vitro, and MAO activity was determined. Aliquots of ethanol were added to all control samples.

Results. Rat hypothalamic MAO activity was uneffected by changes in photoperiod (Table) but was inhibited in vitro when melatonin was added at concentrations of 10^{-4} and 10^{-5} *M* (Table). Rat hypothalamic MAO activity was increased after pinealectomy. Rat pituitary MAO was significantly increased by constant light and pinealectomy and was significantly decreased by constant darkness (Table). Additions of melatonin in vitro at concentrations of 10^{-4} through 10^{-6} *M* decreased pituitary MAO activity (Table).

Discussion. Recent reports suggest that 5-HT as well as other biogenic amine levels increase in the hypothalamus and midbrain after melatonin injections[18,19]. The results from our investigations suggest that this increase could be due to melatonin induced inhibition of MAO activity. This is suggested by the effects of pinealectomy, photoperiod alterations, and melatonin additions in vitro on pituitary and hypothalamic MAO activity. The effects of the treatments on MAO activity were more dramatic in the pituitary than in the hypothalamus. Because the hypothalamus is a mixture of various types of neurons, it may not be as homogeneous a source of MAO as the pituitary. More clear cut results might, therefore, be expected with the pituitary than with hypothalamic preparations. Specific areas of the hypothalamus might undergo changes in MAO activity that could be diluted and therefore not detected with whole hypothalamic homogenate preparations.

Recently it was suggested that pinealectomy enhances LH mobilization via a central serotonergic mechanism, perhaps by depressing serotonin levels of the hypothalamus thus enhancing LH seeretion[21]. Melatonin administration has the opposite effect and increases serotonin levels[18,21]. Our data suggest that MAO activity is effected by melatonin and could represent a target enzyme for this hormone. In this respect, the effect of melatonin on MAO activity could explain the changes in brain biogenic amines after melatonin injections[18,19], and could also explain how melatonin suppresses FSH and LH secretion[15-18]. Melatonin could alter biogenic amine levels in the brain by inhibiting MAO activity and thus effect biochemical and behavioral processes in the animal.

Monoamine oxidase activity in rat hypothalamus and pituitary after melatonin injections, alterations in photoperiod, pinealectomy, or additions of melatonin in vitro

Treatment	No. of animals	Tissue	CPM × 10^3/mg tissue	*P*-value
Photoperiod				
LL	7	Hypothalamus	60.68 ± 3.86[a]	> 0.50[b]
LD	7	Hypothalamus	64.69 ± 3.28	
DD	7	Hypothalamus	63.21 ± 2.68	> 0.50
				> 0.50[c]
LL	7	Pituitary	96.95 ± 6.10	< 0.01
LD	7	Pituitary	74.70 ± 1.17	
DD	7	Pituitary	64.49 ± 2.59	< 0.01
				< 0.001
Pinealectomy				
Control		Hypothalamus	60.41 ± 0.62	
Pinealectomized		Hypothalamus	65.03 ± 0.56	< 0.10
Control		Pituitary	40.30 ± 6.52	
Pinealectomized		Pituitary	60.86 ± 4.19	< 0.02
Melatonin in vitro[d]				
Control		Hypothalamus	81.32 ± 1.20	
10^{-4}*M* Melatonin			58.53 ± 0.83	< 0.001
10^{-5}*M* Melatonin			66.25 ± 0.36	< 0.001
10^{-6}*M* Melatonin			81.22 ± 0.33	> 0.50
Control		Pituitary	9.12 ± 0.37	
10^{-4}*M* Melatonin			6.63 ± 0.12	< 0.05
10^{-5}*M* Melatonin			7.66 ± 0.38	< 0.05
10^{-6}*M* Melatonin			7.39 ± 0.42	< 0.05

Activities are expressed as mean ± standard error of mean.
[a] Standard error of mean values. [b] *P*-value when compared with control animals (pinealectomized control and LD animals, respectively). [c] *P*-value when compared with other treated group. [d] $N=6$ for each assay.

Summary. Rat pituitary MAO activity was reduced by constant darkness and by additions of melatonin in vitro and was increased by constant light and by pinealectomy. Hypothalamic MAO activity followed the same pattern but was less dramatically affected. The data suggest that MAO may be a target enzyme for melatonin.

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Cyclic Nucleotides vs. Adenosine Analogs as Inhibitors of Adenylate Cyclase Activity: Nonidentity of Sites of Action

Previous reports from our laboratories concerning the inhibition of adenylate cyclase (basal) activity from guinea-pig lung by cyclic nucleotide derivatives[1] and adenosine analogs[2] have contrasted the actions of these two classes of inhibitors in at least two ways. First, the inhibition by 2′*O*-palmitoyl cyclic AMP[1] appears competitive from double-reciprocal kinetic plots, whereas the corresponding analysis for the adenosine analog, 9-(tetrahydro-2-furyl)-adenine, reveals inhibition of a noncompetitive type[2]. Second, inhibition of cyclase activity by adenosine analogs is Mg^{2+}-dependent[2], whereas inhibition by cyclic nucleotide derivatives is not[1,2]. In this communication we present the results of additional studies that clearly show that the structural requirements for inhibition by these two classes of compounds are markedly different.

