Muscarnic Agonist Binding and Phospholipid Turnover in Brain*

(Received for publication, December 27, 1982)

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The ability of muscarinic cholinergic agonists to interact with muscarinic receptors in nerve ending preparations and elicit an increased labeling of phosphatidate and phosphatidylinositol from 32P_i has been investigated. Two groups of brain muscarinic agonists are distinguished. Addition of acetylcholine, carbamylcholine, or muscarine resulted in a 2-fold stimulation of phosphatidate and phosphatidylinositol labeling, while bethanechol, pilocarpine, arecoline, and oxotremorine were less effective. Simultaneous addition of two agonists from the more effective group did not result in any further increase in stimulated labeling, while the addition of agonists from the less effective group antagonized the stimulatory effect of carbamylcholine. All of the agonists could completely displace binding of [3H]quinuclidinyl benzilate, a muscarinic antagonist. The displacement of the labeled antagonist by the more effective agonists was more complex than that predicted from a simple mass action isotherm and was compatible with the interaction of the agonists with high and low affinity forms of the receptor. Conversely, the displacement data from less effective agonists did not deviate markedly from those predicted for interaction of the agonists with a single affinity form of the receptor. Dose-response curves for stimulated phosphatidate labeling obtained in the presence of acetylcholine, carbamylcholine, and methacholine were predominantly correlated with occupation of the low affinity form of the muscarinic receptor. These results suggest that the enhancement of phosphatidate and phosphatidylinositol turnover in brain is caused by agonist-mediated conformational changes in the muscarinic receptor and that the ability of an agonist to induce this conversion may be predicted by its differential binding to the high and low affinity forms of the receptor.

Biochemical correlates of muscarinic receptor activation in a variety of tissues include an increased synthesis of cGMP (1), a decreased synthesis of cAMP (2), an increase in Ca^2+ fluxes (3), a decreased K^+ conductance (4), and an increased turnover of two quantitatively minor phospholipids, PhA and PhI (5-7). Of these correlates, only stimulated phospholipid turnover (usually determined as an increased incorporation of 32P into PhA and PhI) consistently accompanies muscarinic receptor activation. While the precise physiological significance of enhanced phospholipid turnover remains unresolved (8), the biochemical mechanism appears to involve an initial phosphodiesteratic breakdown of PhI or its phosphorylated derivatives, phosphatidylinositol 4-phosphate or phosphatidylinositol 4,5-bisphosphate, to yield diacylglycerol, which in turn is rephosphorylated in the presence of [32P]ATP to yield labeled PhA and PhI (9-12).

Known physiological consequences of muscarinic receptor activation, such as contraction or secretion, typically require only a partial receptor occupancy for maximal effect. In contrast, concentrations of agonists approaching those required for full receptor occupancy are necessary to evoke a maximal increase in the turnover of PhA and PhI (13). This observation has prompted the hypothesis that enhanced phospholipid turnover serves in a biochemical amplification step that follows receptor-ligand interaction. However, the relationship between binding of the agonist to the receptor and the initiation of the biochemical response is unclear. For example, while the occupancy hypothesis predicts that the concentration-dependence curves for enhanced phospholipid turnover should be displaced to the right (i.e., higher concentration range) of the physiological curve, this is not always the case (14, 15). Similarly, the addition of partial muscarinic agonists does not invariably result in the predicted smaller increase in phospholipid turnover (16, 17). In addition, while it has been proposed that muscarinic receptors exist in both high and low affinity forms (18), it has yet to be clearly established whether one or both forms of the receptor are coupled to stimulated phospholipid turnover.

In this study, we have examined the relationship between the binding of agonists to brain muscarinic receptors and the initiation of a metabolic response, enhanced PhA and PhI labeling from 32P_i in a nerve ending fraction obtained from guinea pig cerebral cortex.

