MOLECULAR PHARMACOLOGY, 26:51–56

Acute Elevation of Cyclic AMP Does Not Alter the Ion-Conducting Properties of the Neuronal Nicotinic Acetylcholine Receptor of PC12 Cells

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Received December 7, 1983; Accepted March 13, 1984

SUMMARY

The nicotinic acetylcholine receptor of *Torpedo californica* has been shown to be subject to cyclic AMP-dependent phosphorylation, raising the possibility that nicotinic receptors may be regulatable by phosphorylation. To investigate this possibility for a neuronal nicotinic receptor, we have studied the effects of elevation of cyclic AMP on the ion-conducting properties of the nicotinic receptor of PC12 cells. The cyclic AMP content of the cells was altered by exposure to various concentrations of forskolin (an activator of adenylate cyclase) for periods of time ranging from 1 to 40 min. Receptor activation then was measured as agonist-induced influx of $^{86}Rb^+$ into the cells. Throughout a variety of conditions, no changes in agonist-induced ion influx were detected. This was true regardless of the concentration of agonist used, the duration of receptor stimulation that was measured, the concentration of forskolin employed, or the duration of elevation of cyclic AMP prior to receptor activation. Experiments designed to measure receptor desensitization also were unable to detect any differences upon elevation of cyclic AMP. Finally, the antagonism of receptor activation by substance P also was not affected by elevation of cyclic AMP. Thus, no evidence could be obtained in these cells supporting the hypothesis that a neuronal nicotinic acetylcholine receptor can be acutely regulated by changes in cellular cyclic AMP.

INTRODUCTION

Recently, Huganir and Greengard (1) have shown that the *gamma* and *delta* subunits of the nicotinic AChR[2] from *Torpedo californica* are subject to phosphorylation by both endogenous and exogenous cyclic AMP-dependent protein kinases. On the basis of this observation, the logical possibility was raised that phosphorylation and/or dephosphorylation may regulate the receptor in some manner. However, no data relating to this question have appeared. The observation that the muscle-type nicotinic receptor may be regulatable by phosphorylation raises the possibility that nicotinic receptors in general may be subject to phosphorylation-dependent regulation. This question is of particular importance for the neuronal nicotinic receptor; many noncholinergic neurotransmitters are known to alter cellular cyclic AMP and could affect cholinergic excitability in this manner. Unfortunately, since muscle and neuronal nicotinic receptors have both similarities and distinct differences, conclusions based on data obtained with one type of receptor will not necessarily transfer to the other.

In beginning the study of phosphorylation-dependent regulation of neuronal nicotinic receptors, one is faced with the problem that actual phosphorylation of the neuronal receptor cannot be readily assessed at present. For this reason, we have taken a functional approach, determining whether the properties of the receptors are changed by conditions known to alter cyclic AMP-dependent protein phosphorylation. The studies were performed with tissue-cultured pheochromocytoma cells, clone PC12, which possess neuronal-type nicotinic AChRs that are not blocked by α-bungarotoxin (2, 3). Preliminary experiments showed that forskolin (an activator of adenylate cyclase) stimulated cyclic AMP-dependent protein phosphorylation in the cells (4) and indicated that forskolin and adenosine-induced accumulation of cyclic AMP did not affect carbachol-elicited ion influx (5, 6). We report here a continuation of those experiments—a detailed study of the effects of elevated cyclic AMP on several receptor properties.

MATERIALS AND METHODS

Tissue culture methods. The PC12 cells were originally obtained from Dr. Lloyd Greene. The cells were routinely grown in plastic tissue

This work was supported by National Institutes of Health Grant NS 16777.

[1] Recipient of Research Career Development Award NS 00567.

[2] The abbreviations used are: AChR, acetylcholine receptor; DMEM, Dulbecco's modified Eagle's medium; BSS, balanced salt solution.

0026-895X/84/040051-06$02.00/0

culture flasks in DMEM containing 10% heat-inactivated horse serum and 5% fetal bovine serum at 37° in a humidified atmosphere of 90% air:10% CO_2. Animal sera were from K.C. Biologicals (Kansas City, Mo.).

