400 mm, 200 g) and eluted with a MeOH–$CHCl_3$ mixture. Fractions of 100 ml were collected. Chromatog pure product was obtd from fractions 24–47 (2% MeOH in $CHCl_3$), fractions 48–66 (5% MeOH in $CHCl_3$), and from fractions 67–72 (20% MeOH in $CHCl_3$). The eluate was combined and evapd *in vacuo* giving a glass; 2.21 g (50.2%) which would not cryst.

A mixt containing 1.95 g (9.01 mmoles) of the chromatog homogeneous (*S*)-4-chloro-5-amino-6-(1-hydroxy-2-butylamino)-pyrimidine, 150 mg (1.36 mmoles) of $EtSO_3H$, and 10 ml of triethyl orthoformate in 50 ml of $CHCl_3$ was stirred at room temp for 1 hr. The mixt was evapt *in vacuo* and gave a liquid residue which when dissolved in $CHCl_3$ and hexane yielded the crystn product; 1.28 g (62.3%), mp 125–133°. Recrystn from $CHCl_3$–hexane gave the analytical material; mp 139.5–142.5°, yield, 532 mg (25.6%), $[\alpha]^{25}D$ −19.0° (*c* 2.1, 50% EtOH). *Anal.* ($C_9H_{11}ClN_4O$) C, H, Cl, N.

(*S*)-(−)-9-(1-Hydroxy-2-butyl)adenine (8-*S*). Prepd by method C from 6-*S* (R_1 = Et, R_2 = Cl); yield, 59%, mp 160–163° (Me_2CO–C_6H_{14}), $[\alpha]^{25}D$ −24.0° (*c* 1.7, EtOH). *Anal.* ($C_9H_{13}N_5O$) C, H, N.

(*R*)-(+)-6-Chloro-9-(1-hydroxy-2-butyl)purine (6-*R*, R_1 = Et, R_2 = Cl). Prepd by method D from 3 and (*R*)-(−)-2-amino-1-butanol;†† yield, 41%, mp 140–144° ($CHCl_3$–C_6H_{14}), $[\alpha]^{25}D$ +19.2° (*c* 2.1, 50% EtOH). *Anal.* ($C_9H_{11}ClN_4O$) C, H, Cl, N.

(*R*)-(+)-9-(1-Hydroxy-2-butyl)adenine (8-*R*) was prepd from 6-*R* (R_1 = Et, R_2 = Cl) by method C; yield, 53%, mp 160–163° (*i*-PrOH–C_6H_{14}), $[\alpha]^{25}D$ +24.2° (*c* 1.6, EtOH). *Anal.* ($C_9H_{13}N_5O$) C, H, N.

(*RS*)-, (*S*)-, and (*R*)-5-Amino-4-chloro-6-(α-hydroxymethylbenzyl)pyrimidines (5-*RS*, 5-*S*, and 5-*R* where R_1 = C_6H_5) were prepd by the general method A from 3 and (*RS*)-,[9] (*S*)-,[10] and (*R*)-[10,11]1-phenyl-2-hydroxyethylamine; yield of 5-*RS*, 67%, mp, softens at *ca.* 80°, resolidifies and melts at 150–151° (MeOH); yield of 5-*S*, 44%, mp 140–141° (toluene), $[\alpha]^{26}D$ −86.5° (*c* 0.92, 2.5% HCl); yield of 5-*R*, 38%, mp 140–141° (toluene), $[\alpha]^{23}D$ +86.8 (*c* 0.93, 2.5% HCl). *Anal.* for 5-*RS*, 5-*S*, and 5-*R* ($C_{12}H_{13}ClN_4O$) C, H, Cl, N.

