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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

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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,

Table I. Accumulation of Cyclic AMP-^{14}C in Incubated Slices of Guinea Pig Cerebral Cortex. Effect of Various Classes of Depolarizing Agents[a]

Compound[b]	Concentration, mM	% accumulation cyclic AMP-^{14}C
Ouabain Type[c]		
Ouabain	0.05	13.1
Strophanthidin	0.1	4.5
Digitoxin	0.05	13.5
Digitoxigenin	0.1	9.9
Lantosid B	0.05	13.5
Gitoxigenin	0.1	6.6
Scillaren A	0.1	9.7
Convallatoxin	0.1	13.1
Oleandrigenin	0.1	10.6
Cassaine	0.1	6.2
Veratridine Type		
Veratridine	0.05	17.2
Protoveratrine	0.05	11.5
Germine	0.05	0.2
Veracevine	0.05	0.2
Zygadenine	0.05	0.2
Batrachotoxin Type		
Batrachotoxin	0.002	20.6
	0.001	15.9
	0.0001	12.0
Batrachotoxinin A (BTX-A)[d]	0.1	0.4
BTX-A 20-α-2,5-dimethyl-pyrrole-3-carboxylate	0.002	14.6
BTX-A 20-α-(4-bromobenzoate)	0.01	19.9
Batrachotoxin methiodide	0.01	21.7
Miscellaneous		
Papaverine	0.1	1.0, 1.5
5-Benzyloxy-2-imino-hexahydro-pyrimidine (HM-197)	2.0	2.1
Holothurin A	0.1	3.2
Ethanol	500	1.6 ± S. D. 0.6

[a] Assay as described in text. Incubations from 10 to 15 min with ouabain and related compounds, 7–11 min with other agents. Control values are 0.3 ± S.D. 0.1%. [b] A variety of other alkaloids, steroidal alkaloids, etc., were tested at 0.1 mM concentration unless otherwise specified. Significant accumulation of cyclic AMP-^{14}C was not elicited by veratramine, α-solanine. solanidine, conessine, samandarine, tomatillidine, jervine, coniine, strychnine, gelsemine, buphanamine, dehydrobufotenine, reserpine, morphine (0.5 mM), cocaine, yohimbine, dihydroergotamine, scopolamine, theophylline (1 mM), ryanodine (0.05 mM), cortisol, tetrodotoxin (0.05 mM), saxitoxin (0.04 mM), atelopidtoxin (2.5 μg/ml), and apomorphine. Acetycholine either alone or in combination with the acetylcholine esterase inhibitor, physostigmine, did not elicit accumulation of cyclic AMP-^{14}C. The cholinomimetic, carbachol (0.1 mM) was also inactive. [c] Other inhibitors of Na^+-K^+ activated ATPase such as $NiCl_2$ (1 mM), oligomycin (20 μg/ml), and ethacrynic acid (2 mM) were inactive in this system. [d] Batrachotoxinin A at 0.1 mM does not antagonize the effect of batrachotoxin at 0.0001 mM in this system.

a membrane-active saponin with neuromuscular blocking activity,[24] caused accumulation of cyclic AMP-^{14}C. Depolarization-induced accumulation of cyclic AMP-^{14}C is also elicited by EtOH (Table I) and by elevated levels of K^+ or NH_4^+.[8]

Membrane Stabilizers. Compounds that tend to stabilize membrane toward changes in trans-membrane potentials, antagonize the stimulatory effects of depolarizing agents on cyclic AMP-^{14}C formation.[7,8] Such membrane stabilizers include nonspecific agents such as cocaine, and specific agents such as tetrodotoxin which selectively prevent increases in membrane permeability to Na^+.[21] The present test system provides a means to readily assess membrane-stabilizing properties of compounds in brain slices. The compound may be tested either against veratridine which appears to depolarize *via* an elicited increased permeability of membranes to Na^+, against ouabain which apparently depolarizes *via* inhibition of Na^+-K^+ activated ATPase, or against K^+.