Uptake of $^{86}Rb^+$. Activation and ion conductance of the nicotinic AChR of PC12 cells were measured using $^{86}Rb^+$ as the tracer ion and a modification of the techniques described by Huang *et al.* (7) and Stallcup (8). The ion channel of the nicotinic AChR from both muscle and PC12 cells has been shown to be relatively nonselective, especially for monovalent cations. Thus, tracer quantities of $^{86}Rb^+$ can compete with other monovalent cations and enter the cells upon activation of the receptors. We have used $^{86}Rb^+$ rather than the more commonly used $^{22}Na^+$ because $^{86}Rb^+$ is less expensive and it is much less hazardous to use than the high-energy *gamma*-emitting $^{22}Na^+$.

Cells to be used for experiments were replated from flasks to poly-L-lysine-coated 24-well plates (16-mm diameter wells) as previously described (5, 6) and allowed to attach for 8–16 hr before use. To initiate experiments, plates were transferred to a 37° water bath and growth medium was replaced with a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-buffered BSS containing 130 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.8 mM NaH_2PO_4, 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and 20 mM dextrose (pH 7.4). The cells were then allowed to equilibrate for 10 min before use. Pretreatments with forskolin [a specific activator of adenylate cyclase (5, 9)] to alter cellular cyclic AMP levels are described in individual tables and figures. The actual $^{86}Rb^+$ uptake assay was initiated by replacing BSS with BSS containing 2 mM ouabain and incubating for 1 min. Inhibition of Na^+,K^+-ATPase was necessary because $^{86}Rb^+$ behaves much like K^+ and will be pumped into the cells by the ATPase, increasing basal uptake of $^{86}Rb^+$. Following the 1-min pretreatment with ouabain, each well was rapidly rinsed twice with 0.5 ml of a BSS containing reduced Na^+ (NaCl replaced by isosmotic sucrose). The cells then were exposed to 0.2 ml of reduced Na^+ BSS containing $^{86}Rb^+$ [approximately 5 μCi/ml, 2 mM ouabain, and indicated concentrations of carbamylcholine (carbachol)]. Uptake was terminated by rapidly rinsing each well four times with 1 ml of BSS. Cells were dissolved in 0.5 ml of 1 M NaOH, and the amount of $^{86}Rb^+$ and protein was determined for each well. Uptake of $^{86}Rb^+$ always was determined in the presence and absence of agonist, and the difference was taken as receptor-mediated ion flux.

Normalization of $^{86}Rb^+$ uptake to the amount of protein in each well was done in two different ways. Initially, protein was simply measured by the method of Lowry *et al.* (10), using bovine serum albumin as a standard. To decrease the number of protein determinations which had to be performed, later experiments used cells that had been prelabeled with [^{3}H]leucine while they were growing in culture flasks. Routinely, [^{3}H]leucine (0.01 μCi/ml) was added to the DMEM at least 48 hr prior to the time that cells were to be transferred to multiwell plates. At the time of transfer, the growth medium was switched to DMEM without [^{3}H]leucine. At the end of each experiment, 4 wells were randomly selected from each group of 24 for determination of both protein and [^{3}H]leucine content. The amount of protein in each well then was calculated from the amount of ^{3}H in each well. This method was shown to give an accurate reflection of the amount of protein per well for a large range of cell densities.

In addition to normalizing uptake to the amount of protein in each well, it was necessary to normalize for the concentration of $^{86}Rb^+$ in the uptake medium to account for small day-to-day variations in the isotope concentration. Initial experiments demonstrated that carbachol-stimulated uptake of $^{86}Rb^+$ was linearly proportional to the concentration of $^{86}Rb^+$ over at least a 2-fold variation in concentration in the region of 5 μCi/ml. Therefore, all uptake values have been normalized to what they would have been if the concentration of $^{86}Rb^+$ was 5 μCi/ml.