MATERIALS AND METHODS

Carbachol, acetylcholine chloride, muscarine, methacholine, bethanechol, arecoline, oxotremorine, pilocarpine, atropine, and eserine were purchased from Sigma. [32P]Orthophosphoric acid (carrier-free) was obtained from Amersham Corp. [3H]QNB (40.2 Ci/mmol) was obtained from New England Nuclear.

NE1.1 was obtained from guinea pig or rat cerebral cortex as previously described (19). NE1.1 fractions were gently resuspended (4 mg protein/ml) in Buffer A, which contained 142 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl_2, 3.6 mM NaHCO_3, 1 mM MgCl_2, 5.6 mM D-glucose, and 30 mM HEPES-NaCl buffer (pH 7.4). Bovine adrenal chromaffin cells were obtained from the adrenal medulla following enzymatic digestion and mechanical dispersion (20, 21). Cell suspensions were allowed to stand for 24 h, pelleted, and washed three times before resuspension in Buffer A at a concentration of 1 mg of protein/ml.

Phospholipid labeling from 32P_i, in the absence and presence of cholinergic agonists was carried out by incubating aliquots of NE1.1 fraction or chromaffin cells at 37 °C in Buffer A containing 20-50 μCi of γ^32P_i, in a total volume of 0.5 ml. Incubations were terminated after 30 min for NE1.1 fractions and 60 min for chromaffin cells, and

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the lipids were extracted, separated, and quantitated as previously described (2). Under these conditions, the amount of 32P, incorporated into PhA and PhI was proportional to the amount of protein present, and the stimulation (per cent control) of both lipids was independent of protein concentration. Data for stimulated PhA or PhI labeling as a function of agonist concentration was evaluated for evidence of a one- or two-site interaction from the following equations.

\[ f = \frac{F_{\text{max}}[A]}{K + [A]} \]  

(1)

where \( f \) is fractional increase in labeling of PhA or PhI at a concentration \([A]\) of agonist, \( F_{\text{max}} \) is maximum increase in labeling, and \( K \) is affinity constant of the receptor for stimulated PhA or PhI turnover. For a two-site fit, Equation 1 is modified as follows.

\[ f = \frac{F_H[A] + F_L[A]}{K_H + [A] + K_L + [A]} \]  

(2)

where \( F_H \) and \( F_L \) are proportions of high and low affinity sites with affinities \( K_H \) and \( K_L \), respectively, assuming \( F_{\text{max}} = F_H + F_L \).

[3H]QNB binding to NE, fractions was carried out as previously described (23). Briefly, approximately 60 \( \mu \)g of NE, protein were incubated in 2 ml of Buffer A at 25 °C, in the presence of 0.06 \( \mathrm{nmol} \) of [3H]QNB. This temperature assures minimal dissociation of the QNB-receptor complex. Reactions were terminated after 60 min by addition of 3 ml of Buffer A and rapid filtration with a manifold filtration apparatus. Filters were washed twice with 6 ml of chilled Buffer A and radioactivity was determined after the addition of 10 ml of scintillation fluid. Filters were washed twice with 6 ml of chilled Buffer A and radioactivity was determined after the addition of 10 ml of scintillation fluid. Nonspecific binding (<2% of total) was that radioactivity bound to the filter in the presence of 1.0 \( \mu \)M atropine. For displacement studies, agonists were added in varying concentrations prior to the addition of [3H]QNB. The counting efficiency for [3H] was 40%.

Analysis of [3H]QNB binding to NE, fractions and its competition by muscarinic agonists was performed by the method of McKinney and Coyle (24), assuming a competitive agonist-antagonist interaction. The equation describing the displacement of [3H]QNB binding to a single site in the presence of a competing unlabeled agonist \([A]\) with an affinity \( K \) for the receptor may be written as Equation 3.

\[ [QR] = \frac{K_H[A]}{1 + K_H[A] + K[A]} \]  

(3)

where \([QR]\) is [3H]QNB specifically bound to the muscarinic receptor.

