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Antidiuretic hormone involvement in the release of α-melanocyte-stimulating hormone by hyperosmotic stimuli

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Summary. In the normal Wistar rat, the plasma α-MSH level was raised by hypertonic saline injection (as compared with control rats injected with isotonic saline). No such rise in α-MSH followed hypertonic saline administration in the Brattleboro (hereditary diabetes insipidus) animal (compared to isotonic saline injected controls). It is suggested that, in the rat, endogenous antidiuretic hormone is involved in the secretory response of the pars intermedia to osmotic stimuli.
Key words. Antidiuretic hormone; pars intermedia; α-MSH secretion.

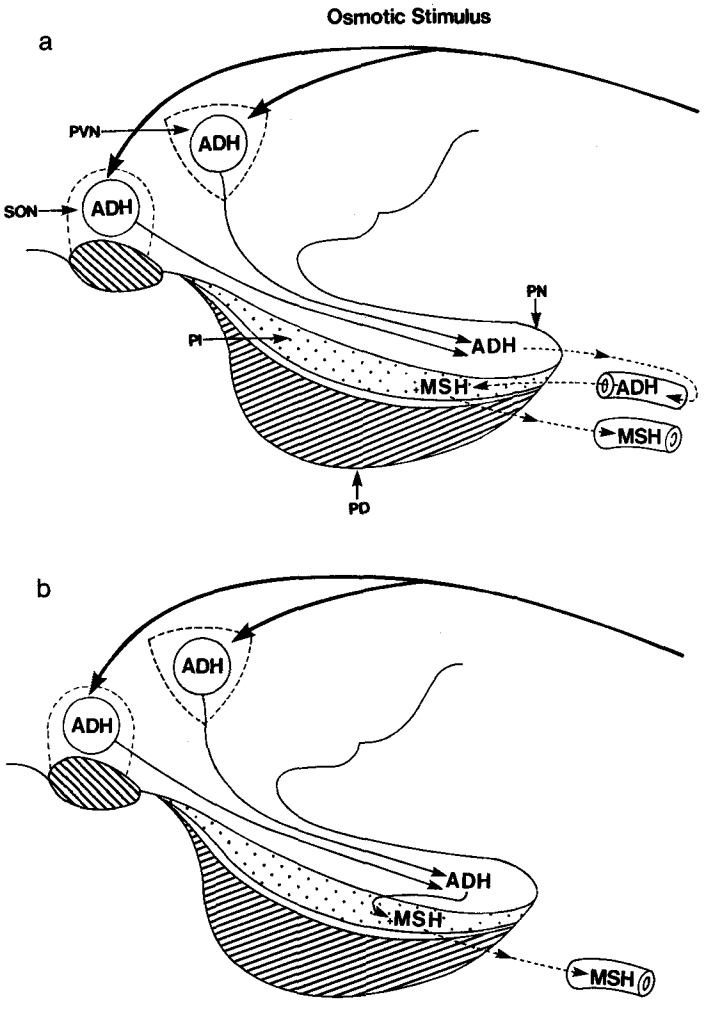
A functional relationship between the pars intermedia of the pituitary gland and the adjacent neurohypophysis has been postulated[1]. This view is based essentially upon the observation that the pars intermedia is activated to release melanocyte-stimulating hormone (MSH) by various forms of osmotic stimuli, such as the injection of hypertonic saline, ingestion of sodium chloride, or deprivation of water[2–5], stresses well known to represent potent stimuli for the release of the neurohypophysial hormones, antidiuretic hormone (ADH) and oxytocin[3–8]. Such stimuli represent useful tools for the experimental investigation of pars intermedia function, but neither the physiological significance of this response nor the mode of activation of the pars intermedia under these conditions is clear.

Whether or not the release of MSH by osmotic stress is dependent on neurohypophysial hormones could be tested by examining the response (MSH secretion) of the pars intermedia to an osmotic stimulus in an animal lacking a functional neurohypophysial system. Experimentally, this poses technical problems; for example it is virtually impossible to destroy either the ADH or the oxytocin system selectively by lesion techniques. However, such a model is available in the form of the genetically abnormal Brattleboro rat, an animal with hereditary diabetes insipidus (D.I.), which is deficient in ADH while possessing a normally functional oxytocin system[8,9]. Experiments were, therefore, carried out to see if the Brattleboro rat would release MSH in response to an osmotic stimulus (hypertonic saline), which was effective in causing the release of MSH in the normal laboratory (Wistar) rat.

