the ventral striatum [2].

With in situ hybridization, expression of mRNA can be evaluated at a cellular level. The mRNA within the substantia nigra is almost exclusively associated with the large pigmented cells (fig.3B), cells previously observed to have high concentrations of tyrosine hydroxylase mRNA, the rate limiting enzyme of dopamine biosynthesis [32]. The vast majority of D2 receptors within the caudate-putamen are synthesized by medium sized intrinsic neurons (fig.3A), contrary to earlier studies which have suggested that descending corticostriatal projections contribute a major portion of these receptors [33]. By combining these methods with immunocytochemistry it may be possible to determine the neurotransmitter phenotype of individual forebrain cells which express D2 receptors, and thus help clarify how dopaminergic systems interrelate with other neurotransmitter systems.

4. DISCUSSION

The distribution of D2 receptor mRNA presented here is in good agreement with both the known pattern of dopaminergic innervation of these forebrain structures and with the distribution of the D2 receptor binding sites in brain as outlined by receptor autoradiographic studies [34,35]. The dorsal striatum possesses the highest levels of D2 receptors, and our results verify that this region expresses the highest levels of D2 receptor mRNA. The fact that we observe a medial to lateral gradient in expression is consistent with the known topographical organization of the ascending dopaminergic projections [2,3]. Further, we see expression of this message in perhaps all of the limbic structures that are known to be dopaminergically

Fig.3. Dark and bright field micrographs of the distribution of D2 receptor mRNA within rat brain. Rat forebrain (A and C) and mesencephalon (B and D) are shown. (A) Caudate-putamen, and (B) the substantia nigra, pars compacta, were both counterstained with cresyl violet. The arrows indicate examples of cells which express the mRNA. Abbreviations used are the same as fig.2. The bar represents 10 μm in length.

innervated. In cortical regions we see significant expression in the piriform and entorhinal cortex, but little in prefrontal and cingulate cortex. Finally, since there are no known intrinsic dopamine containing neurons within these forebrain structures [2,3], and since the mRNA is only present within cell bodies and proximal dendrites (fig.3A,B), these data indicate that this mRNA encodes receptors which are postsynaptic with respect to the nigrostriatal, mesolimbic and mesocortical pathways.

The distribution of D2 receptor mRNA within the mesencephalon is consistent with the distribution of neuronal cell bodies which give rise to the ascending dopaminergic pathways. We have shown that the D2 receptor message is expressed widely throughout the ventral tegmental area and substantia nigra, pars compacta, and not the pars reticulata. Coupled with the observation that the distribution of this D2 receptor message coincides with that of tyrosine hydroxylase mRNA, our results suggest that presynaptic D2 receptors of the various ascending pathways are encoded by the same mRNA as postsynaptic receptors.

Overall, the distribution of this mRNA is consistent with the known distribution of both presynaptically and postsynaptically located D2 receptors of the examined pathways. The widespread distribution of this mRNA has important implications with regard to the pharmacology of dopamine receptors and future prospects for the development of dopaminergic drugs which discriminate between receptors located in these different brain regions. In the case of other G-protein coupled receptors, it has been shown that when the same receptor genes are expressed in different cells, their antagonist pharmacology does not vary with respect to cell type [25,26,36], while agonist pharmacology varies with respect to both cell type and levels of receptor ([28] and unpublished observations). Initial results with the cloned D2 receptors indicate that a similar relationship may hold since D2 receptors expressed in fibroblasts have the same affinity for antagonists and a lower affinity for agonists than do D2 receptors expressed by brain [30].

Thus one may speculate that at least a portion of the D2 receptors expressed by both dopamine neurons and their various postsynaptic targets are likely to have similar antagonist but different agonist pharmacologies. This may explain the differences in agonist pharmacology between presynaptic and postsynaptic D2 receptors [37,38]. In the case of antagonists, the apparent differences in pharmacology between brain regions are inconsistent with the above relationships, and are suggestive of the possible existence of additional receptor subtypes. It should be pointed out however, that these data could also be explained by an interaction with additional receptors, for example, with muscarinic receptors [12].

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