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Intrastriatal adenosine A_1 receptor antisense oligodeoxynucleotide blocks ethanol-induced motor incoordination

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Abstract

Intrastriatal administration of a 21-mer phosphorothioate antisense oligodeoxynucleotide targeting the adenosine A_1 receptor blocked ethanol-induced motor incoordination in the rat and reduced striatal adenosine A_1 receptor content, as judged by specific binding of the A_1-specific ligand 8-cyclopentyl-1,3-dipropylxanthine ($B_{max} = 0.350 \pm 0.07$, $K_d = 1.87 \pm 0.50$ nM). No effect upon striatal adenosine A_2 receptor content was observed ($B_{max} = 0.415 \pm 0.04$, $K_d = 13.13 \pm 1.25$ nM) with the A_2-specific ligand 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine. A mismatched control oligodeoxynucleotide of identical G-C base composition and general sequence structure was without effect on adenosine A_1 receptor ($B_{max} = 0.666 \pm 0.11$, $K_d = 1.32 \pm 0.27$ nM) or adenosine A_2 receptor content ($B_{max} = 0.501 \pm 0.08$; $K_d = 14.65 \pm 1.82$ nM) or ethanol-induced motor incoordination. These results confirm an important role of the striatal adenosine A_1 receptor in mediating certain motor-related physiological effects of ethanol.

Keywords: Adenosine receptor; Motor coordination; Antisense oligodeoxynucleotide

Ethanol abuse contributes significantly to worldwide morbidity and mortality. Alcoholism is thought to contribute to a variety of organic disease processes including hypertension, liver cirrhosis and several forms of cancer (Anderson et al., 1994). Ethanol-induced motor incoordination also contributes substantially to the medical, social and economic burden related to the consumption of alcohol, and approximately half of fatal automobile accidents are ethanol-related (Delauney et al., 1991).

Adenosine has been implicated as a potential mediator of some of the central effects of ethanol (Diamond and Gordon, 1994; Reynolds and Brien, 1995). Meng and Dar (1994, 1995) showed that the rank order of potency of several adenosine receptor ligands favored the involvement of the striatal adenosine A_1 receptor in mediating ethanol-induced motor incoordination in the rat. However, a more definitive identification of the receptor involved requires a more precise ablation of the target than is possible with relatively non-specific small molecule ligands.

In order to more precisely test the hypothesis that the striatal adenosine A_1 receptor mediates (in whole or in part) ethanol-induced motor incoordination, a phosphorothioate antisense oligodeoxynucleotide targeting this receptor was designed. This antisense oligodeoxynucleotide shows completely selective hybridization potential for the adenosine A_1 receptor, with no sequence-dependent hybridization potential for adenosine A_{2a}, A_{2b} or A_3 receptors (GENBANK R95.0). In the present study, the effect of its intrastriatal administration upon ethanol-induced motor incoordination was compared to that of a minimally mismatched control oligodeoxynucleotide of identical G-C base composition and general sequence structure.

A group of 44 rats (300–400 g male Harlan Sprague-Dawley; exposed to 12 h light-dark cycle with lights on at 07:00 h) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and stereotaxically implanted with permanent bilateral stainless steel guide cannulas (24 gauge, length = 10 mm) in the caudate nucleus (from bregma; AP = +0.7 mm, ML = ±2.8 mm, DV = −3.2 mm, in the flat head position).

Rat adenosine A_1 receptor phosphorothioate antisense (2 μg/side; 5′-GAT GTA GGG CGG CAT GGT GGG-3′), mismatch (2 μg/side, 5′-GAA GTT GGC GGG GAA

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GCA GGG-3′) or pyrogen-free artificial cerebrospinal fluid (aCSF) vehicle were injected in 2 μl/side over 2 min, with an additional 30 s delay before the injector was retracted and replaced by a 10 mm long stainless steel stylet. Oligodeoxynucleotides or aCSF were administered twice daily (08:00 h and 20:00 h) for 2 days.

On the morning of the third day (12 h after the last injection), animals were administered 20% ethanol (3.5 g/kg by gastric gavage) or saline and evaluated for motor coordination using a standard rotorod apparatus. Full motor coordination was defined as the ability of the animal to continuously maintain balance on the rotorod for 180 s. Motor coordination times (in seconds) for each rat were recorded at 15, 30, 45 and 60 min time intervals after ethanol administration and were evaluated for overall treatment efficacy using a two-way repeated measures analysis of variance (ANOVA) followed by the Newman-Keuls test. The motor activity ratio was defined as motor coordination times ÷ 180 s.

