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An Agonist That Is Selective for Adenylate Cyclase-Coupled Muscarinic Receptors

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SUMMARY

Compound BM5 [*N*-methyl-*N*(1-methyl-4-pyrrolidino-2-butynyl) acetamide] has previously been described as an agonist at postsynaptic muscarinic receptors and as an antagonist at presynaptic receptors. In the current work, we studied the ability of this compound to selectively stimulate phosphoinositide (PI) turnover in Chinese hamster ovary cells transfected with m1 muscarinic receptors and in SK-N-SH neuroblastoma cells that express only m3 receptors. We also studied the ability of this compound to stimulate adenylate cyclase inhibition in m2 muscarinic receptors from heart tissue and in m4 receptors expressed in NG108-15 cells. BM5 stimulated the two muscarinic receptor subtypes coupled to adenylate cyclase inhibition. In NG108-15 cells, 100 μM BM5 inhibited prostaglandin E_1-stimulated cAMP formation by 36 ± 1.5%, whereas 100 μM of the full agonist oxotremorine-M inhibited cAMP formation by 64.1 ± 1.9%. The half-maximal concentration for BM5 inhibition of cAMP formation was 0.4 ± 0.1 μM. In heart membranes, BM5 inhibited isoproterenol-stimulated adenylate cyclase by 24 ± 2%, whereas oxotremorine inhibited this activity by 34 ± 3%. In contrast to its activity at these receptor subtypes, BM5 did not stimulate the m1 or m3 receptor subtypes, which couple to PI turnover. In these latter two subtypes, BM5 inhibited oxotremorine-M-stimulated PI turnover with IC_{50} values of 10–20 μM. Therefore, BM5 is a partial agonist at adenylate cyclase-coupled muscarinic receptor subtypes and is a pure antagonist at PI turnover-coupled muscarinic receptor subtypes. These studies also suggest that, at least in some parts of the brain, postsynaptic muscarinic receptors are coupled to adenylate cyclase, whereas presynaptic muscarinic receptors are coupled to PI turnover.

The recent cloning and sequencing of five distinct genes for muscarinic cholinergic receptors (1–3) demonstrates the molecular diversity in this family of receptors and raises the possibility of developing receptor subtype-specific ligands. Although several antagonists such as pirenzepine (4), 4-DAMP (5), AF-DX-115 (6), and methoctramine (7) display varying degrees of selectivity, few selective agonists other than McN-A-343 (8) and AF102B (9) are available. Selective muscarinic agonists are of clinical interest in treating the cholinergic deficit caused by the degeneration of the basal forebrain system in patients with Alzheimer's disease (10). Because the postsynaptic muscarinic receptors remain relatively unaffected by this disease (11–13), several clinical studies have attempted to treat the resulting cholinergic deficit with muscarinic agonists (e.g., Ref. 14). These clinical studies have met with little success. One reason for this lack of success may be that these muscarinic agonists stimulate both presynaptic and postsynaptic muscarinic receptors. Stimulation of presynaptic muscarinic receptors has been shown to inhibit acetylcholine release (15, 16), thus possibly rendering this therapy counterproductive by inhibiting the release of acetylcholine from remaining intact basal forebrain fibers. In order for this therapeutic approach to succeed, an agonist that is selective for postsynaptic receptors is needed. Compound BM5 has been shown to be such an agonist (17, 18). We, therefore, studied its mechanism of action in more detail and herein report on these studies.

Activation of muscarinic receptors elicits a number of intracellular responses, including stimulation of PI turnover and inhibition of adenylate cyclase (19). Subtypes[1] m1, m3, and m5 have been shown to couple to PI turnover and not to cyclase inhibition, whereas the other two subtypes have been shown to couple to adenylate cyclase inhibition and not PI turnover (20). When stimulated, muscarinic receptors in SK-N-SH human neuroblastoma cells elicit only PI turnover, whereas those in

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[1] The nomenclature for muscarinic receptor subtypes used throughout this paper is that of Bonner *et al.* (1).

ABBREVIATIONS: BM5, *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide; CHO cells, Chinese hamster ovary cells; DMEM, Dulbecco's modified Eagle medium; HEPES, 4-(2-Hydroxyethyl)-1-piperazine-ethanesulfonic acid; NMS, *N*-methyl scopolamine; QNB, 1-quinuclidinyl benzilate; IBMX, 3-isobutyl-1-methylxanthine; PGE_1, prostaglandin E_1; PI, phosphoinositide; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; GTP-γ-S, guanosine-5′-*O*-(3-thio)triphosphate.

