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Dopamine D_2 receptor ribozyme inhibits quinpirole-induced stereotypy in rats

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Abstract

The injection of a dopamine D_2 receptor hammerhead ribozyme (20 μg) once daily for 5 days into the nucleus accumbens of rats resulted in an inhibition of stereotyped sniffing and locomotor activation produced by the selective dopamine D_2 receptor agonist, quinpirole (0.4 mg kg^{-1} s.c.). The results suggest that ribozymes may be useful in the study of in vivo gene function in the brain.

Keywords: Ribozyme; Dopamine D_2 receptor; Stereotypy

The use of ribozymes, or catalytic RNAs, to inhibit gene expression by sequence-specific cleavage of mRNA can provide an attractive strategy for the study of gene function and they may lead to novel therapeutics. Ribozymes were first characterized in natural systems and synthetic ribozymes have also been shown to be active. There are furthermore opportunities to improve catalytic and stability properties through a variety of chemical modifications (Paolella et al., 1992). However, data regarding the efficiency of ribozymes in living animals are sparse, and the potential of these types of compounds both as experimental tools and as therapeutic agents remains to be shown.

In order to test the efficacy of ribozymes in the living brain, we have designed a heavily chemically modified hammerhead ribozyme directed against rat dopamine D_2 receptor mRNA. Work with selective dopamine D_2 receptor agonists, such as quinpirole, has shown that activation of this dopamine receptor subtype reliably results in stereotyped sniffing and locomotor activation that can be blocked with selective dopamine D_2 receptor antagonists (Koller et al., 1987). Furthermore, a probable site of action for the effects of quinpirole on these two behavioral variables is the nucleus accumbens (Arnt, 1985; Sharp et al., 1987).

In the present study, we have taken advantage of the effects of quinpirole on sniffing and locomotor activation in rats. We have examined whether intra-accumbens injections of a dopamine D_2 receptor ribozyme, but not inactive control ribozyme, antagonized quinpirole-induced sniffing and locomotor activation. For comparisons, a group of rats received intra-accumbens injections of the selective dopamine D_2 receptor antagonist, sulpiride.

Adult male Sprague–Dawley rats (280–320 g) were used. A guide cannula (25-gauge) was stereotaxically mounted in the skull bone and placed against the dura for unilateral intra-accumbens injections (AP +2.1 mm and L −1.2 mm, relative bregma) (Paxinos and Watson, 1998). The injection needle (31-gauge) penetrated 6.8 mm below the dura. Modified 2′-*O*-allyl ribozymes containing ribonucleotides at positions G5, A6, G8 G12, and L15.1 and a 2′-amino-2′-deoxyuridine at position U4 were designed (Ludwig et al., 1998), synthesized and purified according to a new highly efficient procedure (Sproat et al., 1999). The active sequence was 5′-hex-gcucgacc*u*GAuGaggccgugaggccGaaIcugcgcu-iT, where hex is a hexanediol linker, lower case letters represent 2′-*O*-allylribonucleotides, bold upper case letters represent ribonucleotides, *u* represents 2′-amino-2′-deoxyuridine and iT is a 3′-3′ inverted thymidine linkage. This sequence was chosen on its favorable in vitro activity in a cell lysate. The inactive control sequence was 5′-hex-gcucgaccugAuGaggccgugag-

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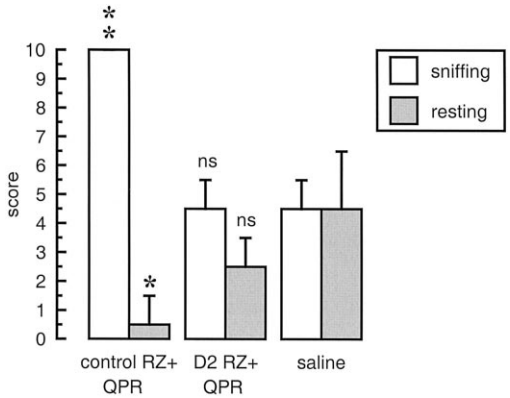

