with a potency order of GTP > GDP = guanosine 5'-[β-thio]diphosphate > ITP > GMP > UTP = CTP confirmed that a guanine nucleotide-binding regulatory protein (G-protein) [for review see Gilman, 1987] mediated the effects of GTP[S]. The G-protein involved, termed G₃z, has yet to be identified.

The effects of GTP[S] (10 μM) were inhibited by spermine and spermidine with IC₅₀ values of approx. 0.25 mM and 2 mM, respectively, and by putrescine at concentrations above 5 mM. Spermine (2.5 mM) blocked the effects of 0.1–100 μM-GTP[S] (Table 1) and additional experiments showed that 1 mM-spermine was equally effective in inhibiting the stimulatory effects of 10 μM, 1 mM- and 2.5 mM-GTP[S]. Thus, spermine does not inhibit the effects of GTP[S] by simple competition. In contrast, inhibitory effects of spermine (5 mM) were only partial in the range 0.3–0.6 mM-Ca²⁺ and were not seen at 1 mM-Ca²⁺ (Table 1). These observations do reflect changes in the rate of phosphoinositide hydrolysis, as spermine (2.5 mM) also blocked the GTP[S]-induced decreases in [³H]polyphosphoinositide levels and the inhibitory effects of 1 mM-Ca²⁺ on [³H]polyphosphoinositide levels were not reversed by 5 mM-spermine.

These findings may be explained in part by binding of the cationic polyamines to polyphosphoinositides. Thus, as observed here and as reported for other systems [Eichberg et al., 1981; Moruzzi et al., 1987], it would be expected that the potency order of polyamines would be spermine (four amino groups) > spermidine (three amino groups) > putrescine (two amino groups). Also, the opposing effects of Ca²⁺ and spermine may be a consequence of competitive binding of these cations to polyphosphoinositides. Similar competition for binding to phosphatidylinerine has been reported [Meers et al., 1986]. While Ca²⁺ and spermine may compete for the same binding site(s), partial reversal of the effects of 5 mM-spermine with 0.6 mM-Ca²⁺ did not result in the reappearance of GTP[S] effects (Table 1). Thus, the interaction of spermine with the phosphoinositide/phospholipase C/Gp system appears to be more complex than simple reversible binding to the polyphosphoinositides and points towards the presence of a second site of interaction. Such an interaction that prevented either binding of GTP[S] to Gp or coupling of Gp with phospholipase C would account for the irreversible inhibition of GTP[S] effects.


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M2 muscarinic cholinergic receptors expressed by a human neuroblastoma SK-N-SH

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Characterization of receptor-mediated inositol phosphate metabolism and associated increases in intracellular calcium concentrations within central neurons have been hampered by the heterogeneity of simple brain slice preparations. Certainly the selective muscarinic antagonist pirenzepine, which binds with high affinity to M1 and low affinity to M2 receptor subtypes (Hammer et al., 1980), suggests mixed M1/M2 receptor populations in cerebrocortical tissue and may be a reflection of the tissue heterogeneity. The development of cell culture models expressing homogeneous M1 and M2 receptor populations would be of great use in characterizing the intracellular events following M1 or M2 receptor activation.

Waelbroeck et al. (1988) have demonstrated 80% M1 muscarinic receptor expression by a human neuroblastoma NB-OK 1. Recent publications with another neuroblastoma cell line, SK-N-SH (Fisher & Heacock, 1988), report M2β receptor expression yet its clone SH-SYSY (Serra et al., 1988) has been reported to express the M1 receptor subtype. The present communication reports the binding characteristics of the labelled non-selective muscarinic antagonist [³H]N-methylscopolamine ([³H]NMS) to whole cell and membrane preparations of the human neuroblastoma SK-N-SH.

Stock cultures of SK-N-SH routinely maintained in minimum essential medium (MEM) supplemented with l-glutamine (2 mmol/l), penicillin (100 i.u./ml), streptomycin (100 μg/ml), amphotericin B (2.5 μg/ml) and 10% (v/v) fetal calf serum were fed twice weekly and passaged (1:3) once a week. [³H]NMS binding to suspension cultures of whole cells and membrane preparations of SK-N-SH cells were performed in Krebs-Henseleit and Mg²⁺/Hepes buffers, respectively. Incubations were performed at 37°C over a 20 min period after which time bound ligand was separated from free ligand by vacuum filtration. Non-specific binding was defined in the presence of atropine sulphate (1 pmol/l). The inhibitor constant IC5₀ and the Hill coefficient, nH, were obtained by computer-assisted curve fitting using ALLFIT (De Lean et al., 1978). The inhibitor constant Kᵢ was calculated according to Cheng & Prusoff (1973).

