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The Effects of Alkylated Xanthines on Cyclic AMP Accumulation in Dog Thyroid Slices Exposed to Carbamylcholine

FRANÇOISE MIOT,[1,2] CHRISTOPHE ERNEUX,[1,3] JACK N. WELLS,[4] AND JACQUES E. DUMONT[1]

Institute of Interdisciplinary Research, Free University of Brussels, School of Medicine, Campus Erasme, B-1070 Brussels, Belgium, and Department of Pharmacology, Vanderbilt University, School of Medicine, Nashville, Tennessee 37232

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SUMMARY

In dog thyroid slices stimulated by thyrotropin (TSH), activation of muscarinic cholinergic receptors leads to a decrease in cyclic AMP levels. Previous studies have established that carbamylcholine enhances cyclic GMP levels and inhibits cyclic AMP accumulation. Several experimental data have suggested that these effects are mediated by an increase in intracellular Ca^{2+} levels. The inhibition of cyclic AMP accumulation results in accelerated catabolism. Dog thyroid phosphodiesterase activity is due to a mixture of three enzyme forms: a calmodulin-sensitive form, a cyclic GMP-stimulated form and a cyclic AMP-specific form. This report is concerned with the comparison of the effects of several phosphodiesterase inhibitors on cell-free phosphodiestease activity and on cyclic nucleotide accumulation in intact cells. Alkylated xanthines, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724), and 2-*O*-propoxyphenyl-8-azapurin-6-one (M & B 22948) were studied as inhibitors of partially purified dog thyroid phosphodieterases and for their ability to alter cyclic AMP and cyclic GMP accumulation in dog thyroid slices that had been stimulated with TSH and/or carbamylcholine. 1-Methyl-3-isobutylxanthine (MIX) and 7-benzyl MIX were the most potent inhibitors of phosphodiesterase activities in the crude soluble and particulate fractions but exhibited no selectivity for inhibiting cyclic AMP or cyclic GMP hydrolysis. In dog thyroid slices stimulated by TSH and in the absence of carbamylcholine, Ro 20-1724 and 1-isoamyl-3-isobutylxanthine (IIX) were the most effective compounds to potentiate the accumulation of cyclic AMP. The rank order of abilities to potentiate cyclic AMP accumulation in dog thyroid slices stimulated by TSH paralleled the rank order of potencies to inhibit the cyclic AMP-specific phosphodiesterase. In the presence of carbamylcholine, the observed decrease in cyclic AMP levels was attenuated by MIX, 8-methoxymethyl MIX (8-MeOMe MIX), 7-benzyl MIX, and M & B 22948, the most potent inhibitors of the calmodulin-sensitive phosphodiesterase. MIX, 8-MeOMe MIX, and 7-benzyl MIX inhibited the cyclic GMP-stimulated phosphodiesterase in the same rank order of potencies as the calmodulin-sensitive enzyme, but M & B 22948 did not significantly inhibit the cyclic GMP-stimulated enzyme activity. IIX and Ro 20-1724 did not alter the carbamylcholine-induced inhibition of cyclic AMP accumulation. The data presented in this study indicate that selective inhibitors of the calmodulin-sensitive phosphodiesterase are able to relieve *in vitro* the carbamylcholine-induced inhibition of cyclic AMP accumulation, whereas selective inhibitors of the cyclic AMP-specific phosphodiesterase are not. This suggests that in the dog thyroid, in the presence of carbamylcholine, the calmodulin-sensitive phosphodiesterase is activated by a rise in the free Ca^{2+} level and becomes the dominant isozyme of cyclic AMP catabolism.

INTRODUCTION

Activation of muscarinic cholinergic receptors leads, in many tissues, to a decrease in cyclic AMP levels (1, 2). It has been demonstrated that in some of these systems, e.g., rabbit myocardium (3) and rat parotid (4), receptor-mediated inhibition of adenylate cyclase is responsible for the observed reduction in cyclic AMP lev-

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[1] Free University of Brussels.

[2] Fellow of the Institut pour la Recherche Scientifique dans l'Industrie et l'Agriculture.

[3] Chargé de Recherches au Fonds National de la Recherche Scientifique.

[4] Vanderbilt University.