The assay described above employed BSS containing reduced Na^+ to diminish competition between Na^+ and $^{86}Rb^+$ for the open ion channels and thus to give an increased signal to noise ratio for the assay. Initial experiments confirmed that qualitatively identical results were obtained if the assay was performed in normal BSS, but the apparent uptake of $^{86}Rb^+$ was much lower and less accurately measured.

Measurements of cyclic AMP. The cyclic AMP content of the cells was determined as previously described (5, 6). Briefly stated, cells were grown and treated identically as for $^{86}Rb^+$ uptake asasys. To terminate experiments, the cultures were rinsed once with BSS, and then cyclic AMP was extracted with 0.5 ml of 0.1 M HCl. The cyclic AMP extracted into the HCl was acetylated with acetic anhydride and triethylamine (11) and then assayed by a radioimmunoassay with [^{125}I]cyclic AMP using the automated Gamma Flow system (12).

RESULTS

Characteristics of agonist-stimulated influx of $^{86}Rb^+$. Most studies of ion fluxes through nicotinic AChR are performed at low temperatures (4–24°) to slow desensitization and increase the linear period of ion flux (8). However, since we were interested in approaching physiological conditions and studying enzymatic processes that would be temperature-sensitive, we were obliged to perform all experiments at 37°. Even at this temperature the rate of carbachol-stimulated $^{86}Rb^+$ uptake was essentially linear for about 15 sec (data not shown). Therefore, most of our studies were performed using this uptake duration. Many other experiments using receptor antagonists (such as *d*-tubocurarine) showed that carbachol-stimulated uptake was due to receptor activation (data not shown).

Effects of elevated cyclic AMP on initial rates of $^{86}Rb^+$ influx. Previous studies had shown that a short pretreatment of PC12 cells with 1 μM forskolin caused a 30- 50-fold increase in cyclic AMP content and maximal facilitation of depolarization-dependent neurosecretion (5). As shown by the data in Table 1, similar conditions caused no change in the initial rate of $^{86}Rb^+$ uptake either in the presence or absence of maximal receptor stimulation with 1 mM carbachol. This was true even if uptake was measured at 6.5 sec, where the initial rate of uptake might be more closely approximated.

Although 1 μM forskolin was known to facilitate secretion maximally, it was possible that the AChR would be responsive to different cyclic AMP concentrations. However, as shown by the data in Fig. 1, similar results were obtained at lower concentrations of forskolin, where the changes in cyclic AMP were much smaller. From Fig. 1 one can see the very large changes in cyclic AMP content which were caused by forskolin. The data in Fig. 1 are

TABLE 1

Effects of forskolin pretreatment on the initial rate of $^{86}Rb^+$ uptake into PC12 cells

Cells were incubated with or without 1 μM forskolin for 12 min and then the uptake of $^{86}Rb^+$ was measured for the indicated times as described under Materials and Methods. Each value is the mean ± standard error of the mean for five or six replicate cultures.

Condition	Uptake time	$^{86}Rb^+$ uptake		
		Basal	Stimulated	Net
	sec	*pmoles/mg protein*		
Control	6.5	6.1 ± 0.4	13.5 ± 0.4	7.4 ± 0.8
Forskolin	6.5	5.2 ± 0.4	13.1 ± 0.5	7.9 ± 0.9
Control	15.0	10.0 ± 0.3	27.9 ± 0.3	17.9 ± 0.6
Forskolin	15.0	8.3 ± 0.3	25.0 ± 0.9	16.7 ± 1.2

