Effects of Therapeutic Agents on Cyclic AMP Metabolism *In Vitro*

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Abstract □ One hundred and fifty-eight compounds representing 49 classes of therapeutic agents were examined for their effects on steroidogenesis in isolated rat adrenal cells, on lipolysis in isolated rat lipocytes, on the activity of guinea pig lung adenylate cyclase, and on the activity of rat brain and cat heart cyclic nucleotide phosphodiesterase preparations. Classes of drugs active in the CNS appeared particularly active in the *in vitro* systems investigated, as did antiparasitic agents. Experience with general screening of compounds for effects on phosphodiesterase activity, along with data reported here, indicated a correlation between compounds with pharmacological activity *in vivo* and inhibition of phosphodiesterase activity *in vitro*. This is *not* to say that the pharmacological activities of the compounds necessarily arise from alterations of adenosine-3′,5′-monophosphate (cyclic AMP) metabolism.

Keyphrases □ Cyclic AMP metabolism, *in vitro*—effects of 158 compounds (49 classes of therapeutic agents) □ Adenosine-3′,5′-monophosphate metabolism, *in vitro*—effects of 158 compounds (49 classes of therapeutic agents) □ Phosphodiesterase activity—49 classes of therapeutic agents □ Cyclase activity—49 classes of therapeutic agents □ Lipolysis activity—49 classes of therapeutic agents □ Steroidogenesis activity—49 classes of therapeutic agents

The role of adenosine-3′,5′-monophosphate (cyclic AMP) as a "second messenger" in various biological processes was the subject of several recent reviews (1–5). These reviews amply documented the wide range of cellular responses to hormone stimulation mediated by this nucleotide. The large variety of processes mediated by cyclic AMP raised the possibility that some compounds which have demonstrated pharmacological effectiveness might act *via* effects on these processes. In this study, members of a variety of drug classes were examined in an effort to identify those agents capable of interacting with cyclic AMP-related systems and/or enzymes.

Forty-nine therapeutic classes were chosen for examination. The agents representing each class were chosen arbitrarily; an effort was made, however, to include as wide a range of chemical structures as possible. All of the drug classes have shown activity in humans, although several experimental compounds that possess pharmacological activities in animals but have not yet been administered to man were included in this survey.

Drugs were evaluated for their effects on the following systems: (*a*) epinephrine-activated lipolysis in fat cells isolated from rat epididymal fat pads, (*b*) ACTH-activated steroidogenesis in cells isolated from the rat adrenal gland, (*c*) cyclic AMP degradation by partially purified preparations of cyclic nucleotide phosphodiesterase from rat brain and cat heart, and (*d*) cyclic AMP synthesis by a particulate fraction from guinea pig lung containing adenylate cyclase activity.

The systems chosen represent an effort to include a spectrum of cellular and subcellular preparations and tissues that would provide an indication of interaction between the drugs of interest and cyclic AMP metabolism in animals. Thus, phosphodiesterase preparations were chosen from CNS and peripheral tissues. The two cell systems utilized are unrelated in function and exhibit widely different characteristics. The hormonally induced responses of both cell types have been shown to be cyclic AMP mediated (6–10). The examination of the effects of drugs on these cells introduces, in addition to influences upon the enzymes that synthesize and degrade cyclic AMP, the factors of plasma membrane permeability, possible cellular compartmentalization of cyclic AMP synthesis and degradation, and interactions of cyclic AMP with intracellular macromolecules.

EXPERIMENTAL[1]

Epinephrine-Stimulated Lipolysis—Lipocytes were prepared from distal segments of rat epididymal fat pads by the method of Rodbell (12), using 2% albumin rather than 4%; glucose was eliminated from all steps in the procedure. Five milligrams of collagenase/g. of fat was used in the digestion of the minced fat. Male Sprague-Dawley rats (125–225 g.), allowed free access to both food and water prior to sacrifice by decapitation, served as the source of the fat. Following three washes, the cells were suspended in seven times the packed cell volume of Krebs-Ringer phosphate-albumin buffer (12). One-milliliter aliquots of this cell suspension were added to plastic scintillation vials containing the buffer, epinephrine (as described later), and the drug to be tested, bringing the final incubation volume to 2.5 ml. After gentle mixing, the vials were capped and incubated at 37° for 1 hr. in a water bath[2], at a shaking rate of approximately 160 c.p.m.

[1] L-Epinephrine bitartrate was purchased from Calbiochem and was dissolved together with an equal weight of ascorbic acid (Calbiochem) to retard oxidation. ACTH was obtained from Sigma, and its potency was estimated to be 112 I.U./mg. by comparison with an international standard, as described previously (11). Isoproterenol (isopropyl arterenol) was a product of Schwartz/Mann. ATP and NAD were products of Calbiochem. α-^{32}P-ATP was obtained from International Chemical and Nuclear Corp. or from New England Nuclear Corp. 8-^{3}H-Cyclic AMP and unlabeled nucleotide were from Schwartz/Mann. Snake venom (*Ophiophagus hannah*) was from Sigma, and glycerolkinase and α-glycerol phosphate dehydrogenase were from Boehringer Mannheim. Collagenase (Code CLS) was obtained from Worthington. Therapeutic agents were obtained from the chemical collection of the Squibb Institute. Buffer constituents and other reagents were of the highest quality generally available.

[2] New Brunswick Scientific Gyrotory shaker.

Following incubation, the reaction was terminated by the addition of 0.5 ml. of 3 M $HClO_4$. The vials were shaken with a Vortex mixer and centrifuged[3] for 15 min. at 2000 r.p.m. and room temperature. The supernatant fractions were then decanted into sample cups of an AutoAnalyzer and assayed for glycerol enzymatically by the method of Ko and Royer (13). Briefly, this method measures conversion of oxidized NAD during the concomitant conversion of glycerol to dihydroxyacetone phosphate, using glycerolkinase, ATP, and α-glycerol phosphate dehydrogenase. The NADH produced was measured fluorometrically wth a spectrophotofluorometer[4]. This method allows quantitation of glycerol in subnanomole amounts.

Each drug was tested at 10, 50, and 100 μM in the presence of sufficient epinephrine (usually about 1×10^{-7} M) to activate lipolysis to about 30% of maximum (200–600 nmoles glycerol released/hr.). In this way, both stimulatory and inhibitory compounds could be detected. Stimulatory compounds were compared each day to theophylline, a potent lipolytic agent (14), and these results were calculated as percent activity of equimolar theophylline. The data for inhibitory compounds are presented as percent inhibition of the epinephrine-activated lipolysis. For compounds that inhibited epinephrine-activated lipolysis by 50% or more at 100 μM, the concentration inhibiting lipolysis 50% (I_{50}) was interpolated from dose–response curves obtained for these compounds.

Every agent was tested for its effect on the fluorometric assay of glycerol, both for intrinsic fluorescence and for quenching of fluorescence. Those compounds that interfered are so indicated in Table I. Water-insoluble compounds were dissolved in dimethyl sulfoxide; solvent controls revealed no effect on lipolysis at the levels added.

ACTH-Stimulated Steroidogenesis—The preparation and incubation of suspensions of isolated rat adrenal cells were performed as described previously (11). Decapsulated adrenal quarters from male Sprague-Dawley rats were suspended in a buffer of Krebs-Ringer bicarbonate–albumin–glucose, pH 7.4, containing bovine albumin (3 g./100 ml.) and glucose (0.2 g./100 ml.). Collagenase (5 mg./ml.) was added to quarters of 32 adrenals in 10 ml. of buffer. The tissue was digested for 1 hr. at 35°, under 95% oxygen–5% carbon dioxide, in a water bath[2] oscillating at 120 c.p.m. After digestion, the tissue was gently dispersed by repeated passage through a Pasteur pipet. The suspended cells were collected by centrifugation at 4° for 10 min. at 480×g, followed by two cycles of washing and recentrifugation in the original volume of buffer. The washed-cell pellet was then resuspended in buffer (1 adrenal/ml.) and filtered through a stainless steel sieve with perforations of 0.2 mm. to remove any large particles of undigested tissue.