### TABLE 1

<table>
<thead>
<tr>
<th>Stimulus of phosphatidate and phosphatidylinositol turnover in nerve ending fractions and chromaffin cells by muscarinic agonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE, fractions (approximately 1 mg of protein) or aliquots of chromaffin cells (approximately 10^6 cells) were incubated for 30 min or 60 min, respectively, in Buffer A containing 30-40 ( \mathrm{pg} ) of 32P, after which time reactions were terminated by the addition of 1.5 ml of chloroform:methanol (12, v/v), and phospholipids were extracted, separated, and quantitated. The results are expressed as a per cent of control incubations (no agonist addition). Values shown are mean ( \pm ) S.E. for the number of separate experiments indicated in parentheses. The values for rat NE, are derived from a single experiment. The concentration of agonists was 1 mM. The incorporation of 32P, into PhA and PhI in NE, fractions in the absence of agonist was approximately 8000 and 2000 cpm, respectively.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PhA*</th>
<th>PhI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>216 ± 4 ( (8) )</td>
<td>199 ± 6 ( (8) )</td>
</tr>
<tr>
<td>155 ± 8 ( (5) )</td>
<td>150 ± 12 ( (3) )</td>
</tr>
<tr>
<td>200 ± 11 ( (3) )</td>
<td>183 ± 13 ( (3) )</td>
</tr>
<tr>
<td>185 ± 8 ( (3) )</td>
<td>187 ± 11 ( (3) )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PhA*</th>
<th>PhI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>113 ± 3 ( (5) )</td>
<td>113 ± 3 ( (5) )</td>
</tr>
</tbody>
</table>

**Group A**

| Carbamylcholine | 216 ± 4 \( (8) \) | 199 ± 6 \( (8) \) |
| Mecamylamine | 155 ± 8 \( (5) \) | 150 ± 12 \( (3) \) |
| Acetylcholine | 200 ± 11 \( (3) \) | 183 ± 13 \( (3) \) |
| Methacholine | 185 ± 8 \( (3) \) | 187 ± 11 \( (3) \) |

**Group B**

| Bethanechol | 146 ± 6 \( (5) \) | 159 ± 9 \( (6) \) |
| Pilocarpine | 133 ± 5 \( (6) \) | 141 ± 8 \( (6) \) |
| Arecoline | 124 ± 5 \( (6) \) | 126 ± 4 \( (6) \) |
| Oxotremorine | 113 ± 3 \( (5) \) | 113 ± 3 \( (5) \) |

* Guinea pig cerebral cortex NE,.
* Rat cerebral cortex NE,.
* Bovine adrenal chromaffin cells; ND, not determined.

### FIG. 1

Concentration-dependence of stimulated PhA and PhI labeling in NE, fractions (approximately 1 mg of protein) for oxotremorine, arecoline, pilocarpine, and bethanechol (Group B agonists). O, agonist alone; ●, agonist plus carbamylcholine (1 mM).

![Concentration-dependence of stimulated PhA and PhI labeling in NE, fractions for various agonists.](http://www.jbc.org/)

The equation describing the displacement of [3H]QNB binding by an unlabeled agonist which has different affinities for two noninteracting sites under conditions in which the concentration of free ligand is essentially the same as total ligand concentration is given as

\[ [QR] = \frac{K_{B}[Q]+K_{A}[A]}{1 + K_{B}[Q]+K_{A}[A]} \]  