Table II. Accumulation of Cyclic AMP-^{14}C in Incubated Slices of Guinea Pig Cerebral Cortex. Antagonism of Stimulatory Effects of Depolarizing Agents by Membrane Stabilizers[a]

Inhibitory agents	% inhibition of stimulatory effects of: Ouabain, 0.05 mM	Veratridine, 0.08 mM	K^+, 43 mM
"Nonspecific" Membrane Stabilizers			
Chlorpromazine (0.5 mM)		55	26
Chlorpromazine methiodide (0.5 mM)			80
Cocaine (1 mM)	79	90	59
Dromoran (1 mM)		95	78
Morphine (1 mM)		14	
Diphenhydramine (0.5 mM)			53
Propranolol (0.5 mM)		88	20
Phenoxybenzamine (0.5 mM)		23	0
Sodium Phenobarbital (1 mM)		25	
Papaverine (0.1 mM)		62	100
Pipradol (0.5 mM)		90	
Prenylamine (0.5 mM)		52	
Specific Membrane Stabilizers			
Tetrodotoxin (0.05 mM)	59[b]	96[c]	12
Saxitoxin (0.04 mM)	45	98[c]	18
Atelopidtoxin (2.5 μg/ml)	47	96	11
(0.25 μg/ml)		54	
Tetraethylammonium ions (3 mM)	11	0	0

[a] Assay as described.[1,2] Incubations from 10 to 15 min with ouabain and from 6 to 10 min with other agents. Control values for ouabain 18.8 ± S.D. 3.1%; veratridine, 19.1 ± S.D. 1.9%; and K^+ (43 mM) 6.9 ± S.D. 1.5%. Control values are 0.3 ± S.D. 0.1%. [b] The effect of 0.1 mM cassaine (another inhibitor of Na^+-K^+ activated ATPase) was inhibited 50% by the presence of 0.001 mM tetrodotoxin. [c] The effect of 0.002 mM batrachotoxin was also completely blocked by tetrodotoxin and saxitoxin.

A variety of compounds including a local anesthetic, cocaine; an analgetic, dromoran; an antihistaminic, diphenhydramine; a tranquilizer, chlorpromazine and its methochloride; and a β-blocking agent, propranolol, were active in antagonizing veratridine- or K^+-elicited accumulation of cyclic AMP-^{14}C (Table II). It is apparent that many of the so-called nonspecific membrane stabilizers such as chlorpromazine are somewhat more selective in blocking the Na^+ channel-mediated effects of veratridine than those mediated by K^+. In agreement with the present data, chlorpromazine has been reported to affect mainly Na^+ conductances.[25] Specific membrane stabilizers such as tetrodotoxin and saxitoxin completely blocked the effects of veratridine and batrachotoxin, partially blocked the effects of ouabain and did not significantly decrease the effect of elevated concentrations of K^+. Tetraethylammonium ions which are known to effect "K^+ channels" rather than "Na^+ channels" in the membrane[26] did not antagonize the accumulation of cyclic AMP-^{14}C elicited by ouabain, veratridine, or K^+.

The antagonistic effect of 10^{-8} M tetrodotoxin against the accumulation of cyclic AMP-^{14}C elicited in 4–6 min in brain slices by varying concentrations of veratridine was investigated. Rather surprisingly for such a complex system, the results gave a straight line on Lineweaver-Burke analysis (Figure 1), suggesting a competitive inhibition by tetrodotoxin of the effects of veratridine in this system.

The blocking of Na^+ channels by tetrodotoxin can be readily reversed by washing the biological preparation[21] while the increased permeability to Na^+ elicited by batrachotoxin is not reversed on washing.[22] A high accumulation of cyclic AMP-^{14}C ensues in brain slices that are first incubated with tetrodotoxin and batrachotoxin[8] and then washed and incubated for an additional period of time, illustrating the irreversible nature of the action of batrachotoxin.

Table III. Accumulation of Cyclic AMP-^{14}C in Incubated Slices of Guinea Pig Cerebral Cortex. Activity of Adenosine Analogs and Related Compounds as Stimulators for Cyclic AMP-^{14}C Accumulation and as Inhibitors of the Stimulation Dye to Adenosine, Histamine, and Veratridine[a]

Agent	Concn, m*M*	% accum cyclic AMP-^{14}C	% inhibition of response[b] due to: Adenosine, 0.1 m*M*	Histamine, 1.0 m*M*	Veratridine, 0.08 m*M*
None		0.3 ± S. D. 0.1			
Adenosine	0.1	3.8 ± S. D. 0.6			
		Purine Modified Analogs[c]			
2-Chloroadenosine	0.1	3.0			
Isoquanosine (2-hydroxyadenosine	0.1	1.8			
2-Aminoadenosine	0.1	1.6			
8-Bromoadenosine	0.1	0.3			
8-Methylaminoadenosine	0.1	1.3			
		Sugar Modified Analogs[d]			
2'-Deoxyadenosine	0.3	0.5	74	30	
3'-Deoxyadenosine	0.3	0.4	89	88	76
5'-Deoxyadenosine	0.5	0.8	84	0	58
Adenine 9-β-L-ribofuranoside	0.3	0.9	35		
Adenine 9-β-D-arabinoriboside	0.3	0.7	82		
Adenine 9-β-D-xylofuranoside	0.3	0.4	89		
		Phosphodiesterase Inhibitors			
Theophylline[e]	1.0	0.4	94	0	74
	0.3		81		
Caffeine	0.5		58		
1-Methyl-3-isobutylxanthine	0.3	0.7	88		
5,5'-Dibromobarbituric acid	0.3		0		
Papaverine	0.1	1.0	0		

