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# Muscarinic Cholinergic Receptors on Cultured Thyroid Cells

## I. Biological Effect of Carbachol and Characterization of the Receptors

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SUMMARY

A muscarinic cholinergic effect on thyrotropin-stimulated cyclic AMP accumulation in cultured porcine thyroid cells is characterized. The muscarinic agonists carbachol, acetylcholine, oxotremorine, and pilocarpine decreased the acute cyclic AMP response to stimulation with thyrotropin (20 mU/ml). A 50% decrease was obtained in the presence of 0.1 mM carbachol. Maximal effects were observed when cells were cultured in the presence of thyrotropin (100 μU/ml) or prostaglandin $E_2$ (1 μM), whereas cells cultured in basal medium or in the presence of dibutyryl cyclic AMP showed a low response to carbachol. Evidence is reported suggesting that carbachol acutely decreases cyclic AMP synthesis. The properties of the muscarinic receptors present in thyroid cells were defined by using the binding of the muscarinic antagonist [$^3$H]quinuclidinyl benzilate to cell homogenates. Scatchard plots revealed a single population of binding sites with a $K_D$ of 0.3 nM. The numbers of binding sites per cell after 4 days in culture were 880 in control cells, 4335 in thyrotropin (100 μU/ml)-treated cells, 5600 in prostaglandin (1 μM)-treated cells, and 1420 in dibutyryl cyclic AMP-treated cells. Therefore the sensitivity to carbachol appeared to be related to the number of antagonist binding sites and to be independent of the sensitivity of the cells to acute thyrotropin stimulation.

### INTRODUCTION

Following the observations of Yamashita and Field (1), the existence of muscarinic cholinergic sensitivity in thyroid epithelial cells has been confirmed by several authors (2, 3). Moreover, the recent description of cholinergic innervation in thyroid gland (4) suggests that this sensitivity is of physiological significance.

As in other systems, the cholinergic agonist carbachol decreases thyrotropin-stimulated (1) cyclic AMP accumulation (3, 5). This effect, which is inhibited by atropine, is mediated by muscarinic cholinergic receptors. Various mechanisms have been proposed to explain such an effect, including changes in intracellular $Ca^{2+}$ (3, 6), modifications of cyclic GMP levels (1, 5, 7), modulation of phosphodiesterase (EC 3.1.4.7) activity (8–10), and alteration of cyclic AMP synthesis (5). None of them is satisfactory, and discrepancies between species, possibly linked to differences in the physiological properties of the stimulated tissue, have been reported (5).

Recent reports concerning the effects of carbachol on stimulated adenylate cyclase (EC 4.6.1.1) activity of particulate fractions (11) derived from neural cells prompted us to investigate the cholinergic effects on a better-defined thyroid system, porcine thyroid cells in primoculture. In order to characterize further the action of cholinergic agonists on thyroid cells, we first studied the biological effect of carbachol at the cellular level and the properties of the muscarinic receptors by using the binding of the labeled antagonist QNB.[3]

We know from previous studies that the sensitivity of cultured thyroid cells to acute stimulation with TSH can be modulated positively and negatively by the chronic presence of TSH in the culture medium (12–14). The sensitivity to carbachol of thyroid cells cultured under these various conditions was therefore studied. We show that carbachol decreased the acute response to TSH independently of the amplitude of this response. In contrast, the number of antagonist binding sites was increased by the chronic presence of TSH or of $PGE_2$ in the culture medium.

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[3] The abbreviations used are: QNB, quinuclidinyl benzilate; TSH, thyroid-stimulating hormone; $PGE_2$, prostaglandin $E_2$; $Bt_2cAMP$, dibutyryl cyclic AMP; MIX, methyl isobutylxanthine.

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## MATERIALS AND METHODS

The preparation and properties of porcine thyroid cells have been described elsewhere (15–17).

*Isolation of cells and culture.* The cells were isolated from adult porcine thyroid glands by a modified discontinuous trypsinization method (16, 18). The finely minced tissue was stirred at 37° in a 0.15% trypsin solution for 15 min and then in a 3 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N'*,N'-tetraacetic acid solution for 30 min. This treatment was repeated four or five times. After several washes, the cells were suspended in Eagle's minimal essential medium containing antibiotics and 10% newborn calf serum. This cell suspension was distributed at a concentration of $2 \times 10^6$ cells/ml in polystyrene Petri dishes not treated for tissue culture (Falcon 1008). Cultures were performed at 37° under a 5% $CO_2$/95% air, water-saturated atmosphere. The culture medium contained one of the following effectors: TSH (0.01–5 mU/ml), $PGE_2$ (1 μM), or $Bt_2$cAMP (1 mM). When the medium was unsupplemented, cells are referred to as control cells. Sustained stimulation during culture is referred to as chronic stimulation.

