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The effect of haloperidol on D2 dopamine receptor subtype mRNA levels in the brain

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Abstract

Chronic neuroleptic treatment induces an increase in the density of D2 dopamine receptors in the striatum. The effect of prolonged administration of haloperidol on mRNA levels of the short (D_{2S}) and long (D_{2L}) isoforms of the D_2 dopamine receptor and the D_3 dopamine receptor in different brain regions was examined. Haloperidol caused a significant rise in both D_{2L} and D_{2S} mRNA levels in the striatum, but had no effect on D_3 mRNA levels. This rise was restricted to the striatum, showing that the effect of haloperidol on dopamine receptor mRNA is both subtype- and tissue-specific.

Key words: Haloperidol; D_{2L} receptor; D_{2S} receptor; D_3 receptor; mRNA

1. Introduction

Dopaminergic pathways in the brain have for some time been associated with a number of neurological disorders, such as schizophrenia, Parkinson's disease and Huntington's chorea [1]. The treatment of schizophrenia by the administration of neuroleptic drugs is frequently complicated by the development of a hyperkinetic movement disorder termed tardive dyskinesia. This is thought to be the result of a supersensitivity of dopamine receptors induced by chronic neuroleptic treatment, and has been associated with an increase in D2 dopamine receptor binding [2]. While the effect of neuroleptics such as haloperidol has been associated with dopamine receptors of the D2 subclass** (i.e. D_2, D_3 and D_4) in the striatum, most studies at the mRNA level have concentrated on the D_2 receptor subtype and have been performed on the striatum and pituitary gland [3–6]. Little is known of whether haloperidol acts similarly on long and short isoforms of the D_2 receptor or of whether it affects the mRNA levels of the more recently cloned D_3 [7,8] or D_4 [9] dopamine receptors. We were interested in investigating whether the effect of haloperidol can be observed in brain regions other than the striatum, and in gaining more information about the subtype-specificity of its action. We have therefore taken advantage of oligonucleotide probes which can distinguish between the long and short isoforms of the murine D_2 dopamine receptor, and a cDNA probe specific for the murine D_3 receptor in order to observe the effect of chronic haloperidol treatment on mRNA levels of D_2 and D_3 receptors in different regions of the mouse brain.

2. Materials and methods

2.1. Animals and tissue preparation

30 Balb/c mice (20–25 g) were injected i.p. daily with 4 mg/kg haloperidol for 16 days. Control mice received an equivalent volume (0.5 ml) of vehicle (PBS, pH 6.5). Three days after the last injection mice were sacrificed and brain regions were dissected out and frozen immediately in liquid nitrogen for RNA preparation. Tissue from each brain region was pooled for haloperidol-treated and control mice, and was used for RNA preparation. Whole brains were taken from two mice from each group to be used for membrane preparation and [^{3}H]spiperone binding as previously described [10].

2.2 Labelling of oligonucleotides and riboprobes

Two antisense oligonucleotides were synthesised according to the sequence of the mouse D_2 dopamine receptor [11]. The D_{2L}-specific probe was based on a sequence from the 87-bp insert (5′-CTCCGCCTGTTCACTGGGAAACTCCCATTAG-3′). The D_{2S}-specific probe was based on the 15 nucleotides flanking the 87-bp insert (5′-CGGCGGGCAGCATCCTTGAGTGGTGTCTTC-3′). The specificity of these probes for the respective subtypes was determined by Northern analysis using RNA prepared from D_{2L} and D_{2S} stably transfected cell lines. The β-actin probe was based on exon 5 of the human β-actin sequence (5′-TGCCACCAGACAGCACTGTGTTGG-3′). Oligonucleotides were labelled at their 5′ termini using T4 polynucleotide kinase and [γ-^{32}P]ATP. For riboprobe preparation, a 293-bp PCR product from the putative 3rd cytoplasmic loop of the mouse D_3 dopamine receptor was generated as described previously [8], subcloned into the *Eco*RI site of pBluescript (Stratagene), and linearised with *Bam*HI. Transcription was carried out according to manufacturer's instructions using T3 RNA polymerase (Boehringer Mannheim).

