Volume 255, number 2, 335–339 FEB 07627 September 1989

Messenger RNA encoding the D_2 dopaminergic receptor detected by in situ hybridization histochemistry in rat brain

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Received 21 July 1989

A 30 base synthetic oligonucleotide probe was used to detect the mRNA encoding the rat D_2 dopaminergic receptor. On Northern analysis, the probe identified a single species of mRNA of approximately 2.9 kb, present at highest levels in the striatum but also found in the brainstem, neocortex and diencephalon. On sections, neurons containing high levels of the mRNA were detected in the striatum, the substantia nigra pars compacta and the ventral tegmental area. Lower levels of signal were seen over neurons in the hypothalamus, the frontal neocortex, and the globus pallidus.

Striatum; Substantia nigra; Dopamine receptor; mRNA; Northern analysis

1. INTRODUCTION

Recently, the gene encoding the rat D_2 receptor has been cloned and sequenced [1]. This allows the technique of in situ hybridization histochemistry (ISHH) to be applied to the localisation of the mRNA encoding the receptor in tissue sections, in order to identify the neuronal cell bodies which synthesise the protein and to allow future studies on the regulation of the synthesis of the receptor to be undertaken.

2. MATERIALS AND METHODS

Adult male Wistar rats were used. For the extraction and isolation of total RNA, 6 animals were stunned and immediately decapitated. The brains were rapidly removed and the following regions dissected out – the neocortex, hippocampus, striatum, diencephalon (thalamus and hypothalamus), cerebellum and remaining brainstem. The tissue obtained from each region was pooled and total RNA extracted according to the method of Chirgwin et al. [2]. Total RNA was electrophoresed (50 μg per lane) on a 1.5% denaturing agarose gel (6.7% formaldehyde), transferred directly to a nylon membrane (Hybond N, Amersham) and fixed by UV irradiation.

For ISHH, three animals were deeply anaesthetised with an intraperitoneal injection of sodium pentobarbitone (Sagatal) and perfused through the heart with phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4) followed by 30% sucrose in 20 mM phosphate buffer. Tissue preparation and ISHH were performed as previously described [3]. Some sections were incubated in a ribonuclease solution (RNase A, 20 μg and 1 U RNase T1 in 0.5 M NaCl/10 mM Tris-HCl, pH 8, 1 mM EDTA) for 30 min at 37°C before fixation as controls.

Synthetic 30 base oligonucleotide probes were purchased (Oswell DNA Service) with a sequence complementary to bases 1111–1140 of the rat mRNA [1] (antisense strand) and with a sequence matching that of the same bases of the message (sense strand). Both probes were 3′ end-labelled with radioactive dATP by 3′ terminal deoxynucleotidyl transferase using a commercially available kit (NEN-DuPont). For Northern analysis, [^{32}P]dATP was used, whereas [^{35}S]dATP was incorporated into the probes for ISHH because of the greater resolution the latter allows on autoradiography. Labelled oligonucleotide probe was added to hybridization buffer comprising 4 × SSC, 20 mM phosphate buffer, 10% dextran sulphate, 1 × Denhardt's solution and 50% formamide, containing denatured salmon sperm DNA, yeast transfer RNA and PolyA (all 100 mg/ml; Sigma). For ISHH, 0.1% DTT was added to the buffer immediately prior to use.

For Northern analysis, 10^6 cpm/ml of radiolabelled antisense oligonucleotide was used, and the blot was hybridized at 35°C for 18 h. Following hybridization, the membrane was washed in 1 × SSC containing 0.1% SDS for 1 h at 50°C followed by

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

40 min at 55°C. The membrane was apposed to X-ray film for 48 h for the generation of an autoradiogram.

For ISHH, sections were covered with 100 μl of hybridization buffer containing 10^6 cpm of labelled probe. Some sections were hybridized with the radiolabelled antisense strand, others concurrently with the same amount of labelled sense strand and still others with radiolabelled antisense probe together with a 1000-fold excess of unlabelled antisense strand. The sections were hybridized for 18 h at 27°C. Following hybridization, the sections were washed in 1 × SSC (4 changes, 15 min each) at 52°C followed by a further 2 h at room temperature. The slides were then air-dried. Half were placed against tritium-sensitive film (Hyperfilm, Betamax, Amersham) for 21 days for the generation of autoradiograms. The remainder were dipped in photographic emulsion (Ilford K5; 1:1 with 2% glycerol at 43°C) and stored for 25 days at 4°C. At the end of this time, the dipped sections were developed and counterstained with cresyl violet.