(4)

where \([B_o]\) and \([B_i]\) are the concentrations of the high and low affinity receptor sites with affinities \( K_{B} \) and \( K_{A} \), respectively, and \([B_{total}] = [B_o] + [B_i]. A computer curve-fitting program was written for Equations 3 and 4 in which the data input consisted of 12-18 pairs of values of \([A]\) and \([QR]\). \([A]\) is known and \([QR]\) experimentally determined. \([Q]\), \( K_{B} \), and \([B_{total}] \) are measured independently. The program generated values of \([B_o]\) and \([B_i]\), \( K_{B} \) and \( K_{A} \), the percentage of high affinity sites, and the ratio of \( K_{B}/K_{A} \). The experimental data were fitted to both a one- and two-site model and best fit determined by a minimum sum of the squared residuals and a Student's t-test on the differences. The data were further examined for systematic deviations from a one- or two-site model by nearest neighbor residual
analysis (18). In addition, plots of occupancy of the receptor by agonists as a function of [A] were visually examined for deviations from predicted one- or two-site models. In some experiments the data were fitted to a three-site model, incorporating the terms for displacement of \(^{3}H\)QNB from a "super high" affinity site by a muscarinic agonist (25). Only in a limited number of experiments was there a statistically significant improvement in fit, so that this data treatment was not routinely performed. Protein was determined by the method of Geiger and Bessman (26).

**Table II**

Nonadditivity in phosphatidate and phosphatidylinositol labeling following the simultaneous addition of two Group A agonists to nerve ending fractions

<table>
<thead>
<tr>
<th>NE(_{11}) Fraction</th>
<th>PhA</th>
<th>PhI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. I</td>
<td>Exp. II</td>
</tr>
<tr>
<td>Carbamylcholine</td>
<td>191</td>
<td>194</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>241</td>
<td>297</td>
</tr>
<tr>
<td>Methacholine</td>
<td>197</td>
<td>187</td>
</tr>
<tr>
<td>Muscarine</td>
<td>189</td>
<td>185</td>
</tr>
<tr>
<td>Carbamylcholine + acetylcholine</td>
<td>227</td>
<td>207</td>
</tr>
<tr>
<td>Carbamylcholine + methacholine</td>
<td>192</td>
<td>188</td>
</tr>
<tr>
<td>Acetylcholine + methacholine</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>Acetylcholine + muscarine</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Muscarine + methacholine</td>
<td>ND</td>
<td>184</td>
</tr>
</tbody>
</table>

*ND, not determined.

![Fig. 2](http://www.jbc.org/) Occupancy of the muscarinic receptor by Group A agonists as a function of agonist concentration. The displacement of \(^{3}H\)QNB by agonists was analyzed by a computer program which generated values of \(K_h\), \(K_l\), \(B_h\), and \(B_l\) for a two-site fit and \(K_1\) site for a one-site fit, as described under "Materials and Methods." From these values, plots of theoretical one-site (○) and two-site receptor occupancy (●) were obtained and compared to the actual receptor occupancy (▲). For all Group A agonists the displacement data was best fitted statistically to a two-site model. Results are from one of five separate experiments that gave similar results.

![Fig. 3](http://www.jbc.org/) Occupancy of the muscarinic receptor by Group B agonists as a function of agonist concentration. See legend to Fig. 2 for details. Group B agonist displacement data fits the one-site model. Each result is from one of three to five separate experiments that gave similar results.

**RESULTS**

**Stimulation by Muscarinic Agonists of PhA and PhI Labeling from \(^{32}P\) in NE\(_{11}\) Fractions and Dissociated Adrenal Chromaffin Cells—**Muscarinic agonists (1.0 mM) differed considerably in their ability to enhance PhA and PhI turnover in the NE\(_{11}\) fraction. Addition of carbamylcholine, acetylcholine, methacholine, or muscarine (Table I, Group A) resulted in a larger stimulation of PhA and PhI labeling than agonists in Group B, i.e. betahanechol, pilocarpine, arecoline, or oxotremorine. This pattern of agonist efficacy was also observed for NE\(_{11}\) fractions obtained from rat cerebral cortex and other areas of guinea pig brain (hippocampus, inferior and superior colliculi, and pons/medulla, not shown). However, in bovine adrenal chromaffin cells, oxotremorine and arecoline were relatively more efficacious, although still less effective than carbamylcholine in stimulation of PhI labeling, while in this tissue pilocarpine was the least efficacious agonist.