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** To avoid confusion of nomenclature, the terms D1 and D2 shall be used to refer to the two classes of dopamine receptor originally defined by pharmacological and biochemical studies at the protein level. The cloned receptor subtypes, defined by their DNA sequence, shall be denoted by a subscript, e.g. D_2.

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2.3. RNA preparation and Northern analysis

Total RNA was prepared from different brain regions according to the guanidium thiocyanate–caesium chloride method [12]. 20 μg total RNA (striatum, olf. tubercle) or 30 μg total RNA (other brain regions) was electrophoresed on a 1% formaldehyde-agarose gel, and blotted onto nylon membranes ('Qiabrane', Qiagen). Northern blots were hybridised as described previously [8], and densitometric analysis of autoradiograms was performed using a Molecular Dynamics 300A Computing Densitometer with the ImageQuant Software to get volume integration.

2.4 RNase protection analysis

RNase protection analysis was performed using the enzyme RNase ONE (Promega) according to manufacturer's instructions. Briefly, 30 μg sample RNA was hybridised with 10^5 cpm of riboprobe overnight at 48°C, and was then RNase digested for 35 min at room temperature using 1 ml RNase ONE. Digested samples were precipitated in the presence of 10 μg carrier yeast tRNA and were run on a 6% polyacrylamide/7 M urea sequencing gel. The gel was dried before overnight exposure to autoradiography at −80°C.

3. Results

To confirm that the haloperidol treatment had been effective in increasing dopamine receptor protein levels, binding assays using [^{3}H]spiperone were performed on membranes prepared from two treated and two control mice. A 25–35% rise in D2 dopamine receptor levels was observed (data not shown), in accordance with previous studies [2]. In order to determine whether the increase in dopamine receptor levels was widespread or whether it showed tissue-specificity, Northern blots of RNA from different brain regions of haloperidol-treated and control mice were hybridised with an oligonucleotide specific for the long isoform of the D_2 receptor, which produces a band at 2.9 kb. A 2.4-fold (2.42 ± 0.26) increase in D_{2L} mRNA levels was observed in the striatum (Fig. 1), as quantitated by densitometric scanning. However, this change in D_{2L} mRNA levels was confined to the striatum, with no change occurring in other D_2-expressing tissues such as olfactory tubercle, hypothalamus, brainstem, olfactory bulb (Fig. 2) and cortex (data not shown). Hybridisation with a β-actin probe produced no change in β-actin mRNA levels between haloperidol-treated and control animals (Figs. 1 and 2), confirming that the effect observed in the striatum was specific to D_{2L} mRNA and was not the result of a general increase in striatal transcription.

In order to determine whether haloperidol also influences expression of the short D_2 isoform, a D_{2S}-specific oligonucleotide was hybridised to the same, de-probed, Northern blots. As with the D_{2L} isoform, a greater than 2-fold (2.2 ± 0.4) increase in D_{2S} mRNA levels was observed in the striatum in haloperidol-treated mice (Fig. 1), with no change occurring in the any other tissues (data not shown).

Since spiperone also binds with high affinity to other members of the D2 dopamine receptor subclass, it is possible that the haloperidol-induced rise in [^{3}H]spiperone binding may be the consequence of increased mRNA levels of more than one subtype of dopamine receptor. Since Northern analysis is not sufficiently sensitive to detect the low levels of D_3 receptor mRNA, we used RNase protection analysis on total RNA from haloperidol-treated and control mice in order to investigate whether haloperidol influences D_3 receptor mRNA expression. Using a probe from the putative third cytoplasmic loop of the D_3 receptor, a specific signal was observed at approximately 285 bp (Fig. 3, arrow), which was not present in control embryo RNA or the carrier yeast tRNA. In contrast to the D_2 receptor, the D_3 receptor mRNA levels did not change upon haloperidol treatment in the striatum, nor was any change observed in tissues where the D_3 receptor is more highly expressed, such as the olfactory tubercle and olfactory bulb (Fig. 3). It should be noted that the lower D_3-specific band may represent the shorter D_3 isoform (D_{3S}), and that this also shows no change upon haloperidol treatment.

