Agonists Differentiate Muscarinic Receptors That Inhibit Cyclic AMP Formation from Those That Stimulate Phosphoinositide Metabolism*

(Received for publication, July 22, 1983)

Joan Heller Brown‡ and Susan L. Brown§

From the Division of Pharmacology, M-013 H, University of California, San Diego, La Jolla, California 92039

Muscarinic receptor stimulation elicits two distinct biochemical responses in embryonic chick heart cells: inhibition of catecholamine-stimulated cyclic AMP formation and stimulation of phosphoinositide (PhI) hydrolysis. We observe two major differences in the effects of agonists on these responses. First, carbachol and oxotremorine both inhibit cyclic AMP formation, but only carbachol stimulates PhI hydrolysis. Second, the dose-response relationships for the cyclic AMP and PhI responses differ; the half-maximal concentrations of carbachol needed to inhibit cAMP accumulation and stimulate PhI hydrolysis are \( 2 \times 10^{-7} \) M and \( 2 \times 10^{-6} \) M, respectively. We carried out radioligand binding studies on intact chick heart cells to determine whether these data could be explained in terms of different agonist binding states of the muscarinic receptor. In intact cells, carbachol competes for \(^{3}H\) quinuclidinyl benzilate-binding sites with high and low affinity, while oxotremorine shows only high affinity binding. We suggest that the receptor state common to both agonists is the state associated with inhibition of adenylate cyclase, while the very low affinity binding site seen only with carbachol is associated with the PhI response. We also consider the possibility that both responses are caused by a single receptor state that is efficiently coupled to adenylate cyclase inhibition and inefficiently coupled to PhI hydrolysis. Whichever mechanism is correct, our findings demonstrate that muscarinic receptors coupled to adenylate cyclase and the PhI response can be differentiated by virtue of their sensitivity to agonist and the efficiency with which some agonists induce receptor change and elicit receptor-mediated biochemical responses.

The concept that there are muscarinic receptor subtypes was first proposed by Burgen and Spero (1) in 1968 on the basis of pharmacological studies comparing the receptors that mediate K⁺ efflux and contraction in guinea pig intestine. Subsequent studies measuring physiological responses also suggested that not all muscarinic receptors are the same (2–4). Progress in the subclassification of muscarinic receptors has been limited, however, in part because there are few generally available drugs with muscarinic receptor selectivity.

The introduction of radioligand binding techniques provided a more direct means for defining subtypes of muscarinic receptors. Radioligand binding studies generally show a uniform population of binding sites for muscarinic antagonists (5–8), a finding that argues against muscarinic receptor heterogeneity. Several nonclassical muscarinic receptor antagonists that appear to show selectivity have recently been studied. Some, like gallamine, probably do not act at the primary antagonist-binding site (8, 9). Another, pirenzepine, has higher affinity for muscarinic receptors in some brain regions and ganglia than for those in peripheral tissues (10), but its selectivity is disputed (11).

In contrast to the heterogeneous binding seen for antagonists, muscarinic agonists appear to bind to muscarinic receptors in membranes with more than a single affinity (12–15). Birdsall et al. (13) have suggested that agonist-binding sites of different affinity result from the conformational coupling of receptors to their effectors. Ehler et al. (15) have likewise expressed the view that the different classes of agonist-binding sites result from the coupling state of the muscarinic receptor.

Muscarinic receptors in the heart couple to and inhibit adenylate cyclase through a process requiring guanine nucleotides (14, 16–18). Cardiac muscarinic receptors also couple to phosphoinositide metabolism (19, 20), through as yet undefined molecular processes. Both of these biochemical responses appear to be direct and primary consequences of receptor occupation, in contrast to the more distal physiological responses that result from agonist binding.

Although it is generally accepted that the process of receptor-effector coupling influences agonist affinity, there are no reports comparing muscarinic receptors coupled to adenylate cyclase and those coupled to phosphoinositide metabolism. The experiments presented here demonstrate that muscarinic agonists have different potencies and efficacies for eliciting cyclic AMP and phosphoinositide responses, and indicate that the relationship between receptor occupancy and response depends on the nature of the coupling. The data presented here also demonstrate that binding parameters obtained in membranes cannot explain the disparate behavior of agonists, while data obtained with intact cells provide information in this regard.