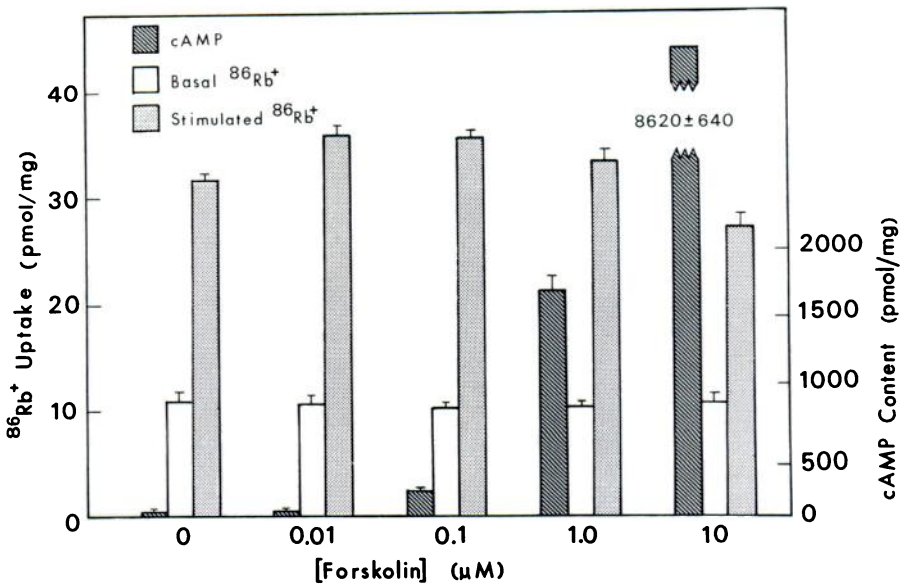

FIG. 1. *Effects of various forskolin concentrations on cellular cyclic AMP content and uptake of $^{86}Rb^+$*

Cells were pretreated with the indicated concentrations of forskolin for 12 min prior to determination of cyclic AMP content and $^{86}Rb^+$ uptake. Both basal and carbachol-stimulated (1 mM carbachol) uptake of $^{86}Rb^+$ were measured for 15 sec as described under Materials and Methods. All values are means ± standard error of the mean for five or six replicate assays.

representative of several repetitions of this experiment, and the apparent small increases in $^{86}Rb^+$ uptake with 0.01 and 0.1 μM forskolin were not reproducibly observed (see also Table 2). The decrease in $^{86}Rb^+$ uptake with 10 μM forskolin was not investigated further, since the elevation of cyclic AMP was so great under these conditions.

All of the experiments described above employed maximal stimulating concentrations of carbachol and would not have detected changes in the sensitivity of the receptor to various concentrations of agonist. The carbachol concentration dependence for $^{86}Rb^+$ uptake is shown in Table 2. Response was first detected above 10 μM carbachol, peaked at 1 mM carbachol, and then declined at higher concentrations. The concentration causing one-half maximal uptake was about 50–100 μM, consistent with other reports for both neural and muscle nicotinic receptors. The pronounced decline of $^{86}Rb^+$ uptake with supermaximal concentrations of carbachol probably reflects an increased rate of desensitization of the receptors, as has been seen previously in *Torpedo californica* and PC12 cells (13, 14). Of greatest importance, however (as shown by the data in Table 2), neither 0.01 nor 1 μM forskolin had any effect on carbachol-stimulated uptake of $^{86}Rb^+$ at any concentration of the agonist.

TABLE 2

Effects of forskolin treatment on the concentration dependence of carbachol-stimulated uptake of $^{86}Rb^+$

Cells were pretreated for 12 min with or without the indicated concentration of forskolin. Uptake of $^{86}Rb^+$ then was determined for 15 sec with the concentrations of carbachol given. Values are means ± standard error of the mean of six assays.

Carbachol concentration	$^{86}Rb^+$ uptake	
	Control	Forskolin-treated
mM	*pmoles/mg protein*	
Expt. 1, 0.01 μM forskolin		
0	10.8 ± 0.7	10.6 ± 0.8
0.03	19.6 ± 0.8	21.5 ± 1.1
0.10	28.0 ± 1.9	31.1 ± 2.0
1.0	31.5 ± 1.8	32.4 ± 1.6
5.0	21.7 ± 1.8	23.4 ± 2.3
Expt. 2, 1 μM forskolin		
0	10.7 ± 0.6	10.4 ± 0.4
0.03	16.8 ± 1.1	17.9 ± 1.4
0.10	26.6 ± 1.5	24.9 ± 0.5
1.0	32.5 ± 1.1	31.4 ± 1.1
5.0	22.0 ± 1.1	22.4 ± 1.2