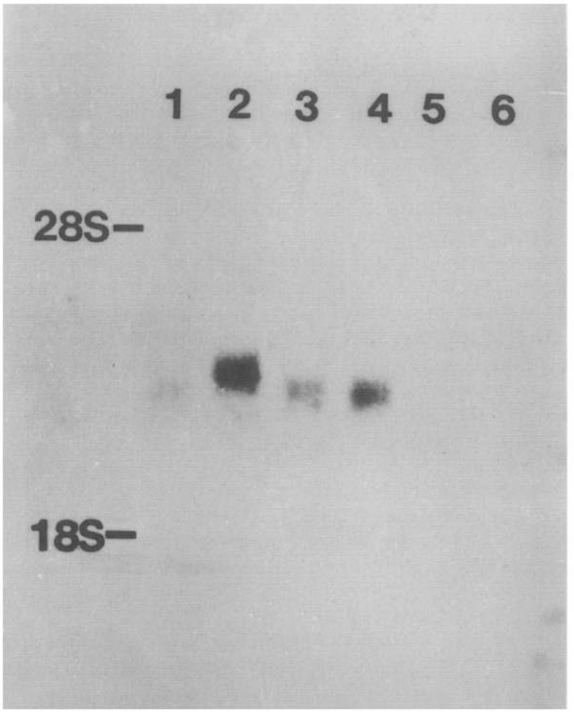


Fig.1. Regional Northern blot of D_2 receptor mRNA. The positions of the major ribosomal bands (18S and 28S) are indicated. Total RNA extracted from 1, neocortex; 2, striatum; 3, diencephalon; 4, brainstem; 5, hippocampus; and 6, cerebellum.

3. RESULTS

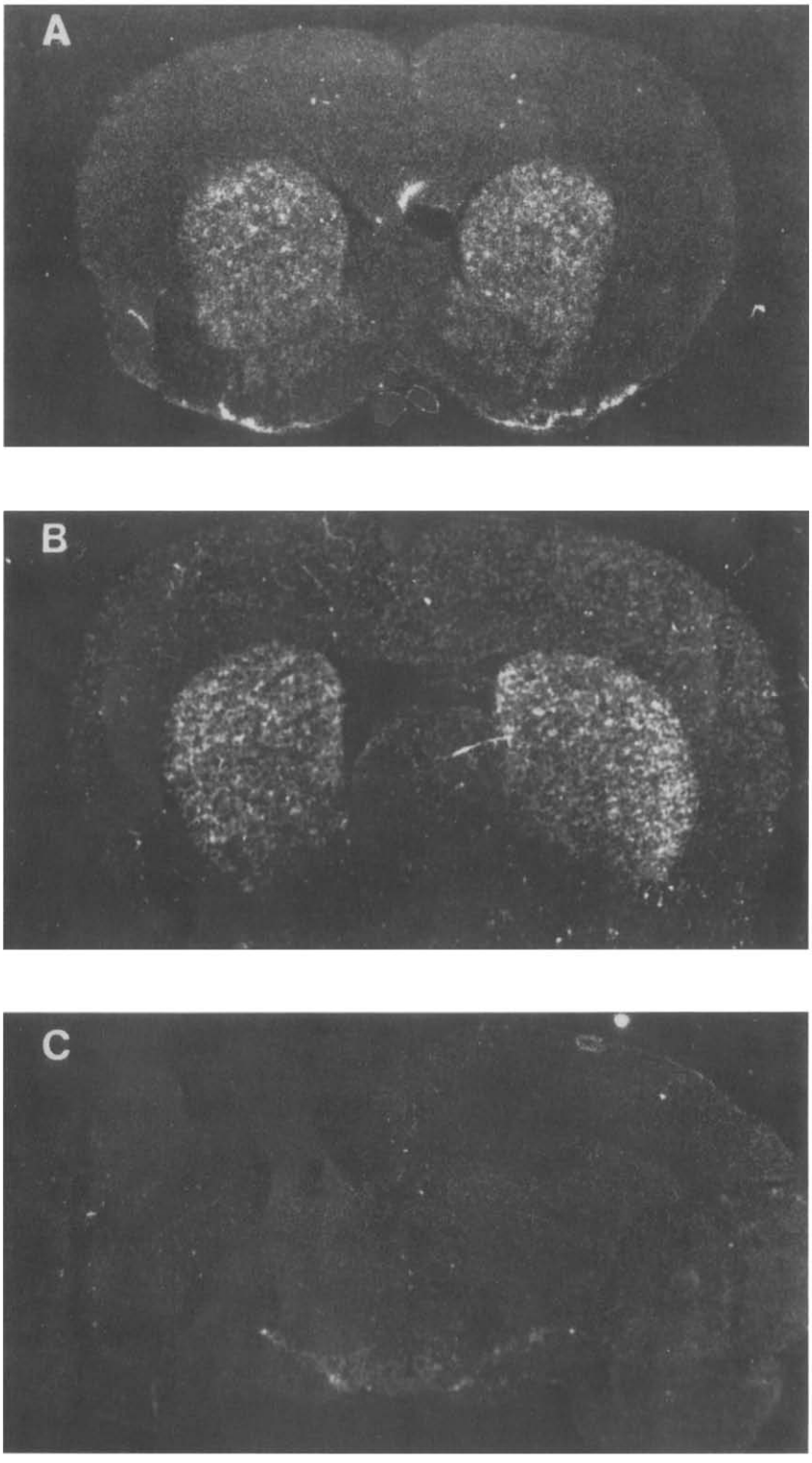
Northern analysis using the antisense oligonucleotide probe showed labelling of a single species of RNA of approximately 2.9 kB. The regional distribution of the mRNA was greatest in the striatum, but also present at lower levels in the brainstem and diencephalon and at a very low but positive level in the neocortex (fig.1). Both the size and regional distribution of the detected mRNA are in agreement with those reported by Bunzow et al. [1], indicating that the mRNA encoding the D_2 dopamine receptor molecule, and no other mRNA species, is being detected.