**Group B Agonists: Dose-Response and Effect on Carbamylcholine-stimulated PhA and PhI Labeling—**To establish that optimum concentrations of agonists in Group B had been employed, dose-response curves for stimulated PhA and PhI labeling were constructed (Fig. 1). Stimulated PhA and PhI labeling was optimum at approximately 1.0 mM concentrations of oxotremorine, arecoline, betahanechol, and pilocarpine. Agonists in Group B antagonized the stimulatory effect of carbamylcholine addition on phospholipid labeling. The rank order potency so obtained was oxotremorine > arecoline = pilocarpine > betahanechol. Conversely, the simultaneous addition of agonists in Group A did not elicit an increase in labeling of either PhA or PhI greater than that produced by each agonist alone (Table II).

**\(^{3}H\)QNB Binding to NE\(_{11}\) Fractions: Displacement by Agonists—**\(^{3}H\)Quinuclidinyl benzilate bound to freshly prepared and frozen NE\(_{11}\) fractions (\(B_{\text{max}} = 1.10 \pm 0.08\) pmol/mg of protein, \(n = 8\)) with an affinity constant \(K_d\) of 48.9 ± 7.0
TABLE III

Displacement of [3H]QNB binding to nerve ending fractions by muscarinic agonists

<table>
<thead>
<tr>
<th>Group</th>
<th>Best fit</th>
<th>n</th>
<th>Kd</th>
<th>Ka</th>
<th>Kf/Kd</th>
<th>Per cent of high affinity sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>2-site</td>
<td>5/5</td>
<td>0.04 ± 0.02</td>
<td>5.91 ± 1.60</td>
<td>148</td>
<td>41.5 ± 1.4</td>
</tr>
<tr>
<td>Carbamylcholine</td>
<td>2-site</td>
<td>5/5</td>
<td>1.41 ± 0.30</td>
<td>76.70 ± 7.37</td>
<td>54</td>
<td>41.8 ± 1.4</td>
</tr>
<tr>
<td>Methacholine</td>
<td>2-site</td>
<td>5/5</td>
<td>0.17 ± 0.08</td>
<td>20.02 ± 5.38</td>
<td>113</td>
<td>36.6 ± 1.9</td>
</tr>
<tr>
<td>Muskacine</td>
<td>2-site</td>
<td>5/5</td>
<td>1.46 ± 0.53</td>
<td>41.48 ± 9.01</td>
<td>28</td>
<td>27.0 ± 1.3</td>
</tr>
</tbody>
</table>

*In one experiment for each agonist, displacement data could be fitted to a two-site model, although <20% of receptors were in the high affinity form. For calculation of the mean affinity constant (Kd), a one-site interaction was assumed for all experiments.

pmol (n = 8). All agonists could completely displace [3H]QNB bound to the NE1, fraction, the most potent being oxotremorine, while the least effective was bethanechol. Plots of receptor occupancy by the agonist as a function of agonist concentration revealed that Group A agonists showed a systematic deviation from the curve predicted for the interaction of an agonist with a single affinity site. The displacement data could, however, be readily fitted to a two-site model in which the muscarinic receptor was proposed to exist in both high and low affinity forms (Fig. 2). In contrast, the displacement data for agonists in Group B could readily be fitted to a one-site model, and there was no significant improvement in fit for a two-site model (Fig. 3). Although the sum of the squared residuals for a two-site model was invariably smaller for agonists in both groups, only for Group A agonists were statistically significant differences in fit between one-site and two-site models observed. Values for the high and low affinity forms of the receptor (Kd and Ka) and percentage high affinity sites are expressed as mean ± S.E. For agonists in Group B, a single affinity constant has been calculated.