4. Discussion

The effect of haloperidol in increasing dopamine receptor levels has previously been studied at the level of the protein using radioligand binding assays. More re-

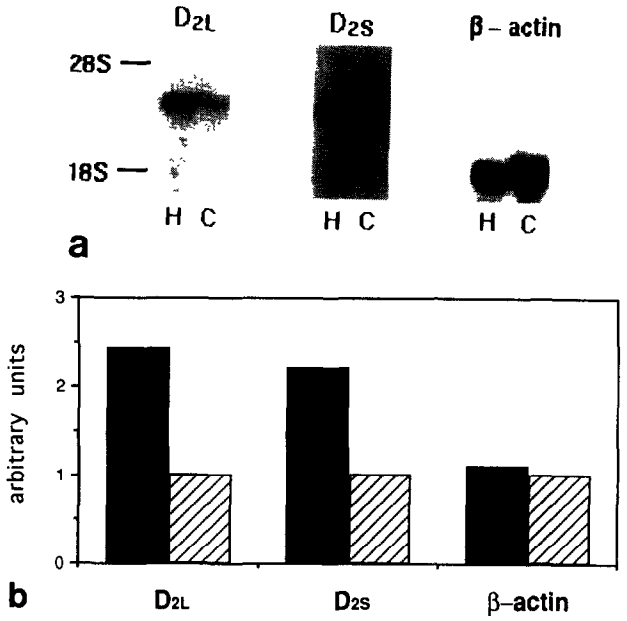

Fig. 1. Northern analysis showing the effect of haloperidol treatment on D_{2L} and D_{2S} receptor mRNA levels in striatum. (a) Northern blot analysis was performed on total RNA from the striatum of haloperidol-treated (H) and control (C) mice. Hybridisation was performed with a D_{2L}-specific oligonucleotide probe (left), a D_{2S}-specific oligonucleotide probe (centre) or a β-actin specific oligonucleotide probe (right). The position of the 18 S and 28 S ribosomal bands are indicated. (b) Densitometric scanning was performed on Northern blots to quantitate the increase in mRNA levels induced by haloperidol (black) compared with control (hatched) levels. The data analysed was obtained from three independent experiments.

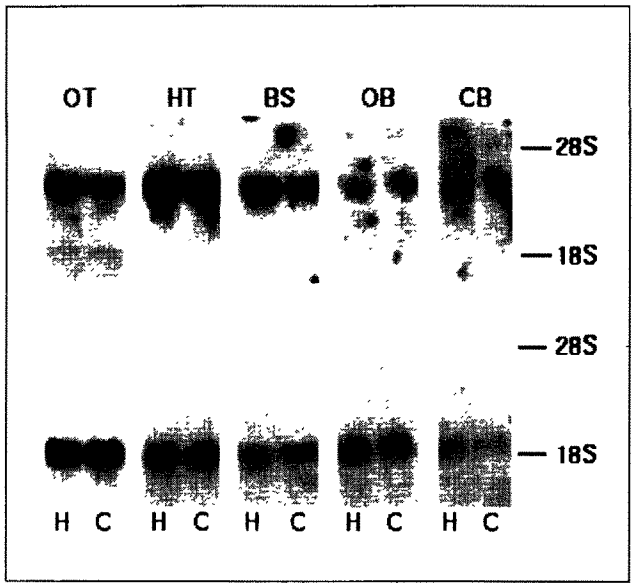

Fig. 2. Northern analysis showing the effect of haloperidol treatment on D_{2L} and D_{2S} receptor mRNA levels in various brain regions. Northern blot analysis was performed on total RNA from various brain regions of haloperidol-treated (H) and control (C) mice. Hybridisation was performed with a D_{2L}-specific oligonucleotide probe (upper panel) or a β-actin specific oligonucleotide probe (lower panel). Lanes representing olfactory tubercle were exposed to autoradiography overnight; other lanes were exposed for 5 nights. The position of the 18 S and 28 S ribosomal bands are indicated. (OT) olfactory tubercle, (HT) hypothalamus, (BS) brainstem, (OB) olfactory bulb, (CB) cerebellum.

cently, its effect on the expression of dopamine receptor mRNA has been investigated by a number of groups. While most studies to date have been performed on rats, we have based our system on mice, and have included relatively large numbers in order to diminish the effect of individual differences.

