the pulmonary artery of monocrotaline-treated rats is apparently related to the elevation of pulmonary arterial blood pressure as indicated by the right ventricular hypertrophy (table 1). According to Kay et al.[9] and Katayama et al.[10] the systolic pressure of the pulmonary artery ranges from 20 to 30 mm Hg in control rats, and after monocrotaline treatment, it can reach 100 mm Hg or more.

Ooshima et al.[5,6] and Iwatsuki et al.[7] have reported that in 2 models of hypertension in rat, there is increased synthesis of collagen in various arteries of the systemic circulation including the aorta, mesenteric arteries and brain arteries, and that such effects are reversed when blood pressure is lowered by antihypertensive drugs. On the basis of these findings, they have postulated that elevation of blood pressure itself initiates the changes in collagen synthesis in the arterial vessels. The present results suggest that this possibility is also applicable in the case of the pulmonary circulation.

Table 2. In vitro incorporation of ^{14}C-labeled proline into collagenase-digestible protein of arterial tissues isolated from monocrotaline-treated rats and control rats

Group	Pulmonary artery	Aorta
Test	2,283 ± 420[a] (6)[b]	2,464 ± 650 (6)[b]
Control	499 ± 149 (5)[b]	3,485 ± 1,680 (9)[b]

[a] $p < 0.01$; [b] the numerals in the parentheses indicate the number of pools of tissue, each comprising 4–10 rats. Rats were killed 21 or 22 days after an injection of 40 mg/kg of monocrotaline. The pulmonary artery and aorta were excised, minced and pooled. Samples of tissue were incubated with ^{14}C-labeled proline, homogenized, dialyzed, and treated with collagenase. Values for incorporation of ^{14}C-labeled proline are expressed as cpm/mg protein. The values represent the mean ± SD.

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Antidepressant drugs elevate rat pineal and plasma melatonin*

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Summary. Acute administration in the mid-light phase of a number of antidepressant drugs of different pharmacological profiles elevated pineal and plasma melatonin (measured by radioimmunoassay). Following chronic treatment with the tricyclic antidepressant clomipramine, the elevation was significantly reduced. This may be an effect of reduced ß-adrenergic receptor sensitivity after chronic clomipramine administration, analogous to other findings of reduced ß-adrenergic receptor binding and reduced noradrenaline-sensitive adenylate-cyclase response.

The development of radioimmunoassay[2] and gas-chromatography-mass-spectography methods[3] for measuring the pineal hormone melatonin has permitted physiological studies of circulating levels in man. Plasma melatonin undergoes circadian variations that reflect variations in pineal β-adrenergic receptor sensitivity. This has led to growing interest in the measurement of melatonin in affective disorders, since both desynchronization of circadian rhythms[4] and β-adrenergic sensitivity changes[5] have been postulated to occur in depression and mania. Preliminary clinical investigations indicate a lowering of melatonin in depression and an increase of it in mania[6,7] that would be in accordance with the latter hypothesis. The antidepressant drug desmethylimipramine can increase the conversion of tryptophan to melatonin[8], as well as increasing the activity of the enzyme N-acetyl-tranferase in vitro and in vivo[9]. We therefore treated animals with a number of different antidepressant drugs (and potential antidepressants) and measured melatonin in the pineal gland and plasma after both acute and chronic administration.

Male albino Wistar rats were kept on an light: dark cycle of 12:12 (lights on at 05.00 h) for 3 weeks prior to use, at a temperature of 25 °C, with food and water ad libitum. In acute drug experiments, the animals were injected i.p. in the middle of the light phase and decapitated 2 h later; in chronic experiments, the animals were injected i.p. daily 15 min before lights off and decapitated 2 h after a final injection. Trunk blood was collected in heparin and centrifuged, and the plasma frozen; pineals were rapidly removed and frozen.

Melatonin concentrations were determined by a previously described radioimmunoassay[10]. Extensive validation using the classical techniques of parallelism of diluted aliquots of extract, cross-reactivity measurements, and chromatographic identity of immunoreactivity with standard melatonin, showed that the antibody used in these studies (K244) shows good specificity and sufficient sensitivity for reproducibility[11]. However, radioimmunoassay specificity is always open to question since every sample cannot be validated. We found in some of the pineal extracts from animals treated with antidepressant drugs that TLC, (in $CHCH_3$:methanol 9:1 using tritiated melatonin as marker) revealed a fast-running immunoreactive spot. The R_f did not correspond to any indoles similar in structure to melatonin that have been tested for cross-reactivity against K244. TLC of plasma from the same animals did not show the fast-running spot found in the pineals. However, TLC of plasma from animals treated with Ro 11-2465 showed about 30% of the immunoreactivity to be slow-running and not associated with melatonin. Thus, certain groups might have pineal melatonin values slightly increased due to this unknown component; this caveat applies only to animals

treated with desmethylimipramine, imipramine, and Ro 11-2465, but not to controls or plasma samples (except for Ro 11-2465).