Finally, to test whether the duration of time during which the cyclic AMP content was elevated made any difference, cells were pretreated with forskolin for times varying from 1 to 40 min. Previous experiments have shown that the cyclic AMP content of the cells treated in this manner plateaus within 4–6 min and then stays constant for prolonged periods of time (5, 6). Again, no effect on basal or carbachol-stimulated uptake of $^{86}Rb^+$ was seen (Fig. 2). This is in contrast to the enhancement of depolarization-dependent secretion produced by elevation of cyclic AMP, which is very time-dependent (5, 6). Taken together, the data support the conclusion that elevation of cyclic AMP does not alter the initial rate of ion influx through the activated AChR.

Effects of elevated cyclic AMP on receptor desensitization. By studying the initial rate of ion flux subsequent to receptor activation, one minimizes the influences of receptor desensitization on observed rates of $^{86}Rb^+$ influx. However, elevation of cyclic AMP (and presumably subsequent phosphorylations) also could alter the desensitization process directly. The observation that elevated cyclic AMP did not alter $^{86}Rb^+$ uptake at very high carbachol concentrations (where desensitization is especially pronounced) suggested that desensitization was not altered by these conditions. However, this question

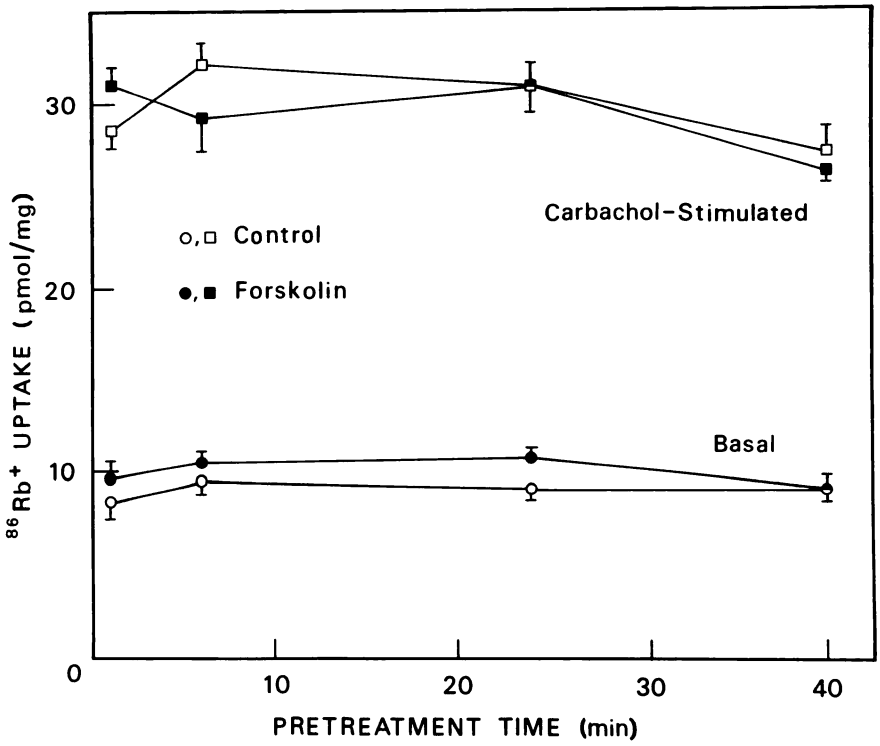

FIG. 2. *Effects of various forskolin pretreatment times on uptake of $^{86}Rb^+$*
Cells were pretreated with 1 μM forskolin for the indicated times prior to determination of both basal and carbachol-stimulated uptake of $^{86}Rb^+$ (1 mM carbachol) for 15 sec. Values are means ± standard error of the mean for six replicate assays.

was addressed directly in three ways. First, cells were pretreated with 1 μM forskolin for 12 min and then carbachol-stimulated uptake of $^{86}Rb^+$ was monitored for an additional 12 min in the presence and absence of forskolin. Carbachol-stimulated uptake of $^{86}Rb^+$ under these conditions would reflect both the initial rapid influx and the subsequent, much reduced, uptake through the predominantly desensitized population of receptors. As shown by the data in Experiment 1 of Table 3, under these conditions basal uptake of $^{86}Rb^+$ was slightly decreased by elevation of cyclic AMP, but carbachol-stimulated uptake was unchanged.