ISHH shows that the mRNA is present at high levels in the basal ganglia and in the pars compacta of the substantia nigra and ventral tegmental area in the midbrain (fig.2). In particular, the neostriatum and ventral striatum shows the highest levels of hybridization, with lesser amounts in the globus pallidus. A low level of signal is also present in the frontal neocortex.

On the dipped sections, the autoradiographic grains are concentrated over neuronal cell bodies (fig.3). In the striatum, all the large cells are heavily labelled together with many of the medium-sized neurons (fig.3a). In the midbrain, virtually every neuron in the pars compacta of the substantia nigra and in the ventral tegmental area shows a significant accumulation of autoradiographic grains (fig.3b). Moderately labelled cells are also seen scattered in the globus pallidus and in parts of the hypothalamus, and slight labelling is seen over some cortical neurons, particularly in the deep layers of the frontal lobe.

In addition to the Northern analysis, specificity of the ISHH was checked by the use of sections pretreated with RNase, by comparison with ISHH using labelled sense strand probe and by the use of an excess of unlabelled probe in the hybridization buffer for cold displacement of the signal. All these gave no significant signal on film or on dipped sections. Only the sense strand control on dipped sections is illustrated (fig.3c).

Fig.2. Distribution of mRNA for D_2 receptor. Autoradiograms of coronal brain sections are presented in a rostral (A) to caudal (C) direction. Increased whiteness in the photographs, printed directly from Betamax film, corresponds to higher levels of hybridization.