The following drugs that are used clinically or claimed to be antidepressants were injected (50 mg/kg) in the mid-light phase 2 h before sacrifice: clomipramine, imipramine, desmethylimipramine, maprotiline, pargyline, L-5-hydroxy tryptophan (L-5HTP) and Ro 11-2465[12].

For chronic experiments lasting 4 weeks, L-5HTP-ester (Ro 3-5940, 100 mg/kg/day) clomipramine (20 and 10 mg/kg/day) and Ro 11-2465 (10 mg/kg/day) were injected i.p. together with saline controls. All animals (including controls) received a final acute dose of the appropriate drug in the mid-light phase 2 h before sacrifice.

Figure 1 summarizes the effects of acute administration (50 mg/kg) on pineal melatonin. Increasing serotonin availability by precursor loading with L-5HTP had little effect. As previously reported[13], the monoamine oxidase inhibitor, pargyline, had the most marked elevation. Of greater interest was the similar and marked elevation of melatonin by antidepressant drugs, both noradrenaline (desmethylimipramine, maprotiline) and/or serotonin (imipramine, clomipramine, Ro 11-2465) uptake inhibitors.

In the table, the corresponding plasma melatonin values following acute drug administration are shown. As in the pineals, the least elevation was achieved by L-5HTP and the greatest with pargyline. Among the different uptake inhibitors, all drugs except clomipramine increased plasma melatonin. In the entire group, a correlation between pineal and plasma melatonin concentrations in each animal was found ($r = 0.50$, $N = 52$, $p < 0.01$). Thus, elevation of pineal melatonin by most drugs was correlated with a corresponding but smaller increase in plasma melatonin. Figure 2 summarizes the effect of chronic drugs on pineal melatonin. Chronic L-5HTP treatment again had no effect. In contrast, 2 dosage regimes of chronic clomipramine resulted in a reduction of the elevated pineal melatonin after the final acute injection of the drug. Chronic Ro 11-2465, in spite of having many pharmacological properties similar to clomipramine, did not cause a reduction of the acute elevation.

These results show that, similar to desmethylimipramine[8,9] a number of other antidepressant drugs can also elevate pineal melatonin. It is very surprising that a selective serotonin uptake inhibitor, Ro 11-2465, (W. Burkard, personal communication) can elevate pineal and plasma melatonin as do the selective noradrenaline uptake inhibitors maprotiline and desmethylimipramine. However, in view of the presence of interfering compounds in both pineal and serum extracts of animals treated with Ro 11-2465 it is possible that the elevation of melatonin is somewhat overestimated.

The experiment was not designed to distinguish between the effects of reuptake inhibition or β-adrenergic receptor stimulation. However, the increase of melatonin after different antidepressants may be understood in the light of evidence suggesting a common β-adrenergic agonist action independent of differences in presynaptic pharmacology. Such a common mechanism of action has been proposed with respect to the effect of different antidepressant modalities to induce β-receptor subsensitivity. This has been documented for β-adrenergic receptor binding[14-19], and for reduction of the noradrenaline-sensitive adenylate-cyclase response[20,21]. The observation that chronic clomipramine reduced the melatonin elevation fits in well with such an interpretation of reduced β-receptor sensitivity. However, it is noteworthy that of the drugs studied, only clomipramine had such an effect. Neither L-5HTP, still controversial therapeutically, nor Ro 11-2465, a drug not yet clinically

Effect of acute antidepressant drugs on rat plasma melatonin

Treatment (50 mg/kg)	N	Melatonin pg/ml mean ± SEM	Significance (t-test)
Controls	14	15.9 ± 1.5	
Maprotiline	5	39.2 ± 4.6	$p < 0.001$
Desmethyl-imipramine	5	40.0 ± 8.5	$P < 0.001$
Imipramine	5	23.8 ± 4.4	$p < 0.05$
Clomipramine	6	13.3 ± 2.5	NS
Ro 11-2465	6	23.7 ± 2.3	$p < 0.01$
Pargyline	6	47.7 ± 5.7	$p < 0.001$
L-5HTP	5	21.2 ± 4.3	NS

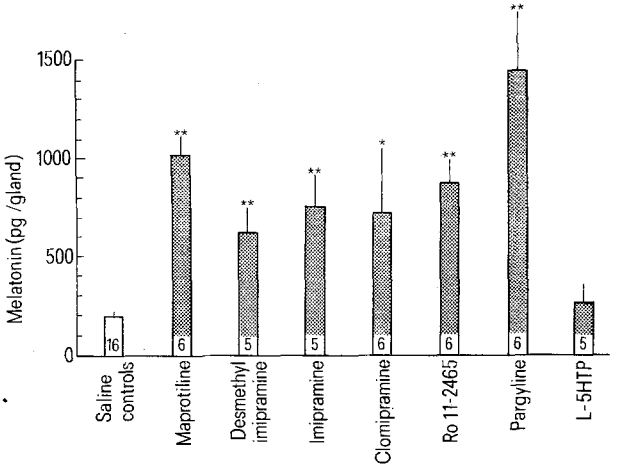

Fig. 1. Melatonin content of pineal glands 2 hours after acute drug injection i.p. (50 mg/kg) in the mid-light phase: means ± SEM of the number of animals indicated in each column. * $p < 0.002$, ** $p < 0.001$.