*Short-term incubations (acute stimulation).* After 1–5 days in culture, the cells were centrifuged at 500 × *g* for 2 min at 5°, the supernatant was discarded, and the cells were suspended in 20 ml of phosphate-buffered saline (pH 7.4) (NaCl/Pi) of the following composition (milligrams per liter): NaCl, 8000; KCl, 200; $Na_2HPO_4 \cdot 2H_2O$, 2890; $KH_2PO_4$, 200; $CaCl_2 \cdot 2H_2O$, 66.6; and $MgCl_2 \cdot 6H_2O$, 100. This procedure was repeated three times and the cells were finally suspended in an NaCl/Pi solution containing 0.1% glucose at a concentration of $5 \times 10^6$ cells/4 ml. Aliquots (0.4 ml) were incubated for 30 sec–15 min at 37° in air, in a final volume of 0.45 ml containing different effectors (MIX, 1 mM; TSH, 20 mU/ml; and carbachol). The incubation was stopped by adding $HClO_4$ (1 N final solution).

*Cyclic AMP and cyclic GMP determination.* After sonication and centrifugation the resulting pellet was assayed for protein concentration (19). The cyclic nucleotides in the supernatant were acetylated by the method of Harper and Brooker (20) and measured by radioimmunoassay according to the method of Cailla and *et al.* (21, 22) with modifications including the use of charcoal to separate free from bound antigen. The cyclic AMP measured represents the total amount present in the cells and in the incubation medium. Assays were performed in triplicate and cyclic AMP determinations in duplicate. The values reported in figures and tables are means ± standard error of the mean.

*[$^3$H]QNB binding studies.* After various culture periods, the cells were washed with NaCl/Pi at 5°, suspended in NaCl/Pi medium at 0° ($5 \times 10^6$ cells/ml), and homogenized in a Dounce homogenizer with a tightly fitting pestle; the homogenate was assayed immediately for [$^3$H]QNB binding. Homogenate (0.1 ml) was added to 0.1 ml of NaCl/Pi containing [$^3$H]QNB at different concentrations (up to 1.5 nM) and other effectors to be tested. Nonspecific binding was measured in the presence of 10 μM scopolamine. After 60 min of incubation at 26°, cold NaCl/Pi (1 ml) was added and the assay mixture was rapidly filtered through a glass-fiber filter (Whatman GF/C) under mild vacuum. After washing, bound radioactivity was measured on the filter by liquid scintillation counting. Since racemic [$^3$H]QNB was used in this study, the concentration of the active isomer was considered to be one-half of that added in the assay. The concentration of the active isomer as thus defined is plotted in the figures and referred to hereafter in the text.

Quadruplicate measurements of total and nonspecific binding were performed. Differences between duplicates were less than 5%. All experiments were performed at least three times with closely agreeing results.

*Other methods and chemicals.* Protein determinations were performed according to the method of Lowry *et al.* (19), using bovine serum albumin as standard. The number of cells was evaluated using a protein/DNA ratio of 7.5 for TSH-, $PGE_2$-, or $Bt_2$cAMP-treated cells; a protein/DNA ratio of 6 for control cells; and a DNA content of 8 μg/$10^6$ cells.

Purchases were made from the following sources: TSH from Armour and Company (Phoenix, Ariz.); racemic [$^3$H]-QNB (specific activity 30 Ci/mmole) from New England Nuclear Corporation (Boston, Mass.); trypsin, Eagle's minimal essential medium, and newborn calf serum from Grand Island Biological Company (Grand Island, N. Y.); and $Bt_2$cAMP, cyclic AMP, and cyclic GMP from Boehringer Mannheim (Mannheim, Federal Republic of Germany). Muscarinic agonists and antagonists were obtained from Sigma Chemical Company (St. Louis, Mo.), with the exception of racemic QNB, which was a gift from Roche (Basel, Switzerland). All other chemicals were of the highest purity commercially available. Antibodies and labeled antigens for cyclic AMP and cyclic GMP radioimmunoassays were kindly supplied by Dr. M. Delaage and H. Cailla (Marseille, France).

