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THE ROLE OF DOPAMINE D_2 RECEPTOR IN THE BEHAVIORAL EFFECTS OF IMIPRAMINE — STUDY WITH THE USE OF *ANTISENSE* OLIGONUCLEOTIDES

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Antisense strategies have a potential to specifically block the production of a given protein, e.g. receptor subtype, thus may help to uncover its behavioral and/or biochemical function. In the present study we demonstrated the utility of this approach for studying the role of dopamine D_2 receptors in the anti-immobility effect of imipramine in the forced swimming test. Following intracerebroventricular (i.c.v.) administration of phosphorothioate oligonucleotide complementary to mRNA encoding for dopamine D_2 receptors (D_2 *antisense* ODN; 1 nmol/1µl H_2O, twice a day for 5 days) to the rats, the decrease in the locomotor activity (shortened total distance travelled and decrease in vertical activity, without differences in the stereotypic movements of animals), as well as the decrease of specific binding of [^{3}H]raclopride in the striatum and limbic forebrain were observed. At the same time, i.c.v. administration of D_2 *antisense* ODN reversed the effect of imipramine in the forced swimming test, what may indicate that the dopamine D_2 receptors play a significant role in the behavioral anti-immobility effects of imipramine.

Key words: *dopamine D_2 antisense oligonucleotides (D_2 antisense ODN), imipramine, locomotor activity, forced swimming test, binding of [^{3}H]raclopride, rats*

INTRODUCTION

The role of dopamine D_2/D_3 receptors in the mechanism of action of antidepressant drugs (ADs) has been shown in our previous studies (1—5). However, a persistent methodological problem associated with these studies is that the dopaminomimetic drugs used may have actions on other CNS receptors (e.g. amphetamine with the indirect effect on α_1-adrenergic receptors) so that their effects may not be specific to their dopaminomimetic activity.

Indeed, the synaptic actions of dopamine are mediated by at least five distinct dopamine receptor subtypes recently cloned (6) which were previously classified into two families (D_1 and D_2) according to their pharmacological profiles (7). Although selective drugs easily discriminate between these two families, drugs that usefully discriminate between family members (D_1 versus D_5; D_2 versus D_3 and D_4) are not yet available. These include one of the classic dopamine D_2 receptor agonist, quinpirole, which now has been shown to bind also to dopamine D_3 receptor (8) as well as several of the most widely used antipsychotics (9).

It is well known that a possible effect in the forced swimming test is produced by various substances which stimulate the locomotor activity. An effect which is not accompanied with the locomotor hyperactivity is regarded as a specific antidepressant-like effect. The ADs-induced reduction of the immobility time in the forced swimming test in rats is considered to be mainly due to activation of the dopamine system. However, despite the studies done by Delini-Stula *et al.* (10), who have shown the antagonism of anti-immobility effect of levoprotiline by haloperidol and sulpiride, but not by prazosin, the critical involvement of dopamine D_2/D_3 receptors was not clearly proved.

Therefore, since the selectivity of widely used drugs acting at the level of dopamine receptors should be reconsidered, the *antisense* oligonucleotides (ODNs), complementary to the specific mRNA sequences encoding dopaminergic receptors have been supposed to become a tool for blocking specific gene expression, thus helping to elucidate the specific role of appropriate proteins (e.g. receptors) in certain experimental paradigms. The major advantage of this approach is the — theoretically — relatively simple, rational design and synthesis of ODNs that should bind only to specific nucleic acid sequence. The problem of rapid degradation of ODNs in tissues has been partially obviated by chemically modifying ODNs. Phosphorothioate ODNs are less likely to be degraded by nucleases (11) or RNAase H (12) than their unmodified counterparts. This modification increases their half-lives to more than 24 h (13). Since ODNs do not pass an intact blood-brain barrier (12, 14), intracerebroventricular (i.c.v.) or intracerebral injection have become a standard in brain research (15).

In the present study we designed the *antisense* sequence complementary to mRNA encoding for dopamine D_2 receptors (phosphorothioate D_2 *antisense* ODN) in order to see how important this subtype of dopamine receptors is for the effect of imipramine in forced swimming test. The *antisense* sequence has been chosen following several published reports indicating its efficacy in decreasing the dopamine D_2 receptor density as well as its function manifested in certain behavioural paradigms (16—19).

MATERIALS AND METHODS

The experiments were carried out on rats (male Wistar, 250—270 g). The animals had free access to food and water before experiment and were kept in a constant room temperature (22 ± 1°C) on a natural day-night cycle. The experiments were performed in accordance with the ethical requirements.