Control and drug incubations were conducted in buffer, including 1 ml. of adrenal cell suspension and 0.05–0.10 mU. ACTH/ml. ($1–2 \times 10^{-10}$ M), a concentration of the hormone sufficient to produce 20–50% of the maximum steroidogenic response of the cells. Final drug concentrations of up to 200 μM were attained by the inclusion of up to 0.1 ml. of aqueous drug solutions in the incubation mixtures. Drugs insoluble in water were prepared as concentrated solutions in dimethyl sulfoxide and were added in volumes of up to 0.01 ml. to the incubation mixtures. Final volumes of the incubation mixtures were adjusted to 2.5 ml. by addition of appropriate volumes of buffer. Cells were incubated for 2 hr. at 35° under 95% oxygen–5% carbon dioxide.

Determinations of corticosterone, by fluorescence in sulfuric acid–ethanol, were performed on 2-ml. aliquots of the incubation mixtures as previously described (11, 15). The possibility of direct interference of the drugs in the corticosterone determinations was ruled out by parallel determination of the effect of analogous drug concentrations on fluorescence of corticosterone standards. Those drugs that significantly raised or lowered the fluorescence of corticosterone standards were considered to interfere with the test and are so indicated.

Cyclic Nucleotide Phosphodiesterase Assay—Cyclic 3′,5′-nucleotide phosphodiesterase from rat brain and cat heart was partially purified by a modification of the procedure of Brooker *et al.* (16). A 1–2-kg. mongrel cat or 10–12 male Sprague-Dawley rats (150–200 g.) were sacrificed by cervical dislocation or decapitation, respectively. The cat heart and rat brains were immediately placed on cracked ice, and the following steps were carried out at 0–4°. The organs were minced and homogenized in 5–10 volumes of 0.05 M imidazole buffer (pH 7.5), also containing 5 mM dithiothreitol in the case of the heart preparations. The homogenates were immediately centrifuged for 15–20 min. at 39,000 ×g. The supernatant fractions were adjusted to 50% saturation with ammonium sulfate, the pH was adjusted to 7.5 with 1 N NaOH, and the mixture was allowed to stand for 1 hr. The solutions were again centrifuged as before, and then the precipitates were taken up in the smallest possible volume of the imidazole buffer and dialyzed against 20 volumes of the buffer. Protein concentration was from 5 to 7 mg./ml. for cat heart phosphodiesterase by the micromodification (17) of the biuret method (18), and it was 15–20 mg./ml. for rat brain phosphodiesterase as determined by the method of Lowry *et al.* (19). Both enzyme preparations were stored at 0–4° until used. In the case of the rat brain enzyme, preparations were stable for up to 6 months. The cat heart enzyme was less stable and was freshly prepared each month.

The hydrolysis of cyclic AMP at near physiological concentrations was measured, in at least duplicate assays, by a modification of the radioactive assay described by Brooker *et al.* (16). This procedure was adapted so that phosphodiesterase activity could be monitored in the presence of potential inhibitors. Briefly, 50 μl. of a solution of ^{3}H-cyclic AMP (0.16 μM) as substrate, 50 μl. of an aqueous solution of inhibitor, and 50 μl. of phosphodiesterase solution containing human serum albumin (1 mg./ml.) and an excess of snake venom nucleotidase (1 mg./ml.) were incubated with shaking in a plastic liquid scintillation vial for 10 min. at 37°. The ^{3}H-cyclic AMP was converted to ^{3}H-5′-AMP by phosphodiesterase. The ^{3}H-5′-AMP, in turn, was converted to ^{3}H-adenosine by the nucleotidase. The reaction was stopped by the addition of Dowex AG 1-X2 resin, which adsorbed unchanged ^{3}H-cyclic AMP and left ^{3}H-adenosine in solution. Only ^{3}H-adenosine was counted after the addition of scintillation fluid due to quenching by the resin of the radiation from adsorbed ^{3}H-cyclic AMP. Potency was measured by determining the micromolar concentration of inhibitor that caused a 50% inhibition of the enzymatic activity (I_{50}).

Adenylate Cyclase Assay—Lung alveolar tissue was obtained from normal guinea pigs sacrificed by decapitation. The tissue was minced and a tissue grinder[5] was used to prepare 20% homogenates in chilled buffer containing 1 mM $MgCl_2$ and 2 mM glycylglycine, pH 7.5 (20). The homogenate was strained through four layers of gauze and centrifuged at 1000×g for 15 min. at 4°. The pellet was resuspended in the original volume of buffer and recentrifuged. The pellet was again resuspended in buffer, and 0.5–1.0-ml. aliquots were sealed in ampuls and stored under liquid nitrogen for future assay of adenylate cyclase activity. Samples stored in this manner exhibited undiminished activity for as long as 3 months. Protein was determined by the method of Lowry *et al.* (19) with crystalline bovine serum albumin as standard.

Adenylate cyclase activity was assayed in duplicate by a modification of previously published methods (21, 22). The total assay volume was 0.59 ml. and contained 1.8 mM $MgCl_2$, 0.8 mM glycylglycine, 32 mM tromethamine (pH 7.8), 1.2 mM ATP, $3–5 \times 10^6$ c.p.m. α-^{32}P-ATP, and particulate enzyme fraction (100–200 mcg. lung protein). The presence of 6–10 mM theophylline *decreased* lung cyclase activity, however, and was omitted from assays of the lung enzyme (23). In some assays, 0.01 mM isoproterenol was included. Activities in the presence of isoproterenol were about 60% higher than basal values.

The incubation mixture was reacted for 15 min. at 37° with shaking and then boiled for 3 min. to inactivate the cyclase. One hundred microliters of a solution containing 4 μmoles ATP, 1.25 μmoles cyclic AMP, and 0.15 μc. ^{3}H-cyclic AMP was added to the reaction mixture. The denatured protein was sedimented by centrifugation, and the supernate was applied to a Dowex 50W-X8 (100–200-mesh) column of approximately 1-cm.^{3} bed volume. The column was eluted with water, and the first 3 ml. collected was discarded except for the blank (no enzyme) assays, for which this fraction provided an accurate measure of the (radioactive) ATP added. The next 4 ml. eluted contained 55–70% of the total cyclic AMP present. This fraction was treated with 0.5 ml. of 0.18 M $ZnSO_4$ followed by 0.5 ml. of an equivalent barium hydroxide solu-

[3] International model 5 centrifuge.
[4] Aminco-Bowman.
[5] Duall.

Table I—Effects of Various Drugs on Cyclic AMP Metabolism *In Vitro*[a]