FIG. 4. Concentration dependence of carbamylcholine-stimulated PhA labeling. NE1, fractions (approximately 1 mg of protein) were incubated in Buffer A containing 30-40 μCi of [32P], and terminated 30 min later. Results are expressed as a fraction of maximal response (obtained at 10 mM carbamylcholine) as a function of agonist concentration. In absolute terms, 10 mM carbamylcholine addition increased PhA labeling to 226 ± 4% of control (mean ± S.E.). Values are mean ± S.E. for eight determinations derived from three individual NE1, preparations. Analysis revealed a two-site interaction with values for Kd and Ka of 0.31 μM and 108.8 μM, respectively. The fraction of the receptors in the low affinity form was 88%.

FIG. 5. Concentration dependence of methacholine- and acetylcholine-stimulated PhA labeling. See legend to Fig. 4 for details. Results shown are derived from single experiments for each agonist. The affinity constants for methacholine and acetylcholine were 30 μM and 20 μM, respectively, assuming a one-site interaction. Ten mM concentrations of methacholine and acetylcholine increased PhA labeling to 232 and 222% of control, respectively.
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was selected as the preferred measure of receptor activation, since it represents an early plasma membrane event, unlike PhI resynthesis, which occurs subsequently at the endoplasmic reticulum (5). In addition, enhanced PhA labeling is of sufficient magnitude to permit accurate detection at low agonist concentrations, in contrast to stimulated inositol lipid breakdown which is often 25% or less for full agonists at optimum concentrations (14, 17, 28, 29). Carbamylcholine stimulation of PhA labeling was detectable at 1.0 μM and was maximal at 10 μM (Fig. 4). While this data could be dissociated into high and low affinity components using computer fits, only 12% of the inferred muscarinic receptors were in the high affinity state. The constant for the low affinity state of the muscarinic receptor (109 μM) as determined from enhanced PhA labeling was in the range of that obtained from the binding data (77 μM). Similarly, dose-response curves for PhA labeling obtained with methacholine and acetylcholine indicated that both of these agonists also interact predominately with a single low affinity form of the receptor (Fig. 5). The values for affinity constants so obtained were 30 μM and 20 μM, respectively, and are in the range of values obtained for the low affinity form of the muscarinic receptor derived from binding studies (20 μM and 6 μM, respectively).

**DISCUSSION**

The present study indicates that muscarinic receptors coupled to enhanced phospholipid turnover in nerve ending preparations from cerebral cortex possess a distinctive profile with regard to agonist efficacy. The ability of Group B agonists to antagonize the stimulatory effect of carbamylcholine indicates that while both groups of agonists bind to the same population of brain muscarinic receptors, Group B agonists are less efficacious and may thus be considered “partial” agonists. In contrast, when muscarinic receptors on dissociated adrenal chromaffin cells are examined, arecoline and oxotremorine are relatively more efficacious agonists than they are in the brain. The categorization of agonists, therefore, appears to be highly dependent on the source of receptors.

Evidence in favor of a tissue-specific profile of muscarinic agonist efficacy for stimulated phospholipid turnover and/or the attendant physiological response has been obtained from other sources. Thus, betanechol addition to the superior cervical ganglion elicits increases in PhI turnover and membrane depolarization comparable to other full agonists in this preparation such as carbamylcholine or muscarine, while the physiological responses (depolarization) due to pilocarpine and oxotremorine addition are characteristic of partial agonism (4, 30). In contrast, the stimulation of amylase release from pancreatic preparations and the accompanying PhI breakdown evoked by the addition of pilocarpine are comparable in magnitude to those produced by acetylcholine or carbamylcholine (31, 32). While these tissue differences might be interpreted to reflect the presence of different receptor molecules, present evidence favors the existence of a single receptor protein in varying molecular environments (33).