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els. In contrast, in three different cases muscarinic receptor-mediated activation of cyclic nucleotide phosphodiesterases may, at least partially, account for decreased cyclic AMP levels: in dog thyroid (5, 6), in human diploid lung fibroblasts (2), and in human astrocytoma cells (7). The mechanism(s) involved in this phosphodiesterase activation are still poorly understood. In the dog thyroid, carbachol exerts several effects *in vitro*: (a) it enhances cyclic GMP accumulation, protein iodination, and glucose oxidation; and (b) it inhibits cyclic AMP accumulation and hormone secretion (8). Several types of experimental data indicate that these effects are mediated by intracellular free Ca^{2+}: the actions of carbachol are abolished in calcium-depleted slices as well as in the presence of calcium competitors; they are mimicked by the divalent cation ionophore A23187 in the presence of Ca^{2+} and by high concentrations of calcium itself (6, 8–10). Finally, with regard to the inhibition of cyclic AMP accumulation, the effects of both carbachol and ionophore A23187 are relieved in the presence of MIX[5] but not Ro 20-1724, two potent phosphodiesterase inhibitors (5, 6).

Dog thyroid contains at least three forms of phosphodiesterases: a calmodulin-sensitive form, a cyclic GMP-stimulated form, and a cyclic AMP-specific form (6). To gain more insight into the functional role of these enzyme forms after exposure of thyroid tissue to cholinergic stimuli, the present report is concerned with the comparison of potencies of several inhibitors on cell-free phosphodiesterase activity and on cyclic nucleotide accumulation in intact cells. Alkylated xanthines, MIX, 7-benzyl MIX, 8-MeOMe MIX, and IIX, were chosen since the calmodulin-sensitive and the cyclic AMP-specific phosphodiesterase activities of pig coronary arteries differ in their susceptibilities to inhibition by xanthines with varied substituents. 7-Benzyl MIX and 8-MeOMe MIX are relatively selective inhibitors of the calmodulin-sensitive isozyme, whereas IIX inhibits selectively the "low K_m" cyclic AMP phosphodiesterase activity (11, 12). These compounds were compared with Ro 20-1724 and M & B 22948, two relatively specific inhibitors of cyclic AMP and cyclic GMP phosphodiesterase activities, respectively (13, 14). Some of these data have been reported elsewhere in preliminary form (15, 16).

MATERIALS AND METHODS

Phosphodiesterase assay. The phosphodiesterase preparation was incubated as previously described (17). The standard incubation mixture contained 40 mM Tris-HCl (pH 7.4), 5 mM $MgSO_4$, 5 mM 2-mercaptoethanol, bovine serum albumin (1 mg/ml), and tritiated cyclic nucleotides. Phosphodiesterase was incubated for 10–30 min to obtain 5–20% hydrolysis of substrate in the absence of inhibitor. 5′-Nucleotidase was used to convert [^{3}H]AMP or [^{3}H]GMP to the corresponding nucleoside. Nucleosides were separated from the cyclic nucleotides by QAE-Sephadex A-25 chromatography. All results are means of duplicate or triplicate determinations. Protein concentrations were measured by the method of Lowry *et al.* (18).

All of the compounds tested, with the exception of 8-MeOMe MIX, which was diluted to 1 mM in water, were prepared in 100% Me_2SO at 40 mM. At the highest concentration used, 2.5%, Me_2SO did not alter any step of the phosphodiesterase assay.

Phosphodiesterase preparation. Dog thyroids, sliced and minced with scissors (17), were homogenized with a motor-driven Teflon-glass homogenizer in Buffer A, containing 20 mM Tris-HCl (pH 7.4), 2 mM $MgCl_2$, 0.4 mM PMSF, 2 mM benzamidine, 5 μM leupeptin, trypsin inhibitor (25 mg/liter), 5 mM 2-mercaptoethanol, and 0.25 M sucrose (1 g of thyroid tissue per 5 ml of buffer medium). The supernatant fraction was obtained by centrifugation at 40,000 × *g* for 60 min at 4°. The particulate fraction was washed twice in the homogenization medium.