(*RS*)-, (*S*)-, and (*R*)-6-Chloro-9-(α-hydroxymethylbenzyl)purine (6-*RS*, 6-*S*, and 6-*R* where R_1 = C_6H_5 and R_2 = Cl). Prepd by general method B; yield of 6-*RS*, 75%, mp 129–130° (toluene); yield of 6-*S*, 78%, mp 114–116° (EtOH, $[\alpha]^{26}D$ −5.15° (*c* 11.1, MeOH); yield of 6-*R*, 74%, mp 114–116°, $[\alpha]^{24}D$ +5.46° (*c* 10.6, MeOH). *Anal.* for 6-*RS*, 6-*S*, and 6-*R* ($C_{13}H_{11}ClN_4O$) C, H, Cl, N.

(*RS*)-, (*S*)-, and (*R*)-9-(α-Hydroxymethylbenzyl)adenine (9-*RS*, 9-*S*, and 9-*R*) were prepd by the general procedure of method C; yield of 9-*RS*, 64%, mp 200–201° (*i*-PrOH); yield of 9-*S*, 57%, mp 211–213°(MeOH), $[\alpha]^{25}D$ −6.55° (*c* 1.8, 2.5% HCl); yield of 9-*R*, 55%, mp 213–214° (*i*-PrOH), $[\alpha]^{23}D$ +6.69° (*c* 1.7, 2.5% HCl). *Anal.* for 9-*RS*, 9-*S*, and 9-*R* ($C_{13}H_{13}N_5O$) C, H, N.

Reagents and Assay Procedures. Adenosine deaminase (Type I, calf intestinal mucosa) was purchased from the Sigma Chemical Co. The procedure for the assay of reversible inhibitors has previously been described[1,2] and is a modification of the method of Kaplan[12] based on the work of Kalckar.[13]

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Accumulation of Cyclic Adenosine Monophosphate in Incubated Slices of Brain Tissue. 1. Structure–Activity Relationships of Agonists and Antagonists of Biogenic Amines and of Tricyclic Tranquilizers and Antidepressants

Minta Huang and John W. Daly*

Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014.
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A radiometric technique, involving the use of brain slices prelabeled by incubation with adenine-^{14}C, provides a simple method to assess the effects of a variety of compounds on the accumulation of cyclic AMP-^{14}C in brain tissue. In guinea pig cerebral cortical slices only catecholamines containing a β-OH group such as norepinephrine, α-methylnorepinephrine, and isoproterenol are active. Dopamine, adrenalone, and 6-hydroxydopamine are inactive, as are most phenolic amines such as tyramine, normetanephrine, and octopamine. Both α and β receptors appear to be involved in the enhanced accumulation of cyclic AMP-^{14}C evoked by catecholamines. Serotonin, α-methylserotonin, and 4-hydroxytryptamine stimulate accumulations of cyclic AMP-^{14}C, while other isomeric hydroxytryptamines are inactive. The effect of serotonin is blocked by methsergide. Histamine and related compounds stimulate accumulation of cyclic AMP-^{14}C. α-Methylhistamine is inactive. The effect of histamine is antagonized by antihistaminics. Accumulation of cyclic AMP-^{14}C is evoked by certain tricyclic tranquilizers and antidepressants, such as chlorpromazine and imipramine. The stimulatory effect of these psychotropic agents is blocked by theophylline.

A variety of substances including norepinephrine, serotonin, histamine, ouabain, veratridine, batrachotoxin, adenosine, and tricyclic antidepressants stimulate the accumulation of cyclic AMP-^{14}C in slices of brain tissue that have been prelabeled by incubation with adenine-^{14}C.[1-8] The present paper provides structure–activity correlations for the effects of catecholamines, serotonins, histamines, antidepressants, tranquilizers, and various biogenic amine antagonists on the cyclic-AMP-^{14}C generating system present in cerebral cortical slices from guinea pig. In the accompanying paper,[9] depolarizing agents, membrane stabilizers, phosphodiesterase inhibitors, adenosine analogs, and their interactions with this system have been investigated.