Drug Class and Drugs	Inhibition of Phosphodiesterase Activity from Rat Brain[b]	Inhibition of Phosphodiesterase Activity from Cat Heart[b]	Effect on Rat Epididymal Fat Cell Lipolysis[c]	Effect on Rat Adrenal Cell Steroidogenesis[d]	Effect on Guinea Pig Lung Adenylate Cyclase Activity[e] S	Effect on Guinea Pig Lung Adenylate Cyclase Activity[e] B
Adrenal corticosteroids						
1. Betamethasone	>100	>100	0	–	NT[f]	
2. Hydrocortisone	170	>100	+	inter[g]	NT	
Amebicides						
3. Iodochlorhydroxyquin	150	>100	– –	0	– –	–
Analeptics						
4. Picrotoxin	>100	>100	0	–	NT	
5. Pentylenetetrazol	>1000	>100	+	–	NT	
6. Dextroamphetamine sulfate	>1000	>100	– –	0	–	0
7. Methylphenidate	>1000	>100	0	–	NT	
Analgesics						
8. Morphine sulfate	>1000	>100	0	0	NT	
9. Meperidine hydrochloride	>1000	>1000	0	0	NT	
10. Propoxyphene	780	>100	0	– –	0	
11. Codeine sulfate	>1000	>100	0	–	NT	
12. Pentazocine	200	180	0	– –	–	0
13. Aspirin	>1000	>100	0	–		0
14. Acetophenetidin	>1000	>100	0	–	NT	
15. Aminopyrine	580	>100	0	0		0
16. Phenylbutazone	110	>100	0	0	NT	
Androgens						
17. Testosterone	190	28	+	0	NT	
18. Testolactone	270	>100	+	–	NT	
Anorexiants						
19. Dextroamphetamine sulfate	>1000	>100	– –	0	–	0
20. Chlorphentermine	>1000	>100	– –	–	0	–
Anthelmintics						
21. Bunamidine	44	80	– –	– –	– –	– –
22. Pyrvinium pamoate	8	100	– –	– –	–	0
Antianxiety drugs						
23. Meprobamate	>1000	>100	0	0	NT	
24. Chlordiazepoxide	110	>1000	+	inter	–	–
25. Diazepam	33	60	+	–	0	0
26. Hydroxyzine	>100	>100	0	– –	–	–
Antibacterial drugs						
27. Chloramphenicol	30	70	0	–	NT	
28. Erythromycin	>100	>100	0	–	NT	
29. Ampicillin	>1000	>100	0	0	NT	
30. Tetracycline	340	>100	0	0	NT	
31. Furazolidone	>1000	>100	+	– –	0	
32. Sulfanilamide	>100	>100	0	0	NT	
33. Isoniazid	>1000	>100	0	0	NT	
Anticoagulants						
34. Heparin	>1000	>100	–	–		– –
35. Acenocoumarol	120	>100	–	0	NT	
36. Anisindione	>100	>100	0	0	NT	
Anticonvulsants						
37. Phenobarbital	>1000	>100	–	–		0
38. Diphenylhydantoin	480	>100	0	0	NT	
39. Phenacemide	300	>1000	++	0		0
Antidepressives						
40. Amitriptyline	460	>100	0	– –	–	
41. Imipramine	700	>100	–	– –	–	
42. Tranylcypromine	>1000	>1000	– –	0	0	
Antiemetics						
43. Scopolamine hydrobromide	>1000	>1000	–	0	NT	
44. Buclizine	>100	>1000	0	–	NT	
45. Cyclizine	>100	>100	–	– –	0	
46. Meclizine	240	>100	0	–	NT	
Antifungal agents						
47. Amphotericin B	58	>100	inter	– –	– –	0
48. Tolnaftate	250	>100	+	0	NT	
Antihistaminics						
49. Cyproheptadine	>100	>100	0	– –	– –	0
50. Diphenhydramine	>100	>100	–	– –	–	0
51. Chlorpheniramine	>100	>100	–	– –	0	
52. Pheniramine	>1000	>100	0	inter	NT	

Table I—(*Continued*)

Drug Class and Drugs	Inhibition of Phosphodiesterase Activity from Rat Brain[b]	Inhibition of Phosphodiesterase Activity from Cat Heart[b]	Effect on Rat Epididymal Fat Cell Lipolysis[c]	Effect on Rat Adrenal Cell Steroidogenesis[d]	Effect on Guinea Pig Lung Adenylate Cyclase Activity[e] S	Effect on Guinea Pig Lung Adenylate Cyclase Activity[e] B
Antihypertensive drugs						
53. Promethazine	360	>100	−	−−	−	0
54. Reserpine	170	280	+	−	NT	
55. Guanethidine	>1000	>100	+	−	NT	
56. Methyldopa	>1000	>100	++	0		0
57. Hydralazine	620	>1000	++	0		0
58. Pargyline	>1000	>100	0	0	NT	
59. Imipramine	700	>100	−	−−	−	
60. Phenoxybenzamine	>100	>100	0	−−	−	−
61. Phentolamine	>1000	>100	−	−	NT	
62. Protoveratrine A	110	>100	0	0	NT	
Anti-inflammatory agents						
63. Chloroquine	>100	>100	0	−	NT	
64. Flufenamic acid	>100	200	0	0	NT	
65. Triamcinolone	>100	>100	0	0	NT	
66. Triamcinolone acetonide	>100	>100	0	−	NT	
67. Benzydamine	>100	>100	−−	−−	−	0
68. Mefenamic acid	>100	>100	0	0	NT	
69. Ibufenac	>100	>100	0	0	NT	
70. Azathioprine	>100	>100	0	0	NT	
71. Aurothioglucose	>100	NT	0	+		−−
72. *N*-(2,6-Dichloro-*m*-tolyl)-anthranilic acid[h]	>100	78	0	0	NT	
73. Niflumic acid	>100	200	0	0	NT	
74. 9α-Fluoro-11β,21-dihydroxy-2′,2′-dimethyl-16α,17α-dioxolano-1,4-pregnadiene-3,20-dione 21-ester with phosphoric acid[i]	>100	>100	0	0	NT	
Antimalarials						
75. Hydroxychloroquine analog[j]	25	>100	−−	−−	−−−	−
76. Quinacrine hydrochloride	130	>100	−	inter	NT	
Antineoplastics						
77. Busulfan	>1000	>100	+	0		0
78. Cyclophosphamide	>100	>100	0	0	NT	
79. Methotrexate	>100	480	++	0		0
80. 6-Mercaptopurine	28	>100	0	0		−
81. 5-Fluorouracil	>100	>100	+	0	NT	
82. Actinomycin F_1	>100	150	0	−−	−−−	0
83. Vincristine	290	>100	0	−−	−	0
84. Vinblastine	160	>1000	++	−−	−	−
85. Prednisone	>100	>100	0	−	NT	
86. Floxuridine	>100	>100	−	0	NT	
Antiparkinsonism drugs						
87. Benztropine mesylate	320	>100	+,−	−−	0	
88. Biperiden	370	>100	0	−	NT	
Antipsychotics						
89. Chlorpromazine	230	180	−	−−	−−	−
90. Triflupromazine	300	180	0	−−	−−	−
91. Fluphenazine	48	40	−−	−−	−−	−
92. Thioridazine	180	90	−	−−	−−	−−
Antiserotonin drugs						
93. Cinanserin hydrochloride[k]	450	>100	0	−	0	0
Antitussives						
94. Codeine sulfate	>1000	>100	0	−	NT	
95. Pipazethate	>1000	>100	0	−−	0	
96. Benzonatate	420	>100	0	−−	0	
Bronchodilators						
97. Ephedrine	>1000	>100	−−	0	−	
98. Isoproterenol	720	>1000	++	−		++
99. Methoxyphenamine	>100	>100	−−	−	0	
Cardioactive agents						
100. 4-(3,4-Dimethoxybenzyl)-2-imidazolidinone[l]	140	170	++	−		+
101. Papaverine	6	2	+	−	NT	
102. Bradykinin	>50	>100[m]	+	−	NT	
Chelating agents						
103. Dimercaprol	>100	>100	−	+	NT	

(*Continued*)