The cyclic GMP-stimulated and the cyclic AMP phosphodiesterase preparations were obtained by DEAE-cellulose chromatography. The crude supernatant fraction (4 ml) was applied to a DEAE-cellulose column (0.9 × 13 cm). Phosphodiesterase activity was eluted with an exponential gradient of 0–0.5 M $(NH_4)_2SO_4$ (400 ml) in Buffer A without sucrose (19). Peak fractions were concentrated with an Amicon PM 10 ultrafiltration cell and dialyzed overnight against Buffer A without sucrose. This step was followed by gel filtration on Sephacryl S-200 (17).

The calmodulin-sensitive phosphodiesterase was obtained according to the method of Klee *et al.* (20). An aliquot (20 ml) of dog thyroid supernatant fraction was applied to a DEAE-cellulose column (2.6 × 40 cm). The column was washed with 1 volume of 0.05 M $(NH_4)_2SO_4$, followed by 1 volume of 0.08 M $(NH_4)_2SO_4$ in a buffer containing 20 mM Tris-HCl (pH 7.5), 5 mM 2-mercaptoethanol, 2 mM benzamidine, 5 μM leupeptin, 20 μM EGTA, trypsin inhibitor (25 mg/liter), 0.4 mM PMSF, and 0.3 mM α-*N*-benzoyl-L-arginine methyl ester. Phosphodiesterase activity was eluted with the same buffer containing 0.15 M $(NH_4)_2SO_4$. This step of purification was followed by affinity chromatography on calmodulin-Sepharose as described earlier (6).

Measurement of cyclic nucleotide accumulation in intact cells. Dog thyroid slices were prepared and incubated as described previously (5). Briefly stated, thyroid glands of dogs that had been treated with thyroid extract (100 mg/10 kg; Thyranon Organon, Oss, The Netherlands) were sliced and incubated at 37° under an O_2/CO_2 (95:5, v/v) atmosphere in 2 ml of Krebs-Ringer bicarbonate buffer enriched with 8 mM glucose and bovine serum albumin (0.5 mg/ml). A preincubation of 1 hr was performed before the test incubation. The phosphodiesterase inhibitors were added to the medium 10 min before the end of the preincubation. The slices were then transferred to fresh medium containing the phosphodiesterase inhibitors and the other agents under study. For assay of the cyclic nucleotides, the incubation was stopped by boiling the slices in water for 5 min. The slices were homogenized and centrifuged, and the supernatant fractions were lyophilized. The lyophilized tissue extracts were resuspended in water. Cyclic AMP and cyclic GMP concentrations were determined by the method of Gilman (21) and by radioimmunoassay, respectively (22).

Materials. Cyclic AMP and cyclic GMP were obtained from Boehringer (Mannheim, Federal Republic of Germany). Tritiated cyclic nucleotides, obtained from the Radiochemical Centre (Amersham, United Kingdom) were purified on a Dowex AG50W-X8 cation-exchange resin column (23). PMSF, 5′-nucleotidase (*Crotalus atrox* venom), trypsin inhibitor from soybean, α-*N*-benzoyl-L-arginine methylester, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′N′*-tetraacetic acid, MIX, benzamidine, leupeptin, and carbamylcholine were purchased from Sigma Chemical Company (St. Louis, Mo.) QAE-Sephadex A-25 and Sephacryl S-200 were from Pharmacia (Upsala, Sweden) DE52 and DE23 were from Whatman (Maidstone, United Kingdom). TSH (bovine thyrotropin, Thytropar) was obtained from Armour (Kankakee, Ill.). Ro 20-1724 was provided by Hoffmann-La Roche Company (Nutley, N. J.). IIX, 7-benzyl MIX, and 8-MeOMe MIX were prepared according to published procedures (11, 12). M & B 22948 was obtained

[5] The abbreviations used are: MIX, 1-methyl-3-isobutylxanthine; 7-benzyl MIX, 1-methyl-3-isobutyl-7-benzylxanthine; IIX, 1-isoamyl-3-isobutylxanthine; 8-MeOMe MIX, 1-methyl-3-isobutyl-8-methoxymethylxanthine; Ro 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; M & B 22948, 2-O-propoxyphenyl-8-azapurin-6-one; Me_2SO, dimethyl sulfoxide; QAE-Sephadex, quaternary diethyl-(2-hydroxypropyl)aminoethyl-Sephadex; TSH, thyroid-stimulating hormone; PMSF, phenylmethylsulfonyl fluoride.

from May & Backer (Dagenham, United Kingdom). Calmodulin was purified from bovine brain according to the method of Yazawa *et al.* (24).