## RESULTS

*Characterization of the effect of carbachol on TSH-stimulated cyclic AMP accumulation.* The acute effect of carbachol was first studied using thyroid cells cultured 4 days in the presence of TSH (100 μU/ml) which had previously been shown to display the maximal cyclic AMP response to acute TSH stimulation (12–14). As shown in Fig 1, a decreased accumulation of cyclic AMP upon incubation in the presence of TSH (20 mU/ml) and carbachol (10 μM) was observed after less than 1 min and was not significantly dependent on incubation time up to 15 min. Five-minute incubations were used for further studies. This effect was dose-dependent, and other muscarinic agonists [acetylcholine (in the presence of 1 μM eserine), oxotremorine, and pilocarpine] also decreased TSH-induced cyclic AMP accumulation (Fig. 2). The antagonists atropine (0.1 μM) and QNB (0.1 μM) abolished the effect of carbachol. A $K_d$ of approximately 1.5 nM was found for atropine, using adenylate cyclase inhibition by carbachol.[4] The effects of carbachol on cells cultured in the presence of TSH were similar when assayed in the presence or absence of a phosphodiesterase inhibitor (MIX, 1 mM). The basal cyclic AMP synthesis measured during 5-min incubations in the presence of MIX was also depressed by carbachol.

[4] B. Verrier, unpublished results.

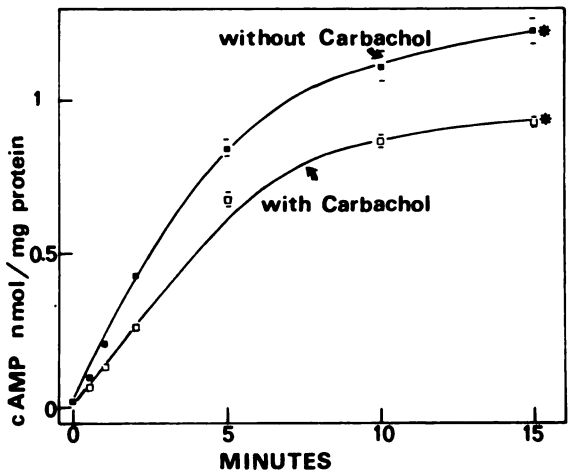



FIG. 1. *Effect of carbachol on acute TSH-stimulated AMP accumulation in cultured thyroid cells*

Thyroid cells were cultured for 4 days in the presence of TSH (100 μU/ml), washed, and challenged for various periods of time (0–15 min) with a maximal concentration of TSH (15 mU/ml) in the presence of MIX (1 mM) and with or without carbachol (10 μM). * Significance of the difference between control and carbachol-treated cells after 15 min: $p < 0.001$.

*Modulation of thyroid cell sensitivity to carbachol.* We have previously shown that the sensitivity of thyroid cells to acute TSH stimulation was increased by the chronic presence in the culture medium of TSH (100 μU/ml), $PGE_2$ (1 μM), or $Bt_2cAMP$ (1 mM). Higher concentrations of TSH induced a partial refractoriness, and after 4 days in culture in the absence of any stimulator (control cells) the cells had a low acute cyclic AMP response to TSH (12–15).

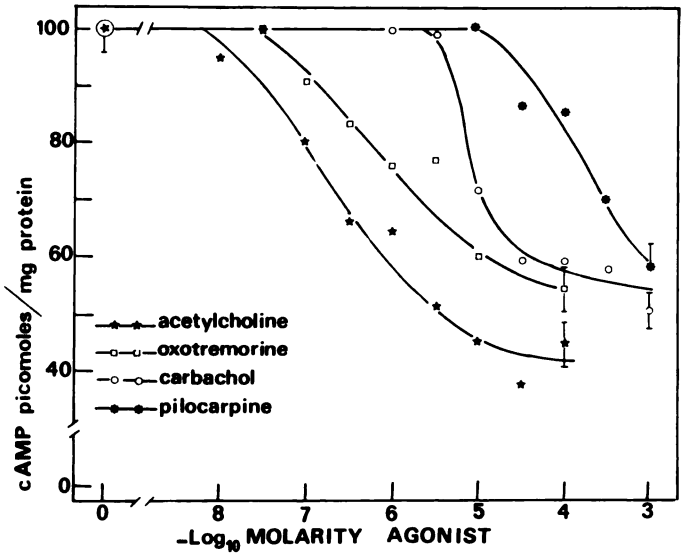



FIG. 2. *Effect of various concentrations of muscarinic agonists on acute TSH-stimulated cyclic AMP synthesis in cultured thyroid cells*

The experiment was carried out as in Fig. 1 except that the cells were acutely challenged for 5 min in the presence of graded concentrations of a series of muscarinic agonists (0–1 mM). Significance of the difference between control and cells treated with 0.1 or 1 mM agonist: $p < 0.001$.