D_2 Antisense *ODN*

Based on the cDNA sequence for the dopamine D_2 receptor (20, 21) a 20-mer phosphorothioate oligodeoxynucleotide was designed and purchased (molecular biology grade) from Biometra GmBH, Germany. The *antisense* was targeted to the area of the dopamine D_2 receptor cDNA sequence that bridges initiation codon (from −10 to +10: 5'-GTG GAT CCA TTG GGG CAG TG—3'). This selected target sequence has relatively low homology with any of the other known cDNA sequences found in the National Center for Biotechnology Information (BLAST 2.0).

Intracerebroventricular administration of D_2 antisense *ODN*

The rats were operated under pentobarbital anaesthesia (30 mg/kg *i.p.*), 24 h before and after the operation the antibiotics (1ml/kg, Linco-Spectin, Upjohn) were administered. They were implanted chronically and bilaterally with stainless guide cannulae 9.0 mm long (0.4 mm o.d.), according to the method described by Paxinos and Watson (22). After 7-day postoperative period, D_2 *antisense* ODN solution (1 nmol in 1 µl of H_2O) was administered 10 times in 12-h intervals to conscious unrestrained animals via an internal cannula (10.6—11.0 mm long; 0.3 mm o.d.) that extended beyond the guide cannula to the lateral ventricle (AP -0.4—0.8, L 1.2—1.5, V 2.0—2.4). Antisense solution or solvent was administered in a volume of 1 µl over 2 min with a 2 microliter Hamilton syringe. The internal cannula was withdrawn 1 min before the termination of the injection. Postoperatively, rats were housed singly with food mash and water provided ad libitum.

[^{3}H] Raclopride binding

For binding experiments the rats were sacrificed 12 h after the last dose of D_2 *antisense* ODN, their brains were quickly removed and striata and limbic forebrains were dissected out, frozen on dry ice and stored until used. Specific binding of [^{3}H]raclopride was performed, according to the method descibed previously (5).

Locomotor activity

At 48 h before the test animals were adapted for 24 h to the experimental room. After adaptation, 12 h after the last i.c.v. administration of D_2 *antisense* ODN, each animal was placed individually into one of four plexiglass cages. Four Animal Activity Meter (Auto-Track System, Columbus Instruments, USA) was used. The animals were observed continously for 30 min.

The Auto-Track system consists of plexiglass monitor cages (40 × 40 × 22 cm) surrounded by horizontal and vertical infrared sensors non-detectable by the animal. The monitor cages are connected to a Digiscan activity monitors. Data for the following variables of locomotor activity detected by the Digiscan, were collected in an IBM PC/XT compatible computer system: DT — total distance travelled by the animal (cm), BSM — number of stereotypic movements (all tiny

movements not linked to the displacement, i.e. grooming, scratching and sniffing around) and V — vertical activity, i.e. single ambulations on cage walls as well as typical rearing with sniffing and looking around.

Forced swimming test

The total immobility time was measured 12 h after the last i.c.v. administration of D_2 *antisense* ODN.

The total immobility time of rats was assessed according to Porsolt *et al.* (23) during a 5-minute observation period. Imipramine (Polfa, Poland; 10 mg/kg) was given three times at 24, 5 and 1 h before the test to naive and operated animals.

Histological analysis

After completion of the experiment, the rats were deeply anaesthetized with pentobarbital (450 mg/kg i.p.), perfused intracardially with isotonic saline solution followed by perfusion with 4% paraformaldehyde. The brains were taken out and stored in the fixative (8% formaldehyde solution). Coronal sections were cut from the frozen tissue at 90 μm, photographed and the position of all injection cannula tips was checked. Only those animals with histologically confirmed proper injection sites were used in the data analysis.

Statistical analysis

The results are expressed as means ± SEM. A statistical evaluation was carried out by Student's t-test, or by an analysis of variance (one-way ANOVA) followed by Newman-Keuls test. In biochemical studies ANOVA followed by Duncan's test was used.

RESULTS

Locomotor activity

I.c.v. administration of D_2 *antisense* ODN decreased all measured parameters of rat locomotor activity, i.e. shortened the total distance travelled (DT) and caused decrease in vertical activity (V). At the same time no differences in the stereotypic movements of animals (BSM) were observed (*Fig. 1*).

Binding studies

Specific binding of [^{3}H]raclopride was decreased following i.c.v. administration of D_2 *antisense* ODN, both in the striatum as well as in the limbic forebrain. The effect was more pronounced in the latter brain region (*Fig. 2*).