RESULTS

Inhibition of crude dog thyroid phosphodiesterases. Phosphodiesterase activity in the 40,000 × *g* supernatant fraction and pellet from a dog thyroid homogenate exhibited the following properties: (a) at 1 μM substrate concentration, cyclic GMP was the substrate preferred over cyclic AMP with either the soluble or particulate fraction as enzyme source (data not shown). At that substrate concentration, the ratio of cyclic AMP degrading activity to that of cyclic GMP was 0.3 ± 0.05 in the soluble fraction and 0.8 ± 0.05 in the particulate fraction (mean of ratio determined from four different preparations); (b) hydrolysis of 3 μM cyclic AMP was stimulated by 3 μM cyclic GMP; (c) in both the soluble and particulate fractions, MIX and 7-benzyl MIX were the most potent inhibitors of cyclic AMP and of cyclic GMP hydrolysis (Table 1). The potencies of the two xanthines to inhibit cyclic AMP and cyclic GMP hydrolysis were similar. In contrast, IIX and Ro 20-1724 appeared to be much less potent inhibitors of both cyclic AMP and cyclic GMP hydrolysis by soluble and particulate fractions than were MIX and 7-benzyl MIX.

Inhibition of partially purified, soluble, dog thyroid phosphodiesterases. Three enzymatic activities have been separated from a 40,000 × *g* dog thyroid supernatant fraction (6): a high-affinity cyclic AMP phosphodiesterase; a calmodulin-sensitive phosphodiesterase hydrolyzing both cyclic AMP and cyclic GMP, but more efficient for cyclic GMP; and a phosphodiesterase hydrolyzing both cyclic nucleotides and stimulated by micromolar cyclic GMP.

We have compared the inhibitory potencies of MIX, 7-benzyl MIX, IIX, 8-MeOMe MIX, Ro 20-1724, and M & B 22948 on the three partially purified phosphodiesterases. The inhibition of the cyclic GMP-stimulated phosphodiesterase was measured with 3 μM cyclic AMP as substrate in the presence of 3 μM cyclic GMP as effector. MIX and 8-MeOMe MIX were the most effective inhibitors of this activity. IIX, Ro 20-1724, and M & B 22948 in the 0.1–30 μM range had no effect, and 7-benzyl MIX appeared to be a weak inhibitor of this enzyme activity (Fig. 1). In a previous study of the rat liver cyclic GMP-stimulated phosphodiesterase (25), we reported that hydrolysis of cyclic AMP, at micromolar concentrations and without added cyclic GMP, was stimulated in the presence of 50 μM MIX. This effect has been reproduced by 8-MeOME MIX but not with the other analogues of MIX (data not shown).

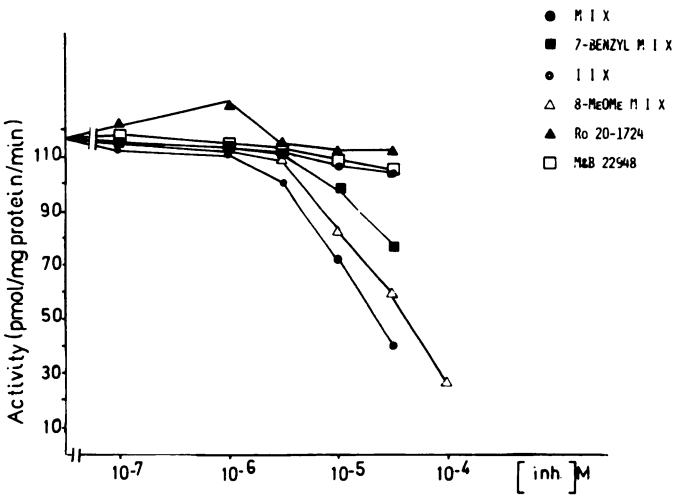

FIG. 1. *Inhibition of the partially purified dog thyroid cyclic GMP-stimulated phosphodiesterase activity*

Phosphodiesterase activity was measured at 3 μM cyclic AMP as substrate in the presence of 3 μM cyclic GMP as effector. The inhibitors were solubilized in 100% Me_2SO at 40 mM and further diluted in water. Results are expressed as picomoles of cyclic AMP hydrolyzed per minute per milligram of protein.