NG108-15 neuroblastoma × glioma cells elicit only adenylate cyclase inhibition (21). The former cell line has been shown to express only m3 receptors (22), whereas the latter expresses only m4 receptors (3). These two cell lines, along with cells transfected with m1 receptors, and rat heart, which expresses only m2 receptors (3), therefore are useful tools with which to study the subtype specificity of various ligands.

In the following report, we demonstrate that BM5 is a partial agonist at adenylate cyclase-coupled receptors (both the m2 and m4 subtypes) but is an antagonist at PI turnover-coupled receptors (both the m1 and m3 subtypes). Because BM5 is a presynaptic antagonist/postsynaptic agonist (17, 18), these findings suggest that, at least in some parts of the brain, adenylate cyclase-coupled receptors are located postsynaptically, whereas PI turnover-coupled receptors are located presynaptically.

Experimental Procedures

Materials. BM5 was from Dr. J. Stephen Kennedy (Neurosciences Research Branch, National Institute of Mental Health). IBMX, PGE_1, creatine phosphate, creatine phosphokinase, thymidine, and hypothanxine were from Sigma Chemical Co. (St. Louis, MO). Oxotremorine-M was from RBI (Natick, MA). GTP-γ-S was from Boehringer Mannheim (W. Germany). [^{32}P]ATP, [^{3}H]NMS, and [^{3}H]QNB were from DuPont-NEN (Boston, MA).

Cell culture. NG108-15 neuroblastoma × glioma hybrid cells were obtained from Dr. M. Nirenberg (National Institutes of Health) and were grown at 37° in DMEM (Advanced Biotechnology, Silver Spring, MD) supplemented with 10% newborn calf serum (Advanced Biotechnology), 0.1 μM hypoxanthine, and 0.016 μM thymidine. Cells of passage 19–26 were used in these studies. SK-N-SH human neuroblastoma cells were obtained from the American Type Culture Collection (Rockville, MD) and were grown in DMEM supplemented with 10% newborn calf serum. Cells of passages 64–77 were used in these studies. All cells were subcultured weekly using a divalent cation-free phosphate-buffered saline solution containing 0.02% EDTA and were maintained for at least 4 days as confluent cultures before being subcultured further. CHO cells transfected with the m1 receptor subtype were obtained from Dr. Craig Venter in this laboratory and were grown in Ham's F12 medium supplemented with 10% fetal bovine serum (Advanced Biotechnology), 50 units/ml penicillin, and 50 μg/ml streptomycin. These cells were subcultured with trypsin.

Inhibition of PGE_1-stimulated cAMP levels in NG108-15 cells. Cells were plated in 24-well plates and were grown to about 90% confluence. The growth medium was replaced with a solution of 1 mM IBMX in DMEM-HEPES (0.5 ml/well) and the cells were incubated for 10 min at 37°. The indicated drugs were then added along with 5 μM PGE_1, and the cells were incubated for another 15 min at 37°. The reaction was terminated by aspirating the medium, and cAMP was extracted by incubating the cells in 0.1 N HCl for 30 min at room temperature. An aliquot (35–50 μl) was removed, lyophilized, and assayed for cAMP by radioimmunoassay (23).

Adenylate cyclase assay. Membranes from rat heart were obtained as previously described (24). Briefly, rat heart was minced and homogenized in 10 mM triethanolamine and 145 mM NaCl (pH 7.4), on ice, in a Brinkman Polytron homogenizer (20 sec, 75% of maximum). The homogenate was centrifuged at 30,000 × *g* for 20 min at 4°. The pellet was suspended in the same buffer and centrifuged again. This process was repeated one more time to yield a crude membrane fraction. The membranes were stored frozen at −70° in 0.6 M sucrose until used. Adenylate cyclase activity was determined by adding 120 μg of membrane to a reaction mixture containing a final concentration of 50 mM triethanolamine hydrochloride (pH 7.4), 5.0 mM $MgSO_4$, 100 μM ATP, 1 mM cAMP, 1 mM IBMX, 100 μM GTP, 0.5 mM EGTA, 0.5 mM dithiothreitol, 240 mM sucrose, 0.5% bovine serum albumin, 5 mM creatine phosphate, 0.7 mg/ml creatine phosphokinase, and 1–2 μCi of [^{32}P]ATP. This mixture was incubated at 30° for 10 min. [^{32}P]cAMP was isolated by column chromatography (25).