We have shown here that the action of the neuroleptic haloperidol in raising D_2 mRNA levels is exclusive in the brain to the striatum, and that it is effective on both the long and short D_2 receptor isoforms to the same degree. This activity, however, is not extended to the D_3 dopamine receptor, at which haloperidol is also known to display potent antagonist activity.

While some groups have found no change in D_2 mRNA levels upon haloperidol treatment [13,14], there are several studies which do show a correlation between neuroleptic treatment and an increase in striatal D_2 receptor mRNA levels [4–6,15]. In addition, Martres et al. [15] see no overall change in D_2 receptor mRNA levels in the cortex or pons-medulla using a probe common to the long and short D_2 isoforms, in agreement with our findings that D_2 mRNA levels did not change in any tissue other than the striatum. Furthermore, the 2-fold increase reported by Buckland et al. [16] in D_{2L} and D_{2S} mRNA levels in whole brain most probably arises from the selective increase in the striatum reported here.

The evidence presented in this study suggests that haloperidol acts differentially at the D_2 and D_3 dopamine receptor subtypes, but that it acts equally at the short and long isoforms of each receptor. The finding that D_3 receptor mRNA levels do not change upon haloperidol treatment is supported by a recent study using in situ hybridisation on D_3 receptor mRNA in the nucleus accumbens [17], which shows no change in the quantity or distribution of D_3 receptor transcripts upon haloperidol treatment in rats.

The mechanism by which haloperidol produces its effect is not clear. It appears, however, that it is the antagonist activity of haloperidol at D_2 receptors which is connected with its ability to augment D_2 mRNA levels. A rise in striatal D_2 mRNA has been found to be induced by other D2 antagonists in addition to haloperidol, such as sulpiride [6], while studies in the pituitary gland show that agonists at D2 receptors such as bromocriptine have no such effect [3]. However, although receptor blockade appears to be related to the increase in D_2 dopamine receptor concentration, there seem to be other factors involved. Haloperidol also acts as a strong antagonist at D_3 receptors, but these levels are not augmented following neuroleptic treatment. In addition, haloperidol acts as an antagonist at D_2 receptors throughout the brain, whereas we have shown here that only in the striatum does this lead to upregulation of D_2 receptor mRNA. It remains to be determined what other factors play a role in determining the tissue- and subtype-specificity of the response to haloperidol treatment.

The haloperidol induced rise in D_2 mRNA levels may be mediated either at the transcriptional level or post-transcriptionally, for example by increasing mRNA stability. The notion that an increase in transcriptional activity is involved is supported by evidence that haloperidol can increase mRNA levels of the transcription factor c-*fos* and that this activity is mediated by D2

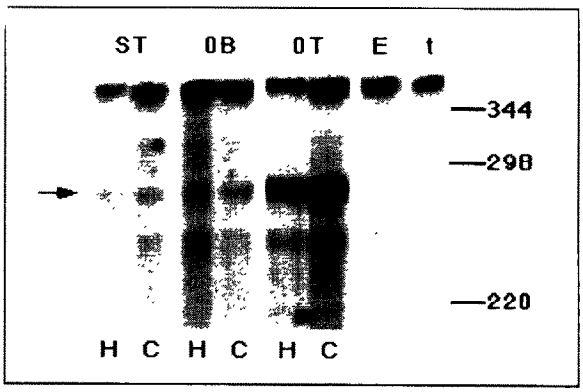

Fig. 3. RNase protection analysis showing the effect of haloperidol treatment on D_3 receptor mRNA levels. RNase protection analysis was performed on total RNA from different brain regions of haloperidol-treated (H) and control (C) mice, using a cRNA probe prepared from a PCR product representing the third cytoplasmic loop of the D_3 dopamine receptor (see section 2). Arrow indicates the position of the specific D_3 mRNA band. (ST) striatum, (OT) olfactory tubercle, (OB) olfactory bulb, (E) embryo, (t) tRNA.

receptors, since it is inhibited by the D2 agonist LY171555 and mimicked by the D2 antagonist (+) butaclamol [18]. It will be interesting to observe what other transcription factors emerge as being involved in controlling the expression of D2 receptors, and whether they act similarly on both short and long isoforms of the D_2 receptor. An improved understanding of the control of dopamine receptor gene expression is essential for the development of highly specific drugs which can be used in the treatment of dopamine receptor-related neurological disorders.

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