EXPERIMENTAL PROCEDURES

Methods—Hearts were removed from 13-day-old chick embryos, the atrial appendages were cut away, and the heart was either sliced open or divided into quarters for incubations. For preparation of heart cells, the tissue was minced more finely and the cells were enzymatically dispersed at 35 °C for 45 min in Ca⁺⁺-Mg⁺⁺-free buffer (21) containing 0.25% trypsin. To free the cells, tissue fragments were triturated in Krebs-Henseleit medium buffered with 20 mM Hepes¹ and containing 1% bovine serum albumin. Heart cells were...

¹ The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PhI, phosphoinositide; Ins-1-P, inositol 1-phosphate; QNB, quinuclidinyl benzilate; Gpp(NH)p, guanylyl-5'-yl imidodiphosphate.
separated from erythrocytes and cell debris by centrifugation in 30% Percoll for 30 min at 20,000 × g. The final cell preparation was 80–90% viable (by trypan blue exclusion), and the yield of cells was 4–8 × 10^6 cells/heart.

All subsequent tissue incubations were performed at 35 °C in Krebs-Henseleit buffer of the following composition (millimolar): NaCl, 118; KCl, 4.7; CaCl₂, 3.0; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25; Na₂EDTA, 0.5; and glucose, 10, pH 7.4. The same buffer was used for chick heart cell incubations except that CaCl₂ was reduced to 1.8 mM and Na₂EDTA was omitted. Tissues and cells were gassed continuously with 95% O₂, 5% CO₂.

In cyclic AMP experiments, tissue samples were equilibrated with 100 μM isobutylmethylxanthine for 20 min prior to a 2-min drug exposure. The effect of carbachol and oxotremorine was assessed in the presence of 3 μM isoproterenol. Following drug incubation, tissues were either frozen or immediately sonicated in 10% trichloroacetic acid. Cyclic AMP was purified and assayed by the competitive protein binding assay of Gilman, as described previously (22). Protein was assayed by the method of Bradford (23).

Phosphoinositide breakdown was monitored by measuring accumulation of the hydrolysis product inositol 1-phosphate. This is a metabolite of phosphatidylinositol that can also be formed through breakdown of the polyphosphoinositides. Tissues were labeled for 1 h with [³H]inositol (4 μCi/ml), and then carbachol or oxotremorine was added along with 10 mM LiCl, which inhibits Ins-1-P breakdown (24). Thirty minutes after hormone addition, tissue samples were either frozen or immediately sonicated in chloroform:methanol:water (5:10:4). A two-phase system was achieved by the further addition of chloroform and H₂O to a final chloroform:methanol:water of 10:10:9. The aqueous phase, containing Ins-1-P, was removed, and [³H]Ins-1-P was separated from [³H]inositol by a modification (19) of the anion exchange chromatography procedure described by Berridge et al. (24).

Binding assays were carried out using either a 20,000 × g, 10 min membrane preparation as described previously (8) or with intact dissociated cells from 13-day-old embryonic chick hearts. Assays were carried out in duplicate or triplicate at 35 °C in Krebs-Henseleit medium buffered with Heps (25 mM for membranes, 20 mM for buffer), pH 7.4. The binding reaction was carried out for either 60 min (membranes) or 75 min (cells). Nonspecific binding (that not inhibited by 10 μM atropine) was <10% for membranes and <20% for intact dissociated heart cells, and was subtracted from total binding to give specific binding. The specific binding of [³H]QNB was saturable, and the [³H]QNB saturation binding isotherm was consistent with a single population of radioligand-binding sites with an averaged site model, occupancy of each site was calculated separately using either the

\[ \text{Occupancy} = \frac{[L]}{[L] + K_d} \]

where \([L]\) is the agonist concentration and \(K_d\) is the agonist dissociation constant. When agonist competition curves were fit by a two-site model, occupancy of each site was calculated separately using either the \(K_1\) or \(K_2\) values.

Materials—Fertilized white leghorn chicken eggs were obtained from McIntyre Poultry and Eggs (San Diego, CA) and incubated at 39 °C in a Leasy 1200 incubator. Carbamylcholine (carbachol) chloride and oxotremorine sesquifumarate were from Sigma. Trypsin 1-300 was from ICN Nutritional Biochemicals. [³H]Inositol (15.8 Ci/mmol) and [³H]cyclic AMP (32.3 Ci/mmol) were from New England Nuclear.

RESULTS

Carbachol and oxotremorine inhibit isoproterenol-stimulated cyclic AMP formation in embryonic chick heart slices (Fig. 1A). Maximal concentrations of either muscarinic agonist cause close to 100% inhibition of the stimulation produced by isoproterenol. The concentration of oxotremorine that half-maximally inhibits cyclic AMP formation (\(K_{act}\)) is 6 × 10⁻⁸ M, while that of carbachol is 2 × 10⁻⁷ M.