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Table I. 6-Substituted cyclic nucleotides as inhibitors of adenylate cyclase activity from guinea-pig lung

Compound		I_{50} (mM) [a]	% Inhibition at 6 mM
A)	1.8 mM Mg^{2+} present in assay		
	Cyclic AMP	>6.0	<15
	N^6,N^6-diethyl cyclic AMP	2.3	75
	N^6-benzyl cyclic AMP	0.3	76 [b]
	8-Benzylthio cyclic AMP	>6.0	<15
	N^6-ethyl-8-benzylthio cyclic AMP	3.5	60
	N^6,N^6-diethyl-8-benzylthio cyclic AMP	0.3	94 [b]
	N^6-butyl-8-benzylthio cyclic AMP	0.6	100
B)	11.8 mM Mg^{2+} present in assay		
	N^6-ethyl-8-benzylthio cyclic AMP	3.5	77
	N^6-butyl-8-benzylthio cyclic AMP	0.5	100
	N^6-phenylcarbamoyl-8-benzylthio cyclic AMP	1.0	50 [b]
	6-Piperidino-8-benzylthio purine cyclic ribotide	0.2	100 [b]

[a] Concentration of inhibitor necessary for 50% inhibition of cyclase activity. [b] Inhibition at 1 mM compound.

Materials and methods. Duplicate assays of adenylate cyclase activity were carried out as reported previously[3,4] with [α-^{32}P]ATP as substrate and an isolation procedure involving the use of [^{3}H]cyclic AMP as a recovery standard, chromatography on Dowex 50 ion exchange resin, and treatment with nascent $BaSO_4$. Adenylate cyclase fractions were suspensions of once-washed pellets obtained by centrifugation (at 1000 $\times g$ for 15 min) of homogenates of lung tissue[1] from male guinea-pigs. Cyclic nucleotide derivatives were supplied by Dr. R. K. Robins of the Nucleic Acid Research Institute of ICN Pharmaceuticals, Inc. Adenosine analogs were contributed by Miss K. A. Losee of the Squibb Institute for Medical Research, except for N^6-(3-methyl-2-butenyl)adenosine (NSC 105546), which was obtained from Dr. H. B. Wood, jr., of the National Cancer Institute.

Results and discussion. Substitution of the N^6-amino function of either cyclic AMP or the 8-benzylthio derivative increases the potency of the resulting compound as an inhibitor of basal cyclase activity, relative to the parent structure (Table I). 6-Piperidino-8-benzylthiopurine cyclic ribotide is most striking in this regard, being more than 30 times as potent as 8-benzylthio cyclic AMP. The I_{50} value (0.2 mM) determined for this compound is, however, significantly higher than that (0.03 mM) found for *2'0*-palmitoyl cyclic AMP[1], the most potent cyclic nucleotide inhibitor we have observed. Similarly, N^6-benzyl cyclic AMP is more than 20 times as potent as cyclic AMP as a cyclase inhibitor. Results for the N^6-ethyl-8-benzylthio and N^6-butyl-8-benzylthio derivatives emphasize the lack of dependence of inhibitory potency upon Mg^{2+} concentration.

In marked contrast to the results for cyclic nucleotide derivatives, substitution of the N^6-amino group of either adenosine or 9-benzyladenine decreases the inhibitory effect relative to the parent compound (Table II). For the 9-benzyladenine series, the decreases in potency amount to more than 30-fold. It is of interest that replacement of the N^6-amino group (as in 9-benzyl-6-chloropurine) does not decrease inhibitory potency any further than, for example, alkyl or dialkyl substitution of the amino group.

These results represent additional evidence that cyclic nucleotide derivatives and adenosine analogs inhibit cyclase activity by different mechanisms. Najjar et al.[5–7] have recently postulated a model for the regulation of adenylate cyclase activity in which the enzyme is inactivated through phosphorylation by a cyclic AMP-dependent protein kinase, possibly present in close proximity to cyclase in the plasma membrane. This model suggests that the potency of a cyclic nucleotide as an inhibitor of lung cyclase activity might be directly related to its ability to stimulate cyclic AMP-dependent protein kinase in the same preparation. Data concerning the ability of cyclic nucleotides to stimulate plasma-membrane-associated protein kinase from lung are not

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Table II. 6-Substituted adenosine analogs as inhibitors of adenylate cyclase activity from guinea-pig lung