Results and Discussion

General. Various biogenic amines such as norepinephrine, histamine, and serotonin cause accumulation of cyclic AMP-^{14}C in brain slices preincubated with adenine-^{14}C.[1,5,10-13] Often the effect of the biogenic amine is potentiated under conditions of membrane depolarization; *i.e.*, 43 m*M* K^+.[5] Indeed with guinea pig cerebral cortical

Table I. Accumulation of Cyclic AMP-^{14}C in Incubated Slices of Guinea Pig Cerebral Cortex. Effect of Amines in Presence of Depolarizing Concentrations (43 mM) of Potassium Ions[a]

	Δ% cAMP-^{14}C
Catecholamines[b]	
L-Norepinephrine	6.2 ± 1.0
L-Epinephrine	10.8 ± 1.8
L-Isoproterenol	3.5 ± 0.5
N-Methylepinephrine	4.2, 2.9, 2.4
N-Methylepinephrine methiodide	Ns
α-Methylnorepinephrine	4.3, 5.0
Phenolic Amines[c]	
N-Methyl-*m*-octopamine (neosynephrine)	6.2 ± 0.5
4-Hydroxy-3-methanesulfonamidophenethanolamine[d]	3.2, 3.1
Phenolic Indoleethylamines	
Serotonin	5.2 ± 1.1
N-Methylserotonin	3.6
7-Chloroserotonin	5.0
Bufotenine	Ns
α-Methylserotonin	3.0, 4.2
4-Hydroxytryptamine	4.1, 4.4
6-Hydroxytryptamine	Ns, Ns
7-Hydroxytryptamine	Ns, Ns
5,6-Dihydroxytryptamine	Ns
Histamines	
Histamine	26.0 ± 3.8
α-Methylhistamine	Ns, Ns
α-Methyl-β-hydroxyhistamine	Ns
N^{ω}-Methylhistamine	20.7
N^{ω}-Isopropylhistamine	Ns
N^{ω},N^{ω}-Dimethylhistamine	18.2
N^{ω},N^{ω}-Dimethylhistamine methiodide	Ns

[a] Assay as described.[1] Incubations were from 6 to 10 min with 0.05 mM amine. Controls with 43 mM K^+ were 6.9 ± S. D. 1.5% and Δ cAMP-^{14}C represents increase elicited by the amine. Ns = not significant with the mean less than 2 S. D. apart. Values are ±S. D. in four or more experiments or individual values. Only norepinephrine, histamine, N^{ω}-methylhistamine, and N^{ω},N^{ω}-dimethylhistamine elicited significant accumulation of cyclic AMP-^{14}C in the absence of depolarizing concentrations of K^+ (*cf.* ref 1, 6, 7). [b] 2-Methyl-, 5-methyl- and 6-methylnorepinephrine, dopamine, adrenalone, 6-hydroxydopamine, and 3,4,5-trihydroxyphenethylamine had no significant activity. [c] Tyramine, candicine, octopamine, normetanephrine, metanephrine, *N-tert*-butyl-3-hydroxymethyl-4-hydroxyphenethanolamine (salbutanol), 3-hydroxymethyl-4-hydroxyphenethanolamine, *m*-tyramine, *m*-octopamine, paranephrine, *N*-isopropyl-3,5-dihydroxyphenethanolamine (orciprenaline), 3-hydroxy-4-methanesulfonamidophenethanolamine, and α-methyl-3,5-dihydroxy-4-methoxyphenethanolamine had no significant activity. [d] Soterenol, the side chain *N*-isopropyl analog stimulates adenyl cyclase in frog erythrocytes.[22]

slices, incubation in the presence of 43 mM K^+ allowed the detection of stimulatory amines such as serotonin, whose effect on accumulation of cyclic AMP-^{14}C was within experimental variation during normal incubations.[3,5] The results presented in Tables I and II were conducted under conditions (43 mM K^+) where this enhanced response to biogenic amines pertains. A similar enhanced response to amines is also elicited by the presence of adenosine in the incubation medium (Table III).[3,5] The time of assay (6-10 min) has been chosen to minimize the effect of rapidly changing amounts of cyclic AMP-^{14}C.

