Stimulation of Growth Hormone Secretion Following Two Successive Intracarotid Injections of Stalk Median Eminence Extract in Urethane Anesthesized Rats[1]

The evidence in favour of the existence in the hypothalamus of a growth hormone releasing factor (GH-RF) has been gathered in different species of animals, and biological and radioimmunological procedures have been used for evaluating the effects of such a principle on the release of GH in vitro and in vivo[2–7]. In vivo studies performed in primates with radioimmunological methods have confirmed the property of GH-RF to release GH into the circulation[7]. This property was first hypothesized by authors who, using rats, have found a depletion of pituitary bioassayable GH after administration of stalk median eminence (SME) extracts[4,5]. Disturbingly enough, however, no consistent results have been obtained when GH-RF containing preparations were tested in vivo in rats with the help of radioimmunological methods[7–9]. Having recently found that adult male rats anesthesized with urethane represent an extremely well suited preparation for the study of GH secretion in this species, using radioimmunological procedures[10], we have used this type of animal preparation to study the effect of intracarotid injections of SME extracts. These extracts have been repeatedly shown to deplete pituitary stores of bioassayable GH after injection into the carotid artery[4].

Materials and methods. Adult Sprague-Dawley male rats, 250–300 g body weight, were anesthesized with urethane (1500 mg/kg. i.p.) around 09.00 h. A polyethylene catheter was threated into the jugular vein, and another one in the left carotid artery. The animals were then allowed to sleep undisturbed and unrestrained for 2 h. Heparine (1.0 mg/0.5 ml) was then injected and a first base-line blood sample collected from the jugular vein (indicated on the table as -10′ value). A 0.75 equivalent of SME extract, obtained from coeval male rats and prepared as previously described[4], was injected into the carotid artery 10 min after the first sample was collected (0′ time). A blood sample was collected 10 min later (10′ value). The animals were then allowed to sleep undisturbed for an additional hour, and a second baseline blood sample was then withdrawn (second -10′ value). A second intracarotid injection of a 0.75 equivalent of the same SME extract used for the first injection was given 10 min later (second 0′ time), and a blood sample collected after an additional 10 min period (second 10′ value). Rat No. 76 received, in the same fashion, 2 successive injections of a cerebral cortex (CC) extract obtained from the same animals from which the SME extract injected to rats No. 72 and 73 was obtained. The CC extract injected was equivalent in weight to the 0.75 SME extract and was treated in the same way. Rat No. 70 received 2 successive injections of 30 mU of vasopressin (Pitressin®, Parke-Davis). Each blood sample (0.5 ml) was replaced with the same amount of saline (0.9%). Blood samples were centrifuged at 4 °C, the plasma was separated and GH measured with the double antibody radioimmunoassay method of SCHALCH and REICHLIN[11].

Results and discussions. The results are shown in the Table. In all rats treated with SME, the first injection did not bring any significant increase of plasma GH. But when, 1 h later, a second injection of the same extract was practiced, a quite remarkable increase in plasma GH was observed in all animals. The amount of GH found to be present in the 0.75 equivalent of SME extract (about 8 ng) is certainly too low to explain the rise of plasma GH observed after the second intracarotid injection. Two successive injections of a CC extract, or of an amount of vasopressin equivalent to that known to be present in a SME[12], did not modify plasma GH.

One possible interpretation for the fact that only the second of two successive injections of a SME crude extract was able to stimulate the release of radioimmunoassayable GH in our animals might be based on the assumption that 2 pools of GH coexist in rat pituitaries. A storage pool (S-GH), which would constitute the 99.9% of the whole pituitary GH, would be in the form of a pro-hormone, immunoassayable but devoid of biological activity, and thus not bioassayable; this pool would not be readily releasable by GH-RF. A small fraction of rat pituitary GH (around 0.1%) would be the releasable pool (R-GH), biologically active and, thus bioassayable, but also immunoassayable. The R-GH would be readily releasable by GH-RF, and thus rapidly and often depleted by many factors (stress, cold, hypoglycemia, etc.), but at the same time constantly and slowly reformed from the S-GH, under the influence of GH-RF or of a hypothetical GH-synthesizing factor (GH-SF). When a first injection of a SME extract is given, it would immediately deplete the R-GH thus inducing a measurable decrease of bioassayable GH; however, the amount depleted, and that released in the blood, would be too small to be easily detected by a radioimmunoassay (assuming a total pituitary content of 500 µg of radioimmunoassayable GH for a rat of 300 g body wt., a depletion of 50% of the releasable pool would induce a decrease of only 250 ng, and an increase in the circulation, 10 min later, of only about 5 ng/ml). The first injection of SME extract would also induce, at the same time, an increased transformation of S-GH into R-GH, which, 1 h later, would be larger than before the injection, due to a compensatory effect. A second injection of the SME extract, given at this time, would then induce an increase of circulating GH which could easily be detected by a radioimmunoassay (if the R-GH pool increases to 0.4%, in the example given above, the plasma GH could increase by about 20 ng/ml) (Figure A, B, C, D). It is also possible that part of this increase is due to the passage into circulation of a certain amount of immunoassayable pro-hormone.