Cells were cultured for 4 days under various conditions and challenged by TSH in the presence or absence of carbachol. In the absence of MIX, thyroid cells cultured under various conditions displayed different responses to carbachol (Table 1). Control cells, which had a very low sensitivity to TSH, showed no response to carbachol. Cells cultured in the presence of $Bt_2cAMP$, which were highly sensitive to acute TSH stimulation, also had a low response to carbachol (11% inhibition). Cells cultured in the presence of low concentrations of TSH (100 μU/ml) or of $PGE_2$ (1 μM) had the highest response to carbachol (50% and 35% inhibition, respectively). The effect of carbachol was therefore not linked to cell sensitivity to acute TSH stimulation. In the presence of MIX, a sensitivity to carbachol was revealed in control cells, and the effect observed in $Bt_2cAMP$ treated cells was higher than in the absence of MIX (Table 1).

*Effect of carbachol on $PGE_2$- and isoproterenol-stimulated cyclic AMP synthesis.* $PGE_2$ and isoproterenol are known as stimulators of thyroid cell cyclic AMP synthesis. Thyroid cells cultured in the presence of TSH (100 μU/ml) are highly sensitive to $PGE_2$ stimulation, and the cyclic AMP response was decreased by carbachol (Table 2). Cells cultured in the absence of stimulators are sensitive to isoproterenol stimulation (15), and carbachol also decreased this response (Table 2). The inhibitory effect of the muscarinic agonist was therefore not specific for TSH-stimulated cyclic AMP accumulation.

*Effect of carbachol on cyclic AMP levels in cells prestimulated by TSH or isoproterenol.* When cells were stimulated by TSH, even in the presence of 1 mM MIX, the intracellular level of cyclic AMP plateaued after 15 min, probably as a result of an incomplete inhibition of phosphodiesterase. The subsequent addition of carbachol resulted in a fall in the cyclic AMP level (30% decrease after 10 min) (Fig. 3A). When isoproterenol was used as a stimulator and if carbachol was added 10 min later together with propranolol (10 μM), the subsequent de-

TABLE 1

*Effect of culture condition on inhibition by carbachol of the cyclic AMP response to TSH stimulation*

Cells were cultured under different conditions, as indicated, for 4 days and then challenged for 5 min with TSH (15 mU/ml) in the presence or absence of MIX (1 mM) with or without carbachol (20 μM), and cyclic AMP was measured. Values in parentheses represent percentage of inhibition produced by carbachol. Basal cyclic AMP levels did not depend on culture conditions and were approximately 10 ± 2 pmoles/mg of protein. Statistical analysis: control versus carbachol.

| Culture condition | Cyclic AMP | | | |
|---|---|---|---|---|
| | Without MIX | | With MIX | |
| | Control | Carbachol | Control | Carbachol |
| | *pmoles/mg protein* | | | |
| Control | 16 ± 1 | 19 ± 2 (0)[a] | 88 ± 1 | 63 ± 2 (28)[b] |
| TSH (25 μU/ml) | 71 ± 5 | 57 ± 1 (20)[c] | 228 ± 33 | 175 ± 1 (23)[a] |
| TSH (100 μU/ml) | 112 ± 1 | 56 ± 2 (50)[d] | 835 ± 40 | 416 ± 9 (50)[d] |
| TSH (1000 μU/ml) | 85 ± 2 | 43 ± 3 (50)[d] | 789 ±70 | 328 ± 5 (58)[d] |
| $Bt_2cAMP$ (1 mM) | 969 ± 55 | 857 ± 49 (11)[a] | 1535 ± 17 | 941 ± 70 (38)[d] |
| $PGE_2$ (1 μM) | 301 ± 4 | 195 ± 1 (35)[d] | 561 ± 25 | 432 ± 30 (22)[e] |

[a] Not significant.
[b] $p < 0.01$.
[c] $p < 0.05$.
[d] $p < 0.001$.
[e] $p < 0.02$.

TABLE 2

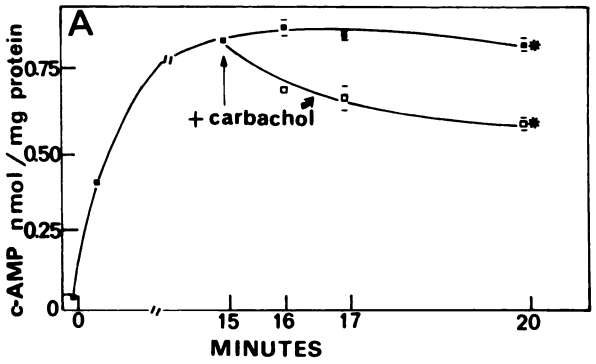
*Effect of carbachol on cyclic AMP response to $PGE_2$ and isoproterenol*

Cells were cultured for 4 days in the presence or absence of TSH (100 μU/ml) and then challenged for 5 min with $PGE_2$ (1 μM) or isoproterenol in the presence of MIX (1 mM) with or without carbachol (0.1 mM). Values in parentheses represent percentage of inhibition produced by carbachol.