Catecholamines. Primary and secondary catecholamines containing a β-OH group stimulate cyclic AMP-^{14}C accumulation in cerebral cortical slices from guinea pig (Table I). Dopamine, adrenalone, 3,4,5-trihydroxyphenethylamine, and 6-hydroxydopamine, which do not contain the β-OH group, are inactive. Dopamine is also inactive in slices from various brain regions of rat, cat, monkey, and rabbit.[5,6,13-15] The tertiary amine, *N*-methylepinephrine, is active in guinea pig cerebral cortex, while the corresponding methiodide is inactive. It is of interest in respect to the role of false transmitters in the CNS, that α-methylnorepinephrine shows marked activity in stimulating accumulation of cyclic AMP-^{14}C. Other potential false transmitters such as the 2-methyl-, 5-methyl-, and 6-methylnorepinephrine are virtually inactive.

Phenolic Amines. Octopamine and related compounds are inactive in this system and do not act as antagonists against norepinephrine or isoproterenol. 4-Hydroxy-3-methanesulfonamidophenethanolamine, however, exhibits significant activity in stimulating accumulation of cyclic AMP-^{14}C (Table I). Its isomer, 3-hydroxy-4-methanesulfonamidophenethanolamine is inactive. Only one *m*-hydroxyphenethanolamine, *N*-methyl-*m*-octopamine (neosynephrine) shows activity in this test system. Since closely related amines such as *m*-octopamine are inactive, the effect of neosynephrine is being investigated further. Nonphenolic amines such as amphetamine, mescaline, phenethylamine, and phenethanolamine are inactive.

Hydroxytryptamines. The effect of serotonin on cyclic AMP-^{14}C accumulation can be demonstrated in incubated slices of guinea pig cerebral cortex only in the presence of a depolarizing agent or adenosine.[3,5] A variety of compounds related to serotonin were tested under depolarizing conditions (Table I). Only serotonin, α-methylserotonin, 4-hydroxytryptamine, and 7-chloroserotonin are active. The tertiary amine, bufotenine, is inactive, as are 6-hydroxytryptamine, 7-hydroxytryptamine, 5,6-dihydroxytryptamine, tryptamine, α-ethyltryptamine, and *N,N*-dimethyltryptamine.

Histamines. Structure-activity relationships among histamines and related compounds, have been reported for accumulation of cyclic AMP-^{14}C in cerebral cortical slices of rabbit and guinea pig.[6] Histamine, N^{ω}-methylhistamine, and N^{ω},N^{ω}-dimethylhistamine are active both in the presence and absence of 43 mM K^+ (Table I and ref 6). α-Methylhistamine, its β-OH derivative, the methiodide of N^{ω},N^{ω}-dimethylhistamine, and N^{ω}-isopropylhistamine are inactive.

Amine Antagonists. The nature of the amine-sensitive regulatory units which govern adenyl cyclase activity in incubated brain slices has been investigated in rabbit cerebellar,[15] human cerebral,[10] and guinea pig cerebellar and cerebral slices[7] with various antagonists. The results indicated that the adenyl cyclase regulatory units responsive to catecholamines in human cerebral and in both rabbit and guinea pig cerebellar tissue are typical β receptors. By contrast, the effect of epinephrine on accumulation of cyclic AMP-^{14}C from prelabeled pools in guinea pig cerebral cortical slices appeared to be mediated by an α-receptor.[7] Chlorpromazine prevented the accumulation of cyclic AMP elicited by norepinephrine in slices of rat hypothalamus.[16]

In the present study, prelabeled slices of guinea pig cerebral cortex have been used to investigate the interaction of biogenic amines in the presence of 43 mM K^+ with a variety of amine antagonists (Table II). β-Blocking agents such as dichloroisoproterenol are particularly active against isoproterenol and relatively ineffective against the other biogenic amines. Methsergides is a very effective antagonist of serotonin. It is important to bear in mind that the results reported in Table II are obtained in the presence of a depolarizing concentration of K^+. Compounds which act as membrane stabilizers in this system will therefore antagonize the synergistic response to K^+ and a biogenic amine. Of the blocking agents tested, only pheniramine and diphenhydramine caused marked inhibition of cyclic AMP-^{14}C formation evoked by 43 mM K^+ in the absence of an amine.[9] It is surprising that none of the β antagonists *appear* to func-

