Effect of surgical denervation on interscapular brown adipose tissue of hypophysectomized rats

	Control rats	Hypophysectomized rats	
		Sham denervated	Denervated
Body weight (g)			
Initial (arrival)	191 ± 4	186 ± 3	179 ± 3
28 °C adaptation	247 ± 6	156 ± 4▲	156 ± 2
Final (at sacrifice)	315 ± 9	157 ± 6▲	152 ± 2
Brown adipose tissue			
Wet weight (mg)			
per rat	410 ± 23	214 ± 20▲	250 ± 15
per 100 g b.wt	133 ± 7	135 ± 9▲	165 ± 9■
Mitochondrial proteins (mg)			
per IBAT	10.4 ± 0.9	7.7 ± 0.6▲	5.4 ± 0.6■
per IBAT/100 g b.wt	3.3 ± 0.3	4.9 ± 0.3▲	3.6 ± 0.4■
GDP binding (nmol) per mg mitochondrial proteins	0.26 ± 0.03	0.66 ± 0.09▲	0.19 ± 0.02■
per IBAT	2.82 ± 0.50	5.00 ± 0.73▲	1.02 ± 0.17■
per IBAT/100 g b.wt	0.88 ± 0.15	3.16 ± 0.37▲	0.66 ± 0.10■

Results are presented as mean ± SEM. Number of animals: 6 per group; ▲ significant effect of hypophysectomy, ■ significant effect of sympathectomy.

absence of thyroid and adrenal hormones, hypophysectomized rats can increase IBAT thermogenesis. In 28 °C hypophysectomized rats, as in normal cold-adapted rats surgical denervation impairs the stimulation of the proton conductance pathway [8]. Thus, this observation might mean that the changes observed in IBAT of hypophysectomized rats are mediated by the sympathetic system. Hypophysectomy did not modify the norepinephrine content of IBAT and did not produce changes in the medullo-adrenal system [12]. However nothing is known about enhanced norepinephrine turnover or enhanced norepinephrine sensitivity in the BAT of hypophysectomized rats.

Some hypotheses remained to be verified concerning the origin of BAT stimulation in hypophysectomized rats. Firstly, it might be suggested that thermal neutrality of hypophysectomized rats is higher than 28 °C and that some stimulation of thermogenesis occurs at that temperature. Secondly, since the hypophysectomized rat is deficient in pituitary, adrenocortical and thyroid hormones, the observed stimulation cannot be accounted for by pituitary-regulated hormones. It is possible that the lack of such hormones enhances the effect of sympathetic activity on the BAT thermogenic process by suppressing some retrocontrol. Rothwell and Stock [5] claimed that hypophysectomy stimulates thermogenesis, probably as a result of decreased adrenal steroid release since chronic treatment of hypophysectomized rats with ACTH lowers purine nucleotide binding to BAT mitochondria. However, these last findings were difficult to relate to the ACTH promoting effect on BAT thermogenesis in normal rats [13, 14]. It is possible that high levels of hypothalamic releasing factors such as corticotropin-releasing factor can stimulate BAT [15].

From this study, it could be concluded that the hypophysectomy-induced increase in BAT activity is dependent on sympathetic system integrity. However, the mechanisms of sympathetic stimulation are still unknown.

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Neither prolactin nor growth hormone restore the nocturnal rise in pineal N-acetyltransferase activity or melatonin content in hypophysectomized rats

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Summary. Hypophysectomy in adult male rats greatly attenuated the nocturnal rise in both pineal N-acetyltransferase (NAT) activity and melatonin content. High nighttime levels of NAT and melatonin were not restored by treating the animals with either prolactin or growth hormone, alone or in combination. Treating intact rats with bromocriptine, which depresses circulating prolactin levels, also was without effect on pineal melatonin synthesis. It appears that neither prolactin nor growth hormone are of major importance in determining pineal melatonin production.
Key words. Pineal gland; hypophysectomy; N-acetyltransferase; melatonin; prolactin; growth hormone.

The pineal gland of virtually all mammals exhibits a nocturnal increase in melatonin content[3]. Whereas light exposure during the normal dark period can totally suppress the nighttime rise in pineal melatonin[4-6], one endocrine manipulation, i.e., hypophysectomy, has been shown to attenuate, although not totally prevent, the nocturnal elevation of melatonin in the pineal gland of the rat[7]. The effect of hypophysectomy on the ability of the pineal gland to produce melatonin has, however, been only preliminarily investigated and studies on the effect of hormone substitution on pineal melatonin production in hypophysectomized animals have not heretofore been attempted.