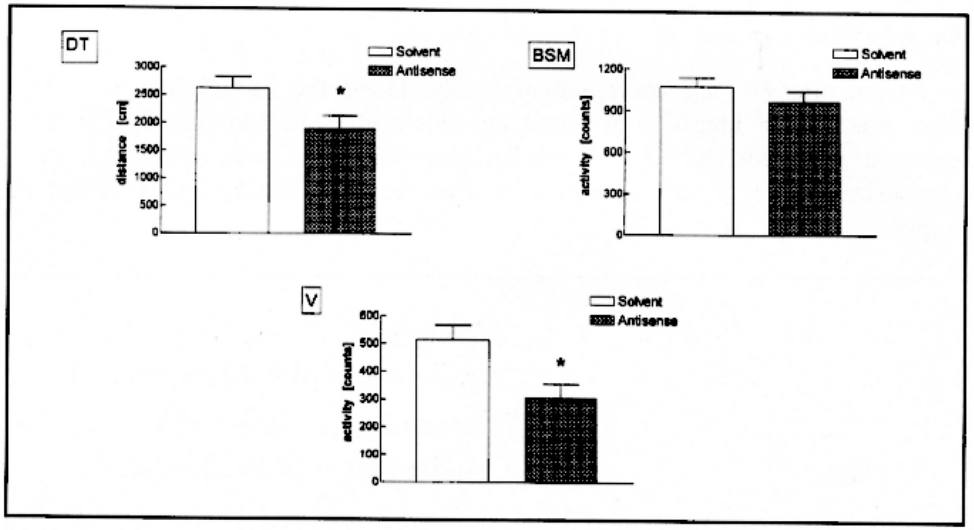

Fig. 1. Effect of bilateral intraventricular (lateral ventricle) injections of D_2 *antisense* ODN (1 nmol/1 µl; n = 5) or solvent (1 µl; n = 9) on spontaneous activity of the rats. DT — distance travelled [cm]; BSM — burst of stereotypic movements (i.e.: grooming, scratching, sniffing around) [counts]; V — vertical activity (i.e.: rearings, climbing up cage walls) [counts]. The total observation time was 30 min.
*$p < 0.05$ vs solvent-receiving group, unpaired t-Student test.

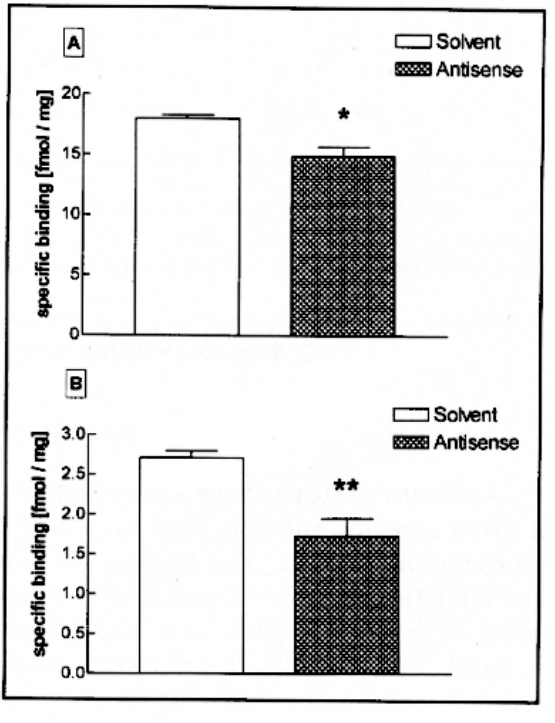

Fig. 2. Effect of bilateral intraventricular (lateral ventricle) injections of D_2 *antisense* ODN (1 nmol/1 µl; n = 6) or solvent (1 µl; n = 7) on specific binding of [^{3}H]raclopride to dopamine D_2 receptors in the rat striatum (A) and limbic forebrain (B).
*$p < 0.05$ vs solvent-receiving group, ANOVA followed by Duncan's test.

Forced swimming test

Imipramine (10 mg/kg) significantly shortened the immobility time. The effect was almost identical in intact animals and in the animals which were operated and received i.c.v. administration of the solvent. However, i.c.v. administration of D_2 *antisense* ODN reversed the anti-immobility effect of imipramine (*Fig. 3*).