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104. Penicillamine	>1000	>1000	−	+	NT	
Coronary vasodilators						
105. Nitroglycerin	>100	>100	0	−	NT	
106. Dipyridamole	4	25	inter	0	NT	
Deficiency anemia drugs						
107. Folic acid	>100	>100	+	+	NT	
108. Vitamin B_{12}	>100	>100	0	0	NT	
Diuretics						
109. Furosemide	350	>100	0	0	NT	
110. Acetazolamide	>1000	>100	−	−	NT	
111. Bendroflumethiazide	130	>100	0	−	NT	
Estrogens, progestogens						
112. Ethinyl estradiol	30	29	++	inter		0
113. Norethindrone	38	>100	+	inter	NT	
Expectorants						
114. Acetylcysteine	>100	>100	0	0	NT	
GI agents						
115. Methantheline bromide	700	>100	0	−	NT	
116. Atropine sulfate	>1000	>100	+	−		0
117. Isopropamide	>100	>100	0	0	NT	
118. Magaldrate	390	>100	+	0	NT	
119. Carboxymethylcellulose, sodium	>1[n]	>1[n]	0	0	NT	
120. Castor oil	>1[n]	>0.33[n]	0	0	NT	
121. Phenolphthalein	>100	32	0	inter	NT	
122. Bisacodyl	78	210	+	0	NT	
123. Diphenoxylate	>1000	>100	+	−−	−	0
General anesthetics						
124. Thiopental, sodium	370	>100	0	0		0
125. Hydroxydione, sodium	>100	>100	0	−	NT	
Glaucoma drugs						
126. Neostigmine methylsulfate	>1000	>1000	+	0	NT	
127. Pilocarpine hydrochloride	>100	>100	0	−	NT	
128. Dichlorophenamide	>100	>100	−	0	NT	
Gout drugs						
129. Colchicine	260	>100	0	−	NT	
130. Indomethacin	92	90	0	0	NT	
131. Allopurinol	460	>100	0	0	NT	
Hypocholesterolemics						
132. I[o]	>100	>100	−−	−−	−−	−
133. 2-Methyl-2-(1-naphthyloxy)propionic acid[p]	>100	>100	+	0	NT	
134. 2-[(5,8-Dihydro-1-naphthyl)oxy]-2-methylpropionic acid[q]	>100	>100	+	0	NT	
135. Triparanol	>100	>100	0	inter	NT	
136. Clofibrate	>100	>100	0	−	NT	
Hypoglycemics						
137. Phenformin	>1000	>1000	0	0	NT	
138. Tolbutamide	610	>1000	++	0		0
Local anesthetics						
139. Dibucaine hydrochloride	260	>100	−	−−	+	
140. Lidocaine hydrochloride	>100	>100	+	−−	0	
141. Procaine hydrochloride	>1000	>100	0	−	NT	
Migraine drugs						
142. Methysergide	250	100	inter	−	NT	
Myasthenia gravis drugs						
143. Neostigmine methylsulfate	>1000	>1000	+	0	NT	
144. Edrophonium bromide	900	>100	+	0	NT	
Narcotic antagonists						
145. Nalorphine	500	>100	0	−	NT	
Oxytocics						
146. Oxytocin	>7	>7	0	0	NT	
Peripheral vasodilators						
147. Isoxsuprine	>1000	>100	++	−−	−	0

Table I—(*Continued*)

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Radiopaque media						
148. Diatrizoate, sodium	>1000	>100	0	0		0
Sedative–hypnotics						
149. Phenobarbital	>1000	>100	−	−		0
150. Secobarbital, sodium	>100	>100	0	0		0
151. Thiopental, sodium	370	>100	0	0		0
152. Chloral hydrate	>1000	>100	0	0	NT	
Skeletal muscle relaxants						
153. Mephenesin	>1000	>100	0	−	NT	
154. Succinylcholine chloride	>100	>100	0	−	NT	
155. Chlorzoxazone	310	660	++	0		0
Thyroid hormones and antithyroid agents						
156. Levothyroxine, sodium	>100	210	+	0	NT	
157. 2-Thiouracil	400	>100	0	0	NT	
Topical nasal decongestants						
158. Phenylephrine hydrochloride	>100	>100	−	−	NT	
159. Oxymetazoline	>100	>100	+	−	NT	
160. Xylometazoline	>100	>100	+	−	NT	
Vasopressors						
161. Angiotensin II amide, 5-valine	>100	>100	+	0	NT	
162. Methoxamine hydrochloride	>1000	>100	−	inter	NT	
163. Metaraminol	>100	>100	++	0		0
164. Phenylephrine hydrochloride	>100	>100	−	−	NT	
165. Mephentermine	>1000	>100	−	0	NT	

[a] Index of agents appended to paper (see text). [b] The concentration of compound (micromolar) that inhibits phosphodiesterase activity by 50% (I_{50}, μM). I_{50} for theophylline is 120 μM against rat brain phosphodiesterase and 50 μM against cat heart phosphodiesterase. The experimental uncertainty in the I_{50} values is ±15%. [c] Effect of compound on lipolysis denoted as follows: − −, inhibition with I_{50} <100 μM; −, inhibition with I_{50} >100 μM; 0, no effect at 100 μM (less than ±15% change); +, stimulation up to 40% that of equimolar theophylline; and ++, stimulation >40% that of equimolar theophylline at 10, 50, and 100 μM. [d] Effect of compound on steroidogenesis: − −, inhibition of >50% at 100 μM; −, inhibition of 11–49% at 100 μM; 0, no effect (less than ±10% change); +, stimulation by 200 μM compound of 11–49% that of equimolar 1-ethyl-4-(isopropylidenehydrazino)-1*H*-pyrazolo[3,4*b*]pyridine-5-carboxylic acid ethyl ester (SQ 20,009, see *Reference 56*); and ++, stimulation by 200 μM compound of ≥50% that of the equimolar pyrazolopyridine. [e] Effect of compound on adenylate cyclase activity in the presence (S) and absence (B) of 10 μM isoproterenol: − −, inhibition of >50% at 100 μM; −, inhibition of 16–49% at 100 μM; 0, no effect (less than ±15% change); +, 16–49% stimulation at 100 μM; and ++, >50% stimulation at 100 μM. [f] Not tested. [g] Interferes with assay. [h] SQ 20,196. [i] SQ 15,935. [j] 7-Chloro-2-(2-chlorostyryl)-4-[4-(diethylamino)-1-methylbutyl]amino]quinoline. [k] SQ 10,643. [l] Ro 7-2956. [m] Compound tested was 6-glycylbradykinin. [n] I_{50} expressed as milligrams per milliliter. [o] 2,2‴-[(1-Methyl-4,4-diphenylbutylidene)]bis(*p*-phenyleneoxy)bistriethylamine. [p] SQ 11,355. [q] SQ 11,354.

tion. The resulting precipitate was sedimented, and the zinc sulfate–barium hydroxide treatment was repeated without disturbing the first precipitate. After centrifugation, 1 ml. of supernate containing ^{32}P-cyclic AMP and ^{3}H-cyclic AMP was mixed with 15 ml. of scintillator (100 g. naphthalene, 14 g. 2,5-diphenyloxazole, and 0.1 g. dimethyl 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene/2 l. dioxane) and counted. The amount of ^{32}P-cyclic AMP formed in each assay was corrected for recovery losses subsequent to the incubation with the aid of the ^{3}H-cyclic AMP found in each case. Activities were computed as picomoles cyclic AMP formed per milligram protein per 15-min. incubation period. Relative activities were determined by comparisons of assays with drug present to those without drug.

RESULTS AND DISCUSSION

The data collected for 158 compounds representing 49 therapeutic classes are displayed in Table I. With the exception of the results on inhibition of phosphodiesterase activity, which are entered explicitly, the large number of data included led to the adoption of a semiquantitative format for the table, which is explained in the accompanying legend. The location of specific compounds in Table I may be facilitated by the use of an alphabetic list of agents indexed to the consecutive numbering employed in the table; such an index is appended to this paper. More detailed results for selected compounds of interest are discussed in this section.

Since many of these drugs possess multiple pharmacological activities, an agent may appear under more than one therapeutic heading; *e.g.*, phenobarbital is entered under "anticonvulsants" and "sedative–hypnotics." On the other hand, the necessarily arbitrary method of selection and classification of these compounds has led to situations where a drug is entered under only one of its possible activities; *e.g.*, cyproheptadine is entered under "antihistaminics" but not under "antiserotonin drugs." These difficulties are probably inevitable and, hopefully, will not prove excessively troublesome.

The sources of the assay systems in which the agents were examined represent different mammalian tissues and species. Strictly speaking, the diversity of these sources limits the confidence with which one can correlate effects on cyclase or phosphodiesterase activity with cellular responses in the systems employed in this study. Experience with inhibitors of phosphodiesterase activity (24), however, has prompted the conclusion that differences in inhibitory potency from tissue to tissue and from species to species are quantitative rather than qualitative; *i.e.*, a compound inhibiting phosphodiesterase activity from one tissue will inhibit the phosphodiesterase activity more or less from any other tissue, with a variation in potency of within one or, rarely, two orders of magnitude. Moreover, this variation will tend to be larger from tissue to tissue from the same species than from species to species for the same tissue. This kind of generalization would also be expected to hold for inhibitors of the activity of the adenylate cyclase catalytic moiety but might *not* apply to tissue-specific hormone receptors on the cyclase complex.