| Culture condition | Incubation condition | Cyclic AMP | |
|---|---|---|---|
| | | Control | Carbachol |
| | | *pmoles/mg protein* | |
| TSH (100 μU/ml) | $PGE_2$ (1 μM) | 639 ± 12 | 514 ± 7 (19)[a] |
| Control | Isoproterenol (10 μM) | 254 ± 3 | 183 ± 8 (28)[a] |

[a] Significantly different ($p < 0.001$) from the corresponding control.

crease in cyclic AMP content was not affected by the presence of carbachol (Fig. 3B). Taken together, these results suggest that carbachol was acting on cyclic AMP synthesis rather than on its degradation.



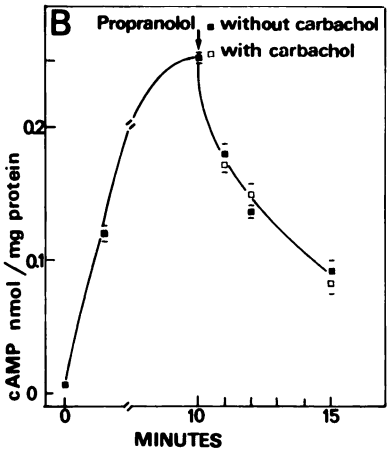




FIG. 3. *Effect of carbachol on the intracellular cyclic AMP content of cultured thyroid cells prestimulated with TSH or isoproterenol*

A. Cells were cultured for 4 days in the presence of TSH (100 μU/ml). After washing, they were stimulated with TSH (15 mU/ml) in the presence of 1 mM MIX. After 15 min the incubation was continued in the absence (■) or presence (□) of 20 μM carbachol. The cellular cyclic AMP content was measured at various incubation times after the addition of carbachol. * Significance of the difference between control and carbachol treated cells: $p < 0.001$.

B. Cells were cultured for 4 days in unsupplemented medium (control cells). After washing they were challenged with 10 μM isoproterenol in the presence of 1 mM MIX. After 10 min of incubation, propranolol (0.1 mM) was added with (□) or without (■) 0.1 mM carbachol.

*Effect of carbachol on modulation of cyclic GMP levels.* An effect of carbachol on intracellular cyclic GMP levels has been reported in other systems (23–25) and in the thyroid gland (3, 5, 7). Under the conditions used for our study of the effects of carbachol on acutely stimulated cyclic AMP accumulation in cultured thyroid cells, no change in cyclic GMP level was detected. The cells were able to synthesize cyclic GMP, since nitroprusside (1 mM) a known stimulator of guanylate cyclase, increased the intracellular cyclic GMP level 5-fold after a 5-min incubation (results not shown). However, after a 15-min preincubation in the presence of 1 mM MIX, the addition of carbachol, after 1 min of incubation, induced a small (1.5-fold) and transient increase in the cyclic GMP level. Our results show that the effect on cyclic AMP accumulation was not a consequence of an effect on the cyclic GMP level.

*Effect of carbachol on iodide metabolism.* In cultured thyroid cells, TSH acutely stimulates iodide efflux and iodide organification. Carbachol had no effect on TSH-stimulated iodide efflux but decreased the stimulation of organification (results not shown). This effect might be a consequence of the lower increase in cyclic AMP level.

*Characterization of muscarinic receptors on cultured thyroid cells.* Muscarinic receptors were first identified using homogenates derived from thyroid cells cultured for 4 days in the presence of TSH (100 μU/ml). The binding was performed at 26°. Nonspecific binding, measured in the presence of 10 μM scopolamine, was linearly dependent on QNB concentration. Total and specific binding were linearly dependent on protein concentration up to 120 μg/assay. The standard assay contained about 80 μg of protein.

Binding kinetics showed that specific binding was maximal after 30 min of incubation in the presence of 1 nM QNB (Fig. 4). In the presence of 0.1 nM QNB the plateau

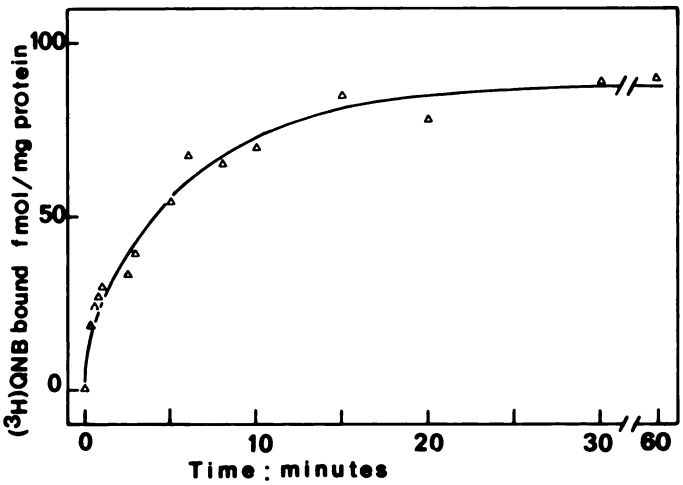



FIG. 4. *Time course of specific [$^3$H]QNB binding to homogenates derived from cultured thyroid cells*