Table II. Accumulation of Cyclic AMP-^{14}C in Incubated Slices of Guinea Pig Cerebral Cortex. Antagonism of Stimulatory Effects of Catecholamines, Serotonin, and Histamine in Presence of Depolarizing Concentrations of Potassium Ions by α- and β-Blocking Agents, Antihistaminics, Antiserotinergics, and Tricyclic Psychotropic Agents[a]

Blocking agent (0.1 m*M*)	Stimulatory conditions: Norepinephrine + 43 m*M* K^+	Epinephrine + 43 m*M* K^+	Isoproterenol + 43 m*M* K^+	Serotonin + 43 m*M* K^+	Histamine + 43 m*M* K^+
	% inhibition of response due to amine				
		α-Blocking agents[b]			
Phenoxybenzamine	100, 85	100	75	94, 94, 90	88, 78, 90
Dibenamine	68			100	30
Dihydroergotamine	72, 71			25, 31	0
		β-Blocking Agents[c]			
Dichloroisoproterenol	16, 28, 34	47	93	54	16
Propranalol	43, 53	27	100	62	0
		Antihistaminics			
Pheniramine[d]	69, 78			100	88
Diphenhydramine[d]	96				100, 100
		Antiserotinergics			
Methsergide	31			100, 100	30
		Tricyclic Psychotropic Agents[e]			
Chlorpromazine	67, 80, 74			100, 100	33, 73
Imipramine	76, 100, 100			54	16, 83

[a] See footnote *a* to Table I. For the increase in cyclic AMP-^{14}C accumulation elicited by these amines in the presence of 43 m*M* K^+, see Table I. [b] The response due to norepinephrine was inhibited 97% with phentolamine. [c] The response due to norepinephrine was blocked by 27% with L-(+)-*N*-isopropyl-4-nitrophenethanolamine (L-INPEA) and 40% by the D-(−) isomer (D-INPEA). It was blocked 47% by sotalol, 40% by bunolol, 18% by pronethalol, 77% by butoxamine, and 0% by the diethylmethylammonium analog of propranolol. [d] Partially blocks effect of 43 m*M* K^+ induced cyclic AMP-^{14}C formation. [e] These tricyclic compds at higher concns elevate cyclic AMP-^{14}C levels (Table IV).

Table III. Accumulation of Cyclic AMP-^{14}C in Incubated Slices of Guinea Pig Cerebral Cortex. Antagonism of Stimulatory Effects of Norepinephrine, Serotonin, and Histamine in the Presence of Adenosine by Various Blocking Agents[a]

Blocking agent (0.1 m*M*)	Stimulatory conditions: Norepinephrine + 0.1 m*M* adenosine	Serotonin + 0.1 m*M* adenosine	Histamine + 0.1 m*M* adenosine
	% inhibition response due to amine		
Phenoxybenzamine	72, 85, 76, 86	54, 65, 49	62, 67
Dichloroisoproterenol	14, 0	0, 0, 0[b]	10, 8
Propranolol[c]	0, 0, 0	0, 0	6, 0
Pheniramine	0, 16	12, 0, 0[b]	88, 83
Chlorpromazine	48, 32	0,[b] 0,[b] 0	24, 38

[a] Assay as described.[1] Incubations from 6 to 10 min with 0.05 m*M* amine. For increase in cyclic AMP-^{14}C accumulations under these conditions, see ref 3. Individual inhibition values are reported. [b] Stimulations observed in these experiments of up to 30%. [c] Neither D- nor L-INPEA significantly blocked the synergistic effect of norepinephrine and adenosine.

