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Daily Propranolol Administration Reduces Pineal Concretion Formation in the Mongolian Gerbil[1] (42354)

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Abstract. Daily afternoon injections of a β-adrenergic blocking agent, propranolol, for 8 weeks inhibited the number of vacuoles and concretions formed in the gerbil pineal gland. The data suggest that sympathetic input to the pineal gland is necessary for the formation of pineal concretions.

The pineal gland of the Mongolian gerbil (*Meriones unguiculatus*) accumulates pineal concretions (brain sand, acervuli, corpora arenacea) with age (1). Interruption of the rich sympathetic innervation of the gland by superior cervical ganglionectomy prevents concretion formation in young animals (2) or drastically reduces the number of concretions in adult animals (3); these data suggest that pineal concretions are labile structures and that they are maintained directly or indirectly by an intact sympathetic innervation. The main purpose of the present study was to evaluate the hypothesis that blockade of β-adrenergic stimulation by propranolol would lead to a decreased number of pineal concretions.

Materials and Methods. All gerbils used in this experiment were bred in our animal facility during the month of June. The room was maintained on a 14:10 light:dark photoperiod (lights on 0600 hr) and a constant (22 ± 2°C) ambient temperature. Animals received food and water *ad libitum*. At 4 weeks of age, pups (n = 8/group) were assigned to either a control group or a group receiving injections of propranolol.

Propranolol was prepared fresh each day. Animals received a subcutaneous injection of saline or propranolol (5 mg/kg body wt) each afternoon between 1600 and 1800 hr for 8 weeks. This time of day was chosen since the pineal indole melatonin also decreased pineal concretion formation and it had been injected 2–4 hr before the onset of darkness. At the termination of the experiment, the animals were weighed and killed. Testes and accessory organs (seminal vesicles + coagulating glands) were excised and weighed. The superficial pineal glands were quickly exposed to Bouin's fluid, embedded in paraffin, sectioned serially and mounted on glass slides. The sections were stained with hematoxylin and eosin and alternate sections were evaluated for vacuoles containing pineal concretions or for empty vacuoles. Results were analyzed by a two-tailed t test.

Results. The results of the histological evaluation are shown in Fig. 1. Treatment with propranolol for 8 weeks significantly inhibited pineal concretion formation; similarly, the number of pinealocytes with large empty vacuoles was significantly reduced. Body weights of males treated with propranolol were significantly reduced ($P < 0.005$) compared to the saline-injected control animals (controls 76 ± 5 g vs propranolol 58 ± 3 g). Regardless of the change in body weight, the absolute weights of the testes were not altered after treatment with propranolol (controls 877 ± 136 mg vs propranolol 1031 ± 18 mg); the absolute weight of the accessory organs tended to be smaller in the propranolol-treated group although this was not statistically significant.

Discussion. Recent evidence suggests that the gerbil pineal receives multiple neural inputs (cholinergic, peptidergic, catecholaminergic) (4–6). The sympathetic projection to the superficial pineal gland originates in the superior cervical ganglia and is transmitted to the pineal gland via the nervi conarii (4, 7). Fluorescence microscopy of the superficial pineal gland reveals a heavy catecholaminergic innervation; these nerve fibers exhibit a green fluorescence with an excitation/emission

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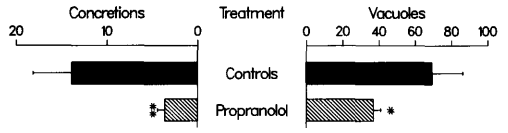

FIG. 1. Total number of vacuoles and concretions of gerbils receiving daily afternoon injections of propranolol for 8 weeks are shown. Means ± SEM are indicated. $^{**}P < 0.01$; $^{*}P < 0.05$ vs controls.

maxima of 415/475 nm typical of norepinephrine (8) as has been found in the pineal gland of numerous other species (9). Classically, it is thought that norepinephrine release from these nerve endings reversibly binds to β-adrenergic receptors located on the pinealocytes thereby starting a chain reaction which ultimately leads to melatonin synthesis and excretion (10). Administration of a β-adrenergic receptor antagonist such as propranolol inhibits the night-time rise in *N*-acetyltransferase (NAT), the rate-limiting enzyme in the synthesis of melatonin in the rat (11).

Calcified inclusions in the pineal gland of the gerbil were first reported by Japha and colleagues (1). They noted that the concretions appeared in pineal cells called chromophobes as early as 3 weeks of age; the concretions increased in number throughout life (1). The ultrastructural morphology of the gland and these interesting structures has been subsequently described in detail and recently reviewed (12, 13). Denervation of the gland by superior cervical ganglionectomy at 4 weeks of age or chronic treatment with the pineal indole melatonin beginning when the animals are 8 weeks old prevents the formation of the calcareous deposits (2, 14). In the present experiment, daily afternoon treatment with the β-adrenergic blocking agent propranolol beginning at 4 weeks of age also prevented concretion formation; these results are very similar to the observations obtained in the above-mentioned denervation studies. Thus, it would seem that β-blockade affects the CNS or the pineal and disturbs the normal balance in neural input to the gland which is necessary to initiate and maintain formation of the acervuli; the effect may be a primary one directly at the level of the pinealocyte or perhaps a secondary one due to lack of sympathetic innervation of some other brain region which affects pineal function. Whether this effect is strictly due to a dampening of the sympathetic input to the gland or is the result of a delicate interplay of sympathetic, cholinergic, and peptidergic input to the vacuolated pinealocytes remains to be investigated.

The origin and biological function of the concretions is a topic under consideration in a number of laboratories at the present time. Much of the older literature maintains that the concretions are the result of degenerative changes in the pineal gland. However, newer theories maintain that the concretions may (1) serve as a storage site for pineal peptide or protein hormones (15) or (2) are the result of a calcium-carrier complex being deposited on an organic matrix after a polypeptide is secreted into the blood vascular system (16). Alternatively, Diehl (17) suggested that pineal concretions occurred due to a reduced drainage of tissue fluids since the gland lacks a well-developed lymphatic system. Which, if any, of the above-mentioned theories is correct is unknown at the present time although the observation that propranolol inhibits the formation of these concretions mitigates against the idea that these are static structures which simply accumulate in the pineal with age.

One interesting but as yet unexplained phenomenon concerns the relatively small number of concretions and vacuoles present in the gerbil pineal glands from the present experiment compared to previous studies from our laboratory (3, 14). One difference between this experiment and those previously published (3, 14) concerns the source from which the animals were obtained. In the present experiment, the animals were bred in our facility; in previous studies (3, 14), the animals were obtained from a commercial supplier. Thus, shipping and the housing of animals initially unfamiliar to each other may have caused unusual stressful conditions and high catecholamine levels which prompt concretion formation; alternatively, other factors (genetic, time of year) may affect this process.

There are interesting clinical applications of the present study. Just like the gerbil gland, the human pineal gland calcifies with age; the chemical composition of the acervuli of both species is nearly identical as determined by ultrastructural and X-ray microprobe techniques (18). Since numerous individuals are

placed on chronic propranolol therapy for the treatment of hypertension, such patients may, like gerbils, fail to form new concretions and actually show regression of the concretions already accumulated; such a precedent for the regression of extant concretions has been previously reported in the gerbil (3).

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