After the completion of the motor coordination tests, rats were killed by sodium pentobarbital (60 mg/kg, i.p.) and decapitated. Brains were either dissected and striatum isolated and frozen at −70°C till use, or cut on a cryostat for histological analysis. Adenosine A_1 and A_2 receptor binding in rat striatal membranes was determined according to established methods (Jarvis et al., 1989; Cunha et al., 1995) using the A_1 antagonist, 8-cyclopentyl-1,3-[^{3}H]dipropylxanthine, and the A_2 agonist, 2-*p*-(2-carboxyethyl-[^{3}H])-phenethylamino-5′-*N*-ethylcarboxamidoadenosine (DuPont NEN). Results of Scatchard analyses (B_{max}) were analyzed using Kruskal-Wallis ANOVA.

Blood ethanol levels in the antisense oligodeoxynucleotide treatment ($n = 4$) and mismatch control oligodeoxynucleotide treatment groups ($n = 3$) taken after the 60 min time period indicated blood alcohol levels of 0.3160 ± 0.023 mg/dl and 0.3592 ± 0.055 mg/dl, respectively. Placement of the guide cannula in the striatum was histologically confirmed.

Ethanol-induced motor incoordination was significantly decreased in the antisense oligodeoxynucleotide-treated animals, but not in animals treated with either the mismatch control oligodeoxynucleotide or aCSF (Fig. 1a). In addition, intrastriatal pretreatment with antisense or mismatch control oligodeoxynucleotides had no effect on the normal motor coordination of the rats (saline + A_1 mismatch/A_1 antisense, Fig. 1a). This attenuation of the motor incoordinating effects of ethanol by the adenosine A_1 receptor antisense was supported by receptor binding assays. The adenosine A_1 receptor density in the striatum was significantly reduced in the antisense-treated group (B_{max} = 0.350 ± 0.07; K_d = 1.87 ± 0.50 nM; $P < 0.05$; Fig. 1b) as compared to the aCSF and mismatch treatment groups. This attenuation of receptor number was also found to be highly specific for the adenosine A_1 receptor as no significant reduction in adenosine A_2 receptor number was observed (B_{max} = 0.415 ± 0.04; K_d = 13.13 ± 1.25 nM; $P >$ 0.05, Fig. 1b). Furthermore, a mismatched control oligodeoxynucleotide was without effect on adenosine A_1 receptor (B_{max} = 0.666 ± 0.11, K_d = 1.32 ± 0.27 nM) or adenosine A_2 receptor content (B_{max} = 0.501 ± 0.08; K_d = 14.65 ± 1.82 nM). These results specifically identify the striatal adenosine A_1 receptor as a central mediator of ethanol-induced motor incoordination in the rat.

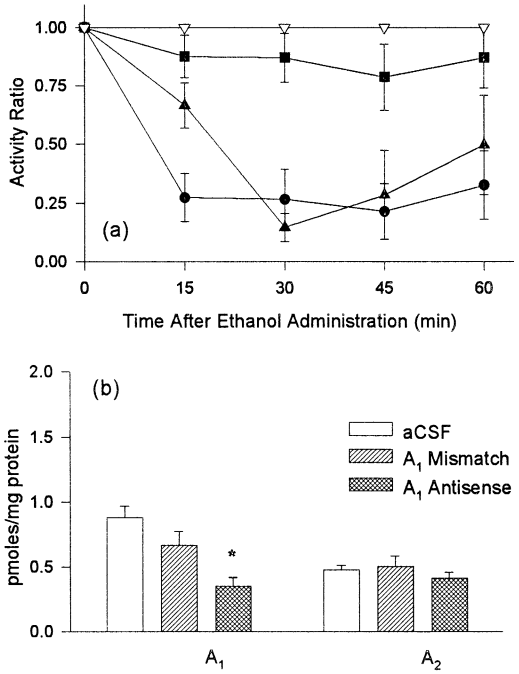

Fig. 1. (a) Effects of adenosine A_1 receptor mismatch control and adenosine A_1 receptor antisense oligodeoxynucleotides on ethanol-induced motor incoordination following their intrastriatal administration (see Introduction for detail). (▽) Saline + A_1 mismatch/A_1 antisense; (●) ETOH + A_1 mismatch; (■) ETOH + A_1 antisense; (▲) ETOH + aCSF. Intrastriatal adenosine A_1 receptor antisense pretreatment significantly attenuated the motor incoordination induced by acute ethanol at all four evaluation time periods ($P < 0.05$), while no such effect was observed in adenosine A_1 receptor mismatch-treated rats. Animals treated with aCSF and gavaged with saline showed complete motor coordination on the rotorod (activity ratio = 1.0; data not shown). Each point represents mean ± S.E.M. ($n = 5$–9). (b) B_{max} values from adenosine A_1 and A_2 binding assays. Asterisk denotes significant difference ($P < 0.05$) between the adenosine A_1 receptor antisense-treated group and the aCSF vehicle and adenosine A_1 receptor mismatch treatment groups. Each bar represents mean ± S.E.M. ($n = 3$–6).

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