A
B
C

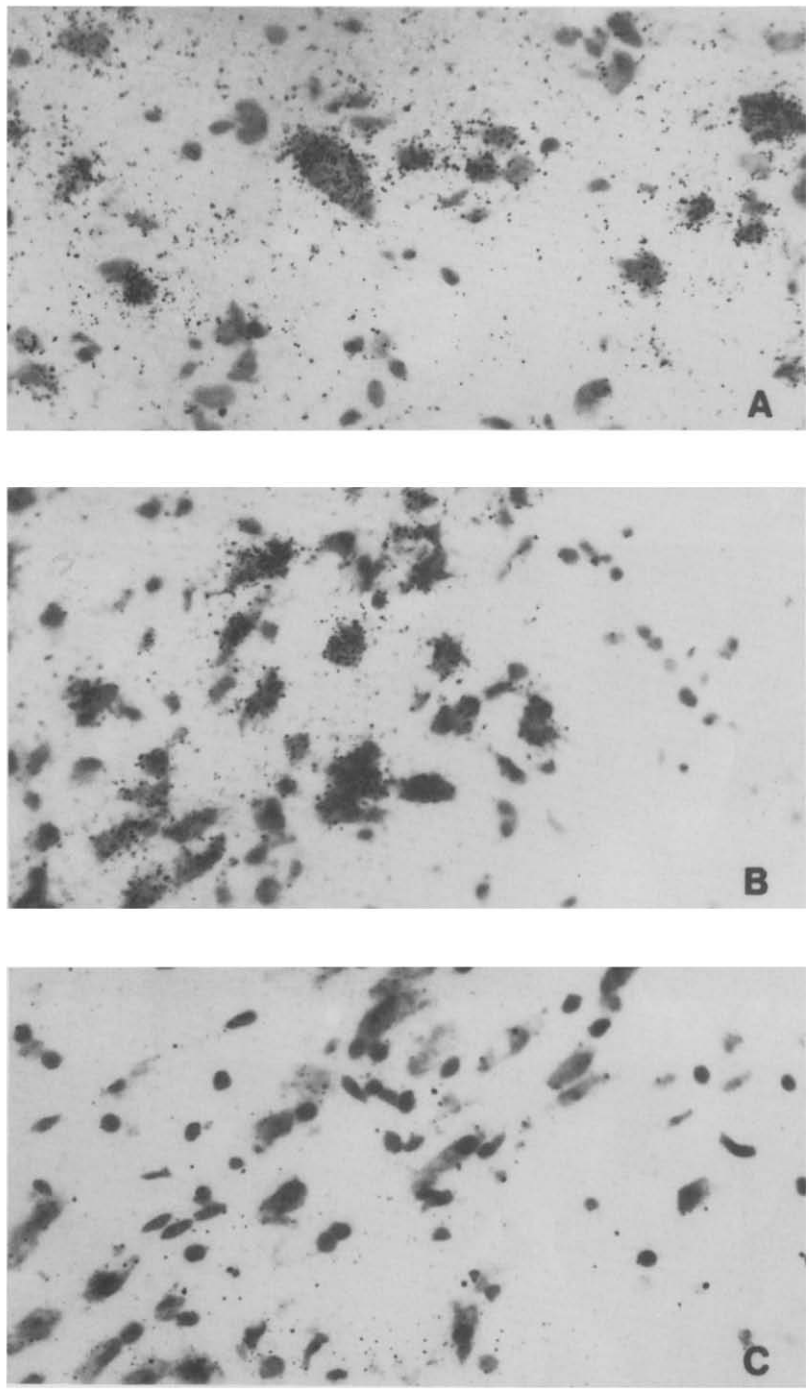


Fig.3. Cellular distribution of hybridization signal for D_2 receptor mRNA. Photomicrographs from emulsion dipped slides of brain sections. Sections were stained with cresyl violet following emulsion development. A, neostriatum; B, substantia nigra; C, substantia nigra following hybridization with the labelled sense strand oligonucleotide probe. × 470.

4. DISCUSSION

The observed distribution of the mRNA encoding the D_2 receptor is in broad agreement with descriptions of the distribution of the receptor itself using autoradiography [4–6], with the highest levels of hybridization in the basal ganglia and in the midbrain dopaminergic cell populations. Some differences are, however, apparent in certain brain areas. No significant ISHH signal was detected in the hippocampal formation, the cerebellum, the tectum or the central grey matter of the midbrain, although the receptor protein has been reported in these sites [5,7]. Of course, levels of mRNA need not be directly related to the quantity of receptor protein in different neuronal populations, and these regions may have very low levels of the mRNA, undetectable by ISHH under the present conditions. Alternatively, the ISHH observations may indicate that the receptors in these areas are localised to the terminals of dopaminergic axons arising elsewhere.

The detection of D_2 receptor mRNA by ISHH represents an additional important technique for the future investigation of the regulation of D_2 receptors in a wide variety of experimental and pathological conditions. ISHH has the advantage when compared with receptor autoradiography that it identifies the neuronal cell somata which synthesise the receptor as opposed to the location of the protein on cell processes. This will allow the contribution to changes in overall D_2 levels made by changes in synthesis in different subpopulations of neurons to be assessed.

Acknowledgements: This work was supported by the Wellcome Trust. A.J.L.B. is a Cottrell Fellow of Research into Aging. P.J.H. is an MRC Training Fellow.

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