tion as membrane stabilizers in this system, since most of these compounds (Table II, footnote *c*), except perhaps sotalol and *N*-isopropyl-4-nitrophenethanolamine, have been reported to have local anesthetic properties.[17] A quaternary derivative of propranolol devoid of β-receptor activity[18] shows no blocking activity against norepinephrine in this system, while propranolol is active, suggesting that this blocking agent is, indeed, inhibiting the synergistic response to amine and 43 m*M* K^+ by interaction with the amine regulatory unit. However, the D and L forms of *N*-isopropyl-4-nitrophenethanolamines are nearly equally effective in antagonizing the response to norepinephrine in the presence of 43 m*M* K^+ (Table II, footnote *c*), while only the D (−) isomer is reported to have β-blocking activity.[19] Neither of the isomers shows significant blocking activity against a combination of norepinephrine and adenosine (Table III, footnote *c*), suggesting that their weak blocking activity in the presence of 43 m*M* K^+ is due more to a *selective* membrane stabilization rather than to β-blocking activity.

The synergism between depolarizing agents and amines apparently involves "release" of adenosine under depolarizing conditions.[5] Thus, an alternate and better method for screening for amine antagonists relies on the synergistic effect of adenosine and amines (*cf.* ref 3). Certain adenosine analogs and xanthine derivatives such as theophylline are the only compounds known to antagonize the effects of adenosine in this system.[9] The results obtained with blocking agents, either against the combination of the amine and adenosine (Table III) or against the combination of the amine and 43 m*M* K^+ (Table II), are usually similar. Chlorpromazine, however, does not antagonize the synergism between serotonin and adenosine, while it completely blocks the synergism between this amine and 43 m*M* K^+, indicating again *selective* membrane stabilization.

The results with the different blocking agents *vs.* the different amines suggest that a variety of regulatory units are involved. It seems reasonable to postulate that a serotonin receptor, a histamine receptor and both α and β receptors for norepinephrine exist in guinea pig cerebral cortical slices and are involved in the regulation of adenyl cyclase activity.

Antidepressants and Tranquilizers. A number of tricyclic antidepressants and tranquilizers have been found to cause accumulation of cyclic AMP-^{14}C (*cf.* ref 8). These include tricyclic antidepressants such as imipramine, protryptyline, and desipramine, and tranquilizing agents such as chlorpromazine. Other antidepressants such as amphetamine and related compounds and the monoamine oxidase inhibitors are inactive or only weakly active (Table IV). Other tranquilizers such as reserpine, tetrabenazine, and droperidol, are inactive. Prenylamine is active. A recent report[8] indicated that only the tricyclic antidepressants enhanced accumulation of cyclic AMP, but in our own studies consistent enhancement by tricyclic tranquilizers is also observed.

The mechanism whereby these psychotropic drugs cause enhanced accumulation of cyclic AMP-^{14}C is under investigation.[20] Present evidence indicates that they, like the depolarizing agents, cause enhanced accumulation of extracellular adenosine which then stimulates accumulation of cyclic AMP-^{14}C. The stimulatory effects of adenosine,[21] depolarizing agents,[2] and the psychotropic drugs are in-

Table IV. Accumulation of Cyclic AMP-^{14}C in Incubated Slices of Guinea Pig Cerebral Cortex. Effect of Antidepressants, Tranquilizers, and Related Compounds[a]

Agent	% accumulation cyclic AMP-^{14}C[b]
Antidepressants	
Imipramine	5.3
Protryptyline	7.7, 7.7
Desipramine[c]	6.8 ± 1.2
Chlorimipramine	14.4, 16.4, 17.4
Opipramol	1.3, 0.6, 0.7
Amitryptyline	5.8
Iprindole	15.9, 14.2
CNS Stimulants	
D-Amphetamine	0.2, 0.3, 0.7
Magnesium pemoline	0.2
4-Ethyl-3-piperidyl phenylcyclopentylglycolate (Ditran)	0.3
Methylphenidate (Ritalin)	1.7
Pipradol	0.3, 0.6
Azcyclonol	0.2
MAO Inhibitors	
Iproniazid	0.2
Pargyline	0.5
Tranylcypromine	0.5
D-α-Methylphenylhydrazine	0.3
Harmaline	0.4, 0.6
α-Ethyltryptamine	0.4, 0.3
Tranquilizers	
Reserpine	0.4
Tetrabenazine	0.3
Droperidol	0.6
Prenylamine (Segontin)	10.9, 11.2, 11.5
Chlorpromazine	11.4 ± 1.1
Chlorprothixene	3.8
Promethazine (Phenargan)	4.0, 12.6, 10.5
Promazine	7.4, 7.7, 7.2