We have studied dog thyroid calmodulin-sensitive phosphodiesterase activity at a 1 μM cyclic AMP substrate level. Ro 20-1724 and IIX did not inhibit this enzymatic form, whereas MIX, 7-benzyl MIX, and 8-MeOMe MIX were potent inhibitors of cyclic AMP hydrolysis, with I_{50} values of 2–6 μM (Table 2). The rank order of potencies to inhibit the calmodulin-sensitive phosphodiesterase activity was the same in the presence or absence of calmodulin (data not shown). In contrast, IIX and Ro 20-1724 were the most potent inhibitors of the cyclic AMP-specific phosphodiesterase activity, with I_{50} values of 7.1 μM and 5.2 μM, respectively (Table 2). MIX and 7-benzyl MIX were about 2-fold and 10-fold less potent, respectively, than IIX and Ro 20-1724. 8-MeOMe MIX and M & B 22948, in the same concentration range (0.1–30 μM), were without effect on the cyclic AMP-specific phosphodiesterase activity.

Effects of phosphodiesterase inhibitors on cyclic nucleotide accumulation in dog thyroid slices. IIX and Ro 20-1724 were particularly potent in enhancing cyclic AMP levels in tissue stimulated by TSH (1 mU/ml). In the typical experiment shown in Fig. 2, 50 μM IIX and 50 μM Ro 20-1724 elicited 10-fold increases in the TSH-stimulated cyclic AMP accumulation. The rank order of abilities to potentiate cyclic AMP accumulation in TSH-stimulated tissue was also maintained at lower concentrations of agents (1–10 μM, data not shown). Cyclic GMP accumulation was measured in dog thyroid slices

TABLE 1

Inhibition of cyclic nucleotide phosphodiesterase activities in supernatant and particulate fractions of dog thyroid homogenates

The I_{50} value is the concentration of the agent required to produce 50% inhibition of the hydrolysis of 1 μM substrate. Some of these agents are insoluble above 100 μM; for those, the percentage inhibition at 100 μM is given if I_{50} was above 100 μM. Values are means ± standard error of the mean of triplicate determinations with four different preparations.

Inhibitor	I_{50}			
	Supernatant fraction		Particulate fraction	
	Cyclic AMP	Cyclic GMP	Cyclic AMP	Cyclic GMP
	μM	μM	μM	μM
MIX	7.7 ± 0.5	7.4 ± 0.5	75 ± 12	37 ± 8
7-Benzyl MIX	9.5 ± 1.8	9.0 ± 1.7	87 ± 12	54 ± 6
IIX	>100 (13%)	>100 (13%)	>100 (33%)	>100 (15%)
Ro 20-1724	>100 (14%)	>100 (7%)	>100 (16%)	>100 (0%)

TABLE 2

Inhibition of partially purified dog thyroid phosphodiesterases

Phosphodiesterase activity was measured at 1 μM cyclic AMP as substrate in the presence of 1 μg of calmodulin purified from bovine brain and 2 mM $CaCl_2$ for the calmodulin-sensitive phosphodiesterase. I_{50} was determined graphically. Values are means ± standard error of the mean of triplicate determinations; the number of different preparations is indicated in parentheses.

Inhibitor	I_{50}	
	Calmodulin-sensitive enzyme	Cyclic AMP-specific enzyme
	μM	μM
MIX	3.2 ± 0.8 (5)	12.8 ± 0.3 (4)
7-Benzyl MIX	6.3 ± 1.2 (3)	62 ± 6 (4)
IIX	>100 (3)	7.1 ± 1.9 (4)
8-MeOMe MIX	2.1 ± 0.1 (2)	>100 (2)
M & B 22948	11.0 ± 1.0 (2)	>100 (2)
Ro 20-1724	>100 (3)	5.2 ± 0.6 (4)

stimulated by 10 μM carbamylcholine. Representative data from experiments with four different dog thyroids showed that the cholinergic-induced increase in cyclic GMP levels (Fig. 3) was more potentiated in the presence of 7-benzyl MIX than with any of the other tested compounds.