Cyclic Nucleotide Phosphodiesterase Activity—Compounds were tested for their inhibitory effects on phosphodiesterases from rat brain and cat heart. Most of the compounds were tested for activity at concentrations of up to 100 μM, although several were tested at concentrations as high as 1 mM. Of the 158 compounds examined, 14 compounds inhibited rat brain phosphodiesterase activity with I_{50} values <100 μM; 12 of the drugs lowered phosphodiesterase activity of the cat heart enzyme equally as well. Eleven agents (7%) inhibited the rat brain enzyme activity by 50% or more at 50 μM; six compounds (4%) were similarly effective against catalysis by cat

Table II—Effects upon Lipolysis of Drugs that Inhibit Phosphodiesterase (PDE) Activity

Drug	Class	I_{50}, μM Rat Brain PDE	I_{50}, μM Cat Heart PDE	Lipolytic Effect as Percent of Equimolar Theophylline at 50 μM	Lipolytic Effect as Percent of Equimolar Theophylline at 100 μM
Dipyridamole	Coronary vasodilator	4	25	Interferes	
Papaverine	Cardioactive	6	2	44	38
Pyrvinium pamoate	Anthelmintic	8	100	Inhibits[b]	
Hydroxychloroquine analog[a]	Antimalarial	25	>100	Inhibits[c]	
6-Mercaptopurine	Antineoplastic	28	>100	Inactive	
Chloramphenicol	Antibacterial	30	70	Inactive	
Ethinyl estradiol	Estrogen	30	29	77	49
Diazepam	Antianxiety	33	60	57	43
Norethindrone	Progestogen	38	>100	71	39
Bunamidine	Anthelmintic	44	80	Inhibits[d]	
Fluphenazine	Antipsychotic	48	40	Inhibits[e]	
Amphotericin B	Antifungal	58	>100	Interferes	
Bisacodyl	GI	78	210	56	26
Indomethacin	Gout	92	90	Inactive	
Anisindione	Anticoagulant	100	>100	Inactive	
Thioridazine	Antipsychotic	180	90	Inhibits[f]	
Testosterone	Androgen	190	28	32	24
Phenolphthalein	GI	>100	32	Inactive	
N-(2,6-Dichloro-*m*-tolyl)anthranilic acid[g]	Anti-inflammatory	>100	78	Inactive	

[a] 7-Chloro-2-(2-chlorostyryl)-4-{[4-(diethylamino)-1-methylbutyl]amino}quinoline. [b] Inhibited lipolysis 100% at 50 and 100 μM. [c] Inhibited 94% at 100 μM and 78% at 50 μM. [d] Inhibited 100% at 100 μM and 99% at 50 μM. [e] Inhibited 62% at 100 μM and 20% at 50 μM. [f] Inhibited 30% at 100 μM. [g] SQ 20,196.

heart phosphodiesterase preparations. These numbers of active agents are significant in light of experience with *random* testing of large numbers of compounds for inhibition of phosphodiesterase activity. Under those conditions, 1% or less of the compounds tested show inhibition of 50% or more at 50 μM. Accordingly, inhibitors of phosphodiesterase activity *in vitro* seem especially likely to possess pharmacological activities.

Table II shows the effects of potent phosphodiesterase inhibitors ($I_{50} \leq 100$ μM against the enzyme from either brain or heart) on lipolysis in the isolated fat cell. Inhibitors are arranged in order of decreasing potency against the rat brain enzyme. Of the 19 agents listed in Table II, six show lipolytic properties. These include the steroid hormones (ethinyl estradiol, norethindrone, and testosterone), bisacodyl, papaverine, and diazepam. The effects of these drugs on the isolated lipocytes appear consistent with their ability to inhibit phosphodiesterase activity in other tissues, since agents capable of inhibiting phosphodiesterase activity might be expected to stimulate lipolytic activity in the presence of submaximally activating amounts of epinephrine (14). Five compounds inhibit (pyrvinium pamoate, bunamidine, and 7-chloro-2-(2-chlorostyryl)-4-{[4-(diethylamino)-1-methylbutyl]amino}quinoline[6], an analog of hydroxychloroquine, inhibit strongly), six are inactive, and two interfere with the assay. The compounds that inhibit lipolysis may be acting *via* effects on systems other than lipocyte phosphodiesterase and it is of interest that three of the five possess clinical antiparasitic activity.

Table II also points out broad therapeutic areas in which more than one representative drug is a good phosphodiesterase inhibitor. The steroid hormones and antiparasitic agents were already mentioned. Several drugs active in the CNS also inhibit phosphodiesterase activity substantially. The phenothiazines, fluphenazine and thioridazine, and the benzodiazepine anxiolytic, diazepam, are examples selected from Table II. Findings on the inhibition of phosphodiesterase activity by phenothiazines are in agreement with an earlier report (25). Another anxiolytic agent, chlordiazepoxide, is very nearly as potent an inhibitor ($I_{50} = 110$ μM) of rat brain phosphodiesterase activity as the compounds listed in Table II. A correlation between antianxiety activity and inhibition of rat brain phosphodiesterase activity was recently noted (26).

Chlordiazepoxide is also of interest because it is much more active against rat brain phosphodiesterase than against the cat heart enzyme. Other potent phosphodiesterase inhibitors that display a clear tissue specificity (>threefold difference in I_{50} values) are the hydroxychloroquine analog, 6-mercaptopurine, testosterone, and phenolphthalein (Table II). Of this group, phenolphthalein and testosterone are more active against the cat heart phosphodiesterase.

Of the drugs that have been tested as phosphodiesterase inhibitors in the 100–1000-μM concentration range, 51 showed I_{50} values in this range against either or both enzyme preparations. Many of the remaining agents active in the CNS inhibited in this range: anticonvulsants (diphenylhydantoin and phenacemide), antidepressants (amitriptyline and imipramine), antipsychotics (chlorpromazine and triflupromazine), sedative–hypnotics (thiopental), and analgesics (propoxyphene, pentazocine, aminopyrine, and phenylbutazone). The relatively weak inhibition of phosphodiesterase activity (less than theophylline) observed here for amitriptyline, imipramine, and promethazine contrasts with the finding of Muschek and McNeill (27) that these compounds were approximately 15 times as active as theophylline against rat brain phosphodiesterase.

The antiparasitic drugs (iodochlorhydroxyquin and quinacrine) remaining from Table I were active, with I_{50} values of 150 μM or less. Similarly, two of the three remaining steroids that were tested (testolactone and hydrocortisone) inhibited phosphodiesterase with I_{50} values < 300 μM. Some of the other drug classes contained several representatives that inhibited phosphodiesterase activity

Table III—Effects on Phosphodiesterase and Adenylate Cyclase Activity of Drugs that Stimulate Lipolysis

Drug	Class	Phosphodiesterase Inhibition, I_{50}, μM Rat Brain	Phosphodiesterase Inhibition, I_{50}, μM Cat Heart	Percent Change in Cyclase Basal Activity by 100 μM Drug
Isoproterenol	Bronchodilator	720	>1000	+60
4-(3,4-Dimethoxybenzyl)-2-imidazolidinone[a]	Cardioactive	140	170	+21
Metaraminol	Vasopressor	>100	>100	+15
Chlorzoxazone	Muscle relaxant	310	660	+14
Hydralazine	Antihypertensive	620	>1000	+10
Ethinyl estradiol	Estrogen	30	29	+10
Isoxsuprine	Vasodilator	>1000	>100	+6
Tolbutamide	Hypoglycemic	610	>1000	+4
Methyldopa	Antihypertensive	>1000	>100	+3
Methotrexate	Antineoplastic	>100	480	−1
Phenacemide	Anticonvulsant	300	>1000	−8
Vinblastine	Antineoplastic	160	>1000	−31

[a] Ro 7-2956.

[6] SQ 18,057.