Materials and methods. A total of 14 adult male Wistar rats (b.wt 160–210 g) and 11 homozygous adult rats (both male and female) of the Brattleboro strain (b.wt 175–240 g and exhibiting marked diabetes insipidus), were used and were maintained under standard conditions of lighting and temperature. The animals were anesthetized with urethane (15% w/v in distilled water, at a dose of 1.5 g/kg b.wt, i.p.). Both the Wistar and

The effect of i.v. injection of hypertonic (2%) saline on the plasma α-MSH level in anesthetized Wistar and Brattleboro rats

Animal	Injection (i.v.)	Number of animals	Plasma α-MSH (pg/ml) 12 min after NaCl Mean	± SEM	Significance
Wistar rats	Hypertonic (2%) NaCl	9	4598	294	Significant ($p < 0.01$)
	Control-isotonic (0.9%) NaCl	5	1053	111	
Battleboro rats	Hypertonic (2%) NaCl	5	922	81	Not significant
	Control-isotonic (0.9%) NaCl	6	1265	271	



Scheme to illustrate two possible mechanisms whereby ADH is involved in the release of MSH in response to an hyperosmotic stimulus. *a* ADH is released from the pars nervosa into the general circulation, whence it reaches the pars intermedia via the pituitary blood supply and in turn activates the release of MSH. *b* ADH reaches the pars intermedia from the adjacent pars nervosa. This could be via nerve fibers which *either* directly impinge on the MSH-secreting cells, or release ADH close to the pars intermedia, whence it reaches the MSH-secreting cells by some form of local transport process. ADH = antidiuretic hormone; MSH = melanocyte-stimulating hormone; ⌒⓪ = systemic circulation; PVN = paraventricular nucleus; SON = supraoptic nucleus; PI = pars intermedia; PN = pars nervosa; PD = pars distalis.

Brattleboro animals were divided into control and experimental groups, which were treated as follows:

In each of the experimental rats, 2 ml hypertonic (2%) saline were injected i.v. and, 12 min later, blood was withdrawn from the heart by syringe after opening the chest. The same procedure was carried out on the control animals, using 2 ml isotonic (0.9%) saline in place of the hypertonic solution. Immediately after collection, the blood samples were centrifuged at 2500 rpm and the separated plasma stored at −20 °C, until assayed. The level of immunoreactive α-MSH in plasma was measured by radioimmunoassay method[10].

Results and discussion. Compared with the corresponding control group, plasma α-MSH levels were significantly elevated in the Wistar, but not in the Brattleboro rats, injected with hypertonic saline (table). Since a hypertonic stress, capable of causing the discharge of MSH in the normal rat, failed to release MSH in the ADH-deficient Brattleboro animal, it seems likely that ADH is an essential component of the control system whereby the pars intermedia (and MSH release) is so activated. Moreover, since it is well-known that a hypertonic stress of this type causes the release of oxytocin as well as of ADH from the neurohypophysis[11,12], and, as the oxytocin system is known to be functionally normal in the Brattleboro rat[8,9], it may also be concluded that oxytocin is probably not involved in the release of MSH from the pars intermedia by hyperosmotic stimuli. Two possible ways in which MSH might be released by an osmotic stimulus, involving the mediation of endogenous ADH, is depicted diagrammatically (fig.).

If this hypothesis is correct, it should follow that exogenous ADH would cause the release of MSH. Some previous workers have tested the effect of administered ADH on MSH release in the normal rat, measuring plasma MSH by biological assay[13-15]. The dose levels of ADH employed by these workers were unphysiologically high and there is some disagreement between the results of the two groups. Nevertheless, the concensus of opinion is that administered ADH is capable of releasing MSH and we have observed this to be so in both normal Wistar and Brattleboro rats (unpublished observations), using quite low doses of ADH and measuring plasma α-MSH by radioimmunoassay. Moreover, directly applied ADH releases α-MSH from the superfused neurointermediate lobe in vitro, both of Wistar and Brattleboro rats (Howe and Ray, submitted for publication). We therefore conclude that, under conditions of osmotic stimulation at least, the release of α-MSH in ADH-mediated.

