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Brown adipose tissue activity in hypophysectomized rats: involvement of sympathetic system

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Summary. At thermal neutrality, hypophysectomy enhanced interscapular brown adipose tissue (IBAT) activity (increase of purine nucleotide binding) in the rat. This stimulation is dependent on sympathetic system integrity since surgical denervation of IBAT impairs its thermogenic response.
Key words. Hypophysectomy; sympathectomy; brown adipose tissue.

Brown adipose tissue (BAT) is the major site of both non-shivering and diet-induced thermogenesis. It has recently been found to be a common effector for both thermic and weight regulation [1]. It has been shown that the same modifications of the composition of BAT which are normally induced following cold stimulation are also observed in hypophysectomized rats acclimated at 28 °C [2]. Some enzymes known to modulate the energy supply to that organ showed enhanced activities [3] and binding of purine nucleotides in BAT mitochondria (an indicator of the thermogenic state of the tissue) was higher at thermal neutrality in hypophysectomized rats supplemented with thyroxine and corticosterone [4] or at ambient temperature [5]. The development of thermogenesis in BAT is under the control of hypothalamic thermoregulatory centers and the effector pathway involves the sympathetic nervous system via the release of norepinephrine [6], but its activity can be modified by a number of hormonal factors [7]. It remains to be demonstrated that the subsequent changes in BAT induced by hypophysectomy at thermal neutrality [2] also requires an intact sympathetic nervous system, as has been demonstrated for cold-induced stimulation [8]. In the present study the possible involvement of the sympathetic nervous system was investigated by surgical denervation of hypophysectomized rats.

Materials and methods. Male Long-Evans rats, 6–7 weeks old, were hypophysectomized by the parapharyngeal route. The effectiveness of hypophysectomy was verified by the following criteria: no weight gain, testicular atrophy, absence of pituitary remnant upon examination of the sella turcica in the sphenoid bone under binocular microscopy post mortem. The operation was simulated in another group. Only those hypophysectomized rats were used which did not gain weight over a period of at least 3 weeks. The control and hypophysectomized rats were acclimated to 28 °C for 5 weeks. 10 days before sacrifice, interscapular BAT (IBAT) of a group of hypophysectomized rats was surgically denervated: animals were anesthetized with chloral 200 mg/kg i.p., the five nerves supplying each lobe of IBAT were isolated and cut without damage to the tissue. It has already been established that such denervated IBAT is severely depleted in norepinephrine [9]. The animals were fed on a standard laboratory diet (UAR 03), manufacturer's data: 23.5% proteins, 5% lipids, 49.8% carbohydrates. All rats had free access to food and water and the lights were on from 07.00 to 19.00 h. IBAT mitochondria were prepared and purine nucleotide binding was assessed by the method described by Nedergaard et al. [10]. Various concentrations of GDP were used (0.1, 1, 3 μM) to test the linearity of a Scatchard plot and to obtain a satisfactory estimation of high affinity binding sites. The mitochondrial protein yield to IBAT mitochondria was determinated by spectrophotometric assay of cytochrome C oxidase [11] in the homogenate and in the mitochondrial fraction. Results are expressed as mean ± SEM. Statistical analysis used an unpaired t-test.

Results and discussion. Hypophysectomized rats lost some weight during the first week after operation but then maintained a stable weight of about 155 g. The weight of IBAT was significantly lower in hypophysectomized rats (214 mg vs 410 mg). However in terms of body weight (per 100 g b.wt) the amount of IBAT was the same in hypophysectomized and control rats. Hypophysectomy led to a significant decrease of 28% in the mitochondrial protein content (7.7 mg vs 10.4 mg). But when expressed per 100 g b.wt, mitochondrial proteins were significantly (50%) higher in hypophysectomized rats (4.9 mg vs 3.3 mg). Specific GDP binding (expressed per mg mitochondrial proteins) showed a significant 2.5-fold increase in the hypophysectomized rats (0.66 nmol vs 0.26 nmol). An estimate of total GDP binding per IBAT can be obtained if mitochondrial yield is combined with specific GDP binding. In this case a considerable increase of 1.8-fold was still observed for the hypophysectomized group (5.00 nmol vs 2.82 nmol).

In the hypophysectomized rat with denervated IBAT 10 days before sacrifice, body weight was the same as in hypophysectomized control rats. Expressed per 100 g body weight, denervated IBAT weight was significantly higher (165 mg vs 135 mg). Lipid accumulation explained the difference (results not shown). Total IBAT mitochondrial proteins showed a significant diminution compared to hypophysectomized rats (5.4 mg vs 7.7 mg) and to controls (5.4 mg vs 10.4 mg). In terms of body weight, mitochondrial proteins were the same in denervated IBAT of hypophysectomized rats and in normal controls. Surgical denervation completely abolished the specific GDP binding increase observed in hypophysectomized rats. Total GDP binding was five-fold lower in denervated rats than in hypophysectomized control rats (1.02 nmol vs 5.00 nmol). Expressed per 100 g body weight, total GDP binding was comparable in denervated hypophysectomized rats and in normal controls.

Our results obtained at thermal neutrality agree with Fellenz's study on hypophysectomized rats supplemented with thyroxine and corticosterone [4]. We show that even in the

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.