[a] Assay as described.[1] Incubations from 6 to 10 min. Concentration of agents, 0.5 m*M*. [b] Normal controls: 0.3 ± S. D. 0.1%. Values are average of four or more experiments ± standard deviation or results from single experiments. [c] The effect of desipramine is unaffected by the presence of 0.1 m*M* norepinephrine or histamine or 1 m*M* cocaine. It is antagonized >50% by the presence of 1 m*M* theophylline.

hibited by theophylline. The psychotropic drugs do not, however, appear to function as depolarizing agents, since their effects on cyclic AMP are not antagonized by membrane stabilizers such as cocaine (*cf.* ref 9). The possible significance of elevated levels of cyclic AMP to the clinical efficacy of these psychotropic drugs is unknown.

Conclusions

The present paper has attempted to delineate the versatility of a simple radiometric method for investigation of the effects of biogenic amine agonists, antagonists, and other psychotropic drugs on the formation of cyclic AMP in brain tissue. The use of other species, in addition to the guinea pig, would provide an additional breadth. In rat and mouse, the responses of cyclic AMP-^{14}C to norepinephrine in the presence and absence of antagonists may be readily assessed without the presence of 43 m*M* K^+.[11-13] Other brain regions such as cerebellum may also be used for such studies, since they also differ in their responsiveness to various stimulants.[7,13]

The cyclic-AMP-generating system in brain slices is also sensitive to the effects of depolarizing agents, presumably *via* "release" of adenosine, which then interacts with an adenosine-sensitive regulatory unit for adenyl cyclase. The versatility of the present radiometric method in screening for and investigating depolarizing agents and specific and nonspecific membrane stabilizers and the role of the postulated adenosine receptor in these effects is the subject of the accompanying paper.[9]

Experimental Section

The method for assessing accumulation of cyclic AMP-^{14}C in slices of guinea pig cerebral cortex is essentially as described.[1] However, it has proven more convenient to collect slices by decanting the medium through a 400-mesh nylon net supported on several layers of absorbant tissue paper rather than through the use of Büchner funnels. Two modifications[7,13] of this method have now been described.

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Accumulation of Cyclic Adenosine Monophosphate in Incubated Slices of Brain Tissue. 2. Effects of Depolarizing Agents, Membrane Stabilizers, Phosphodiesterase Inhibitors, and Adenosine Analogs

Minta Huang, Hirotoshi Shimizu, and John W. Daly*

Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014.
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A radiometric assay involving the use of brain slices prelabeled by incubation with adenine-^{14}C provides a simple method to assess the effects of depolarizing agents, membrane stabilizers, and adenosine agonists and antagonists on the accumulation of cyclic AMP-^{14}C in brain tissue. The stimulatory effects of depolarizing agents, such as ouabain, cassaine, veratridine, and batrachotoxin, on cyclic AMP-^{14}C accumulations can be blocked by nonspecific membrane stabilizers such as cocaine or by specific membrane stabilizers such as tetrodotoxin, saxitoxin, and atelopidtoxin. Adenosine and certain analogs stimulate accumulation of cyclic AMP-^{14}C and are antagonized by other adenosine analogs or by xanthine derivatives such as theophylline. Interactions between agents provides a means of assessing their mechanism of action in this cyclic-AMP-generating system.

A simple radiometric technique[1] for assaying the accumulation of cyclic adenosine 3,5′-monophosphate (cyclic AMP) from a prelabeled pool of adenine nucleotides in brain slices provides a means to assess the activities of a variety of compounds in terms of inhibition or stimulation of the accumulation of cyclic AMP-^{14}C in the CNS.[2-9] The preceding paper[2] dealt with biogenic amines, their agonists and antagonists, and the tricyclic psychotropic drugs. The present paper investigates the effects of membrane-depolarizing agents, membrane stabilizers, phosphodiesterase inhibitors, and adenosine analogs.