[a] Assay as described in text. Incubations from 6 to 11 min. Control values for adenosine 3.8 ± S. D. 0.6%; histamine 2.2 ± S. D. 0.7%; veratridine, 19.1 ± S. D. 2.1%. [b] Preincubated with inhibitor for 3–4 min prior to addition of stimulant. [c] Tubercidine, 1-methyladenosine, 6-*N*,*N*-dimethyladenosine, 8-azadenosine, and inosine were inactive as stimulants at 0.1 m*M* and were inactive as adenosine antagonists at 0.3–0.5 m*M*. [d] 5'-Deoxy-5'-aminoadenosine, 5'-deoxy-5'-azidoadenosine, adenosine-5-nicotinate, 3'-*C*-methyladenosine, 3'-*O*-methyladenosine, and 2',3'-isopropylideneadenosine were inactive as stimulants at 0.1 m*M*. [e] 7-Methylxanthine, 8-azaxanthine, 1-methylxanthine, and 8-chlorotheophylline were inactive as adenosine antagonists at 0.5 m*M*.

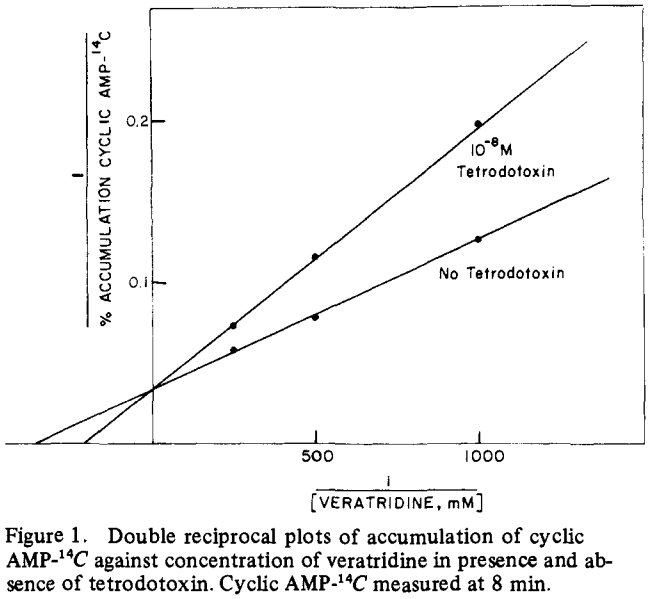

Figure 1. Double reciprocal plots of accumulation of cyclic AMP-^{14}C against concentration of veratridine in presence and absence of tetrodotoxin. Cyclic AMP-^{14}C measured at 8 min.

A much lower accumulation of cyclic AMP-^{14}C ensues in slices incubated with tetrodotoxin and veratridine, washed and incubated for an additional period, indicating the reversible nature of the action of veratridine.[8] In a similar experiment, slices were incubated with the 4-bromobenzoate of batrachotoxinin A (0.01 m*M*) and tetrodotoxin (0.5 m*M*) for 6 min, washed and incubated for an additional 8 min in Krebs–Ringer solution. Tetrodotoxin initially blocked the action of the 4-bromobenzoate of batrachotoxinin A. Eight minutes after washing the preparation, a low accumulation of cyclic AMP-^{14}C of 3.7% was found. A control incubation with the 4-bromobenzoate for 8 min resulted in an accumulation of 17.8%. Thus, in marked contrast to batrachotoxin, the action of the 4-bromobenzoate of batrachotoxinin A is readily reversible upon washing.

The compound, 5-benzyloxy-2-iminohexahydropyrimidine (I) has been reported to be a depolarizing agent.[27] Its neuromuscular blocking activity has, however, been compared to tetrodotoxin.[27,28] In brain slices, I *did* elicit a significant accumulation of cyclic AMP (Table I) and this effect could be partially blocked by 1 m*M* cocaine or by 1 m*M* theophylline, suggesting a depolarization-linked accumulation of cyclic AMP-^{14}C. In contrast to tetrodotoxin or saxitoxin, I potentiated rather than blocked the effect of veratridine. The combined effect of I and 43 m*M* K^+ on cyclic AMP-^{14}C accumulation was nearly additive. The results suggest that in brain slices the membranal action of I is quite different from that of tetrodotoxin.