Table IV—Potent Inhibitors of Lipolysis and Their Effects on Adenylate Cyclase Activity

Drug	Class	Inhibition of Lipolysis, I_{50}, μM	Percent Inhibition (at 100 μM) of Cyclase Stimulation by 10 μM Isoproterenol	Inhibition of Cyclase Basal Activity, I_{50}, μM
Pyrvinium pamoate	Anthelmintic	0.023	49	I[a]
Bunamidine	Anthelmintic	4	100	23
Iodochlorhydroxyquin	Amebicide	20	100	>1000
Hydroxychloroquine analog[b]	Antimalarial	30	100	90
Chlorphentermine	Anorexiant	30	20	I
Ephedrine	Bronchodilator	35	24	NT[d]
I[c]	Hypocholesterolemic	40	100	>100
Benzydamine	Anti-inflammatory	60	27	I
Fluphenazine	Antipsychotic	65	93	230
Tranylcypromine	Antidepressive	70	0[e]	NT
Methoxyphenamine	Bronchodilator	100	9	NT
Dextroamphetamine sulfate	Anorexiant	100	26	I

[a] No significant inhibition at 100 μM. [b] 7-Chloro-2-(2-chlorostyryl)-4-{[4-(diethylamino)-1-methylbutyl]amino}quinoline. [c] 2,2′′′-[(1-Methyl-4,4-diphenylbutylidene)bis(*p*-phenyleneoxy)]bistriethylamine. [d] Not tested. [e] Insignificant stimulation (<15%) observed.

between 100 and 1000 μM or were small groupings in which every remaining entry inhibited in this range. These were: antihypertensives (reserpine, hydralazine, protoveratrine A, and imipramine), antiparkinsonism drugs (benztropine and biperiden), gout drugs (colchicine and allopurinol), thyroid hormones and antithyroid agents (levothyroxine and 2-thiouracil), and antineoplastics (actinomycin F_1, vincristine, vinblastine, and methotrexate).

The ability of antineoplastic agents to inhibit phosphodiesterase activity should be considered along with the recent report that exogenous dibutyryl cyclic AMP or cyclic AMP plus the phosphodiesterase inhibitor, theophylline can restore normal morphology to cultured sarcoma cells (28). It is at least conceivable that the antineoplastic properties of the drugs discussed arise in part from their phosphodiesterase activity. Such a hypothesis, though highly speculative, may deserve further examination.

Lipolysis—Twelve drugs stimulated lipolysis to at least 40% of the levels reached with equimolar theophylline, at each of three concentrations: 10, 50, and 100 μM. As mentioned previously, stimulation of lipolysis might reflect an inhibition of phosphodiesterase activity by the agent; it might also arise from stimulation of the adenylate cyclase complex consisting of hormone receptor and catalytic moiety. These compounds are collected in Table III, together with their effects on the phosphodiesterase and basal cyclase activities of the preparations employed.

Of the lipolytic agents listed in Table III, isoproterenol stimulated as expected from its activity as a potent β-adrenergic agonist; it was active in the absence of epinephrine and at concentrations as low as 0.1 μM. The actions of ethinyl estradiol and 4-(3,4-dimethoxybenzyl)-2-imidazolidinone[7] are consistent with their inhibition of activity of phosphodiesterase preparations from other tissues. The remaining compounds may stimulate lipolysis *via* effects on cellular metabolism subsequent to the synthesis and/or degradation of cyclic AMP. These compounds might possibly be lipolytic due to inhibition of lipocyte phosphodiesterase activity, but this appears unlikely, except in extreme and unusual circumstances, because of the weak activity these compounds show against the enzymes from brain and heart.

The inhibition of phosphodiesterase activity by certain of these lipolytic agents deserves some comment. 4-(3,4-Dimethoxybenzyl)-2-imidazolidinone is 12–14 times as potent an inhibitor of rat erythrocyte phosphodiesterase activity (29) as of rat brain or cat heart phosphodiesterase activity (Table I). This is an illustration of the quantitative differences in inhibition of enzymes from different mammalian tissues and species that may be observed for a particular compound. The weak phosphodiesterase inhibition seen for tolbutamide is qualitatively consistent with the findings of Brooker and Fichman (30), who found a very weak inhibition of rat kidney phosphodiesterase activity by this sulfonylurea derivative.

Of the drugs in Table III, only isoproterenol clearly stimulated basal adenylate cyclase activity significantly at 100 μM. This was expected, since isoproterenol was the catecholamine used to generate hormone-stimulated cyclase activity. Vinblastine was a potent lipolytic agent which, nonetheless, inhibited lung cyclase basal activity significantly at 100 μM. This antineoplastic agent also strongly inhibited adrenal cell steroidogenesis and isoproterenol-stimulated cyclase activity. Concentrations of tolbutamide above 1 mM were previously shown to inhibit either hormone-stimulated or cyclic AMP-stimulated lipolysis (31–36). It is now shown that, at lower concentrations, tolbutamide stimulated hormone-stimulated lipolysis in isolated lipocytes (Table I). For instance, at 50 μM, it was 65% as active as equimolar theophylline in stimulating lipolysis. Tolbutamide at 100 μM neither inhibited nor stimulated guinea pig lung cyclase activity. Levey *et al.* (37) recently reported the stimulation of rabbit and human heart cyclase activity by tolbutamide. This stimulation is likely to be tissue specific, inasmuch as we confirmed (unpublished experiments) the stimulation of heart cyclase activity by histamine demonstrated previously (38) but did not detect an effect of histamine upon the lung enzyme of similar magnitude (unpublished results).

Thirteen agents stimulated lipolysis well enough relative to theophylline at concentrations of 10 and 50 μM to be rated (++) in Table I, but they were not sufficiently stimulatory relative to theophylline at 100 μM. These drugs are worthy of mention because of their potency at the lower concentrations. The group includes steroids (hydrocortisone, testolactone, and norethindrone), GI agents (atropine, magaldrate, and bisacodyl), and antineoplastics (busulfan and 5-fluorouracil) as well as furazolidone, guanethidine, papaverine, lidocaine, and edrophonium bromide. Of these, papaverine, norethindrone, and bisacodyl are good inhibitors of phosphodiesterase activity (I_{50} < 100 μM *versus* either preparation).

Twelve therapeutic agents inhibited lipolysis with I_{50} values ≤ 100 μM; they are given in Table IV together with their effects on cyclase activity. The four most potent inhibitors of lipolysis tested are all antiparasitic agents. Both of the antimalarial agents tested, the hydroxychloroquine analog and quinacrine, inhibited lipolysis in isolated fat cells in the presence of epinephrine. The hydroxychloroquine analog (Table IV) was more potent than quinacrine (Table I). Antimalarial drugs were reported (39) to inhibit lipolysis in adipose tissue. Ephedrine, methoxyphenamine, and amphetamine sulfate all possess sympathomimetic properties and might inhibit lipolysis by competing with epinephrine for the β-adrenergic receptor site.

The site of action of several of these inhibitors of lipolysis was clarified by further studies in which the effects of these compounds (excluding benzydamine) on ACTH-activated and cyclic AMP-activated lipolysis were compared with the effects given in Table IV for epinephrine-activated lipolysis (Table V). These experiments confirmed that ephedrine, methoxyphenamine, and amphetamine inhibited only epinephrine-activated lipolysis. Iodochlorhydroxyquin behaved similarly. 2,2′′′-[(1-Methyl-4,4-diphenylbutylidene)-bis(*p*-phenyleneoxy)]bistriethylamine[8] (I), the hydroxychloroquine

[7] Ro 7-2956.

[8] SQ 10,591.