When dog thyroid slices were incubated in the presence of TSH (1 mU/ml) and 10 μM carbamylcholine, cyclic AMP concentrations dropped nearly to control values (slices incubated without TSH). This inhibitory effect of carbachol was relieved by 80–100% by MIX, 7-benzyl MIX, and 8-MeOMe MIX at 50 μM (Fig. 2) but persisted with IIX and Ro 20-1724. However, the latter two compounds slightly potentiated cyclic AMP accumulation in the presence of TSH and carbamylcholine. M & B 22948 had little, if any, effect to potentiate TSH-induced increases in cyclic AMP concentration. The cholinergic-induced inhibition of cyclic AMP accumulation was relieved, however, by 50 μM M & B 22948.

DISCUSSION

In dog thyroid slices stimulated by TSH, carbamylcholine lowers the intracellular cyclic AMP concentration (5). We have previously reported that the inhibitory effect of carbamylcholine is mediated by an increase in free intracellular Ca^{2+} concentration (9, 10) and is related to an activation of phosphodiesterase activity rather than an inhibition of adenylate cyclase (5, 27). The cholinergic effect is not mediated by adenosine (26) but is relieved by MIX (5).

Increased phosphodiesterase activity could result from at least three distinct mechanisms: (a) an activation of

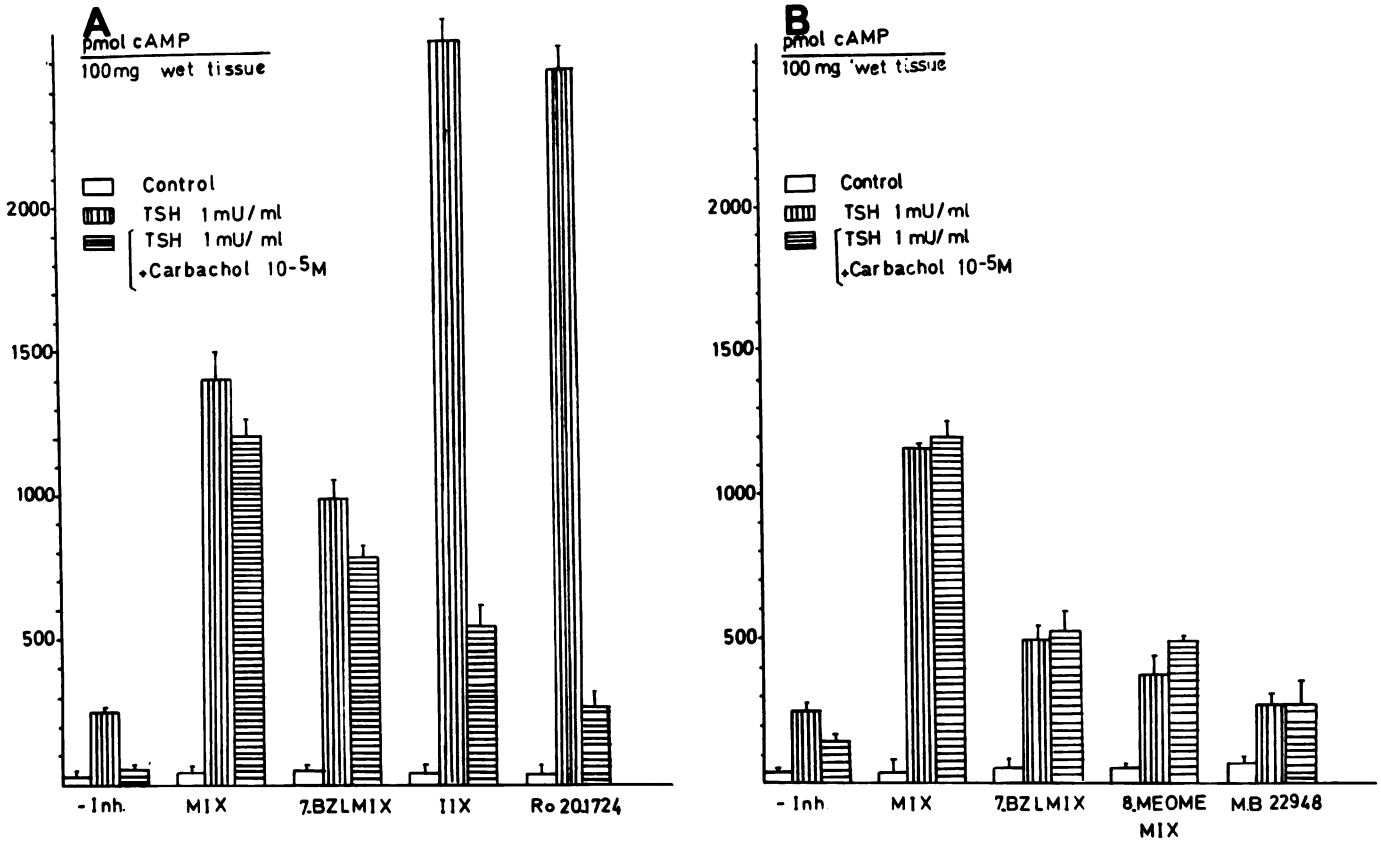

FIG. 2. *Cyclic AMP accumulation in dog thyroid slices incubated in the presence of TSH (1 mU/ml) + 10 μM carbamylcholine*

A. A preincubation of 1 hr in Krebs-Ringer bicarbonate buffer with the tested agents at 50 μM for the last 10 min was followed by a 1-hr incubation in the presence of the tested agents at 50 μM. All of these agents were diluted from an initial solution of 20 mM in 100% Me_2SO so that the final concentration of Me_2SO in the media did not exceed 0.25%. The control media were also supplemented with 0.25% Me_2SO.