Atelopidtoxin (II), a potent highly polar cardiotoxin recently isolated from a Panamanian frog,[29] has been reported to have little activity in blocking action potentials in nerve axons.[30] It was, however, extremely active in blocking the effect of veratridine in brain slices (Table II). In this regard, it possessed approximately 0.1 the activity of tetrodotoxin. II had virtually no effect on K^+-induced or histamine-induced accumulation of cyclic AMP. The blocking effect of II did not appear to be reversible. Thus, when slices had been incubated with II (2.5 μg/ml) for 6 min, washed, and then incubated with veratridine (0.08 m*M*), the accumulation of cyclic AMP during the second incubation by veratridine is almost completely blocked. In this regard, II *differs from tetrodotoxin*, since the effects of the latter compound are readily reversible on washing.

Adenosine and Related Compounds. Adenosine has been

postulated as the intermediary[31] in the mechanism whereby depolarizing agents elicit cyclic AMP-^{14}C accumulation.[3,7-9] Theophylline because of its antagonism of adenosine-mediated mechanisms partially or completely blocks the effects of depolarizing agents.[8] Thus, in studies on membrane stabilizers, it must be ascertained that the compound does not directly antagonize the effects of adenosine. As yet, the only compounds to be found that specifically antagonize the effect of adenosine on cyclic AMP-^{14}C formation are xanthine derivatives such as theophylline, and certain adenosine analogs (Table III). Previously it has been shown that the response to adenosine is highly specific.[7,32] Only adenosine derivatives such as 5′-AMP, ADP, ATP, and adenosine *N*-oxide that could be enzymatically or chemically converted to adenosine during incubations caused enhanced accumulations of cyclic AMP-^{14}C. Certain ring substituted analogs of adenosine such as 2-chloroadenosine, isoguanosine, and 8-methylaminoadenosine have now been found to elicit a significant accumulation of cyclic AMP-^{14}C. The majority of analogs of adenosine modified in the purine ring (tubercidine, 8-azaadenosine) are, however, relatively ineffective in either eliciting or preventing adenosine-induced formation of cyclic AMP-^{14}C. By contrast, analogs modified in the ribosyl moiety strongly inhibit the formation of cyclic AMP-^{14}C as elicited by adenosine, but they do not cause accumulation of cyclic AMP-^{14}C. 5′-Deoxyadenosine antagonizes only adenosine-mediated stimulation of the cyclic AMP-^{14}C generating system, while the 2-deoxy and 3-deoxy isomers also antagonize histamine-mediated stimulation (Table III). The mechanism by which such analogs block formation of cyclic AMP is under investigation.

Theophylline and analogous xanthine derivatives inhibit adenosine-mediated, but not histamine-mediated formation of cyclic AMP (Table III). These compounds also inhibit the 3′,5′-cyclophosphodiesterase that hydrolyzes cyclic AMP to 5′-AMP. An analog such as 1-methyl-3-isobutylxanthine, however, which is some 15 times as active as theophylline as a phosphodiesterase inhibitor[33] is only slightly more active in inhibiting the response of the cyclic AMP-generating system to adenosine. Other potent phosphodiesterase inhibitors such as papaverine, dipyridamol, and hexobendine[34] and 5,5′-dibromobarbituric acid[35] do not or only slightly inhibit the effect of adenosine on cyclic AMP-^{14}C accumulation. Thus, activities of these compounds as phosphodiesterase inhibitors or as adenosine antagonists are clearly different.

The effect of adenosine on cyclic AMP-^{14}C accumulation might be expected to be potentiated by agents which either prevent the degradation or uptake of adenosine. Various adenosine-potentiating substances which are specific coronary vasodilators[36,37] were tested; lidoflazine, dipyridamole, and hexobendine at 0.1 m*M* had no significant effect on stimulation of cyclic AMP-^{14}C accumulation evoked by adenosine at the same concentration.

Conclusions

The present study provides new information on compounds which can influence the accumulation of cyclic AMP-^{14}C in brain and demonstrates the usefulness of the present technique for probing the underlying mechanisms of pharmacological activity for a broad spectrum of compounds.