A limitation with the experimental design described above was that the measured uptake represented a composite of the initial, linear rate of uptake and the subsequent uptake associated with the desensitized population of receptors. A second method which avoids this problem is to examine the actual rate of desensitization by varying the duration of time cells are pretreated with carbachol (in the absence of $^{86}Rb^+$) and then determining the initial rate of $^{86}Rb^+$ uptake during the next 15 sec of receptor activation. Experiment 2 of Table 3 depicts such an experiment. The concentration of carbachol used in this experiment was 0.5 mM in order to decrease the rate of desensitization to a point where the process could be better studied. As shown by the data, with 20 sec of exposure to carbachol, desensitization had decreased the initial rate of uptake by about 50% whether the cells had been pretreated with forskolin or not. Similarly, 35 sec of exposure to carbachol decreased uptake by about 70% in the presence or absence of elevated cyclic AMP. Thus, forskolin pretreatment did not appear to alter the rate of receptor desensitization.

TABLE 3

Effects of forskolin treatment on desensitization of receptors

In all cases, cells were first pretreated with or without 1 μM forskolin for 12 min. For Experiment 1, cells then were incubated for 12 min (forskolin continued where appropriate) with $^{86}Rb^+$, with or without 1 mM carbochol. In Experiment 2, cells then were pretreated with 0.5 mM carbachol for the indicated time, and then immediately exposed to $^{86}Rb^+$ and 0.5 mM carbachol for 15 sec. For Experiment 3, cells were then incubated for 12 min in the absence (basal) or presence (stimulated) of 1 mM carbachol; forskolin was continued where appropriate. At the end of this time, $^{86}Rb^+$ uptake was determined for an additional 15 sec. Values are means ± standard error of the mean for six assays.

Treatment	Carbachol pretreatment time	Uptake time	$^{86}Rb^+$ uptake		
			Basal	Stimulated	Net
			pmoles/mg		
Expt. 1					
Control	0	12 min	208 ± 8	291 ± 8	83 ± 16
Forskolin	0	12 min	180 ± 3	268 ± 7	88 ± 10
Expt. 2					
Control	0	15 sec	7.2 ± 0.4	32.2 ± 1.0	25.0 ± 1.4
	20 sec	15 sec		21.8 ± 1.2	14.6 ± 1.6
	35 sec	15 sec		14.5 ± 0.4	7.3 ± 0.8
Forskolin	0	15 sec	9.4 ± 0.3	32.6 ± 1.1	23.2 ± 1.4
	20 sec	15 sec		21.8 ± 1.1	12.4 ± 1.4
	35 sec	15 sec		16.7 ± 0.6	7.3 ± 0.9
Expt. 3					
Control	12 min	15 sec	8.3 ± 0.5	11.0 ± 1.1	2.7 ± 1.6
Forskolin	12 min	15 sec	7.7 ± 0.5	10.2 ± 0.6	2.5 ± 1.1

Finally, an attempt was made to look at the equilibrium distribution of active and desensitized receptors by exposing the cells to carbachol for a long period of time (12 min) prior to assessment of carbachol-stimulated

uptake of $^{86}Rb^+$. As seen by the data in Experiment 3 of Table 3, even after 12 min of exposure to carbachol, a measurable uptake of $^{86}Rb^+$ was seen during the next 15 sec of exposure. However, elevation of cyclic AMP with forskolin during this time had no effect on carbachol-stimulated uptake. Taken together, these three experiments appear to indicate that elevation of cyclic AMP does not alter desensitization of these nicotinic AChRs.