Cells were cultured for 4 days in the presence of TSH (100 μU/ml). After washing, they were homogenized in NaCl/Pi. Aliquots of the homogenate were incubated with [$^3$H]QNB (1 nM) at 26° for various times in the presence or absence of 10 μM scopolamine. Samples were filtered on glass discs. Binding in the presence of scopolamine was subtracted from total binding, giving saturable specific binding.

was obtained between 30 and 60 min. Binding studies were therefore performed after a 60-min incubation at 26°. Saturation curves showed that maximal specific binding was obtained with a QNB concentration between 1 and 1.5 nM (Fig. 5). A Scatchard plot (Fig. 6) of the saturation data allowed the determination of a single equilibrium constant ($K_D$ = 0.32 nM) and of the binding capacity, which can be calculated as a number of binding sites per cell ($N$ = 4335 sites/cell).

A series of muscarinic agonists (acetylcholine, carbachol, oxotremorine, arecholine, and pilocarpine) and the muscarinic antagonists atropine and scopolamine completed with [$^3$H]QNB for the specific binding sites (Fig. 7). Calculated Hill coefficients, derived from competition experiments, were 1 for the antagonists and approximately 0.7 for the agonists.

*Modulation of the QNB binding capacity.* Cells were cultured in the presence or absence of TSH, and the QNB binding sites were characterized on the corresponding homogenates. A small change in the equilibrium dissociation constant was consistently observed between control (0.20 nM) and TSH-treated cells (0.32 nM) (Fig. 6). Control cells had a low number of muscarinic receptors (800 receptors/cell), and the QNB binding capacity was highly increased by chronic treatment of cells with TSH or $PGE_2$ (4300 and 5600 receptors/cell, respectively) (Table 3). The maximal effect of TSH on the QNB binding capacity was obtained with TSH (100 μU/ml). No further increase was observed when the TSH concentrations present in the culture medium was increased up to 10 mU/ml. The effect of $PGE_2$ occurred in the 0.01–1 μM concentration range, as did the effect of $PGE_2$ on TSH sensitivity previously reported (14). In contrast, $Bt_2$cAMP treated cells, which were highly sensitive to acute TSH stimulation but displayed a relatively low response to carbachol, had a small number of QNB binding sites (1420 receptors/cell) as compared with TSH-treated cells. Nonspecific QNB binding was not affected by culture conditions.

Similar differences were observed when washed, 10,000

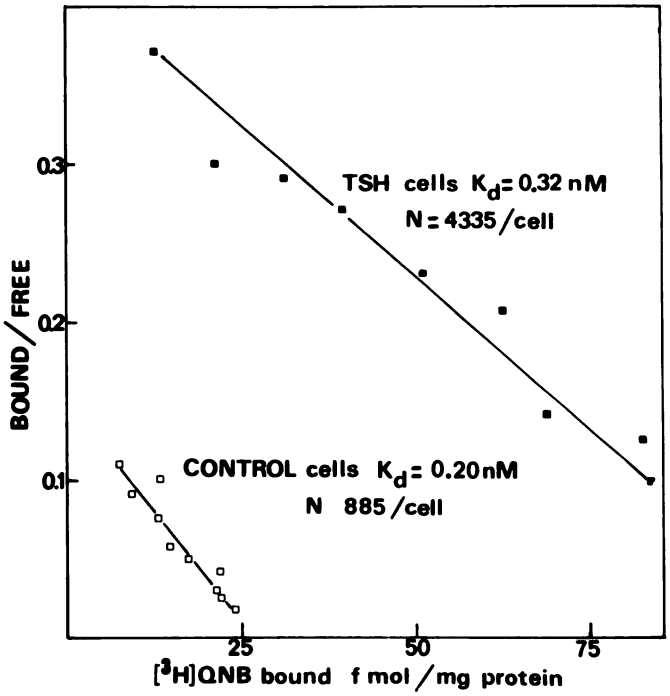



FIG. 6. *Scatchard plot of saturation binding data*
Thyroid cells were cultured with (■) or without (□) TSH (100 μU/ml) for 4 days, and [$^3$H]QNB binding was measured as in Fig. 5.