Stimulation of Pi turnover. Cells were plated into 24-well culture dishes at about 100,000 cells/well. After 24 hr, they were labeled overnight with 2 μCi/well [^{3}H]myo-inositol (15 Ci/mmol) (American Radiolabeled Chemicals, Inc., St. Louis, MO). The cells were then rinsed with 10 mM LiCl in DMEM-HEPES and then incubated in this solution for 5 min at 37°. The indicated drugs were then added and the cells were incubated for 30 min at 37°. The reaction was stopped by aspirating the solution and adding cold methanol to the cells. After the cells were transferred to a glass tube and sonicated for 10 sec, chloroform and water were added to make a two-phase system. The upper aqueous phase was applied to 0.6-ml anion exchange columns (AG X8; Bio-Rad). The columns were washed and eluted as described (26).

Inhibition of [^{3}H]NMS binding. Membranes from cells were prepared as follows. Cells were rinsed with phosphate-buffered saline, lysed in a solution of 10 mM Tris·HCl, pH 7.2, 1 mM EDTA, for 15 min at 2°, and harvested. After a brief homogenization (Polytron, 10 sec, 75% of maximum), the cells were centrifuged at 400 × *g* for 5 min at 2°. The supernatant was centrifuged at 40,000 × *g* for 20 min. The resulting pellet was suspended in the Tris-EDTA solution and centrifuged as above. The final pellet was suspended in phosphate-buffered saline and stored at −70° until needed.

Binding studies were accomplished as follows. Portions of membranes (100–250 μg of protein) were incubated either with various concentrations of [^{3}H]NMS (from 0.01 to 10 nM) or with various concentrations of BM5 (from 0.01 to 300 μM) and 1 nM [^{3}H]NMS in DMEM-HEPES, for 90 min at 37°. The reaction was initiated by adding the membranes and was terminated by rapid filtration using a Brandel cell harvester and GF/B glass fiber filters. The filters were washed three times with 2 ml of ice-cold 0.9% NaCl and then were equilibrated in scintillation counting cocktail for at least 4 hr before being counted at 47% efficiency in a Beckman LS5801 liquid scintillation counter. Nonspecific binding was determined by coincubation with 1 μM atropine, amounted to less than 15% of total binding, and was routinely subtracted from the total binding. The binding data were analyzed using the LIGAND computer program (27).

The GTP shift experiments were performed by incubating portions of membrane with various concentrations of either BM5 or carbachol, in the presence or absence of 50 μM GTP-γ-S and 0.5 nM [^{3}H]QNB, in a buffer composed of 50 mM sodium phosphate, pH 7.4, and 2 mM $MgCl_2$. The membranes were incubated for 30 min at room temperature and then were filtered and processed for scintillation counting as above. The data were analyzed using the ALLGRF program (28).

Other methods. Protein was determined using the assay described by Lowry *et al.* (29), with bovine serum albumin as standard. Unless otherwise stated, K_i values were calculated from the Cheng-Prusoff equation (30).

Results

Effects of BM5 on cAMP levels in NG108-15 cells. NG108-15 cells have m4 muscarinic receptors coupled only to adenylate cyclase inhibition (21). In order to demonstrate activation of these receptors, adenylate cyclase was stimulated with PGE_1, and the consequent muscarinic receptor-mediated inhibition of cAMP levels was measured. As shown in Fig. 1, BM5 partially inhibited PGE_1-stimulated cAMP levels in NG108-15 cells. At a concentration of 100 μM, BM5 inhibited PGE_1-stimulated cAMP levels by 36 ± 1.5% (six experiments), whereas exposure of these cells to 100 μM concentrations of the full muscarinic agonist oxotremorine-M inhibited PGE_1-stimulated cAMP levels by 64.1 ± 1.9% (eight experiments). The inhibitory action of both BM5 and oxotremorine-M was completely prevented by preincubating the cells with 1 μM atropine,

