Hypothalamic and Cerebral Cortical Inhibitors of a Melanocyte-Stimulating Substance Secreted by the Pars Distalis of the Frog Pituitary Gland

Complete dark adaptation by the frog requires secretions from both the pars distalis and the pars intermedia[1]. As a consequence of the greater relative importance of melanocyte-stimulating hormone (MSH) secreted by the pars intermedia, the investigation of the neuro-endocrine control of background adaptation has centered on the relationship of the pars intermedia and the hypothalamus. The hypothalamus exerts an inhibitory control on MSH secretion from the pars intermedia[1–6], probably through the secretion of a chemically unidentified MSH inhibiting factor (MIF)[7,8].

Regulation of the secretion of melanocyte-stimulating substances (MSS) from the pars distalis has not been investigated. This study was conducted to determine whether or not the frog hypothalamus contains factors which could regulate the secretion of the MSS by the pars distalis.

Materials and methods. Male frogs (*Rana pipiens*, 30–50 g, Steinhilber and Sons, Oshkosh, Wis., USA) were maintained wet and illuminated on a light (L) or dark (D) background for 48 h at 22–26 °C. At the end of the 48 h background adaptation, the frog tissues were collected, prepared and employed in the 2 h in vitro frog skin assay for MSS and in the in vitro assay of hypothalamic (H) and cortical (C) extracts (X) for an inhibitor of MSS secretion from the pars distalis[7].

Results. The pars distalis secreted significant amounts of MSS in vitro (Table I; none vs PDD; $P < 0.01$). Aqueous extracts of the cerebral cortex and hypothalamus of light and dark adapted frogs did not stimulate the frog skin significantly (Table I; none vs CXL, HXL, CXD and HXD; NSD, all comparisons). The extracts of both the cerebral cortex and hypothalamus significantly inhibited the secretion of the MSS from the pars distalis (Table I; PDD vs PDDCXL, PDDHXL, PDDCXD and PDDHXD; $P < 0.01$, all comparisons). There was no significant difference in the inhibitory ability of either the cerebral or hypothalamic extracts (Table I; PDDCXL vs PDDHXL; NSD and PDDCXD vs PDDHXD; NSD) or of the extracts from either light or dark adapted frogs (PDDCXL vs PDDCXD; NSD and PDDHXL vs PDDHXD; NSD).

The second experiment was designed to determine if the inhibition indicated by the results of the first experiment was an inhibition of MSS secretion from the pars distalis or an inhibition of the action of MSS on the melanocytes of the frog skin[4]. The frog skin fragments, the hypothalamic and cortical extracts and all of the partes distalis were incubated for 2 h simultaneously, but separately. At the end of the 2 h pre-assay equilibration period, the partes distalis were removed from the beakers into which they had secreted the MSS, the extract equivalent of one hypothalamic or cortical fragment was added to the beaker and finally a fragment of skin was added to measure the effect of the extracts on the action of the MSS. The beakers contents were incubated for another 2 h. Thus, the extracts were not permitted to interfere with the *secretion* of MSS, only the *action* of MSS.

The MSS secreted by the pars distalis caused a significant increase in the MI score (Table II; none vs MSS; $P < 0.01$). The cortical extract added to the MSS did not interfere with the action of the MSS on the skin (Table II; MSS vs MSSCXL; NSD), whereas the addition of hypothalamic extract to MSS resulted in a significant increase in the MI score (Table II; MSS vs MSSHXL; $P < 0.05$) indicating that the hypothalamic extract of the light adapted frog contains a MSS that acts directly on the melanocyte.

Discussion. The results of this study indicate that the pars distalis does contain a melanocyte-stimulating sub-

Table I. The effect of extracts of the frog hypothalamus and cerebral cortex on the secretion of melanocyte-stimulating substance (MSS) from the pars distalis

Group No.	Treatment components[a]	No. of observations	Mean MI score[b]
1	None	6	1.10
2	PDD	6	4.30
3	CXL	6	1.48
4	HXL	6	1.85
5	CXD	6	1.25
6	HXD	6	2.17
7	PDDCXL	6	2.68
8	PDDHXL	6	2.73
9	PDDCXD	6	2.03
10	PDDHXD	5[c]	1.97

[a] Key to treatment component abbreviations: PDD, pars distalis (PD) of dark (D) adapted frogs; CXL, HXL, CXD and HXD, extracts (X) of the cortex (C) and hypothalamus (H) of light (L) and (D) adapted frogs. Treatment beakers of group 1 contained only 3.1 ml of frog Ringer's solution and a piece of frog skin. The treatment beakers of all other groups contained 3.1 ml frog Ringer's solution, plus a piece of frog skin and the indicated treatment components. All beakers were incubated for 2 h. [b] See text for significance of differences between means. [c] One observation lost.