Fig. 1. Effects of dopamine D_2 receptor ribozyme on quinpirole-induced sniffing and locomotor activation. The dopamine D_2 receptor ribozyme (20.0 μg μl^{-1}) and the inactive control ribozyme (20 μg μl^{-1}) were administered once daily for 5 days into the left nucleus accumbens of rats. On day 6, quinpirole (0.4 mg kg^{-1} s.c., −20 min) were administrated, and sniffing and resting was scored by direct observation as described in text. The results are shown as medians ± semi-interquartile range based on 4–6 animals per group. Kruskal–Wallis one-way ANOVA followed by Mann–Whitney *U*-test for pair-wise comparisons was used to determine significance. Shown in the figure are comparisons with appropriate saline-treated controls. $^{NS}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$.

gccgaaIcugcgcu-iT. Ribozymes, including inactive (non-catalytic) control ribozymes, were injected into the nucleus accumbens once daily for 5 days, while sulpiride was injected only on the day of the experiment (day 6). Both ribozymes and sulpiride (including vehicle) were infused in a volume of 1 μl over 90 s and the injection needle was left in situ for another 120 s. On the day of experiments, rats were placed individually in Makrolon Type IV cages for direct behavioral observations by an observer unaware of the treatments. Behavior was observed on the minute, for a total of 10 min and scored if sniffing or resting were present. A low incidence of resting was interpreted as an increase in locomotor activity. The incidence of sniffing and resting was expressed as the cumulative score over 10 min.

As expected, the administration of quinpirole (0.4 mg kg^{-1} s.c., −20 min) produced stereotyped sniffing and increased locomotor activation that was blocked by intra-accumbens injections of sulpiride (4 μg) ($P < 0.01$, quinpirole vs. sulpiride + quinpirole, Mann–Whitney *U*-test). Treatment with the dopamine D_2 receptor ribozyme (20 μg, once daily for 5 days) also blocked the quinpirole-induced behaviors, whereas the inactive control ribozyme had no effects either on sniffing or locomotor activity (Fig. 1).

The present results demonstrate site-specific effects of a dopamine D_2 receptor ribozyme injected into the nucleus accumbens of rats. As quinpirole has about the same affinity for both dopamine D_2 and D_3 receptors, the results also suggest that dopamine D_2 receptors specifically have a critical role in dopamine-mediated stereotypy in rats. This is in agreement with a recent study that used antisense oligonucleotides in order to knock-down dopamine D_2 receptors in vivo (Rajakumar et al., 1997). We suggest that ribozymes may have potential for extensive use in gene function studies in the living brain.

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References

Arnt, J., 1985. Antistereotypic effects of dopamine D1 and D2 antagonists after intrastriatal injection in rats. Pharmacological and regional specificity. Naunyn Schmiedeberg's Arch. Pharmacol. 330, 97–104.

Koller, W., Herbster, G., Anderson, D., Wack, R., Gordon, J., 1987. Quinpirole hydrochloride, a potential anti-parkinsonism drug. Neuropharmacology 26, 1031–1036.

Ludwig, J., Blaschke, M., Sproat, B.S., 1998. Extending the cleavage rules for the hammerhead ribozyme; mutating adenosine$^{15.1}$ to inosine$^{15.1}$ changes the cleavage site specificity from $N^{16.2}U^{16.1}H^{17}$ to $N^{16.2}C^{16.1}H^{17}$. Nucleic Acids Res. 26, 2279–2285.

Paolella, G., Sproat, B.S., Lamond, A.I., 1992. Nuclease resistant ribozymes with high catalytic activity. EMBO J. 11, 1913–1919.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. 4th edn. Academic Press, London.

Rajakumar, N., Laurier, L., Niznik, H.B., Stoessl, A.J., 1997. Effects of intrastriatal infusion of D2 receptor antisense oligonucleotide on apomorphine-induced behaviors in the rat. Synapse 26, 199–208.

Sharp, T., Zetterström, T., Ljungberg, T., Ungerstedt, U., 1987. A direct comparison of amphetamine-induced behaviours and regional dopamine release in the rat using intracerebral dialysis. Brain Res. 401, 322–330.

Sproat, B.S., Rupp, T., Menhardt, N., Keane, D., Beijer, B., 1999. Fast and simple purification of chemically modified hammerhead ribozymes using a lipophilic capture tag. Nucleic Acids Res. 27, 1950–1955.