Results and Discussion

Depolarizing Agents. Only a few classes of compounds are known that can interact with electrogenic membranes so as to evoke membrane depolarization. The cardioactive glycosides represent one such class of compounds. They are presumed to act by inhibition of the membranal Na^+-K^+ activated ATPase, resulting in a slow increase in intracellular Na^+ concentration and a concomitant gradual drop in transmembrane potential.[10] The time course for cyclic AMP formation elicited by ouabain in brain slices is, indeed, much slower than with other agents.[11] The effects of ouabain on cyclic AMP accumulation require Ca^{2+} [11] and may be antagonized by membrane stabilizers such as cocaine and tetrodotoxin.[7] The effect of ouabain on the cyclic AMP-generating system is also antagonized by theophylline, providing evidence for the intermediacy of adenosine.[7,11] A variety of cardioactive steroids have now been tested for effects on the formation of cyclic AMP-^{14}C (Table I). The aglycones, strophanthidin, digitoxigenin, and gitoxigenin were less active than the corresponding glycosides. Cassaine which has been shown to inhibit the Na^+-K^+ activated ATPase[12,13] also stimulated cyclic AMP accumulation in brain slices. Both ouabain[14] and cassaine[15] inhibit rather than stimulate the cylic-AMP-generating system of adipose tissue. Other reported inhibitors of the Na^+-K^+ activated ATPase, such as oligomycin[16] and ethacrynic acid,[17] had no effect on the accumulation of cyclic AMP in brain slices. Oligomycin has, however, been reported to differ from ouabain in its effects on ATPase systems in incubated brain slices.[18]

Another class of compounds, the veratridine alkaloids, appear to cause membrane depolarization by increasing permeability of electrogenic membranes to Na^+.[19] Certainly their effects in biological systems can be prevented by tetrodotoxin,[20] an agent which is known to specifically block increases in the permeability of membranes to Na^+.[21] In brain slices, veratridine elicits accumulation of cyclic AMP-^{14}C. This activity is antagonized by tetrodotoxin, cocaine, and theophylline and requires the presence of Ca^{2+}.[7,8,11] Only one other alkaloid of this class, protoveratrine, exhibited activity in the brain slice (Table I). The inactive analogs of veratridine, that is germine, veracevine, and zygadenine, differ from the active compounds in containing a free 3-hydroxy group rather than an ester.

Another class of compounds which cause depolarization of electrogenic membranes are the steroidal alkaloids related to batrachotoxin. Batrachotoxin in a variety of preparations appears to selectively and irreversibly increase the permeability of electrically excitable membranes to Na^+.[22] Its activity can be antagonized by tetrodotoxin. In brain slices, batrachotoxin elicits cyclic AMP-^{14}C accumulation.[11] This effect is antagonized by tetrodotoxin and by theophylline, and requires the presence of Ca^{2+}.[3,8,11]

Batrachotoxinin A, a much less toxic congener of batrachotoxin was inactive in brain slices in eliciting an accumulation of cyclic AMP-^{14}C (Table I). Certain analogs with differing ester moieties (2,5-dimethylpyrrole-3-carboxylate and 4-bromobenzoate) were active in brain slices, as is the methiodide of batrachotoxin. The effect of the 4-bromobenzoate analog was, in contrast to that of batrachotoxin, readily reversible (see below).

A variety of natural products including steroidal alkaloids, such as α-solanine, were inactive in brain slices with respect to accumulation of cyclic AMP-^{14}C (footnote to Table I). Solanine is reported to be similar in action to cardiac glycosides with respect to its effect on the heart.[23] 5-Benzyloxy-2-iminohexahydropyrimidine caused an accumulation of cyclic AMP-^{14}C, probably by a mechanism involving depolarization as will be discussed below. Holothurin,