Carbachol also increases the hydrolysis of phosphoinositides in the embryonic chick heart. In contrast, oxotremorine causes little increase over the basal level of phosphoinositide breakdown (Fig. 1B). The \(K_{act}\) for carbachol is 2 × 10⁻⁵ M. That for oxotremorine is apparently lower but is difficult to estimate from these data. The comparisons shown in Fig. 1 demonstrate two major differences in the ability of agonists to induce muscarinic receptor-mediated responses. One is that oxotremorine and carbachol are equally efficacious in regulating cyclic AMP metabolism but not in stimulating Phl hydrolysis. The second is that the concentration of carbachol needed to half-maximally stimulate Phl hydrolysis is 100 times greater than that needed to half-maximally inhibit cyclic AMP formation.

In these studies we assayed both responses using the same tissue preparation, incubation medium, temperature, and drugs. There were some minor differences that should be discussed. One is that LiCl was present only in the Phl incubation and isoproterenol only in the cyclic AMP incubation. We have determined in separate experiments that neither of these factors contributes to the differences in agonist activity for the two responses (data not shown). Other differences were the time of agonist exposure and agonist dose; Ins-
were incubated with 100 various concentrations of oxotremorine. The response to carbachol.

To determine whether oxotremorine interacts with receptors coupled to phosphoinositide hydrolysis, we asked if oxotremorine could block the stimulatory effect of carbachol on PhI hydrolysis. Oxotremorine produces a complete and concentration-dependent inhibition of the PhI response to carbachol (Fig. 2). This finding suggests that oxotremorine binds at the same site as carbachol but, like an antagonist, cannot cause the receptor changes needed to stimulate PhI hydrolysis.

The muscarinic receptor antagonist [3H]QNB was used to study the binding properties of muscarinic receptors in membranes prepared from chick hearts. Both carbachol and oxotremorine compete for all [3H]QNB-binding sites. Oxotremorine competition curves are consistently steeper than those for carbachol, but competition curves for both agonists in membranes are always best fit by a model assuming two, rather than one, agonist-binding sites; the binding parameters derived from these experiments are shown in Table I. In the presence of 10-4 M Gpp(NH)p (not shown), both agonist, competition curves are monophasic with the agonist KD value approximating that of the lower affinity site.

Since agonist binding to membranes may not reflect the receptor state in the intact cell, we also examined the relationship between binding and biochemical responses in freshly dissociated cells prepared from 13-day-old embryonic chick hearts. In these studies we have confirmed the basic observations made in the chick heart slices. In the dissociated cells, the concentration of carbachol that half-maximally inhibits cyclic AMP formation is 2 x 10-7 M, while that needed to stimulate PhI hydrolysis is 2 x 10-9 M (Fig. 3); these values are identical to the KN values obtained in the experiments presented above. Oxotremorine is a potent and complete inhibitor of cyclic AMP formation but is virtually inactive in stimulating phosphoinositide hydrolysis in the dissociated heart cells (Fig. 4).

The radioligand [3H]QNB binds homogeneously to muscarinic receptor sites on the intact chick heart cell. Scatchard plots are linear, and the KN values obtained by equilibrium and kinetic measurements are equal. Competition curves for carbachol are biphasic and best fit by a two-site model; the KN and KL values established by computer curve fitting (Table II).

**Table I**

**Differences in oxotremorine effect on PhI and cyclic AMP responses assayed under comparable conditions**

Embryonic chick heart slices were incubated as described under "Methods" except that for either assay agonists were present for 15 min. All cyclic AMP values are from incubations performed in the presence of 3 μM isoproterenol. The basal cyclic AMP concentration was 8.0 ± 0.8 pmol/mg of protein.

| Cyclic AMP | PhI | Cpm/mg, wet wt |
|------------|-----|----------------|
| Control    | 40.2 ± 6.7 | 23.6 ± 0.52 |
| 10-7 M carbachol | 10.8 ± 1.2 | 4.25 ± 1.07 |
| 10-4 M oxotremorine | 10.4 ± 1.2 | 2.47 ± 0.67 |

**Table II**

**Dissociation constants and per cent of high and low affinity agonist-binding sites in chick heart cells and membranes**