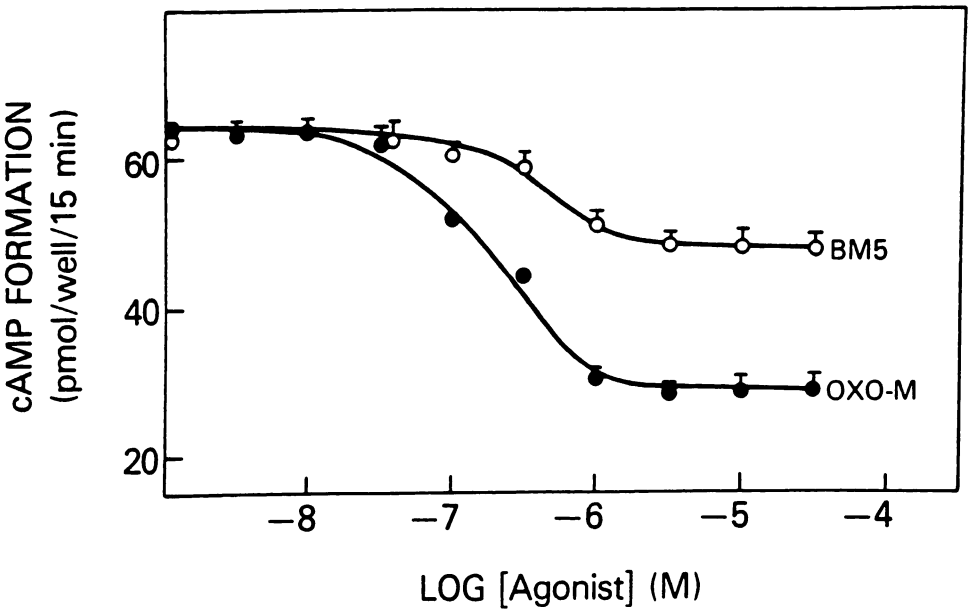

Fig. 1. BM5 as a partial muscarinic agonist in NG108-15 cells. Cells were preincubated with 1 mM IBMX for 10 min at 37°. PGE_1 (5 μM final concentration and the indicated concentrations of BM5 or oxotremorine-M (OXO-M) were then added. After another 15-min incubation at 37°, the intracellular cAMP was extracted with 0.1 N HCl and quantitated by radioimmunoassay. The data represent means ± standard errors of triplicate determinations from a single experiment that was repeated three times (see text).

thus demonstrating that these effects were mediated through muscarinic receptors. BM5, therefore, is a partial muscarinic agonist in this system. The concentration of BM5 that elicited half the maximal inhibitory response was 0.4 ± 0.1 μM (four experiments). BM5 did not inhibit PGE_1-stimulated cAMP accumulation either in SK-N-SH cells or in the CHO cells transfected with m1 receptor.

In order to determine whether BM5 could compete with oxotremorine-M for the same m4 muscarinic receptors in NG108-15 cells, we measured PGE_1-stimulated cAMP levels in cells that had been treated with 100 μM oxotremorine-M and various concentrations of BM5 (Fig. 2). The results show that increasing concentrations of BM5 did partially inhibit the effect of oxotremorine-M. At high concentrations of BM5 (around 100 μM), the cAMP levels in cells treated with BM5 plus oxotremorine-M were the same as those in cells treated only with BM5, indicating that at those concentrations BM5 had displaced oxotremorine-M from the receptors.

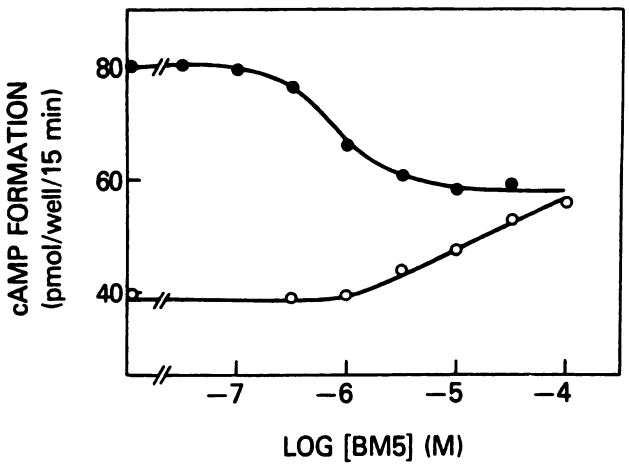

Fig. 2. BM5 inhibition of oxotremorine-M-mediated cAMP formation in NG108-15 cells. Cells were pretreated with 1 mM IBMX and then the indicated concentrations of BM5 were added either alone (●) or with 100 μM oxotremorine-M (○). The cells were stimulated with 5 μM PGE_1 for 10 min and the cAMP was assayed by radioimmunoassay.