B. MIX and 7-benzyl MIX were used as reference compounds.

Results are expressed as picomoles of cyclic AMP per 100 mg of wet tissue ± standard error of the mean.

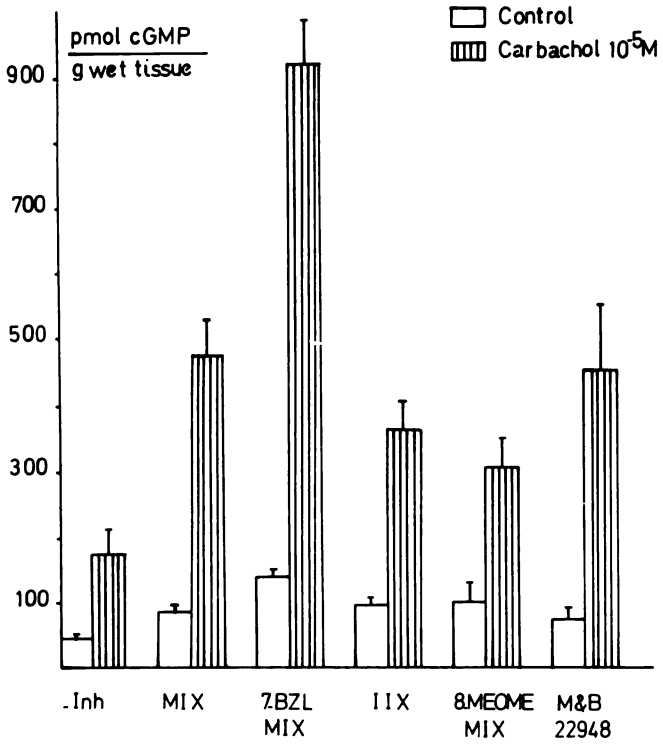

FIG. 3. *Cyclic GMP accumulation in dog thyroid slices incubated in the presence of 10 μM carbamylcholine*

A preincubation of 1 hr in Krebs-Ringer bicarbonate buffer with the tested agents at 50 μM for the last 10 min was followed by a 20-min incubation with the tested agents at 50 μM diluted as explained in the legend to Fig. 2. Results are expressed as picomoles of cyclic GMP per gram of wet tissue ± standard error of the mean.

the calmodulin-sensitive phosphodiesterase by changes in cytoplasmic calcium concentration or changes in the distribution of calmodulin (28); (b) an allosteric cyclic GMP-dependent stimulation (29); or (c) an activation of a membranous cyclic AMP phosphodiesterase after phosphorylation as reported upon insulin addition to hepatocytes (30). This study addressed the question of whether or not substituted xanthines with different inhibitor potencies against phosphodiesterase isozymes could be used as a tool to identify the mechanism(s) by which MIX suppresses the negative control of carbamylcholine on cyclic AMP accumulation in TSH-stimulated dog thyroid slices. We made the hypothesis that a single form of the dog thyroid phosphodiesterases would be directly implicated in this mechanism and that some of the alkylated xanthines would selectively depress the activity of the phosphodiesterase involved.

