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RAPID COMMUNICATIONS

REDUCTION IN MUSCARINIC RECEPTORS BY ANTISENSE OLIGODEOXYNUCLEOTIDE

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Abstract-Treatment of rat superior cervical ganglion cells in culture for 4 days with an antisense oligodeoxynucleotide corresponding to a partial sequence of the mRNA coding the m1 muscarinic receptor decreased m1 receptor mRNA by 64% without affecting m2 receptor mRNA. [^{3}H]Pirenzepine binding to M_1 receptors was decreased by 43%. Intraventricular infusion of this antisense oligodeoxynucleotide reduced muscarinic receptor density in M_1 but not M_2 receptor-rich brain regions.

Key words: pirenzepine; superior cervical ganglia; brain.

Muscarinic receptor subtypes were classified previously into two families (M_1 and non-M_1) based on their affinity for PZ[+]. Molecular cloning studies, however, have revealed the existence of five molecularly distinct muscarinic receptor subtypes (termed m1-m5) [1]. The lack of completely selective receptor subtype agonists or antagonists, as well as the heterogeneous distribution and co-expression of these muscarinic receptor subtypes, hampers the determination of their functional significance. Antisense oligodeoxynucleotides designed to duplex with the mRNA coding for a specific protein have been

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[+]Abbreviations: PZ, pirenzepine; SCG, superior cervical ganglia; and QNB, quinuclidinyl benzilate.

shown to have the ability to block specifically the translation of the selected protein both *in vitro* and *in vivo* [2,3]. Such studies suggest that exogenous nucleotides can enter cells and bind to the specific mRNA, arresting protein synthesis either by stimulating ribonuclease H-mediated degradation or by preventing the translocation of the ribosome along the targeted mRNA sequence.

MATERIALS AND METHODS

An antisense 16-mer phosphorothioate oligodeoxynucleotide (S-oligo) was designed to correspond to nucleotides 4-19 [1] of the m1 muscarinic receptor mRNA (Oligo *Etc.*, Inc., Wilsonville, OR). SCG from 1- to 2-day-old rat neonates were dissected, enzymatically dissociated into single cells, and plated on collagen-coated dishes as previously described [4]. The SCG tissue culture was treated with 0.1 mM S-oligo for 4 days. Untreated cultures served as a control. The levels of m1 and m2 muscarinic receptor subtype mRNAs were measured using a RNase protection assay with rat m1 (390 nucleotides) or m2 (425 nucleotides) receptor riboprobes directed against the third cytoplasmic loop. M_1 muscarinic receptor binding sites were assayed using 20 nM [^{3}H]PZ ± 1 μM atropine. We also investigated whether antisense S-oligo could reduce binding to brain muscarinic receptors following *in vivo* treatment. Male Sprague-Dawley rats weighing 200-250 g were anesthetized with chloralhydrate (400 mg/kg) and subcutaneously implanted with Alzet micro-osmotic pumps that continuously delivered either saline or S-oligo (5 μg/μL/hr) into the lateral cerebral ventricle for 3 days via an implanted cannula. The rats were killed on day 4, the brains were dissected and muscarinic receptors were assayed using the receptor ligand [^{3}H]QNB (concentration range between 0.06 and 2.0 nM).

RESULTS AND DISCUSSION

In SCG cultured cells, the antisense S-oligo decreased the steady-state concentration of m1 receptor mRNA, whereas the concentration of m2 receptor mRNA was not affected. In accordance with these results, the binding of [^{3}H]PZ to muscarinic receptors was decreased by 43% as compared with the control cultures (Fig. 1). Scatchard analyses revealed a decreased binding capacity (B_{max}) in the cerebral cortex, hippocampus and striatum of antisense S-oligo-treated rats (Fig. 2), while the dissociation constant (K_d) was not affected (data not shown). In contrast, [^{3}H]QNB binding to muscarinic receptors in the brainstem was unaltered. The majority of [^{3}H]QNB binding in the cortex, hippocampus and striatum is to pharmacologically defined M_1 receptors (expressed by the m1, m3 and m4 receptor subtype genes), while binding to non-M_1 receptors (expressed by the m2 receptor subtype gene) predominates in the brainstem [5].