The pattern of agonist efficacy in brain is related to the characteristics of agonist displacement of [3H]QNB binding in nerve ending preparations. Agonists that were most effective in enhancing PhA and PhI turnover displaced [3H]QNB in a complex manner, with binding curves deviating markedly from a simple mass action isotherm (Fig. 2). This binding behavior of the agonists can be explained by postulating the presence of two noninteracting forms of the muscarinic receptor with large (30- to 150-fold) differences in $K_p$ and $K_H$, as has been observed in rat brain preparations (18). In contrast, less effective agonists displaced [3H]QNB in a simpler manner, with minor deviation from a single mass action isotherm and relatively small differences in values of $K_L$ and $K_H$. These results are consistent with a model for muscarinic receptor activation proposed by Birdsall and Hulme (34) in which agonists, unlike antagonists, are assumed to induce a conformational change in the receptor, and differences in agonist efficacy arise as a result of conformational restraints imposed by the coupling state of the receptor. From this, it can be predicted that the efficacy of an agonist is a function of $K_p/K_H$. In the present study this prediction is met; full agonists with regard to phospholipid turnover also possess large values for $K_p/K_H$ while the partial agonists, betanechol, pilocarpine, arecoline, and oxotremorine, display lower $K_p/K_H$ ratios. The fact that muscarine ($K_p/K_H = 28$) was as effective as acetylcholine ($K_p/K_H = 148$) indicates a requirement for a minimum value of $K_p/K_H$ for full agonist status, below which a reduced response is seen. While full agonists typically possess a high $K_p/K_H$ ratio, the low affinity form of the brain muscarinic receptor rather than the high affinity form appears in general to be coupled to phospholipid turnover. Although computer fits of carbamylcholine-stimulated PhA labeling could be dissociated into high and low affinity components, almost 90% of the receptors coupled were in the L-form. Similarly, dose-response curves for acetylcholine- and methacholine-stimulated PhA labeling also revealed the sole involvement of a low affinity form of the receptor. Other studies have also indicated the importance of the L-form of the receptor, e.g. cGMP stimulation in neuroblastoma cells (1), inhibition of adenylate cyclase in heart (2), smooth muscle contraction in the ileum (35), and stimulation of PhI turnover by methacholine in pancreas (17). In contrast, enhancement of PhI turnover in guinea pig ileum correlates with the occupancy of both L- and H-forms of the receptor (13), and stimulation of Ca2+ influx into smooth muscle reflects the activation of the H-form (36). While little information exists as to the molecular nature of these high and low affinity forms of the receptor, recent photoaffinity probe studies suggest that the muscarinic receptor exists in oligomeric forms, with the L-form being a dimer of molecular weight 86,000 Da, while the H-form of the receptor is a 160,000-Da tetramer (37).

The observation that the L-form of the muscarinic receptor is preferentially coupled to phospholipid turnover in brain is in complete accord with the model proposed by Birdsall and Hulme (34). The present results also indicate that stimulated phospholipid turnover in brain is tightly coupled to the occupancy of the muscarinic receptor, with little or no “receptor reserve,” in agreement with observations made on enhanced PhI turnover in the ileum (13) and pancreas (17) but in contrast to recent results for methacholine stimulation of PhI turnover in rat parotid (14). Michell and Kirk (38) have suggested that this apparent discrepancy may arise from measurement of enhanced PhI turnover by means of labeling experiments, rather than by inositol lipid breakdown. However, results from the same laboratory have indicated a good correlation between the concentrations of agonists required to elicit a maximal increase in inositol phospholipid breakdown and increased PhI labeling (28).

In conclusion, the results from this study suggest that the profile of agonist efficacy for stimulated PhA and PhI turnover is a reflection of the relative abilities of muscarinic agonists to induce a conformational change in the brain receptor. The low affinity form of the receptor is coupled to PhA and PhI turnover, and full receptor occupancy is required for a maximum effect.

Acknowledgment—We thank Dr. R. W. Holz for preparing the bovine adrenal chromaffin cells.
REFERENCES