Effects of elevated cyclic AMP on substance P inhibition of $^{86}Rb^+$ influx. A final property of the AChR of PC12 cells is its sensitivity to inhibition by substance P; it has been suggested that substance P produces its inhibitory action by increasing the rate of receptor desensitization (15). The inhibitory effect of substance P also was observed using our $^{86}Rb^+$ influx assay (Table 4), but again, pretreatment of the cells under conditions that should promote cyclic AMP-dependent protein phosphorylations had no effect on the inhibition produced by substance P.

DISCUSSION

Of the many different ACh receptors that have been identified, the neuronal nicotinic receptor has been the most resistant to study. Significant advances recently have been made with the renewed use of receptor-binding techniques applied to brain tissue (16). However, as yet, little progress has been made in the development of an assay of receptor function, i.e., ion conduction. For this reason, we are focusing our attention on the neuronal receptor of PC12 cells as a model system in which regulation of the functioning receptors can be studied. It should be recognized that the nicotinic AChR of PC12 cells is a model of *a* neuronal receptor, not *the* neuronal receptor; as yet it is unclear just how many different nicotinic receptors exist in neuronal tissues.

Our initial characterization of the influx of $^{86}Rb^+$ upon stimulation with carbachol showed clearly that the response can be studied at a physiological temperature. This is important if one wishes to study various perturbations meant to mimic physiological changes. However, taken as a whole, the results presented here indicate that the ion-conducting properties of the neuronal nicotinic AChR of PC12 cells are not acutely regulated by increases in cyclic AMP-dependent phosphorylations. This conclusion is based on the lack of systematic changes in agonist-induced ion flux through the receptor-linked ion channel throughout a broad range of cellular cyclic AMP levels maintained for times from 1 to 40 min. Our ability to make this conclusion obviously is dependent on our previous observation that cyclic AMP-dependent protein phosphorylation generally is increased under these conditions (4), and that a functional change in cell behavior (i.e., secretion) also can be detected (5, 6). One possible explanation for our inability to detect any change in receptor function is that the receptor is maximally phosphorylated even at resting cyclic AMP levels. If this is the case, then at least we can conclude that increases in phosphorylation do not appear to play an important role in acute regulation of receptor function. It must be emphasized, however, that our results do not provide evidence that the AChR actually is phosphorylated in PC12 cells. We can conclude for now only that, if phosphorylations occur, we can find no evidence that they alter the function of the receptor. Finally, our analysis of receptor activation has been limited to times greater than 5 sec. Although influx of $^{86}Rb^+$ during the first 15 sec was linear, it still is possible that elevation of cyclic AMP could cause changes observable at the single-channel level. Experiments to test this possibility are in progress.

At present we can find no evidence that conditions that greatly favor cyclic AMP-dependent phosphorylations alter functioning of the nicotinic receptor of PC12 cells. These studies strengthen our earlier conclusion (5, 6) that facilitation of carbachol-stimulated neurosecretion from PC12 cells by elevation of cyclic AMP is not a result of increased depolarization; some step subsequent to receptor-mediated ion influx must be involved. However, the results reported here have been directed only toward the acute effects of cyclic AMP elevation. A separate possibility is that changes in cyclic AMP content for longer periods of time could cause changes in receptor properties or receptor number. Evidence supporting this possibility was provided by a recent study in which exposure of PC12 cells to 1 mM dibutyryl cyclic AMP blocked β-nerve growth factor-induced increases in nicotinic receptor responsiveness (17). Thus, even though our studies failed to detect any effects of elevated cyclic AMP on receptor function, some elements of the relationship between cell cyclic AMP content and neuronal nicotinic receptors remain to be characterized.

TABLE 4

Effects of forskolin treatment on inhibition of receptor activation by substance P

Uptake of $^{86}Rb^+$ was determined in cells pretreated for 12 min in the presence or absence of 1 μM forskolin. Basal and carbachol-stimulated uptake (1 mM carbachol) was determined either with or without the simultaneous addition of substance P. Values are means ± standard error of the mean of four to six assays.