Effects of BM5 on adenylate cyclase activity of heart membranes. Rat heart expresses only the m2 muscarinic receptor subtype (3). In order to determine whether BM5 stimulates this receptor subtype, adenylate cyclase was stimulated with 2 μM isoproterenol, and the consequent inhibition of this response was studied. Under the conditions of this assay, 100 μM oxotremorine-M inhibited adenylate cyclase by 34 ± 3% (three experiments). In contrast, 100 μM BM5 inhibited this activity by 24 ± 2% (three experiments), indicating that as previously observed (31) BM5 is a partial agonist in this system.

Effects of BM5 on inositol phosphate turnover. SK-N-SH cells express m3 muscarinic receptors that are coupled to PI turnover (22). Therefore, in order to determine whether BM5 stimulated muscarinic receptors in these cells, the cells were prelabeled with [^{3}H]myo-inositol and then exposed to muscarinic agents. As shown in Fig. 3 (*bottom*), when these prelabeled cells were exposed to the full agonist oxotremorine-M, a dose-dependent PI turnover response was elicited. No detectable PI hydrolysis, however, could be found in response to BM5 in six separate experiments. Similarly, when CHO cells that had been transfected with the gene for the rat m1 receptor were prelabeled with [^{3}H]myo-inositol and exposed to these agents, oxotremorine-M elicited a large release of [^{3}H]inositol phosphates, although BM5 up to 100 μM was inactive in this respect (Fig. 3, *top*).

In order to determine whether BM5 behaved as an antagonist in these two cell lines, prelabeled cells were first exposed to the indicated concentrations of BM5 and then stimulated with 100 μM oxotremorine-M. As shown in Fig. 4, BM5 inhibited the effect of oxotremorine with half-maximal values of 10–20 μM in both the m1-transfected cells (Fig. 4, *top*) and the SK-N-SH cells (Fig. 4, *bottom*). Therefore, BM5 behaves as an antagonist (K_i of 0.15–0.3 μM) at these muscarinic receptor subtypes.

BM5 inhibition of binding to membranes from each cell line. As shown in Table 1, the dissociation constants for [^{3}H]NMS binding to membranes from each cell type were different. [^{3}H]NMS bound to membranes from NG108-15 cells with an affinity that was 7-fold higher than that for [^{3}H]NMS