Table V—Effects of Potent Inhibitors on Activation of Lipolysis by Epinephrine, ACTH, and Cyclic AMP

Compound	—I_{50}, μM *versus* Activation[a] by— 0.14 μM Epinephrine	10 mU./ml. ACTH	10 mM Cyclic AMP
Pyrvinium pamoate	0.023	1	0.1
Bunamidine	9	6	35
Fluphenazine	65	40	60
I[b]	40	40	Inactive[c]
Tranylcypromine	70	37	Inactive
Hydroxychloroquine analog[d]	25	17	100
Chlorphentermine	30	75	Inactive
Ephedrine	35	Inactive	Inactive
Dextroamphetamine sulfate	100	Inactive	Inactive
Iodochlorhydroxyquin	100	Inactive	Inactive
Methoxyphenamine	100	Inactive	Inactive

[a] Hormones and cyclic AMP present at concentrations giving approximately 50% of maximal activation. [b] 2,2′′′-[(1-Methyl-4,4-diphenylbutylidene)bis(*p*-phenyleneoxy)]bistriethylamine. [c] Less than 20% inhibition at 100 μM. [d] 7-Chloro-2-(2-chlorostyryl)-4-{[4-(diethylamino)-1-methylbutyl]amino}quinoline.

analog, tranylcypromine, and chlorphentermine inhibited epinephrine- and ACTH-activated lipolysis but not cyclic AMP-induced lipolysis. These drugs may act at the locus of the hormone receptors, upon the cyclase catalytic moiety, or through interference with the receptor–catalytic unit relationship. Bunamidine, pyrvinium pamoate, and fluphenazine inhibited all three stimulations by 50% or more at 100 μM, behavior consistent with inhibition, at least, at a metabolic point subsequent to formation of cyclic AMP. For this latter group of compounds, however, the data of Table V do not rule out inhibition also of adenylate cyclase.

The effects of the lipolysis inhibitors on lung adenylate cyclase activity were generally consistent with the considerations of the preceding paragraph. Five compounds (bunamidine, iodochlorhydroxyquin, the hydroxychloroquine analog, I, and fluphenazine) inhibited isoproterenol-stimulated cyclase activity by more than 50% at 100 μM (Table IV). In addition, pyrvinium pamoate inhibited hormone-stimulated cyclase activity by approximately 50% at 100 μM. Of these six cyclase inhibitors, only bunamidine inhibited *basal* cyclase activity about as strongly as hormone-stimulated activity and, hence, would appear to act on cyclase primarily at the catalytic subunit. The hydroxychloroquine analog, iodochlorhydroxyquin, and I inhibited hormone-stimulated activity more strongly than basal cyclase activity, an indication that they affect both the catalytic moiety and the β-adrenergic receptor system. Fluphenazine and pyrvinium pamoate inhibited basal activity relatively weakly or not at all at 100 μM; therefore, at the level of cyclic AMP synthesis, they inhibit primarily the hormone stimulation. Two of the three sympathomimetics (amphetamine and ephedrine) inhibited catecholamine-stimulated cyclase activity significantly, while the third, methoxyphenamine, did not inhibit significantly at 100 μM. Chlorphentermine showed some inhibition of hormone-stimulated, but not basal, cyclase activity.

Table VI—Relative Potency of Selected Inhibitors of Adrenal Steroidogenesis

Compound	Inhibition of ACTH Activation, I_{50}, μM	Inhibition of Activation —by 3 mM Cyclic AMP— Concentration, μM	Percent Inhibition
Pyrvinium pamoate	0.15	0.20	87
Hydroxychloroquine analog[a]	2	2.5	8
Promethazine	2.5	2.0	0
Cyproheptadine	3	20	0
Thioridazine	5	10	0
I[b]	7	10	38
Chlorpromazine	11	15	5
Vinblastine	12	20	71
Imipramine	13	20	6
Amitriptyline	15	20	0
Bunamidine	16	20	84
Phenoxybenzamine	30	40	30
Diphenhydramine	35	50	0
Triflupromazine	40	50	3
Fluphenazine	70	100	10
Chlorpheniramine	80	100	30

[a] 7 - Chloro - 2 - (2 - chlorostyryl) - 4 - {[4 - (diethylamino) - 1 - methylbutyl]amino}quinoline. [b] 2,2′′′-[(1-Methyl-4,4-diphenylbutylidene)bis-(*p*-phenyleneoxy)]bistriethylamine.

Steroidogenesis—Thirty-two of the drugs listed in Table I inhibited ACTH-activated steroidogenesis in isolated adrenal cells by at least 50% at 100 μM. A number of these agents were examined at lower concentrations to assess their relative potencies. The I_{50} values for these strong inhibitors are summarized in Table VI, in order of decreasing inhibitory potency. In an effort to identify potential inhibitors of adrenal adenylate cyclase among the compounds in Table VI, effects of the compounds on cyclic AMP-activated steroidogenesis were also measured. Inhibitor concentrations approximating the I_{50} levels for ACTH-activated steroidogenesis were included in adrenal cell incubations containing 3 mM cyclic AMP, a nucleotide concentration approximating the half-maximal activation level for isolated cells prepared by collagenase treatment (11). Among the drugs that inhibited both cyclic AMP-activated and ACTH-activated steroidogenesis were pyrvinium pamoate, vinblastine, and bunamidine. Pyrvinium and bunamidine thus behaved similarly in the lipocyte and adrenal cell and, therefore, appear to inhibit cellular metabolism at points subsequent to cyclic AMP formation in both cell types. A second group of compounds, in contrast, including the hydroxychloroquine analog, promethazine, cyproheptadine, thioridazine, chlorpromazine, imipramine, amitriptyline, diphenhydramine, triflupromazine, and fluphenazine, was characterized by failure to inhibit cyclic AMP-activated steroidogenesis. It was concluded that compounds in the latter group inhibited steroidogenesis in the intact adrenal cell at a point preceding the appearance of intracellular cyclic AMP, *i.e.*, at the level of adenylate cyclase. It is of interest that eight of the compounds in this group are tricyclic in structure and include five phenothiazines. The activity of the phenothiazines was consistent with recent reports indicating that chlorpromazine inhibits adenylate cyclases from adrenal, thyroid, and brain tissue (40–43).

Moreover, Wolff and Jones (40) demonstrated that trifluoperazine and prochlorperazine inhibit the hormonal stimulation of thyroid adenylate cyclase, while Uzunov and Weiss (43) implicated trifluoperazine, in addition to chlorpromazine, as an inhibitor of norepinephrine-stimulated brain cyclase. In a report that contradicts our findings, Haksar and Peron (44) recently observed that chlorpromazine inhibits the steroidogenic response of rat adrenal quarters to cyclic AMP as well as to ACTH. One possible reason for this difference may lie in inhibitor concentration levels; chlorpromazine concentrations between 100 and 250 μM were required for 50% inhibition of steroidogenesis in the adrenal quarters, whereas our experiments were performed using concentrations of 15 μM or lower.

The 32 strong inhibitors of steroidogenesis were tested for effects on cyclase activity (Table VII). Of the 10 compounds identified as probable adenylate cyclase inhibitors in the adrenal cell, only amitriptyline and imipramine failed to demonstrate at least moderate inhibition of lung cyclase activity. Twelve of the drugs in Table VII inhibited stimulation by isoproterenol by approximately 50% or more when tested at 100 μM. This group included all five of the compounds that were strong inhibitors of *both* lipolysis and steroidogenesis (bunamidine, the hydroxychloroquine analog, I, fluphenazine, and pyrvinium pamoate). All four of the antipsychotic phenothiazines tested (thioridazine, triflupromazine, chlorpromazine, and fluphenazine) were also strong cyclase inhibitors. Hence fluphenazine, thioridazine, and triflupromazine represent additional phenothiazines capable of inhibiting hormone-stimulated cyclase activity. Promethazine also inhibited lung cyclase activity, though more weakly than the other phenothiazines.