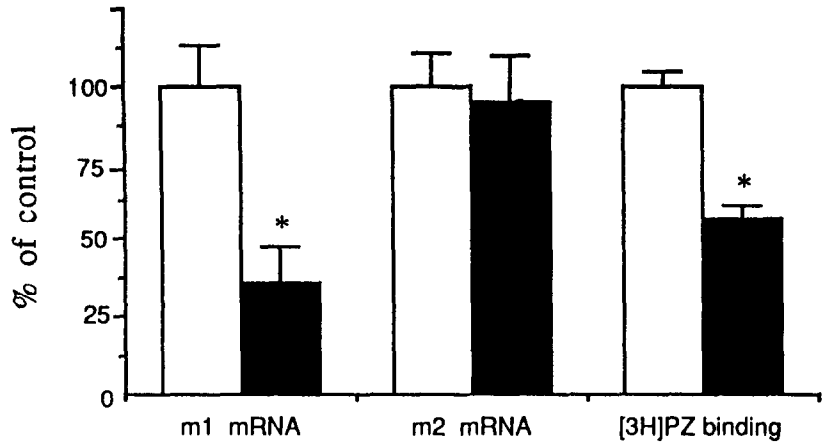

Fig. 1. Effect of m1 receptor antisense S-oligo treatment on muscarinic receptor and mRNA levels in SCG cells. The data are expressed as % of controls (mean ± SEM of 9 dishes). Absolute control values were: 4.5 amol/μg total cellular RNA (m1 mRNA), 7.1 amol/μg total cellular RNA (m2 mRNA) and 124 fmol/mg protein ([^{3}H]PZ). (*: $p < 0.05$; Student's two-tailed t-test).

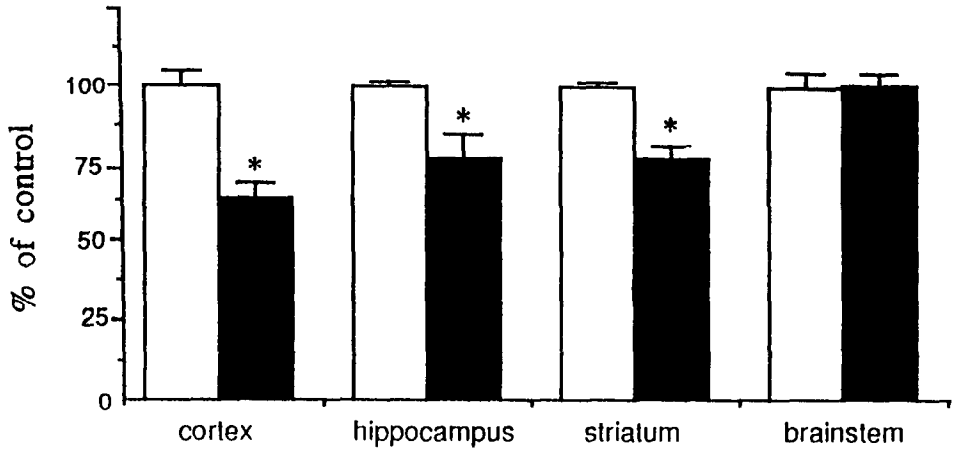

Fig. 2. Effect of m1 receptor antisense S-oligo administration on [^{3}H]QNB binding in rat brain regions. The data are expressed as % of controls (mean ± SEM, N=6). Absolute control values were: 117 fmol/mg tissue (cortex), 122 fmol/mg tissue (hippocampus), 108 fmol/mg tissue (striatum) and 57 fmol/mg tissue (brainstem). (*: $p < 0.05$; Student's two-tailed t-test).

Thus, the *in vivo* data support the *in vitro* findings concerning antisense selectivity. However, we cannot exclude the possibility of a limited diffusion of the S-oligo, although our previous work [3] using a dopamine D_2 antisense S-oligo showed a reduction in D_2 receptors throughout the brain. Finally, [^{3}H]QNB binding in rats treated with either a random or a dopamine D_2 receptor specific S-oligo was not affected [3], providing further evidence for the specificity of the m1 antisense oligo. Interestingly, locomotor activity of the treated rats, as measured by recording photocell interruptions for 5 min in an Omnitech photocell cage, was significantly higher after a 30-min habituation period than the activity of the control rats (333 ± 69 vs 147 ± 43: $P < 0.05$, Student's *t*-test). This observation is consonant with reports showing hyper-locomotion after administration of the non-selective muscarinic receptor antagonist scopolamine [6]. In conclusion, we suggest that the antisense strategy may provide a new approach to study the physiological and pharmacological function of muscarinic receptor subtypes both *in vitro* and *in vivo*.

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