Cell treatment	Substance P concentration	$^{86}Rb^+$ uptake		
		Basal	Stimulated	Net
	μM	*pmoles/mg protein/15 sec*		
Control	0	6.7 ± 0.4	27.1 ± 1.0	20.4 ± 1.4
	5	7.0 ± 0.2	18.9 ± 0.5	11.9 ± 0.7
Forskolin-treated	0	7.9 ± 0.4	28.6 ± 0.7	20.7 ± 1.1
	5	7.9 ± 0.3	21.5 ± 0.7	13.6 ± 1.0

REFERENCES

1. Huganir, R. L., and P. Greengard. cAMP-Dependent protein kinase phosphorylates the nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. U. S. A.* **80**:1130–1134 (1983).
2. Patrick, J., and W. B. Stallcup. Immunological distinction between acetylcholine receptor and the alpha-bungarotoxin-binding component on sympathetic neurons. *Proc. Natl. Acad. Sci. U. S. A.* **74**:4689–4692 (1977).
3. Patrick, J., and W. B. Stallcup. alpha-Bungarotoxin binding and cholinergic receptor function on a rat sympathetic nerve line. *J. Biol. Chem.* **252**:8629–8633 (1977).
4. Zabrenetzky, V., R. McGee, and W. Lovenberg. Cyclic AMP-dependent protein phosphorylation in PC12 cytosol. *Fed. Proc.* **41**:658 (1982).
5. Rabe, C. S., J. Schneider, and R. McGee. Enhancement of depolarization-dependent neurosecretion from PC12 cells by forskolin-induced elevation of cyclic AMP. *J. Cylic Nucleotide Res.* **8**:371–384 (1982).
6. Rabe, C. S., and R. McGee. Regulation of depolarization-dependent release of neurotransmitters by adenosine: cyclic AMP-dependent enhancement of release from PC12 cells. *J. Neurochem.* **41**:1623–1634 (1983).

7. Huang, L. M., W. H. Catterall, and G. Ehrenstein. Selectivity of cations and nonelectrolytes for acetylcholine-activated channels in cultured muscle cells. *J. Gen. Physiol.* **71**:397–410 (1978).
8. Stallcup, W. B. Sodium and calcium fluxes in a clonal nerve cell line. *J. Physiol. (Lond.)* **286**:525–540 (1978).
9. Seamon, K. B., and J. W. Daly. Forskolin: a unique diterpene activator of cyclic AMP-generating systems. *J. Cyclic Nucleotide Res.* **7**:201–224 (1981).
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
11. Harper, J. F., and G. Brooker. Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2'O acetylation by acetic anhydride in aqueous solution. *J. Cyclic Nucleotide Res.* **1**: 207–218 (1975).
12. Brooker, G., W. L. Terasaki, and M. G. Price. Gammaflow: a completely automated radioimmunoassay system. *Science (Wash. D. C.)* **194**:270–276 (1976).
13. Lindstrom, J., R. Anholt, B. Einarson, A. Engel, M. Osame, and M. Montal. Purification of acetylcholine receptors, reconstitution into lipid vesicles, and study of agonist-induced cation channel regulation. *J. Biol. Chem.* **255**:8340–8350 (1980).
14. Leprince, P. Chemical modification of the nicotinic cholinergic receptor of PC-12 nerve cell. *Biochemistry* **22**:5551–5556.
15. Stallcup, W. B., and J. Patrick. Substance P enhances cholinergic receptor desensitization in a clonal nerve cell line. *Proc. Natl. Acad. Sci. U. S. A.* **77**:634–638 (1980).
16. Schwartz, R. D., R. McGee, and K. J. Kellar. Nicotinic cholinergic receptors labelled by [^{3}H]-acetylcholine in rat brain. *Mol. Pharmacol.* **22**:56–62 (1982).
17. Amy, C. M., and E. L. Bennett. Increased sodium ion conductance through nicotinic acetylcholine receptor channels in PC12 cells exposed to nerve growth factors. *J. Neurosci.* **3**:1547–1553 (1983).

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