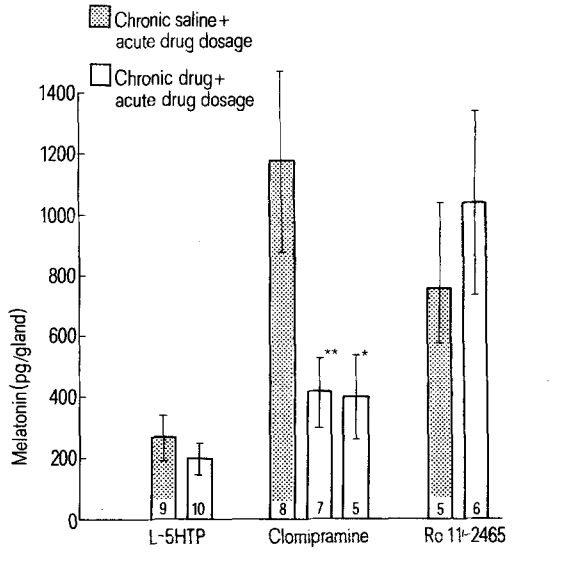

Fig. 2. Melatonin content of pineal glands 2 hours after a final drug injection i.p. in the mid-light phase. Controls stippled columns) had received saline injections daily for 4 weeks previously, and received a single acute injection of the active drug. Animals treated with chronic doses of drugs (open columns) received daily injections of L-5HTP-ester (50 mg/kg), 2 doses of clomipramine (20 mg/kg or 10 mg/kg), Ro 11-2465, (10 mg/kg) before the final acute injection of active drug. *$p < 0.05$, **$p < 0.02$.

tested, had such acute-chronic differences. The latter drug could be compared with fluoxetine, also a selective serotonin uptake inhibitor, that following chronic treatment neither reduced the noradrenaline-sensitive adenylate-cyclase response[22] nor β-adrenergic receptor binding[23]. The correlation between pineal and plasma melatonin is useful in considering clinical studies. Although in the rat plasma melatonin appears to be entirely pineal in origin[24], this is not yet clarified for other species, including man. However, post-mortem pineal synthetic enzymes in man do follow the circadian pattern of circulating melatonin[25]. Thus, these animal data showing melatonin increase following the administration of antidepressant drugs, indicate the possible potential of plasma melatonin measurements in man.

A number of questions arise from these observations: is there a common mechanism of action of antidepressant drugs related to their ability to acutely elevate pineal melatonin? Is this elevation of melatonin related to their β-adrenergic agonist properties? If so, is β-adrenergic stimulation a necessary and/or sufficient pharmacological characteristic of an antidepressant drug, as has recently been suggested[26]? Furthermore, is the difference between chronic clomipramine, which reduced the pineal melatonin response, and L-5HTP and Ro 11-2465 which did not, meaningful with respect to differences in clinical antidepressant efficacy? The answers to these questions may lead to a better understanding of the mechanism of action of antidepressant drugs.

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Dexamethasone protection against the acute lethality of ethanol in mice[1]

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Summary. The administration of dexamethasone (DXM, 2.00 mg/kg) 1 h prior to the injection of lethal doses of ethanol was found to offer complete protection against ethanol toxicity at doses up to 5.25 g/kg and partial protection using higher doses. It is suggested that DXM central action might be involved in the protection against ethanol toxicity.

Ethanol toxicity in mice which is mainly due to respiratory depression[3], can be reversed by the administration of a β-adrenoceptive antagonist[3,4]. On the other hand dexamethasone (DXM) (9- α fluoro-16,α methyl prednisolone, DXM) was suggested to act via inhibiting β-receptors activity[5]. The reversal of ethanol central depression would imply therapeutic potential but β-antagonist usually have deterimental cardiovascular effects. Therefore, it was of interest to study the protective effect of a drug that antagonize ethanol toxicity with minimum side effects.

Materials and methods. In this experiment Swiss-Webster male mice, 25–35 g, had food and water available ad libitum. They were maintained under controlled temperature conditions (23±1 °C) and 12:12 light-dark cycle (lights were on 09.00 h). Animals were kept in these conditions for a period of at least 3 weeks prior to experimentation. Various doses of ethanol were chosen randomly in attempting to find the lethal dose. DXM (2.0 mg/kg, Sigma) was injected 1 h prior to the administration of ethanol. The vehicle for DXM was an equal mixture of propylene glycol and saline and was injected i.p. Likewise ethanol was diluted with saline and was injected using 25% ethanol concentration. Animals treated with ethanol was injected with DXM vehicle to be used as control. Animals were