The compounds in Table VII that inhibited isoproterenol-stimulated cyclase activity by 90% or more at 100 μM may well have I_{50} values of 10 μM or less. Thus, they would appear to inhibit hormone-stimulated activity more strongly than basal activity. This is particularly true for agents with I_{50} values against basal activity >100 μM, such as triflupromazine, chlorpromazine, and fluphenazine. Four other potent inhibitors of steroidogenesis inhibited hormone stimu-

Table VII—Effects on Adenylate Cyclase Activity of Inhibitors of Steroidogenesis

Compound	Drug Class	Percent Inhibition (at 100 μM) of Stimulation by 10 μM Isoproterenol	Inhibition of Basal Activity, I_{50}, μM
Bunamidine	Anthelmintic	100	23
Thioridazine	Antipsychotic	100	80
Hydroxychloroquine analog[a]	Antimalarial	100	90
Triflupromazine	Antipsychotic	100	250
I[b]	Hypocholesterolemic	100	>100
Chlorpromazine	Antipsychotic	96	270
Fluphenazine	Antipsychotic	93	230
Amphotericin B	Antifungal	84	I[c]
Cyproheptadine	Antihistaminic	82	I
Actinomycin F_1	Antineoplastic	50	I
Vinblastine	Antineoplastic	49	420
Pyrvinium pamoate	Anthelmintic	49	I
Isoxsuprine	Vasodilator	40	I
Diphenhydramine	Antihistaminic	39	I
Hydroxyzine	Antianxiety	38	>100
Phenoxybenzamine	Antihypertensive	36	>100
Promethazine	Antihistaminic	34	I
Diphenoxylate	GI agent	31	I
Vincristine	Antineoplastic	29	I
Benzydamine	Anti-inflammatory	27	I
Pentazocine	Analgesic	22	I
Amitriptyline	Antidepressive	16	NT[d]
Imipramine	Antidepressive	16	NT
Benztropine	Antiparkinsonism	10	NT
Lidocaine	Local anesthetic	8	NT
Pipazethate	Antitussive	7	NT
Propoxyphene	Analgesic	7	NT
Cyclizine	Antiemetic	0[e]	NT
Benzonatate	Antitussive	0[e]	NT
Chlorpheniramine	Antihistaminic	0[e]	NT
Furazolidone	Antibacterial	0[e]	NT
Dibucaine	Local anesthetic	0[f]	NT

[a] 7 - Chloro - 2 - (2 - chlorostyryl) - 4 - {[4 - (diethylamino) - 1 - methylbutyl]amino} quinoline. [b] 2,2′′′-[(1-Methyl-4,4-diphenylbutylidene)bis-(*p*-phenyleneoxy)]bistriethylamine. [c] No significant inhibition at 100 μM. [d] Not tested. [e] Insignificant stimulations (<15%) were observed. [f] A stimulation of 22% was observed.

lation, but not basal activity, at 100 μM; these were amphotericin B, cyproheptadine, actinomycin F_1, and pyrvinium pamoate. The effect of amphotericin B is understandable, since its mechanism of action is thought to involve perturbation of membranes (45). Cyproheptadine is a histamine and serotonin antagonist that could also antagonize stimulation by isoproterenol. Of the remaining steroidogenesis inhibitors, diphenhydramine, an antihistamine, and phenoxybenzamine, an α-adrenergic antagonist, produced, at the relatively high concentration of 100 μM, lesser but still significant decreases in the β-adrenergic stimulation generated by isoproterenol.

None of the drugs tested was a potent stimulator of steroidogenesis, although four compounds (folic acid, penicillamine, dimercaprol, and aurothioglucose) stimulated somewhat.

Miscellaneous Adenylate Cyclase Effectors—Several drugs which were not potent effectors of lipolysis or steroidogenesis were, nonetheless, investigated for effects on adenylate cyclase activity. Two of the compounds were strongly inhibitory: heparin (I_{50} = 2 μM) and aurothioglucose (I_{50} = 10 μM). The apparently very potent inhibition by heparin is based on a molecular weight of 20,000, so that the concentration of heparin causing 50% inhibition is 40 mcg./ml. Even with these qualifying remarks, it is perhaps surprising that this anticoagulant inhibits adenylate cyclase, because the inhibition of platelet aggregation is associated with a *stimulation* of platelet cyclase (46–48). On the other hand, the inhibition by aurothioglucose is not surprising. Aurothioglucose is a heavy metal-containing compound which probably interacts with the sulfhydryl group(s) of adenylate cyclase in a manner similar to that of organic mercurials (49–55). 6-Mercaptopurine was a weaker cyclase inhibitor (I_{50} = 420 μM). It is clearly related structurally to the natural substrate for the enzyme (ATP), in addition to possessing a reactive sulfhydryl function.

CONCLUSIONS

A large number of drugs were examined for their effects upon several *in vitro* systems related to cyclic AMP metabolism, including the isolated lipocyte and adrenal cell as well as adenylate cyclase and cyclic nucleotide phosphodiesterase enzyme preparations. Many of the drugs showed some activity in these systems, and a fraction of these were potent inhibitors or stimulators of the enzymic or cellular response. Of the broad therapeutic classes represented, agents effective in the CNS appeared to be especially active in the *in vitro* systems examined. Antiparasitic drugs were also quite active and were generally inhibitory. Inasmuch as the representatives of the therapeutic classes were assigned arbitrarily and without conscious bias, there is reason to believe the general results would be similar with other agents representing the drug classes selected. Despite the large number of therapeutic agents that show activity in the test systems utilized, the data presented here do not provide adequate evidence to decide whether or not the pharmacological properties of any particular drug in man or other animals can be related to an effect on cyclic AMP metabolism as evidenced in these *in vitro* systems. The determination of cyclic AMP levels in tissues after administration of the agent *in vivo* would be helpful, although not definitive, in arriving at a conclusion for a given agent.

APPENDIX: ALPHABETIC INDEX OF AGENTS APPEARING IN TABLE I

Agent	Number of Table I
Acenocoumarol	35
Acetazolamide	110
Acetophenetidin	14
Acetylcysteine	114
Aspirin	13
Actinomycin F_1	82
Allopurinol	131
Aminopyrine	15
Amitriptyline	40
Amphotericin B	47
Ampicillin	29
Angiotensin II amide, 5-valine	161
Anisindione	36
Atropine sulfate	116
Aurothioglucose	71
Azathioprine	70
Bendroflumethiazide	111
Benzonatate	96
Benztropine mesylate	87
Benzydamine	67
Betamethasone	1
Biperiden	88
Bisacodyl	122
Bradykinin	102
Buclizine	44
Bunamidine	21
Busulfan	77
Carboxymethylcellulose, sodium	119
Castor oil	120
Chloral hydrate	152
Chloramphenicol	27
Chlordiazepoxide	24
Chloroquine	63
Chlorpheniramine	51
Chlorphentermine	20
Chlorpromazine	89
Chlorzoxazone	155
Cinanserin hydrochloride	93
Clofibrate	136
Codeine sulfate	11,94
Colchicine	129
Cyclizine	45
Cyclophosphamide	78
Cyproheptadine	49
Dextroamphetamine sulfate	6,19

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Influence of Power on Quality of Emulsions Prepared by Ultrasound

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Abstract □ The effect of ultrasonic power on a mineral oil–surfactant–water emulsion system was demonstrated. An optimum amount of energy was required to produce the best emulsion. Exceeding the optimum requirements produced coalescence phenomena. The best emulsions resulted with optimal surfactant concentrations, at optimal HLB values, and at highest power levels. In the emulsions studied, the HLB and surfactant parameters were more important than the power parameters.

Keyphrases □ Emulsions, mineral oil–surfactant–water—effect of ultrasound, HLB, surfactant, and power parameters □ Ultrasound—effect on mineral oil–surfactant–water emulsions, HLB, surfactant, and power parameters

In recent years, many workers have utilized various types of ultrasonic generators to form emulsions. Beal and Skauen (1) investigated the effect of exposure time and sample geometry on the quality of the emulsion system. Haavisto and Hagner (2) studied the efficiency of emulsification with ultrasound with and without emulsifiers. Myers and Goodman (3), Singiser and Beal (4), Marshall (5), and McCarthy (6) all described experiments using a liquid whistle generator. In these studies, the main parameters considered were the emulsion systems themselves and the length of insonation. Kann and Tester (7) utilized a step-horn transducer with fixed frequency and variable power. However, they concerned themselves with the emulsion rather than instrument parameters.

Since little attention has been focused upon the effect of power on the quality of emulsions manufactured by step-horn ultrasonic generators, this study was designed to determine what effects changes in ultrasonic power might have upon an emulsion system.

EXPERIMENTAL

Sonifier—The ultrasonic instrument[1] used in this study utilizes a power supply, a sonic converter, and a step-horn transducer. It

[1] Branson Sonifier model J-17V, Branson Sonic Power Co., Danbury, Conn.