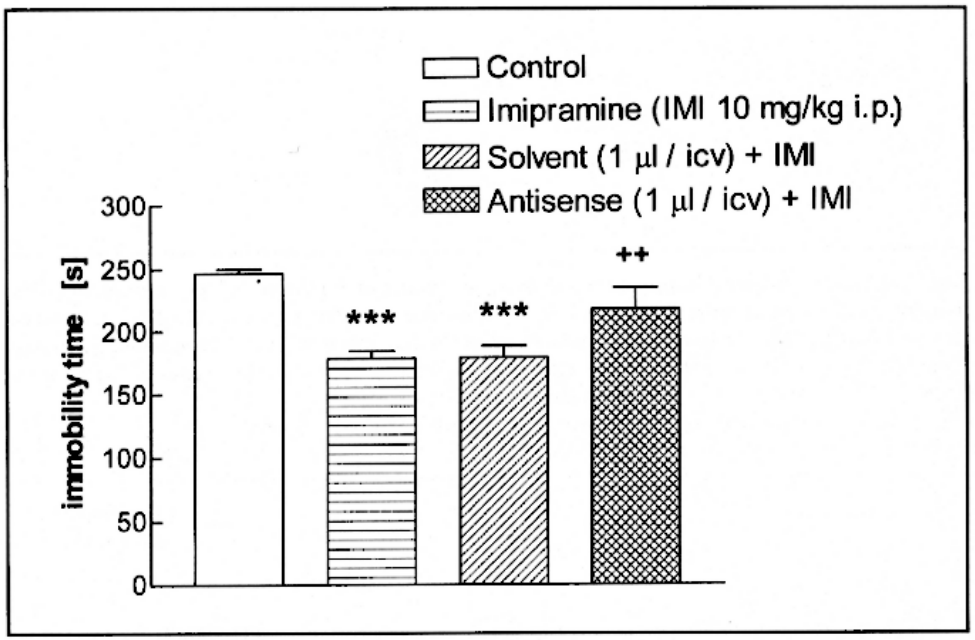

Fig. 3. Effect of bilateral intraventricular (lateral ventricle) injections of D_2 *antisense* ODN (1 nmol/1 µl; n = 7) or solvent (1 µl; n = 9) on the immobility time of rats treated with imipramine (IMI, 10 mg/kg i.p.; n = 16). Control — animals receiving solvent (2ml/kg i.p.) without imipramine (n = 16).

***$p < 0.001$ vs control, $^{++}p < 0.01$ vs solvent-receiving group, ANOVA (number of groups = 4; F = 21.57, R squared = 0.5952), followed by Newman-Keuls test.

DISCUSSION

Antisense strategies have a potential to specifically block the production of a given receptor subtype, thus may help to uncover its behavioral and/or biochemical function. The number of studies using antisense strategy for clarifying the behavioral function of dopamine D_2 receptors increases in recent years. It has been shown that i.c.v. administrating the D_2 *antisense* ODN reduced spontaneous locomotor activity, inhibited quinpirole-induced locomotor activation and elicited catalepsy in rats (24), inhibited rotations

induced by quinpirole in 6-OH-DA lesioned mice (16, 17), supressed — after intrastriatal administration — stereotyped sniffing elicited by apomorphine in the rat (25) and significantly influenced — after intranigral administration — the motor actions of cocaine (26).

It is widely accepted that normal, physiological functions of dopamine D_2 receptors (also pharmacological blockade or stimulation of these receptors) are responsible for locomotor activation (so called "forward" locomotor activity, measured as distance travelled) and for exploratory activity (measured as a number of rearings). On the other hand, behavioral activity such as grooming or scratching and sniffing, are believed to be regulated by dopamine D_1 receptors. This notion seems to be confirmed in the present study, since we observed significant decrease in locomotor (DT) and exploratory (V) activity, without any changes in BSM, after i.c.v. administration of D_2 *antisense* ODN.

The results obtained in the present work demonstrate the utility of this approach for the studying the role of dopamine D_2 receptors in anti-immobility effect of imipramine in the forced swimming test. This test, initially described by Porsolt (23), although bears oversimplification of a highly complex illness as depression is, has been widely used in the studies of action of ADs as well as in order to find compounds with novel antidepressant activity. It has been shown that various compounds which stimulate the locomotor activity are also active in the forced swimming test, and the effect which is not accompanied with locomotor hyperactivity is regarded as specific antidepressant effect. The i.c.v. administration of D_2 *antisense* ODN induces locomotor hypoactivity, therefore it is justified to interprete its reversal of anti-immobility effect of imipramine as a result of diminished synthesis of dopamine D_2 receptor. Especially, since in biochemical experiments we observed the decreased specific binding of [^{3}H]raclopride, D_2 receptor antagonist, following i.c.v. administration of D_2 *antisense* ODN, which may be interpreted as a reduction in the level of D_2 receptors. The degree of this decrease is relatively small compared to the reduction in behavioral changes. However, other investigators have already reported that small changes in the density of dopamine receptors may be associated with profound changes in dopamine-mediated behavioral responses (19). Since administration of D_2 *antisense* ODN causes a decreased synthesis in D_2 receptors, it would cause a proportionally greater decrease in the functional receptors compared with the total pool of receptors, thereby resulting in a large decrease in biological function but a relatively small decrease in the total pool of receptors, which is — in fact — detected by the binding of an antagonist used as radioligand.

Therefore, it may be concluded that, indeed, the dopamine D_2 receptors play a significant role in the behavioral anti-immobility effects of imipramine.

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