Experimental Section

The assay of cyclic AMP-^{14}C accumulation in guinea pig cerebral cortical brain slices was carried out as described.[1,2]

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Relationship between Antihistamine and Antidepressant Activity in Hexahydroindenopyridines

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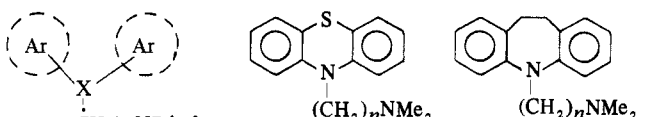
The antihistamine phenindamine 4 (R = Me) has been converted to two epimeric hexahydroindenopyridines **5b** and **6b**, and the pharmacological profile of both of these products shown to be similar to that of the antidepressant desmethylimipramine. These findings do not support the postulate that a relationship exists between antihistamine and antidepressant drugs which depends on the closeness of approach of basic and aromatic centers. Two short series of N-substituted and analogous hexahydroindenopyridines were also made but no activity of note was found. The structural requirements for antihistamine activity in phenindamine and the present compounds are discussed.

It has been recognized for some time that a degree of sedation accompanies the action of most therapeutically effective antihistaminic agents (H_1-receptor antagonists).[1] The intensity of this central effect varies appreciably among the numerous structural types of these drugs, and in the case of phenindamine, central stimulation is usually observed.[2,3] Central depression, therefore, is not necessarily a corollary of peripheral H_1-antagonist activity.

The structural pattern **1**, which can be identified in some form in most antihistaminic agents, can also be recognized in the phenothiazine tranquilizers (*e.g.*, promazine, **2a**) and in the tricyclic antidepressants (*e.g.*, imipramine, **3a**). Both promazine and imipramine exhibit H_1-antagonist properties but these are much more marked in their lower homologs, **2b** and **3b**.[4,5] Several groups of workers have observed that antihistaminic agents possess to varying degrees the pharmacological properties of antidepressant agents, *e.g.*, potentiation of the cardiovascular effects of catecholamines,[6,7] antagonism of reserpine-induced hypothermia,[8] potentiation of amphetamine-induced excitation,[9] and blockade of amine uptake in central and peripheral neurones.[10]

In an attempt to reconcile the structural similarities of the H_1-receptor antagonists of general formula 1 and the psychotherapeutic drugs **2a** and **3a** with their respective biological



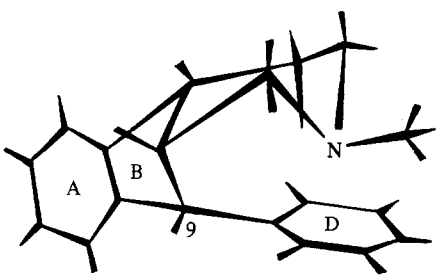
1
Ar = aromatic ring
X = CH, N or CH–O–

2
a, $n = 3$
b, $n = 2$

3
a, $n = 3$
b, $n = 2$

activities, it occurred to us that differing lengths, or preferred conformations of side chains, with consequent variations in the degree and type of interaction of the polarizable aromatic π electrons with the polar N (which will be protonated at the biological pH of 7.4), could result in affinity for different receptors or in altered transport and binding properties. If this hypothesis is correct, it follows that minor molecular modifications of the basic side chain might convert an antihistaminic drug to a substance with predominantly "antidepressant" properties as is observed in the change from **3b** to **3a**. Phenindamine 4 (R = Me) was the antihistaminic drug chosen for such an investigation since its basic center is part of a fused ring system and small structural changes should result in predictable spatial variations of the required type. Its mild central stimulant properties were also of interest in the antidepressant context.

Phenindamine is a rigid molecule in which close approach of the basic center to either aromatic ring is impossible. It is already known that hydrogenation to dihydrophenindamine results in virtually complete loss of H_1-antagonist activity, but the stereochemistry of the product was not studied by the original workers.[11,12] Two of the present authors have shown that the product of hydrogenation is all cis isomer **5a** (see Table I) which is readily epimerized in alkali at C_9 to the H_{4a},H_{9a}-cis; H_9,H_{9a}-trans isomer **6a**.[13] The rings B/C trans fused isomers were not encountered. Examination of Dreiding framework and Corey-Pauling-Koltun space-filling models show that both of the B/C cis fused isomers are flexible molecules, identical with regard to the nearest possible approach of the N to ring A. However, if interaction with the C_9 phenyl ring is considered, there is a clearcut difference between **5a** and **6a**. In the latter, approach of the N to the aromatic center is impossible, but in the former, an N-ring D distance of 2-2.5 Å is attainable (see diagram).



Dreiding molecular model of **5a**

Clearly, the series **4**, **5a**, and **6a** provide an excellent opportunity for testing the hypothesis that different N-aromatic separations are responsible for changes from antihistaminic to antidepressant properties. Initial encouragement was obtained when it was shown that, in contrast to phenindamine, **5a** possesses a pharmacological profile similar in some respects to that of desmethylimipramine (DMI), and this paper reports the synthesis and structure-activity relationships of a short series of indenopyridines in which