The data given are the molar KN values ± S.E. and, in parentheses, the per cent of the total sites having that KN value. The values are averages from three separate experiments in which agonist competition curves with 15-18 agonist concentrations were generated.

| Membranes | Intact cell |
|-----------|------------|
| Carbachol | 2.8 ± 0.9 x 10-7 (48) |
|           | 5.4 ± 1.6 x 10-6 (52) |
|           | 3.2 ± 1.1 x 10-5 (58) |
|           | 5.5 ± 0.7 x 10-4 (42) |
| Oxotremorine | 7.6 ± 1.2 x 10-9 (40) |
|           | 6.6 ± 1.3 x 10-7 (80) |
|           | 5.2 ± 0.9 x 10-7 (100) |
Muscarinic Agonists on PhI and Cyclic AMP Responses

II) were used to derive the theoretical receptor occupancy curves for the high and low affinity binding sites shown in Fig. 3. The competition curves for oxotremorine binding to intact cell receptors are, however, best fit by a one-site model. The theoretical curve for muscarinic receptor occupancy by oxotremorine is shown as the dashed line in Fig. 4.

Agonist binding parameters for receptors in chick heart membranes and those in chick heart cells are compared in Table II. For carbachol the higher affinity site seen in the intact cell has the same $K_0$ as that of the lower affinity site in membranes. Analogously, the $K_0$ of the single oxotremorine-binding site in the intact cell is equal to that $K_0$ of the lower affinity membrane binding site.

**DISCUSSION**

The experiments presented here are the first in which muscarinic receptor-mediated effects on cyclic nucleotide formation and on PhI hydrolysis have been directly compared in a single system. Our data show that there are differences in agonist potency and efficacy for stimulating these two responses and suggest that the agonist-induced receptor changes that lead to the two responses are not the same. Specifically, we observe three discriminatory aspects of agonist behavior: the 100-fold greater potency of carbachol in inhibiting cyclic AMP formation than in stimulating PhI hydrolysis, the low efficacy of oxotremorine as an agonist of inhibiting cyclic AMP formation than in stimulating PhI hydrolysis, and the difference in carbachol and oxotremorine receptor binding properties in intact cells.

In the intact cell, carbachol interacts with two states of the muscarinic receptor, while oxotremorine interacts with only one state. The dissociation constant for oxotremorine in the intact cell ($5 \times 10^{-7} \text{ M}$) is approximately 100-fold lower than the dissociation constant of the higher affinity carbachol-binding site seen in the intact cell ($3 \times 10^{-5} \text{ M}$). This is the same relative difference in oxotremorine and carbachol affinities as is seen in membranes; thus, we believe that the single-oxotremorine-binding site is analogous to the higher affinity carbachol-binding site. Since both oxotremorine and carbachol produce changes in cyclic AMP metabolism, we propose that it is this receptor state, common to both agonists, that is involved with agonist-mediated inhibition of cyclic AMP formation.

A very low agonist affinity binding site is induced or seen by carbachol but not by oxotremorine in intact cells. Carbachol, which induces formation of the very low affinity state, stimulates PhI hydrolysis, while oxotremorine, which does not induce formation of the low affinity binding state, does not stimulate PhI hydrolysis. These data suggest that this is the receptor state that mediates agonist effects on PhI hydrolysis. Fisher et al. (26) recently reported that muscarinic agonists differ in their ability to stimulate PhI metabolism in brain synaptosomes; they also found that a low affinity binding state was induced by muscarinic agonists capable of stimulating PhI hydrolysis but not by agonists that were ineffectual in causing a PhI response.

The intact cell binding data therefore support the idea that the two biochemical responses we have studied are mediated through different states of the muscarinic receptor: a high affinity state associated with inhibition of adenylate cyclase and a low affinity state associated with the PhI response. According to this theory, the disparity in the dose-response relationships for the effect of carbachol on cyclic AMP and PhI responses results from differences in the agonist affinity of the receptor states coupled to these responses. The failure of oxotremorine to stimulate PhI hydrolysis results from its inability to induce the receptor changes that are reflected in lower agonist affinity and are necessary for production of the PhI response.