In a crude soluble or particulate fraction of a dog thyroid homogenate, MIX and 7-benzyl MIX, the most potent inhibitors of phosphodiesterase activity, do not exhibit selectivity for inhibition of cyclic AMP or cyclic GMP hydrolysis (Table 1). Such a selectivity has been observed in pig sperm crude soluble fraction (11). The data presented clearly establish that no correlation can be made between inhibitor potencies of crude phosphodiesterase activity and the abilities of those inhibitors to potentiate cyclic AMP accumulation in intact thyroid cells stimulated by TSH. IIX and Ro 20-1724, the least potent inhibitors of crude phosphodiesterase activity, appeared to have pronounced effects in potentiating cyclic AMP (Fig. 2). It was therefore necessary to study inhibitor potencies on each isolated isozyme. In the absence of carbachol, the rank order of ability of agents to potentiate cyclic AMP accumulations corresponded exactly and exclusively to the rank order of potency to inhibit the cyclic AMP-specific phosphodiesterase (Table 2). In contrast, in the presence of carbachol, IIX and Ro 20-1724 were much less efficient in potentiating cyclic AMP accumulation and did not relieve the carbachol-induced reduction of cyclic AMP concentration in TSH-stimulated slices. These data suggest that, in intact cells and with basal free Ca^{2+} levels, the cyclic AMP levels are mainly controlled by the cyclic AMP-specific phosphodiesterase and that the calmodulin-sensitive enzyme exhibits little activity.

It is possible that increased phosphodiesterase activity, after the thyroid cells are exposed to carbamylcholine, could be provoked by cyclic GMP through an activation of the cyclic GMP-stimulated isozyme. However, several indirect arguments presented earlier bear against this hypothesis (6, 9). As shown in this study, one of the inhibitors, M & B 22948, was able to relieve the carbachol-induced cyclic AMP inhibition but, at concentrations up to 30 μM, had no effect on the cyclic GMP-stimulated phosphodiesterase (Fig. 1), suggesting that inhibition of the latter enzyme is not necessary for the relief of carbachol inhibition.

We have observed that the abilities of compounds to relieve the negative control of carbamylcholine on cyclic AMP accumulation in intact tissue appeared to be predictable from the relative abilities of the compounds to inhibit the calmodulin-sensitive phosphodiesterase. Those inhibitors which potently inhibit this isozyme, i.e., 8-MeOMe MIX, MIX, 7-benzyl MIX, and M & B 22948, also relieve the carbachol-induced decrease in cyclic AMP. In contrast, IIX and Ro 20-1724, two relatively potent inhibitors of the cyclic AMP-specific phosphodiesterase activity but relatively weak inhibitors of the calmodulin-sensitive phosphodiesterase activity, did not relieve the carbachol effect on cyclic AMP. The data are therefore consistent with the involvement of the calmodulin-sensitive phosphodiesterase in the carbachol-dependent stimulation of intact thyroid cell cyclic AMP phosphodiesterase activity.

Cholinergic agonists decrease cyclic AMP accumulation either by an inhibition of adenylate cyclase activity (1, 4), by an activation of phosphodiesterase activity (5), or both (31). This is largely dependent on the cell type under investigation. The data of this study suggests that the carbachol-induced free Ca^{2+} rise stimulates the calmodulin-sensitive phosphodiesterase and consequently the hydrolysis of cyclic AMP in dog thyroid. It also suggests that the latter enzyme form becomes the dominant cyclic AMP-degrading enzyme in cells incubated with carbachol. On the contrary, studies with the same alkylated xanthines in bovine coronary artery strips relaxed by isoproterenol establish that the calmodulin-sensitive phosphodiesterase is not involved in the catabolic pathway of cyclic AMP (32). However, the carbachol effect on human astrocytoma cells, which exhibits characteristics similar to those described in dog thyroid, may be an example of a mechanism similar to that demonstrated in this study (31).

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Send reprint requests to: Dr. Françoise Miot, Institute of Interdisciplinary Research, Free University of Brussels, School of Medicine, Campus Erasme, B-1070 Brussels, Belgium.