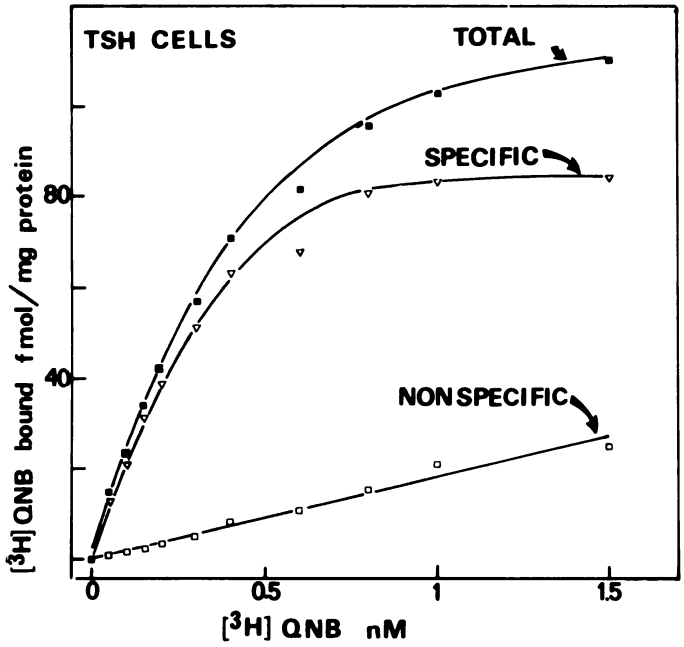



FIG. 5. *Saturation of QNB binding sites in homogenates derived from thyroid cells cultured in the presence of TSH (100 μU/ml)*
Cells were cultured for 4 days with TSH. After washing, they were homogenized in NaCl/Pi, and aliquots of the homogenate were incubated at 26° for 1 hr in the presence of increasing concentrations of [$^3$H]QNB. Bound radioactivity was measured after filtration on glass-fiber discs. Nonspecific binding was evaluated by incubation in the presence of 10 μM scopolamine and subtracted from total binding. A maximum of 440 cpm counted over 10 min was measured for the specific binding.

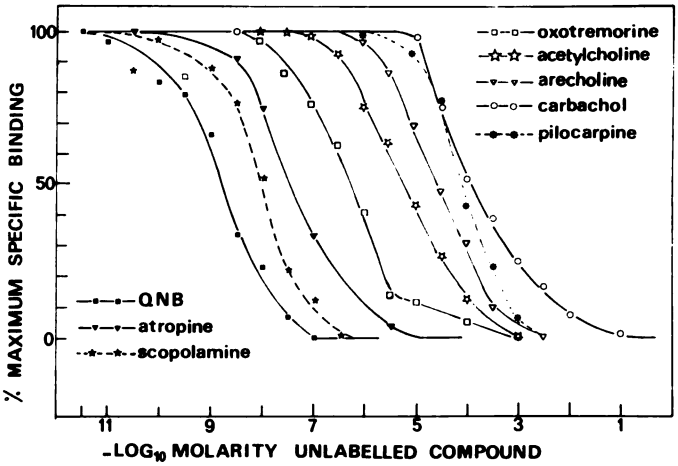



FIG. 7. *Competition by various agonists and antagonists of [$^3$H] QNB specific binding*
Homogenates derived from thyroid cells cultured for 4 days in the presence of TSH (100 μU/ml) were incubated at 26° for 1 hr in the presence of 1 nM [$^3$H]QNB and of various concentrations of unlabeled muscarinic agonists or antagonists. Nonspecific binding was subtracted.

TABLE 3

*Influence of culture condition on the number of [$^3$H]QNB binding sites*

Cells were cultured for 4 days in the presence or absence of various stimulators. The [$^3$H]QNB binding capacity of the corresponding homogenates was measured. Significance of observed differences: control or $Bt_2$cAMP versus TSH or $PGE_2$, $p < 0.001$; control versus $Bt_2$cAMP, $0.02 < p < 0.05$.

| Culture condition | [$^3$H]QNB binding sites/cell |
|---|---|
| Control | 880 ± 103 |
| TSH (100 μU/ml) | 4335 ± 333 |
| $PGE_2$ (1 μM) | 5600 ± 430 |
| $Bt_2$cAMP (1 mM) | 1420 ± 118 |

× *g* pellets derived from cell homogenates were used for binding studies and the same QNB binding parameters were obtained using plasma membranes purified from cultured cells (not shown). The evolution of QNB binding capacity as a function of time in culture is shown in Fig. 8. Almost no binding was detected on freshly isolated cells, likely as a result of the action of trypsin. Control cells progressively regained muscarinic receptors within 4 days in culture. The relative effect of TSH was already maximal on day 1, but the absolute number of receptors increased continuously for up to 4 days in culture. This evolution was not similar to that previously described for TSH receptors in control cells, which decreased between day 1 and day 4 in culture (26).