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Regulation of juvenile hormone titer in African locust

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Summary. In the African locust, the titer of C-16 JH in hemolymph (determined by GC-MS), reflects the rate of hormone biosynthesis (determined by RCA) in normal adult females. Severance of the nervi corporis allati-I (NCA-I) results in a low C-16 JH biosynthesis without affecting physiological events dependent on JH. In NCA-I-transectioned animals, JH titer is higher than in control locusts. JH catabolism does not seem to be involved in this high titer of hormone associated with a very low rate of JH production. In sham-operated females, the bulk of injected [^{3}H]C-16 JH quickly disappeared from the hemolymph but JH was retained in the bloodless body. After severance of the NCA-I, the remaining radiolabeled JH in the hemolymph increased. These results suggest the role of tissue JH-binding or of JH excretion in regulating its level in the locust.
Key words. Juvenile hormone; biosynthesis; catabolism; tissue binding; titer; disconnected copora allata, locust.

Many physiological events in insect life are affected by juvenile hormone (JH). Hemolymph JH titers reflect the net difference between JH biosynthesis, JH catabolism JH excretion and JH binding; events whose relative contribution to the regulation of JH titer remain uncertain. A major mechanism for the regulation of JH titers in larval insects is believed to be catabolism in the hemolymph[1,2]. On the other hand, several authors[3,4] have suggested that changes in the rate of biosynthesis are a major contributor to JH titer change. We have now studied both biosynthesis and JH titer in *Locusta migratoria* females, and our data support the second view in normal adult females. Nevertheless, in adult female locusts whose corpora allata (CA) were denervated, we have shown that neither JH biosynthesis nor JH catabolism were major contributors to changes in JH titer. We suggest that binding of JH by tissues and/or JH excretion could be important factors in controlling JH hemolymph titer.

The pattern of biosynthetic activity of the CA of adult female locusts has been studied with a radiochemical assay in vitro[5] and shown to correlate with ovarian events during the first gonadotrophic cycle[6,7]. After transection of nervi corporis allati I (NCA-I) on the first day of adult life, CA showed a very low rate of synthetic activity[7]. This activity was constant within the 15 days of the observation period. The low biosynthetic activity of the CA after NCA-I transection did not affect ovarian development[7], whereas allatectomy of the young females suppressed ovarian development[8]. Severance of the NCA-I at the beginning of the fourth-larval stadium prevented an increase in JH biosynthesis during the whole of the stadium; but this decreased synthesis did not alter the nature of the next molt which was typically larval[7].

To investigate more fully the apparent discord between CA activity and JH-induced physiological changes after NCA-I severance, we investigated other physiological situations which have been shown to be JH dependent. So, we compared male sexual behavior, male accessory gland activity, and the yellowing that accompanies sexual maturation in operated and sham-operated locusts. We observed that after the CA had been denervated on the first day of adult life, JH production was much lower than in sham-operated males (fig. 1), but all the physiological events under the control of JH occurred at the same time in operated and sham-operated males. Secretions of the male accessory gland began on the 4th day. Mating behavior occurred on days 10–12, but spermatophores containing spermatozoa began to be produced only on the 16th day; inseminated females

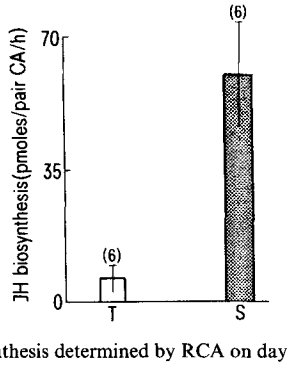

Figure 1. JH biosynthesis determined by RCA on day 21 in NCA-I-transectioned males (T) and sham-operated males (S). The operation was performed on the first day of adult life. Mean ± SEM. Number of animals in parentheses.

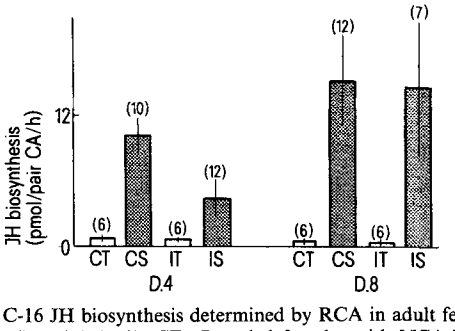

Figure 2. C-16 JH biosynthesis determined by RCA in adult females on days 4 (D.4) and 8 (D.8). CT, Crowded females with NCA-I-transectioned on the first 3 h of the fourth larval stadium. CS, Crowded sham-operated females. IT, Isolated females with NCA-I transectioned in the first 3 h of the fourth larval stadium. IS, Isolated females, sham-operated in the first 3 h of the fourth larval stadium. Means ± SEM. Number of animals in parentheses.