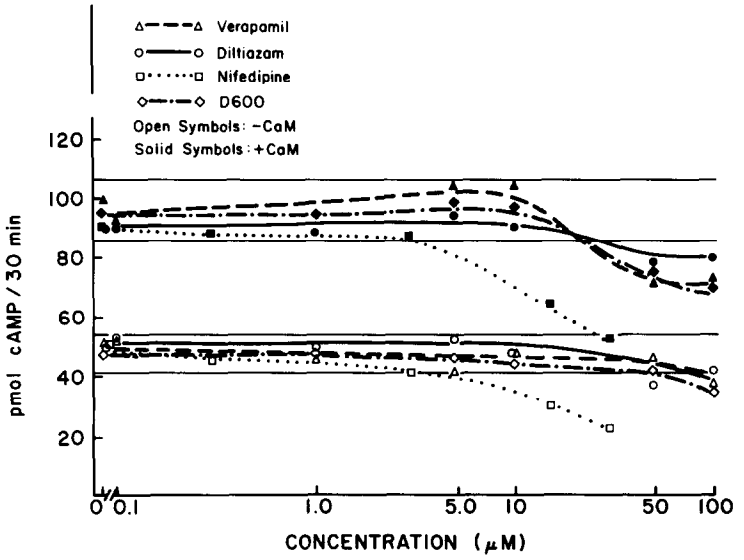

Fig. 3. Effect of increasing concentrations of calcium blockers on the activity of phosphodiesterase in the absence and presence of CaM. Each point represents the mean of three different experimental determinations.

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Effect of superior cervical ganglionectomy on melatonin stimulation by specific MAO-A inhibition

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Monoamine oxidase (MAO) (EC 1.4.3.4) inhibitors have been shown to increase melatonin content of the rat pineal gland [1]. Direct or indirect stimulation of β-adrenergic receptors as well as increased availability of the substrate (serotonin) for melatonin synthesis have been proposed as explanations for this effect of MAO inhibitors [2, 3]. We have observed recently that inhibition of MAO-A, but not MAO-B, increases rat pineal melatonin [4]. The rat pineal is innervated solely by sympathetic nerves that originate in the superior cervical ganglia [5]. MAO-A is found within these sympathetic nerve endings [6, 7], while MAO-B is found within pinealocytes [7]. Considering that superior cervical ganglionectomy almost completely eliminates MAO-A from the pineal gland [6, 7], it is of interest to explore whether ganglionectomy will alter the clorgyline-induced increase of rat pineal melatonin.

Methods

Male Sprague–Dawley rats (200–250 g) were kept two per cage under constant temperature (22°) and a 12 hr light–12 hr dark schedule with free access to food and water for at least 1 week before the experiment.

Superior cervical ganglionectomy (bilateral) was performed according to procedures described elsewhere. Two weeks after the operation (time necessary for completion of the denervating process—the development of ptosis was observed in all animals), saline or clorgyline (2.5 mg/kg, i.p.; a dose which almost completely inhibits MAO-A activity in rat brain basal ganglia [4]) was injected at 10:00 a.m. into sham-operated and ganglionectomized animals. Pineals were removed 2 hr later.

Each pineal was used for the determination of melatonin, serotonin (5-HT), *N*-acetylserotonin (NAS), tryptophan

Table 1. Pineal indole concentrations*

	Melatonin	5-HT	5-HIAA	TRP	NAS
Sham operated (control)	0.18 ± 0.5	110.7 ± 19.2	6.0 ± 3.1	7.0 ± 1.9	ND†
Ganglionectomy	0.19 ± 0.05	69.2 ± 17.0‡	2.9 ± 0.5	5.5 ± 1.6	ND
Sham operated + clorgyline	1.09 ± 0.36§	128.1 ± 32.1	0.8 ± 0.3§	7.7 ± 5.9	0.7 ± 0.4
Ganglionectomy + clorgyline	0.38 ± 0.08§,‖	84.2 ± 27.9	1.4 ± 0.4‡	7.8 ± 2.6	ND

* Each value is the mean ±S.D.; N = 4, except for the sham-operated + clorgyline animals where N = 6.
† Not detectable.
‡,§ Significantly different compared to control (Student's *t*-test): ‡ $P < 0.05$ and § $P < 0.01$.
‖ Significantly different compared to sham-operated plus clorgyline treatment (Student's *t*-test): $P < 0.01$.

(TRP), and 5-hydroxyindoleacetic acid (5-HIAA) by a high performance liquid chromatographic-fluorometric procedure [8].

Results

Levels of pineal indoles for the sham-operated animals in this study (Table 1) were comparable with those of intact control rats from previous reports [4, 9]. The effects observed as a result of ganglionectomy on daytime indoles are in accordance with the literature data [9].

Melatonin content in rat pineals was unaffected by superior cervical ganglionectomy (Table 1). Clorgyline increased melatonin content in both sham-operated (control) and ganglionectomized rats. After clorgyline administration, however, the melatonin increase was much greater in the sham-operated animals (505% above control) than in the ganglionectomized animals (100% above control) (Table 1).

N-Acetylserotonin (NAS) (the intermediate compound between melatonin and serotonin), which is not usually detected during daytime, was observed only in the sham-operated plus clorgyline animals (Table 1).

Discussion

Superior cervical ganglionectomy did not affect basal levels of melatonin, apparently because our experiments were performed during daytime when β-adrenergic stimulation of the pineal is minimal. Ganglionectomy, however, dramatically diminished the clorgyline-induced increase in pineal melatonin and NAS. Considering that ganglionectomy almost completely eliminates MAO-A from the pineal gland, our results suggest that clorgyline increases pineal melatonin primarily through inhibition of MAO-A and consequently prevents monoamines from degradation. Both serotonin and noradrenaline are substrates for MAO-A. Therefore, both increased availability of the substrate (serotonin) for melatonin synthesis and indirect stimulation of β-adrenergic receptors (by noradrenaline) may have been responsible for the clorgyline-induced increase of rat pineal melatonin.

The small increase in melatonin seen in clorgyline-treated ganglionectonized rats is explicable if some of the postsynaptic cell bodies of nerve fibers that innervate the pineal are distal to the ganglia and therefore survived the surgical procedure. This argument is supported by the observation that clorgyline was capable of reducing the content of 5-HIAA in pineals of ganglionectomized rats. Presumably this clorgyline-sensitive enzyme is the MAO-A in some residual sympathetic nerve endings.

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