## DISCUSSION

Our results show that cultured thyroid cells respond to acute carbachol stimulation by a decrease of their cyclic AMP response to thyrotropin as previously known from studies using thyroid gland slices (1–3). This result confirms the existence of a cholinergic sensitivity on thyroid epithelial cells themselves, since only these cells were present in our cultures. The characteristics of the effect favor an action on cyclic AMP synthesis rather than on degradation. This possibility is further supported by studies using particulate fractions derived from cultured thyroid cells (27).

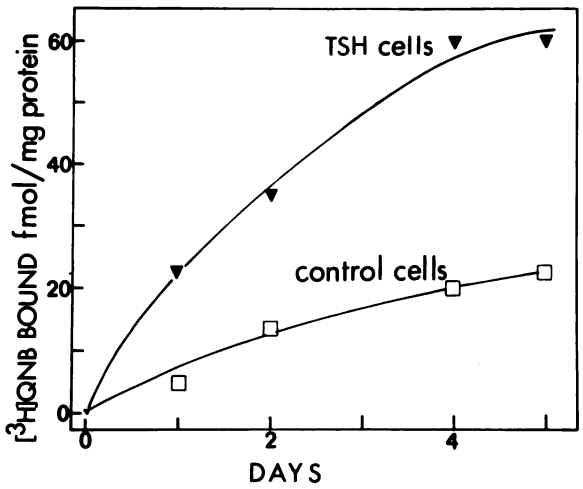



FIG. 8. *Evolution of thyroid cell muscarinic receptors during 4 days in culture*

Freshly isolated cells were cultured in the presence or absence of TSH (100 μU/ml). Specific binding of [$^3$H]QNB under saturating conditions was measured after different times in culture.

In various cell systems, carbachol has been shown to induce a large and transient increase in the intracellular cyclic GMP level (24, 25). Similar results have been reported using dog thyroid glands (5, 7). Our results show that the muscarinic receptor-mediated effect of carbachol on cyclic AMP level was not a consequence of modulation of cyclic GMP levels, since no effect on this parameter was observed in any of the cells tested. Either this lack of effect on cyclic GMP level is specific for the porcine thyroid cell or the conditions for the expression of the effect of carbachol on cyclic AMP and on cyclic GMP synthesis are different. Further studies are in progress using other culture conditions which allow a better expression of thyroid cell differentiation by the use of collagen rafts (28).

The concentration of carbachol necessary for half-maximal effect is in good agreement with previous results on thyroid gland slices and other systems. The thyroid cell muscarinic receptor has properties comparable to those of other similar receptors as defined by QNB binding studies. The equilibrium dissociation constant varies from 0.06 nM for NIE 115 neural cell lines (29) to 0.35 nM for rat parotid (30).

The QNB binding capacity is dependent on cell differentiation: maximal capacity is observed in cells derived from the nervous system (29). There is little evidence for a modulation of the binding capacity in a given system by compounds other than the muscarinic agonists themselves, which induced a desensitization (31). Chronically low acetylcholinesterase activity or parasympathetic denervation induce a reduced binding of QNB in brain and parotid respectively (30, 32).

Our results show that the thyroid cell stimulators TSH and $PGE_2$ increased the number of muscarinic receptors. The maximal number of sites obtained (5600/cell) in $PGE_2$-treated cells was far below values observed for cells derived from the nervous system ($2.5 \times 10^4$ receptors/cell) (29). The possible physiological significance of this effect of thyrotropin is not clear.

The number of muscarinic receptors present on thyroid cells is not correlated with the sensitivity of the adenylate cyclase system to acute TSH stimulation. The 5-fold TSH-induced and 6-fold $PGE_2$-induced increase in QNB binding capacity was not mimicked by $Bt_2$cAMP treatment (1.6-fold increase). The effects of TSH and $PGE_2$ on this parameter are therefore not a consequence of sustained stimulation of cyclic AMP synthesis by TSH or $PGE_2$. The relationship between the number of QNB binding sites and the acute response to carbachol remains to be clarified. The affinity differences observed between receptors present on cells cultured in the presence or absence of TSH are small as defined by QNB binding. However, these receptors may differ by other properties not revealed by this technique; indeed, agonist binding to rat brain muscarinic receptors showed the existence of three populations of receptors with super-high, high, and low affinities which have the same affinity for antagonists (33, 34).

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