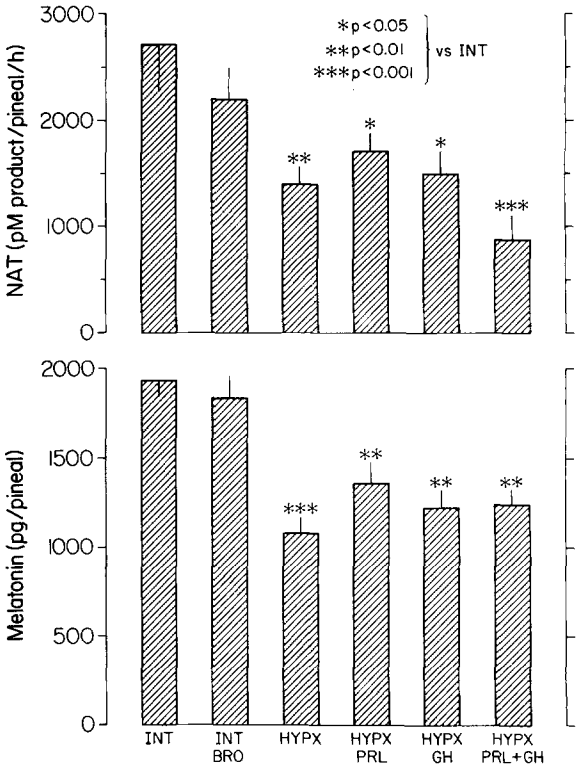
The purpose of the present study was to re-examine the effect of surgical removal of the pituitary gland on pineal synthetic activity by measuring both the pineal content of melatonin and the activity of N-acetyltransferase (NAT); the activity of this enzyme is often a reliable index of melatonin formation[8,9]. Additionally, hypophysectomized rats were treated with either prolactin (PRL) or growth hormone (GH), alone or in combination, to determine their effects on pineal melatonin production. These drugs were chosen for administration since their efficacy had not previously been examined in reference to their ability to alter pineal biosynthetic activity in rats lacking their pituitary, although judging from ultrastructural changes in the pineal these hormones may have a substantial metabolic effect on this organ[10,11].

Materials and methods. Intact (25 each) and hypophysectomized (50 each) male rats were purchased from Harlan-Sprague Dawley (Houston, TX). The body weights at the time of hypophysectomy averaged 150 g. After their arrival in the laboratory, the animals were housed 5–6 per cage and supplied with food and water ad libitum. In the case of the hypophysectomized rats tap water was replaced with physiological saline to compensate for the reduced adrenocortical function. The animals were kept under a photoperiodic regimen of 14:10 with light on at 06.00 h daily. They were maintained under these conditions for 12 days prior to the study as well as during the actual experiment.

The hypophysectomized animals were divided into 4 groups (12 or 13 rats each). One group of rats received twice daily (between 08.00 and 09.00 h and between 16.00 and 17.00 h) a s.c. injection of 150 µg sheep PRL (Sigma Chemical Corporation) while another group received 150 µg GH (Raben type, Nutritional Biochemical Corporation) s.c. at the same times; a third group received both PRL and GH while the final group served as hypophysectomized control animals and received a s.c. injection of physiological saline twice daily. These doses of hormones were selected because of their common usage in other studies[12].

The 25 intact rats were divided into two groups and were treated as follows. One group received two daily s.c. injections of 150 µg bromocriptine (2-bromo-α-ergocriptine methan sulfonate, Sigma); the drug was dissolved in saline acidified with several drops of 5 N acetic acid. Bromocriptine is a well-known suppressor of plasma PRL[13]. The final group of rats received the acidified saline twice daily and served as the intact control rats. The injections in both hypophysectomized and intact rats were given for 4 consecutive days.

Results. At 24.00 h (4 h after darkness onset) on the night after the last injection the rats were weighed and killed, under dim red light, by decapitation. This light is of the improper wavelength to suppress pineal melatonin[10]. The pineal gland was dissected and frozen on solid CO_2. On the day of assay, pineals were sonicated in 100 µl 0.05 M phosphate buffered saline (pH 6.8). Pineals were individually assayed for NAT activity and melatonin content[14]. Data were statistically analyzed using an ANOVA and Student Newman-Keuls multiple range test. The animals that received either GH and GH plus PRL for 4 days had body weights that averaged 26 g more than hypophysectomized rats that received either PRL alone or diluent.



Pineal N-acetyltransferase activity (upper panel) and melatonin content (lower panel) in intact (INT) and hypophysectomized (HYPX) rats treated with either bromocriptine (BRO), prolactin (PRL) or growth hormone (GH). The animals were killed at 24.00 h, 4 h after darkness onset.