Whole cell and membrane preparations of SK-N-SH specifically bound [³H]NMS. The binding was saturable with a Kₛ of 0.188 and 0.186 nM (P < 0.05), a B₅₀ of 11.32 nM and

Abbreviation used: NMS, N-methylscopolamine.
The muscarinic receptor employs various effector mechanisms resulting in stimulation of phosphoinositide turnover, inhibition of adenylate cyclase, accumulation of cyclic GMP and modulation of ion channels. These functions appear to be mediated by interactions of the agonist with the receptor through guanine nucleotide binding proteins (G proteins; for review see Gilman, 1987). It is of great interest to consider whether different subtypes of receptors exist which are linked to individual effector mechanisms, possibly through distinct G proteins.

In rat pituitary GH$_3$ cells, thyrotropin releasing hormone (TRH) stimulates prolactin secretion via the activation of a phosphoinositide-specific phospholipase C (Martin, 1983; Rebecchi & Gerloshengorn, 1983). The products of phosphoinositide breakdown, inositol phosphates and diacylglycerol, are believed to be responsible for the regulation of a biphasic elevation of the intracellular Ca$^{2+}$ ion concentration which is thought to trigger prolactin secretion (Martin et al., 1984; Drummond, 1985). It has also been reported that in GH$_3$ cells muscarinic receptors mediate the attenuation of prolactin release via the inhibition of adenylate cyclase (Martin & Kowalchyk, 1984; Drummond, 1985).

GH$_3$ cell membranes have single affinity binding sites ($K_{d}$, 27 fmol/mg of protein, $K_{d}$, 76 pm) for $[^3]$H-methylscopolamine ($[^3]$H[NMS]). The result of a direct $[^3]$Hpirenzipine binding study (data not shown) does not show any evidence for the presence of high-affinity binding sites. In addition, a study on the competition for $[^3]$H[NMS] binding by pirenzipine shows that the sites are primarily of a putative M$_2$ subtype (Watson et al., 1986); computer analysis of the competition binding curves revealed that a subpopulation of sites which shows a high affinity ($K_{d}$, 4 nm) for pirenzipine is minimal (less than 10%), and that the majority of sites show a low affinity ($K_{d}$, 22 pm) for this antagonist.

It has also been reported that TRH activates a low $K_{d}$ GTPase in membrane preparations from rat pituitary tumour cells (Hinkle & Phillips, 1984). Since the muscarinic receptor in GH$_3$ cells seems likely to be associated only with the inhibition of adenylate cyclase, we have examined the effect of pertussis toxin, which is known to block G$_i$-mediated signal transmission by ADP-riboylation of G$_i$, on the GTPase activity of GH$_3$ cell membranes stimulated with TRH, carbachol or TRH plus carbachol (Table 1). Our data shows a 23% increase in a high-affinity GTPase in cell membranes stimulated with 100 $\mu$m-carbachol. TRH (100 nm) also stimulated the membrane GTPase activity by 20%, and TRH plus car-

Table 1. Displacement of specific $[^3]$H[NMS] binding to whole cell and membrane preparations of a human neuroblastoma SK-N-SH by atropine, pirenzipine and carbachol

Data represent the mean of three independent experiments.

<table>
<thead>
<tr>
<th>Displacer</th>
<th>Preparation</th>
<th>$IC_{50}$ (mol/l)</th>
<th>$K_n$ (mol/l)</th>
<th>$n_h$</th>
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</tbody>
</table>

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Response to muscarinic stimulation in rat pituitary GH$_3$ cells in relation to thyrotropin releasing hormone-induced phosphoinositide turnover and activation of membrane GTPase

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We have recently indicated that carbachol, at concentrations as high as 1 mm, is unable to alter either basal or TRH-stimulated production of inositol phosphates in these cells, while the same agonist showed a dose-dependent inhibition of the accumulation of basal cyclic AMP (in the presence of isobutylmethylxanthine) with an $IC_{50}$ of approximately 1 mm (Yagisawa et al., 1988). The fact that the muscarinic receptor of the GH$_3$ cells, which is able to attenuate adenylate cyclase, is unable to couple to phosphoinositide specific phospholipase C where another receptor (the TRH receptor) can, led us to examine subtypes of the muscarinic receptor by pharmacological methods and its mode of coupling with G proteins.

GH$_3$ cell membranes have single affinity binding sites ($B_{max}$, 27 fmol/mg of protein, $K_{d}$, 76 pm) for $[^3]$H-methylscopolamine ($[^3]$H[NMS]). The result of a direct $[^3]$Hpirenzipine binding study (data not shown) does not show any evidence for the presence of high-affinity binding sites. In addition, a study on the competition for $[^3]$H[NMS] binding by pirenzipine shows that the sites are primarily of a putative M$_2$ subtype (Watson et al., 1986); computer analysis of the competition binding curves revealed that a subpopulation of sites which shows a high affinity ($K_{d}$, 4 nm) for pirenzipine is minimal (less than 10%), and that the majority of sites show a low affinity ($K_{d}$, 22 pm) for this antagonist.

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Abbreviations used: TRH, thyrotropin releasing hormone; NMS, N-methylscopolamine.

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