In vitro Formation of a MSH-Releasing Agent by Hypothalamic Extracts

Several authors[1–3] have demonstrated in hypothalamic extracts peptidases which inactivate neurohypophysial hormones. Recently, in extracts of rat hypothalamus, we have shown the existence of an enzyme attached to microsomes which, incubated with oxytocin, produced a factor that inhibits the release of melanocyte-stimulating hormone (MSH) from the pituitary gland[4]. L-prolyl-L-leucylglycinamide has been identified as the active product inhibiting MSH secretion both in vivo and in vitro[5]. Since it is known that hypothalamic extracts, beside having an agent that blocks the secretion of MSH, also possesses a factor that promotes the release of the hormone[6], we decided to investigate whether the latter factor can also be formed by an enzymic degradation of the neurohypophysial hormones.

Fractions from median eminence (ME), supraoptic nuclei (SON) and paraventricular nuclei (PVN) were obtained from hypothalamic of male rat as previously described[4]. Fractions from several animals were homogenized at 4°C in 0.25 *M* sucrose using 0.2 ml per hypothalamus. The homogenate was centrifuged for 5 min at 1000 *g* and the supernatant re-centrifuged for 60 min at 17,000 *g*. The precipitate were resuspended in 1 ml of phosphate buffer (0.1 *M*, pH 7.0) and then dialyzed against distilled water for 24 h at 4°C. The non-dialyzed material was used for further studies. Aliquots of this material equivalent to 3 hypothalami were incubated in 0.05 *M* phosphate buffer (pH 7.0: final volume 2 ml) at 37°C with 300 mU synthetic oxytocin. The reaction was stopped after 2.5 h by heat treatment. After centrifugation at 1000 *g* for 10 min the supernatant was injected i.v. into 2 male rats of 180–200 g, anesthetized with ether, and the animals were killed 20 min later. The drop in pituitary MSH content in the pooled glands, induced by the injected material, was taken as index of the releasing activity[6]. MSH was measured by an in vitro assay[6].

Table I shows that a drop in the pituitary MSH content was observed in rats injected with an incubate of the PVN extract with oxytocin. Since this effect was not found either by injecting the non-diffusible fraction incubated alone or of non-incubated oxytocin[6], it is probable that an agent which can deplete pituitary MSH content was formed by the system on incubation. The possibility also exists that such an agent could be a fraction of the oxytocin molecule, since it was reported that the hormone is inactivated by the incubation with PVN extracts[7]. Furthermore, incubation of heated extract with oxytocin had no effect on the pituitary MSH content. These results may suggest an enzyme action of the non-diffusible and thermolabile material and support for this presumption was obtained by measuring some of the kinetics of the system. The Figure shows that the amount of MSH-releasing agent yielded increased with the concentration of the hypothalamic extract in the system and that the optimum pH was between 7 and 8.

The enzyme seems to be specific of the hypothalamus, since similar prepared fractions from cerebral cortex incubated with oxytocin failed to induce changes in pituitary MSH content (Table I). Studies of its localization in the hypothalamus showed that fractions obtained from extracts of median-eminence were also effective to produce the MSH-releasing agent when incubated with oxytocin, while no such agent was formed by SON extracts (Table I).

Table I shows also that lysine-vasopressin is likewise capable of serving as substrate for the enzyme to form the MSH-releasing agent.

The subcellular localization of the enzyme was studied. The cellular fractions from PVN extracts, obtained according to the procedure described by GRAY and WHITTAKER[8], were incubated with oxytocin and then the MSH-releasing activity was measured. Table II shows that such activity was observed only in the incubate of mitochondrial fractions with oxytocin.

One requirement for an agent to be considered a releasing factor is for it to act directly on the pituitary. To fullfil that requirement, the incubated material was

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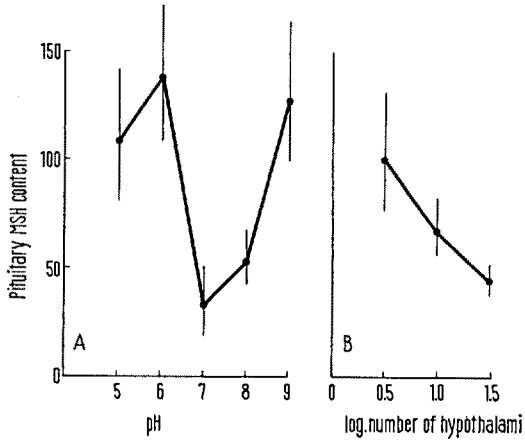

Dependence of the formation of MSH-releasing agent on pH (A) and amount of PVN extract (B). Each point shows the content in pituitary MSH as percent of content in controls and the vertical lines are 95% limits.