The theory provides a straightforward explanation for our data, but it is not the only possible interpretation of our findings. An alternative is that both responses are mediated by a single receptor state and that the discrepant agonist effects result from differences in the efficiency of receptor coupling rather than from differences in receptor affinity. Our data demonstrate that the occupancy curves for the receptor state common to both oxotremorine and carbachol are several log units to the right of the cyclic AMP dose-response curves (Figs. 3 and 4). This suggests that only a fraction of muscarinic receptors need to be occupied to cause inhibition of adenylate cyclase, i.e. that there is efficient receptor-effector coupling. A similar relationship between occupancy of cardiac β-adrenergic receptors and cyclic AMP formation appears to result from efficient coupling between β-adrenergic receptors and adenylate cyclase (27, 28).

While there is evidence that not all receptors need to be occupied to regulate adenylate cyclase, activation of the PhI response appears to require full receptor occupancy (26, 29). The evidence for this is that the dose-response curves for agonist stimulation of PhI turnover lie close to those for agonist binding. In chick heart cells, the dose-response relationships for the PhI response and occupancy of the higher affinity carbachol-binding site are coincident (Fig. 3). Thus, the receptor state coupled to adenylate cyclase could also be coupled to PhI hydrolysis, but less efficiently. Under these conditions, a higher extent of receptor occupancy (higher agonist concentrations) would be necessary to stimulate PhI hydrolysis than to inhibit cyclic AMP formation, thus the disparity in the dose-response relationship for these two responses. Moreover, while an agonist such as oxotremorine, which apparently has lower efficacy than carbachol (13, 26), would occupy enough receptors to inhibit cyclic AMP formation, even full receptor occupancy would not be adequate to stimulate the PhI response.

We have proposed two feasible explanations for why muscarinic receptor-mediated cyclic AMP and PhI responses show different dose-response relationships and different oxotremorine responsiveness. Development of both hypotheses was concentrated heavily on intact cell binding data rather than membrane binding data for several reasons. First, the binding parameters obtained for carbachol and oxotremorine in membranes show no predictable correlation with the $K_{M}$ values for the two responses. Second, it is in the intact cell, not in membranes, that the responses of interest are differentially elicited. Third, only the intact cell data show differences in the binding of carbachol and oxotremorine.

We feel, however, that some caution must be exercised in interpreting intact cell binding data because the nature of the intact cell binding sites and their relationships to binding sites seen in membranes is not yet known. There are several studies in which agonist affinities of muscarinic receptors in intact cells have not been noted to be low relative to those seen in membranes (12, 30, 31). One explanation for this phenomenon, as discussed by Galper et al. (30), is that conditions in the intact cell (e.g. the presence of endogenous guanine nucleotides) favor formation of the low affinity agonist-binding site seen in membranes. Our finding that there is an apparent equivalence between the predominant intact cell binding state and the low affinity membrane binding site (which predominates in the presence of Gpp(NH)p) supports this notion. A second explanation for the finding that intact cells show low agonist affinity is that agonist affinity de-
creases during the course of the radioligand binding assay. This has been observed for agonist binding to intact cell β-adrenergic receptors and may reflect receptor desensitization (32–34). In this regard the very low affinity receptor state recognized by carbachol in intact chick heart cells could be a desensitized state of the receptor which oxotremorine is unable to induce or distinguish from the higher affinity state.

Additional experiments are needed to precisely define the relationship of the intact cell receptor states to the cyclic AMP and PhI response. The notion that coupling efficiency differs for the two responses can be tested by assessing changes in the dose-response relationships which occur following various degrees of muscarinic receptor alkylation (1, 35). The question of whether the very low affinity receptor state seen in the intact cell is formed in the course of the intact cell binding assay can be tested using kinetic rather than equilibrium binding studies (32–34). Regardless of the resolution of these questions, our data demonstrate that the receptor coupled to adenylate cyclase and that coupled to PhI hydrolysis do not behave identically. Molecular differences in the receptors or in the conformational changes that underlie receptor-effector coupling are detected by agonists, especially those of low efficacy. Further studies using agonists that discriminate between receptors coupled to the cyclic AMP and PhI response should be of value for defining the biochemical events involved in receptor-effector coupling and may provide a means for investigating the physiological function of receptor-mediated phosphoinositide hydrolysis.

Acknowledgments—We thank David Goldstein for excellent technical assistance, Dr. Harvey Motulsky for assistance in computer analysis, Drs. Palmer Taylor and Paul Insel for critical discussion, and Sandy Dutky for capable preparation of this manuscript.