Compound	I_{50} (mM) [a]	% Inhibition at 6 mM
Adenosine	0.15	86
N^6-(3-methyl-2-butenyl)adenosine	ND [c]	57
9-Benzyladenine	0.2	81 [b]
9-Benzyl-N^6, N^6-dimethyladenine	>6.0	34
9-Benzyl-N^6-ethyladenine	~6.0	49
9-Benzyl-6-hydrazinopurine	>6.0	22
9-Benzyl-6-chloropurine	~6.0	49

[a] Concentration of inhibitor necessary for 50% inhibition of cyclase activity. Assays carried out in the presence of 11.8 mM Mg^{2+}. [b] Inhibition at 3 mM compound. [c] Not determined.

available in the literature. However, cyclic AMP at concentrations from 10^{-9} M to 10^{-3} M did not affect lung cyclase activity ($\leq 7\%$ change), as might have been expected if kinase activity regulated cyclase activity in these fractions (see also reference[4]). The results of this experiment suggest that cyclic nucleotides do not inhibit lung cyclase activity via effects on an associated protein kinase, and that adenosine analogs and cyclic nucleotides may inhibit cyclase activity by distinct interactions with the cyclase moiety.

Zusammenfassung. Substitution einer N^6-Aminogruppe eines zyklischen Nukleotids führt zu einer erhöhten Hemmwirkung des Nukleotids gegenüber Adenyl-Cyclase von Meerschweinchenlungen, während die N^6-Amino-Substition von Adenosin-Analogen eine herabgesetzte Inhibitionswirksamkeit gegenüber demselben Enzym zur Folge hat. Die experimentellen Daten führen zu dem Schluss, dass der Inhibitionsmechanismus gegenüber Cyclase für beide Verbindungstypen verschieden ist.

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Influence of Methylxanthines on Aniline Disappearance and Metabolism in Rats

A change in microsomal enzyme activity may result in intoxication[1,2] or partial inactivation of drug action[3] and hence be hazardous to animals or man. Therefore, this study was undertaken to compare the in vivo and in vitro drug metabolism of methylxanthines which have been found to stimulate[4,5] or inhibit[6] microsomal enzyme activity when measured in vitro.

Materials and methods. For both assays, in vitro and in vivo measurement of microsomal enzyme activity, male rats weighing 315 $\pm$ 10 g were divided between 1 control and 8 test groups. Animals were pretreated with caffeine 150 mg/kg or 37.5 mg/kg, or theobromine 150 mg/kg or 37.5 mg/kg; instant tea, or instant coffee containing 75 mg/kg of caffeine. All these substances were diluted in water and administered per os daily for 3 days. 2 additional groups were injected i.p. either with 75 mg/kg of phenobarbital, a classic inducer[7] or 100 mg/kg of SKF 525-A a known inhibitor[8]. An in vivo and in vitro assay was also carried out using male rats (320 $\pm$ 20 g) which were pretreated with 75, 46 or 27 mg/kg of phenobarbital or 100, 56 or 32 mg/kg of SKF 525-A together with a control group.

The in vivo and in vitro measurements were made 24 h after the last administration of all test substances, except in the case of SKF 525-A measured after 1 h. To determine the microsomal metabolism in vivo, rats were injected i.p. with 50 mg/kg of aniline and after approxi-

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Table I. In vitro measurement of microsomal enzyme activity

	Aniline hydroxylase (pmoles p-amino phenol/mg protein/min incubation)	Cytochrome P-450 (nmoles/mg protein)
Control (water)	39.8 $\pm$ 4.0	0.831 $\pm$ 0.126
Theobromine (150 mg/kg)	67.1 $\pm$ 7.4[c]	0.783 $\pm$ 0.097
Theobromine (37.5 mg/kg)	38.8 $\pm$ 4.2	0.814 $\pm$ 0.051
Caffeine (150 mg/kg)	45.6 $\pm$ 3.9[a]	0.787 $\pm$ 0.163
Caffeine (37.5 mg/kg)	36.7 $\pm$ 5.6	0.833 $\pm$ 0.127
Instant coffee (containing 75 mg of caffeine/kg)	44.7 $\pm$ 4.6	0.824 $\pm$ 0.093
Instant tea (containing 75 mg of caffeine/kg)	51.7 $\pm$ 5.4[c]	0.830 $\pm$ 0.152
SKF 525-A (100 mg/kg once)	28.5 $\pm$ 5.7[b]	0.785 $\pm$ 0.095
Phenobarbital (75 mg/kg)	127.3 $\pm$ 15.9[c]	1.080 $\pm$ 0.106[b]

Theobromine, caffeine, instant coffee, instant tea and water for controls were administered per os daily for a 3-day pretreatment. Phenobarbital was injected i.p. daily for 3 days and SKF 525-A once only. Each group contained 8 male rats and mean values with confidence limits at 95% were given. Significant difference (t-test) between control and treated groups is indicated as: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.