Table II. The effect of extracts of the frog hypothalamus and cerebral cortex on the action of frog pars distalis MSS

Group No.	Treatment components[a]	No. of observations	Mean MI score[b]
1	None	12	1.38
2	MSS	12	3.12
3	MSSCXL	12	3.44
4	MSSHXL	12	4.18

[a] Key to treatment component abbreviations: MSS, melanocyte-stimulating substance secreted by frog pars distalis of dark adapted frogs; CXL and HXL, extracts (X) of the cortex (C) and hypothalamus (H) of light (L) adapted frogs. Treatment beakers of group 1 contained only 3.1 ml of frog Ringer's solution and a piece of frog skin. The treatment beakers of all other groups contained 3.1 ml frog Ringer's solution, a piece of skin and the indicated treatment components. [b] See text for significance of difference between means.

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stance of unknown identity. Available indirect evidence would suggest that the MSS is not ACTH[1] because (1) ACTH is only about $^{1-2}/_{100}$ as active as MSH in stimulating melanin dispersion[9,10], (2) corticoids do not influence background adaptation although they are known to inhibit ACTH secretion[11] and (3) it is difficult to understand the survival advantage of activating the adrenals each time the frog is required to dark adapt, and vice versa. Furthermore, our results suggest an inhibitory regulation of the secretion of the frog MSS, whereas ACTH secretion in the mammal is regulated by a releasing factor. Since the regulation of MSS secretion appears to be inhibitory and similar studies have provided identical evidence for the inhibitory regulation of MSH secretion by the pars intermedia[7,8] it is possible that the melanocyte-stimulating substance of the pars distalis is MSH.

The presence of a MSS in the hypothalamus has been reported previously[7,8,12] and we[7] have suggested that the accumulated MSS is indicative of a feedback control loop in which the circulating MSS regulates the secretion of the inhibitor to MSH secretion. The lack of MSS in the cortical extracts lends further support to this suggestion. The ability of cortical extracts to inhibit MSS secretion could be due to the catecholamines found in the brain[4].

Further study of the neuroendocrine control of frog background adaptation will require the determination of the identity of the MSS of the pars distalis and the identity of the inhibiting substance(s) of the hypothalamus and cortex.

Zusammenfassung. Nachweis, dass die pars distalis der Hypophyse des Leopardfrosches *Rana pipiens* eine Melanozyten stimulierende Substanz enthält, die in vitro abgesondert wird. Wässerige Extrakte aus Hypothalamus und Kortex des Frosches hemmen die Ausscheidung der die Melanozyten stimulierenden Substanz in vitro.

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In Vitro Metabolism of Progesterone by the Adrenals of Spontaneously Hypertensive Rats

Spontaneous hypertension (SH) has been induced in rats by prolonged inbreeding or cross-breeding[1,2]. The incidence of hypertensive disease in these rats was reported originally to be as high as 100%, and according to Laverty and Smirk[3] the levels of blood pressure become progressively higher with successive generations. The etiology of SH is unknown, although is not dependent on high salt intake, unlike other forms of experimentally produced hypertension, such as hypertension induced by high saline intake, by adrenal regeneration, and by administration of deoxycorticosterone (DOC)[4,5]. Bilateral adrenalectomy lowers the blood pressure in both normal and SH rats but does not modify the preoperative pressure difference between the 2 groups[6].

We have compared the conversion of labelled progesterone by incubated adrenal glands from normal and SH rats in order to establish whether the corticosteroid biosynthetic pattern differs in the 2 groups.

Materials and methods. SH rats, Wistar descendants, were generously donated by Dr. C. T. Hansen, NIH. Female first generation descendants from these animals were used, and Wistar female rats of similar weight obtained from a local breeder served as controls. The mean blood pressure of the hypertensive rats was 200 ± 4 mm Hg (SE; $n = 6$). The animals were decapitated after Nembutal anesthesia (5 mg/100 g) and the adrenals quartered, preincubated for 45 min and then incubated for 4 h in 2 ml Krebs-Ringer bicarbonate glucose supplemented medium, under an atmosphere of 95% O_2 – 5% CO_2, in the presence of 4.84×10^5 dpm of 4-^{14}C-progesterone (SA 2 mC/mg, New England Nuclear). Incubations were performed in quadruplicate. The media were extracted 3 times with dichloromethane, the extracts were chromatographed in the toluene-propylene glycol system[7] and the effluent containing DOC and progesterone was developed in the Bush B3 system[8]. The radioactivity present in the steroid fractions corresponding to 18-hydroxycorticosterone (18-OH-B), aldosterone, 18-hydroxydeoxycorticosterone (18-OH-DOC), corticosterone, DOC and unmetabolized progesterone, was measured by liquid scintillation counting, after derivative formation and multiple chromatography. The methods used for steroid purification have been published elsewhere[9,10]. All the reported values have been corrected for procedural losses from the recovery, at the last stage of purification, of non-radioactive standards added immediately after incubation. The recovery of 18-OH-B was assumed to equal that of 18-OH-DOC, since no pure standard was available.

Results and discussion. Table I gives the organ weights of rats of approximately the same body weight (SH 214 ± 4 g, controls 238 ± 2 g, $n = 6$). No significant differences were observed in kidney, thymus or adrenal weight, but the heart weights were increased in SH rats ($P < 0.001$).

Table II presents the per cent radioactivity recovered in 6 steroid fractions, which was remarkably similar for both groups of rats. These results are in marked contrast with previous findings on other forms of experimental

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