Table I. Decrease of pituitary MSH content in rats after injecting the incubate of mitochondrial fractions from different sources with neurohypophysial hormones

Source of mitochondrial fraction	Neurohypophysial hormone	Decrease of pituitary MSH content/% of control; mean (95% limits)
PVN	oxy	37.8 (26.6–47.6)[a]
ME	oxy	23.8 (17.1–31.1)
SON	oxy	8.2 (+0.7–18.2)
Cerebral cortex	oxy	3.2 (+4.2–10.3)
PVN	—	+13.2 (+42.2–10.5)
PVN heated	oxy	+11.4 (+23.3+1.2)
PVN	L-VP	38.5 (49.2–25.2)

[a] Each value is the weighted mean of two experiments. Controls were a pool of 2 glands from rats injected with a volume of phosphate buffer equal to that of the experimental groups. Oxy, oxytocin; L-VP, Lysine-vasopressin.

tested in rats with electrolytic lesions in the median eminence. The animals were used 15 days after the lesions, and only when they had daily water intake of 100 ml or more. In such animals it is supposed that the neural connections to the hypophysis were cut and any change in pituitary MSH content is by a direct action on the gland. The injection of the incubate of PVN with oxytocin resulted in a decrease in pituitary MSH content of 54.3% (46.1–61.3) (95% limits) (mean of 4 rats), while the same incubate heated prior to the incubation induced a decrease of 5.2% (+6.2 15.3) (mean of 4 rats) indicating that the agent formed by the incubation acts directly on the pituitary to induce the release of MSH.

From this and previous papers[4,5] it can be inferred that peptidases present in the hypothalamus are capable of acting on neurohypophysial hormones, giving rise to products which effect pituitary MSH secretion. Preliminary studies show that the activity of the enzymes that form MSH-releasing and the MSH-inhibiting agents vary under different experimental conditions, together with simultaneous changes in the content of these agents in the hypothalamus, suggesting that both enzymes may be involved in the hypothalamic mechanisms that normally control the secretion of MSH[9,10].

Table II. Decrease of pituitary MSH content after the injection of the incubate of subcellular fractions from PVN extracts with oxytocin

Subcellular fraction incubated with oxytocin	Decrease of pituitary MSH content/% of control; mean (95% limits)
Nucleus	5.4 (15.9–+6.4)
Mitochondrias	45.2 (51.9–37.8)
Microsomes	4.5 17.8–+10.7)
Supernatant	1.4 (11.7–+10.4)

Résumé. La fraction mitochondriale d'extraits du noyau paraventriculaire du rat incubée avec de l'oxytocine produit la formation d'un agent capable de libérer MSH de l'hypophyse. Les résultats suggèrent que la formation de l'agent qui libère la MSH hypophysaire résulte de la dégradation enzymatique des hormones neurohypophysaires.

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[10] Synthetic oxytoxin and LVP was kindly provided by Sandoz A.G. Basel.

Neuronenaktivität hypothalamischer Kerngebiete von Kaninchen nach intraventrikulärer Applikation von Vasopressin und Oxytocin[1]

Das Vorkommen antidiuretisch und oxytocisch wirksamer Substanzen in der Cerebrospinalflüssigkeit (CSF) von Säugetieren[2–5] sowie die Beeinflussung des zentralen und peripheren Nervensystems durch i.v., i.m. und intracisternal appliziertes Vasopressin und Oxytocin[6–13] lassen einen direkten Zusammenhang zwischen dem Vorkommen und einer unmittelbaren Einwirkung dieser Substanzen vom Ventrikelraum aus auf ventrikelnahe Hirnstrukturen als möglich erscheinen. Zur Klärung dieses Problems wurde die Wirkung intraventrikulär verabreichten Oxytocins und Vasopressins auf das Impulsfrequenzverhalten ventrikelnaher Kerngebiete untersucht.

Über stereotaktisch implantierte Stahlsemimikroelekden wurde die Impulsaktivität des Nucleus paraventricularis (PV) und des N. supraopticus (SO) narkotisierter Kaninchen abgegriffen und die Anzahl der Spikes/min automatisch gezählt und registriert. Die Wirkung von synthetischem Liquor nach MERLIS[14], synthetischem Oxytocin (Richter – Budapest) und Lysin-8-Vasopressin (Sandoz – Basel), in synthetischem Liquor gelöst, wurde nach Injektion von jeweils 0,1 ml über eine im rechten Seitenventrikel befindliche Spezialkanüle über einen Zeitraum von 60 min verfolgt. Um die infolge biologischer Variabilität schwankenden Absolutwerte der einzelnen Versuchstiere miteinander vergleichen zu können, wurden die nach Wirkstoffgabe eintretenden Veränderungen in Prozent zur Ausgangslage angegeben. Die Ergebnisse stützen sich auf 123 Versuche an 35 Tieren. Ihre statistische Absicherung erfolgte nach Mittelwertbildung und Berechnung des mittleren Fehlers des Mittelwertes durch den FISHER-Test.

Vasopressin (100 μE) steigert die Impulsfrequenz der Neuronen im Bereich des PV und SO. Obwohl der Effekt der Impulsfrequenzerhöhung in den beiden Kerngebieten quantitativ gleichartig ist (SO: PV = 132:129), scheint ein qualitativer Unterschied hinsichtlich der Reaktionszeit zu bestehen. Der PV reagiert früher und intensiver (bis zur 30. min) als der SO, bei dem die Phase stärkster Aktivierung erst nach der 30. min auftritt. Im Verlaufe von 60 min nähert sich die Impulsfrequenz wieder weitgehend der Ausgangslage (Figur 1a).

Oxytocin (3000 μE) führt unmittelbar nach intraventrikulärer Applikation zu einer Abnahme der Impulsfrequenz in beiden Kerngebieten. Die Abnahme ist jedoch

[1] Diese Arbeit wurde mit Mitteln des Ministeriums für Wissenschaft und Technik der DDR durchgeführt.
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