Intact control rats had high pineal NAT activity and melatonin levels at 24.00 h (fig. 1); both these values were significantly ($p < 0.001$) depressed in hypophysectomized rats not given hormone treatment. Neither PRL nor GH treatment alone restored either the NAT activity or the melatonin values in hypophysectomized rats. Likewise, the combination of PRL and GH had no restorative value in terms of either the activity of the acetylating enzyme or the melatonin content. Thus, the pineal glands of all hypophysectomized rats had statistically equivalent levels of both NAT and melatonin. Bromocriptine treatment, which suppresses circulating levels of PRL, also was without a statistically significant effect on either pineal NAT activity or melatonin levels in pituitary-intact rats.

Discussion. These findings confirm the marked suppressive effect of hypophysectomy on the nocturnal elevation in pineal melatonin levels[7]. This reduction is most likely a result of the concomitant drop in pineal NAT activity. NAT converts serotonin to N-acetylserotonin in the pineal gland and its activity is often, but not always, highly correlated with the melatonin content of the gland[15,16]. With the reduction in NAT activity, presumably insufficient quantities of N-acetylserotonin are available for its conversion to melatonin. It is clear from the present as well as earlier results[7] that the pineal gland of the hypophysectomized rat can synthesize some melatonin at night.

The present findings suggest that the reduction in nocturnal pineal melatonin production after hypophysectomy is not related to the loss of either PRL or GH. Replenishing these hormones, either alone or in combination, failed to significantly restore the ability of the pineal gland to produce mela-

tonin at night. Likewise, when PRL levels in intact rats were depressed by treating the animals with bormocriptine, there was no change in either high nighttime NAT activity or melatonin levels. Thus, the earlier observations showing that PRL changes the ultrastructure of the pineal gland [10, 11] suggests that the alterations observed relate to something other than indoleamine metabolism in these cells.

It is possible, however, that hypophysectomy merely phase-shifted the melatonin rhythm and thus, peak melatonin levels occurred earlier or later in the dark phase. Another study in which hypophysectomized rats were used indicates this procedure does not shift the rhythm, it merely dampens it [7]. Thus, this explanation seems untenable. On the other hand, if hypophysectomy merely shifted the NAT and melatonin rhythms in the present study, neither PRL nor GH was able to re-establish the normal phasing of these cycles.

Hypophysectomy, of course, causes a marked reduction in a number of other pituitary-derived hormones as well. Of these, however, neither thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone, nor adrenocorticotrophin hormone seem to be involved in any substantial way in determining pineal melatonin production. The effect of α-melanocyte stimulating hormone, which derives from the intermediate lobe of the pituitary and which is reduced after hypophysectomy, has not been tested in terms of its ability to influence the conversion of serotonin to melatonin in the pineal gland.

Rather than relying on a single hormone, the nocturnal rise in NAT activity and melatonin production in the pineal gland likely relies on a combination of pituitary and non-pituitary hormones. Furthermore, it would require extensive testing to uncover what this combination of factors may be. The possibility exists that hypothalamic damage as a result of the surgical procedure, rather than the loss of the pituitary gland, accounted for the reduced NAT activity and melatonin level in the pineals of hypophysectomized rats. This explanation seems unlikely considering the diaphragma sellae is quite tough in the rat and numerous studies have shown that this procedure results in no serious damage of the hypothalamus [17]. Finally, had the GH and/or PRL injections been given at some other points thoughout the 24 h, possibly they would have promoted pineal melatonin production.

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Effects of multiple injections of luteinizing hormone-releasing hormone on the induction of pregnancy in androgenized female rats

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Summary. A study of the effect of cyclic (every 4 days) administration of gonadotropin releasing hormone on reproductive performance of the androgenized female rat was carried out. The responses measured were indirect indices of increased gonadotropin output; ovulation rate, uterine decidualization, mating and implantation.
Key words. Androgen-sterilized rat; luteinizing hormone-releasing hormone (LHRH); ovulation; uterine decidualization; mating; implantation.

Administration of androgen to neonatal female rats results in infertility accompanied by persistent vaginal cornification in adulthood. This syndrome may result from disorders of the hypothalamus and the subsequent alteration of the cyclic release of luteinizing hormone (LH) from the pituitary gland [1, 2]. Although ovulation in such androgenized female rats is readily brought about by a single injection of luteinizing hormone-releasing hormone (LHRH) [3, 4]. LH [5–7] or human chorionic gonadotropin (hCG) [5, 8], the rats rarely become pregnant. Hahn and McGuire [4] have suggested that the failure of embryos to become implanted results from a deficit of progesterone from the induced corpora lutea. Implantation failure in androgenized female rats has also been explained by the lowered decidual response in the uterus [9]. However, the reasons for the failure of androgenized female rats to become pregnant are still unknown. In a previous report it was suggested that the uterine sensitivity to blastocyst implantation of androgenized immature rats may be normal [10]. The endocrine environment in androgenized female rats following injections of LH every 4 days is similar to that of normal rats [11, 12]. The pituitary glands of androgenized female rats can release LH in response to LHRH stim-