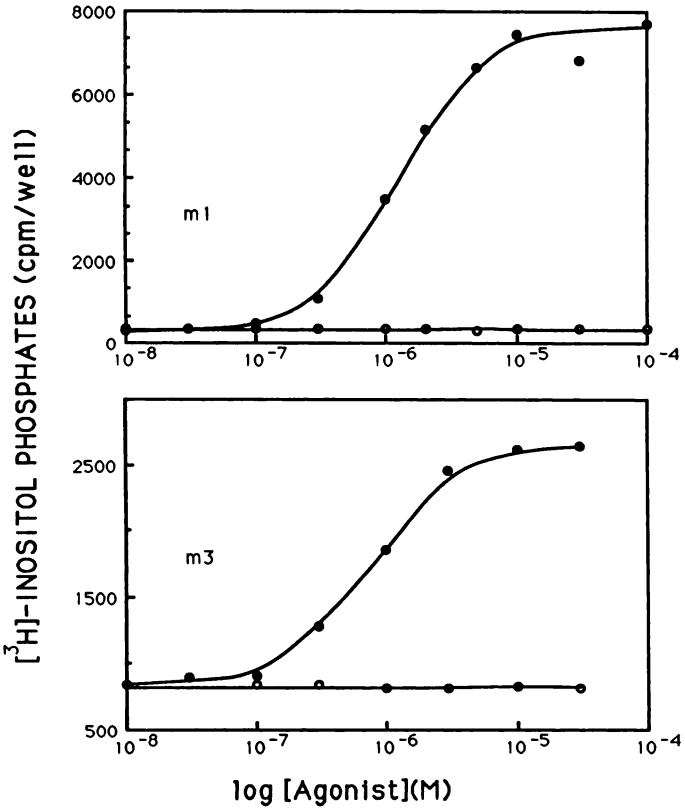

Fig. 3. Lack of effect of BM5 on PI turnover. Transfected CHO cells expressing m1 receptor subtype (*top*) or SK-N-SH cells expressing m3 receptors subtype (*bottom*) were prelabeled with [^{3}H]myo-inositol (2 μCi/well) for 18–24 hr, rinsed, and incubated with 10 mM LiCl on DMEM-HEPES for 10 min at 37°. The indicated concentrations of BM5 (○) or of oxotremorine-M (●) were added and the cells were incubated for another 30 min at 37°. The [^{3}H]inositol phosphates were extracted and separated on ion exchange gels as described in Experimental Procedures. The data represent averages of duplicate determinations from experiments that were repeated three times with similar results (see text).

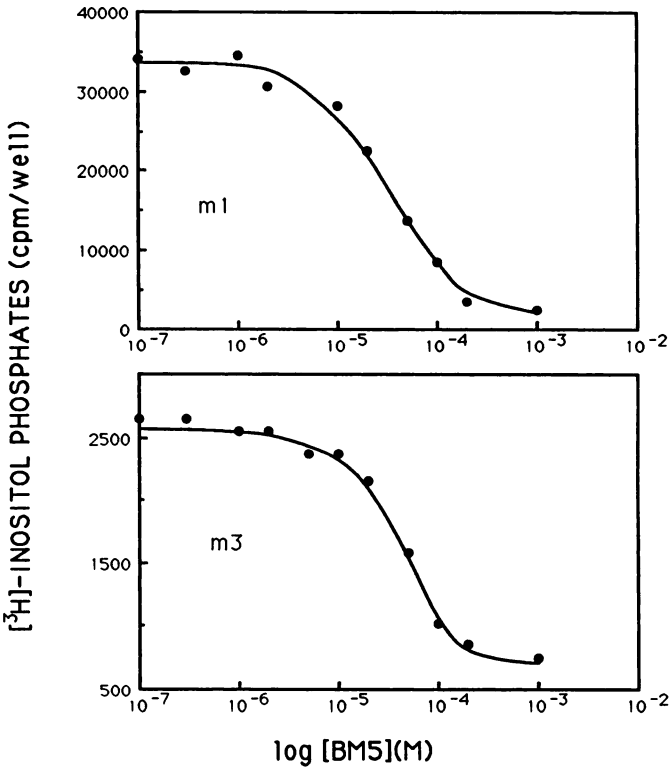

Fig. 4. BM5 inhibition of oxotremorine-M-stimulated PI turnover in transfected CHO cells (*top*) and in SK-N-SH cells (*bottom*). Cells were prelabeled and treated with 10 mM LiCl as described in the legend to Fig. 2. The indicated concentrations of BM5 were then added, along with 100 μM (final concentration) oxotremorine-M, and the cells were incubated for 30 min at 37°. The [^{3}H]inositol phosphates were extracted and separated on ion exchange columns, as described in Experimental Procedures. The data represent averages of duplicate determinations from one experiment that was repeated three times with similar results (see text).

binding to rat heart membranes. [^{3}H]NMS bound to the two PI turnover-coupled receptors with almost identical affinity. Compound BM5 inhibited [^{3}H]NMS binding to each of the receptor subtypes with almost equal affinity. The displacement curves for BM5-mediated inhibition of [^{3}H]NMS binding were best fit by a one-site model on the LIGAND computer program (27). The K_i values for BM5 inhibition obtained from these displacement curves are shown in Table 1.

The binding of muscarinic agonists in some tissues has been shown to be sensitive to guanine nucleotides (32). In order to determine whether compound BM5 also displayed such a GTP shift, we looked at BM5-induced displacement of [^{3}H]QNB binding to membranes from each of the four tissues. As shown in Fig. 5, BM5 displacement of [^{3}H]QNB binding was most sensitive to GTP-γ-S in heart membranes, where 50 μM GTP-γ-S induced an 11.9–13.7-fold increase in the IC_{50} of the displacement curve. In membranes from NG108-15 cells, GTP-γ-S induced a smaller 2.4–3.1-fold shift and, in membranes from the other two cell lines, BM5 binding was insensitive to GTP-γ-S. However, the displacement of [^{3}H]QNB binding by carbachol was also insensitive to GTP-γ-S in these membranes expressing m1 and m3 receptors (data not shown), even though carbachol is a full agonist in these tissues. It is interesting to note that, in heart and in NG108-15 membranes, the shift in the carbachol displacement curve was larger (208-fold in heart, 5-fold in NG108-15) than the shift in the BM5 displacement curves, confirming previous observations that, in tissues that do display GTP shifts, the magnitude of the GTP shift is proportional to the potency of the agonist.

