The deacetylation of N-acetylmethionine occurred more rapidly than that of N-acetylethionine. Deacetylation of N-acetylmethionine was followed by assaying for the formation of methionine (Figure 2). A linear relationship resulted with 1.8 μmoles of methionine being formed by 30 min. These results show that N-acetylmethionine and N-acetylethionine are deacetylated by liver microsomes, but are not converted to the sulfoxide derivative. N-acetylethionine sulfoxide and N-acetylmethionine sulfoxide incubated under the same conditions with liver microsomes were not deacetylated. Carcinogenic arylacetamides have also been reported to be deacetylated by microsomes from dog liver[11].

BENNEDETTI et al.[12] reported that the growth rate of rats fed a methionine-deficient diet (12% casein or enzymatic casein hydrolysate) supplemented with N-acetylmethionine was as good as if the diet was supplemented with methionine. They suggested that the hydrolysis of the acetyl group of N-acetylmethionine took place gradually so that the methionine released mixed with the amino acids released by the hydrolysis of casein. The present study suggests that the rat handles N-acetylethionine in a similar manner.

Summary. Rat liver microsomes deacetylated N-acetylethionine and N-acetyl-methionine to ethionine and methionine. The deacetylation of N-acetylmethionine was more rapid than the deacetylation of N-acetylethionine. Ethionine was slowly converted to ethionine sulfoxide by the microsomal preparations. N-Acetylethionine and N-acetylmethionine were not oxidized by the microsomes.

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Monoamine Oxidase Activity of the Hypothalamus and Pituitary: Alterations after Pinealectomy, Changes in Photoperiod, or Additions of Melatonin in vitro[1]

The pineal gland appears to be involved in the control of gonadal function, possibly acting through the hypothalamic-hypophyseal axis, by altering the synthesis and/or release of gonadotropins[2,3]. The search for an antigonadal agent from the pineal gland has resulted in the isolation of melatonin as well as other compounds[2]. Melatonin fulfills many of the requirements of an antigonadal agent[4]: it reduces ovarian[5–7] and testicular size[8–11]; affects androgen synthesis in vitro[12,13]; and inhibits the release of LH[14,15] and FSH[16,17]. It has been suggested that this inhibition is mediated in part through the hypothalamus[15].

Injections of melatonin increase brain serotonin (5–HT) concentrations[18] as well as other brain biogenic amines[19]. Moreover, 5–HT has been reported to suppress the release of both LH[15] and FSH[17] and can decrease testicular size and alter testicular morphology[19]. Because of the similarities of action between melatonin and 5–HT and the increase in tissue levels of 5–HT caused by melatonin, we decided to ascertain whether melatonin could alter monoamine oxidase activity (MAO, monoamine: O_2 oxidoreductase [deaminating] E.C.1.4.3.4. – The enzyme that metabolizes 5–HT to 5-hydroxyindole acetaldehyde) of neurogenic tissues both in vivo and in vitro.

Materials and methods. The effects of photoperiod on MAO activity were determined by exposing 24-day-old rats (Sprague-Dawley derived strain, 7 animals per group) to 3 different lighting schedules for 10 days prior to sacrifice: the 1st group was placed in constant light (LL); the 2nd group received 12 h light followed by 12 h dark (LD); the 3rd group was placed in constant darkness (DD). At the time of sacrifice, organs were removed, weighed and frozen for later assay.

Another group of seven 24-day-old rats (Sprague-Dawley strain) was pinealectomized, while the control group was sham-operated. Both groups were sacrificed 9 days after sugery. Hypothalami and pituitary glands were assayed for MAO activity as previously described[20]. Statistical comparisons were accomplished by use of the students *t*-test.

In vitro effects of melatonin on MAO activity were determined by using rats from our small animal colony (Holtzman strain). Aliquots (2 mg) of pituitary, or hypothalami were weighed, homogenized, and assayed for MAO activity[20]. Various concentrations of melatonin (dissolved in 95% ethanol) were added to homogenates in

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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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