REFERENCES

1. Burgen, A. S. V., and Spero, L. (1968) Br. J. Pharmacol. 34, 99–115
2. Barlow, R. B., Berry, K. J., Glenton, P. A. M., Nikolau, N. M., and Soh, K. S. (1976) Br. J. Pharmacol. 58, 613–620
3. Fisher, A., Weinstock, M., Gitter, S., and Cohen, S. (1976) Eur. J. Pharmacol. 37, 329–338
4. Gardier, R. W., Tsevdos, E. J., Jackson, D. B., and Delaunois, A. L. (1978) Fed. Proc. 37, 2422–2428
5. Huilme, E. C., Birdsall, N. J. M., Burgen, A. S. V., and Mehta, P. (1978) Mol. Pharmacol. 14, 737–750
6. Fields, J. Z., Roeske, W. R., Morkin, E., and Yamamura, H. I. (1978) J. Biol. Chem. 253, 3251–3258
7. Hosey, M. M., and Fields, J. Z. (1981) J. Biol. Chem. 256, 6395–6399
8. Dunlap, J., and Brown, J. H. (1983) Mol. Pharmacol. 24, 15–22
9. Stockton, J. M., Birdsall, N. J. M., Burgen, A. S. V., and Huilme, E. C. (1983) Mol. Pharmacol. 25, 551–557
10. Hammer, R., Berrie, C. P., Birdsall, N. J. M., Burgen, A. S. V., and Huilme, E. C. (1980) Nature (Lond.) 283, 90–92
11. Laduron, P. M., Leysen, J. E., and Gorissen, H. (1981) Arch. Int. Pharmacodyn. 249, 319–321
12. Ward, D., and Young, J. M. (1977) Br. J. Pharmacol. 61, 189–197
13. Birdsall, N. J. M., Burgen, A. S. V., and Huilme, E. C. (1978) Mol. Pharmacol. 14, 723–736
14. Harden, T. K., Scheer, A. G., and Smith, H. M. (1982) Mol. Pharmacol. 21, 570–580
15. Ehler, J. F., Roeske, W. R., and Yamamura, H. I. (1981) Fed. Proc. 40, 155–159
16. Brown, J. H. (1979) J. Cyclic Nucleotide Res. 5, 423–433
17. Watanabe, A. M., McConnaughey, M. M., Strawbridge, R. A., Fleming, J. W., Jones, L. R., and Besch, H. R., Jr. (1978) J. Biol. Chem. 253, 4833–4836
18. Biegon, R. L., and Pappano, A. J. (1980) Circ. Res. 46, 353–362
19. Brown, S. L., and Brown, J. H. (1984) Mol. Pharmacol. 24, in press
20. Quist, E. E. (1982) Biochem. Pharmacol. 31, 3130–3135
21. Hermemeyer, K., and Robinson, R. (1977) Am. J. Physiol. 233, C172–C179
22. Brown, J. H. (1979) Mol. Pharmacol. 16, 841–850
23. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
24. Berridge, M. J., Downes, C. P., and Hanley, M. R. (1982) Biochem. 206, 587–595
25. Munson, P. J., and Rodbard, D. (1980) Anal. Biochem. 107, 229–239
26. Fisher, S. K., Klinger, P. D., and Agranoff, B. W. (1983) J. Biol. Chem. 258, 7388–7363
27. Terasski, W. L., Linden, J., and Brooker, G. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 6401–6405
28. Venter, J. C. (1979) Mol. Pharmacol. 16, 429–440
29. Michell, R. H., Jaffari, S., and Jones, L. (1976) FEBS Lett. 69, 1–5
30. Galper, R. L., Dziekan, L. C., O’Hara, D. S., and Smith, T. W. (1982) J. Biol. Chem. 257, 10344–10356
31. Putney, J. W., Jr., and Van de Walle, C. M. (1980) J. Physiol. (Lond.) 299, 521–531
32. Pittman, R. N., and Molinoff, P. (1980) J. Cyclic Nucleotide Res. 6, 421–435
33. Toews, M. L., Harden, T. K., and Perkins, J. P. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 3553–3557
34. Insel, P. A., Mahan, L. C., Motulsky, H. J., Stoolman, L. M., and Koachman, A. M. (1983) J. Biol. Chem. 258, 13687–13695
35. Puchgott, R. F., and Bruskin, F. (1967) Ann. N. Y. Acad. Sci. 144, 882–889
Agonists differentiate muscarinic receptors that inhibit cyclic AMP formation from those that stimulate phosphoinositide metabolism.

J H Brown and S L Brown

J. Biol. Chem. 1984, 259:3777-3781.

Access the most updated version of this article at http://www.jbc.org/content/259/6/3777

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/6/3777.full.html#ref-list-1