TABLE 1

Ligand binding to muscarinic receptor subtypes

A crude membrane fraction from each of the cell lines was obtained as described in Experimental Procedures. Binding studies were carried out in DMEM-HEPES by incubating membranes, for 90 min at 37°, with various concentrations of [^{3}H]NMS for the B_{max} and K_d determinations and with various concentrations of BM5 and 1 nM [^{3}H]NMS for the K_i determinations. The data represent means ± standard errors of three determinations, each performed in duplicate. The displacement data were fit by a one-site model using the LIGAND program.

Subtype	Origin	[^{3}H]NMS		BM5, K_i
		B_{max}	K_d	
		fmol/mg	*nM*	*μM*
m1	Transfected CHO	350 ± 45	0.37 ± 0.04	0.27 ± 0.02
m2	Rat heart	125 ± 15	0.99 ± 0.02	0.23 ± 0.03
m3	SK-N-SH	185 ± 15	0.35 ± 0.02	0.33 ± 0.03
m4	NG108-15	50 ± 19	0.13 ± 0.01	0.58 ± 0.1

Discussion

The results of the present study indicate that compound BM5 is a partial muscarinic agonist at adenylate cyclase-coupled receptors and a pure antagonist at muscarinic receptors coupled to PI turnover. Therefore, BM5 is a selective musca-

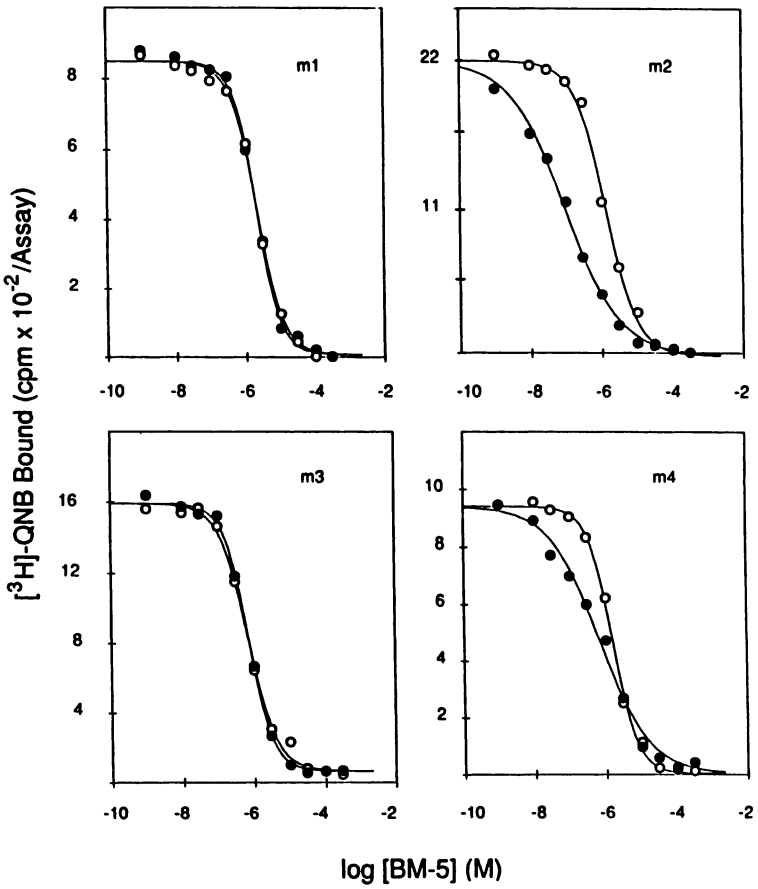

Fig. 5. GTP-γ-S sensitivity of BM5 displacement of [^{3}H]QNB binding to each receptor subtype. Membranes from each of the four tissues were incubated with the indicated concentrations of BM5 and 0.6 nM [^{3}H]QNB, in either the presence (○) or absence (●) of 50 μM GTP-γ-S, in 50 mM sodium phosphate buffer containing 2 mM $MgCl_2$. Following a 30-min incubation at room temperature, the mixtures were rapidly filtered, as described in Experimental Procedures. The data represent means of triplicate determinations from one experiment that was repeated twice with similar results.

rinic agonist, specific for adenylate cyclase-coupled muscarinic receptors. Because compound BM5 has also been described as a postsynaptic agonist/presynaptic antagonist (17), the present findings suggest that, at least in some parts of the brain, postsynaptic muscarinic receptors are coupled to adenylate cyclase, whereas receptors located presynaptically (autoreceptors) are coupled to PI turnover.

Although the results of the functional studies demonstrate that BM5 is a partial agonist only at cyclase-coupled muscarinic receptors, the antagonist properties of this compound did not display an analogous differential potency. In the binding studies shown in Table 1, BM5 inhibited [^{3}H]NMS binding to each of the four receptor subtypes with similar K_i values. The affinity for [^{3}H]NMS binding, however, was significantly lower for the m2 receptor subtype than for the other three subtypes. An analogous finding has been previously reported for the K_i of atropine inhibition of [^{3}H]QNB binding to each of the four human muscarinic receptor subtypes expressed in transfected cells (3).

In the present study, we compared the pharmacological effects of BM5 in cells and tissues from a variety of sources and animal species (see Table 1). Because the DNA sequence of muscarinic receptors has been shown to be highly conserved between species (1), it is unlikely that the differences we observe in the effects of BM5 are due to species variation. In order to address this issue more directly, however, we tested the effect of BM5 in A9 L cells (obtained from Dr. M. Brann, National Institutes of Health) that had been transfected with and express m1 and m3 muscarinic receptors from rat. As with the transfected CHO and the SK-N-SH cells described herein, BM5 did not stimulate PI turnover in A9 cells transfected with m1 or with m3 receptors (data not shown). The other two tissues used in this study (rat heart and NG108-15 cells) also express muscarinic receptor subtypes from rodent (rat and mouse, respectively). Thus, these results further confirm that BM5 is a selective muscarinic agonist, a finding that is not attributable to putative differences in drug-receptor interactions from species variation.

The m1 and m3 muscarinic receptor subtypes have been shown to preferentially couple to PI turnover, whereas the other two subtypes couple preferentially to adenylate cyclase inhibition (20). The m1 subtype displays high affinity for pirenzepine, whereas the m3 subtype displays a lower affinity for pirenzepine (20). Several lines of evidence indicate that presynaptic muscarinic receptors also display a lower affinity for pirenzepine; muscarinic receptors having a low affinity for pirenzepine are preferentially reduced in density either following experimental destruction of the cholinergic afferent pathway or in brain from patients with Alzheimer's disease (33). Furthermore, the release of acetylcholine from cholinergic terminals is relatively insensitive to pirenzepine (34), suggesting therefore that these presynaptic muscarinic receptors are not of the m1 subtype.

It is interesting to note that activation of presynaptic muscarinic receptors inhibits acetylcholine release, despite the stimulation of PI turnover and consequent calcium mobilization. Two explanations could account for this apparent contradiction. First, the calcium mobilized as a result of muscarinic receptor stimulation may be compartmentalized within the cytoplasm and, hence, be unable to stimulate secretion. Second,

despite this rise in intracellular calcium, neurotransmitter release may be inhibited as a consequence of the hyperpolarization known to occur upon stimulation of m3 receptors (35).

Brown and Brown (36) have shown that, in chick heart, both oxotremorine and carbachol are full agonists at adenylate cyclase-coupled muscarinic receptors, whereas only carbachol is an agonist at PI turnover-coupled receptors. In chick heart, therefore, oxotremorine is a selective muscarinic agonist. Oxotremorine has been shown to display a similar, although less definitive, selectivity in other tissues (21, 37). Thus, the selectivity of BM5 observed in the present study has been previously observed with the structurally related compound oxotremorine.

As indicated in the Introduction, compounds that are presynaptic antagonists and postsynaptic agonists may be of clinical use in treating the cholinergic deficits of Alzheimer's disease. Because the efficacy of BM5 is considerably less than that of the full agonist oxotremorine-M, this particular compound may be of limited clinical utility. However, the pharmacological profile of this compound demonstrates the feasibility of making more efficacious subtype-specific muscarinic agonists.

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