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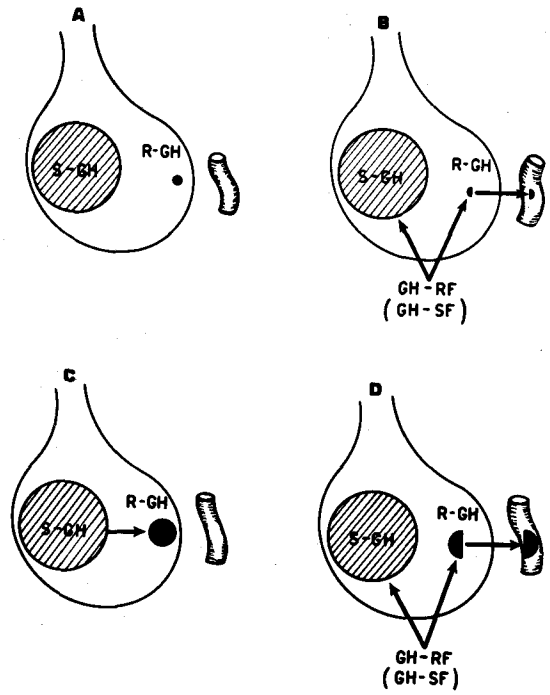
Plasma growth hormone (GH) in urethane anesthesized ♂ rats after intracarotid injections of stalk-median eminence (SME) extract, cerebral cortex (CC) extract, or vasopressin

Groups	Rat No.	GH (ng/ml) First Injection[a]		Second Injection[a]	
		−10′	10′	−10′	10′
SME 0.75 equiv./0.2 ml	49	7.0	8.0	5.9	52.0
	65	7.6	7.0	5.6	20.0
	72[b]	14.2	13.5	9.0	30.0
	73[b]	12.5	17.0	13.0	32.0
CC 0.75 equiv./0.2 ml	76[b]	12.4	13.0	12.4	17.0
Vasopressin 30 mU/0.2 ml	70	13.0	10.0	9.8	9.8

[a] The first injection was given 2 h after the beginning of the anesthesia, the second injection 1 h after the first injection. Both injections were given at 0′. [b] SME and CC extracts were obtained from the same animals



Schematic representation of a rat anterior pituitary and of a pituitary vein. A) Pituitary GH is represented in the form of a storage pool (S-GH), the most abundant (99.9%), and a releasable pool (R-GH), the least abundant (0.1%). Both pools would be measurable by a radioimmunoassay, but a bioassay would detect only the R-GH. Proportions are not respected in the figures.

B) Under the influence of GH-RF, contained in a first injection of a SME extract, half the R-GH is immediatedly released in the circulation, thus halving the pituitary content of bioassayable GH, but leaving practically unchanged the total content of immunoassayable GH. The amount of GH liberated in the blood would be too small to be easily detected by a radioimmunoassay.

C) Under the influence of the same GH-RF, or of a hypothetical GH-SF, the R-GH is slowly reformed, possibly from a pro-hormone existing in the storage pool. By a compensatory mechanism, the new pool of R-GH would be larger than before the injection (0.4% of total pituitary GH).

D) The GH-RF contained in a second injection of a SME extract, given 1 h after the first one, halving again the R-GH pool, would release in the circulation an amount of GH, easily detectable, this time, by a radioimmunoassay.

Interestingly enough, SPITZ et al.[13] have very recently found that a single intravenous pulse injection of arginine is incapable of stimulating the release of human GH, while a second injection given 20 min later is highly effective. Data obtained with the electron microscope[14,15] seem also to favor the hypothesis that injections of hypothalamic extracts induce not only the release, but also the resynthesis of GH from a biologically inactive precursor and that under certain circumstances the resynthesis can be more pronounced than the release[15]. Two or more successive injections of a hypothalamic extract have also been administered by other authors in order to study the effects on the secretion of rat follicle-stimulating hormone (FSH) or luteinizing hormone (LH). It is interesting to notice that plasma LH levels were increased by every injection[16], and that pituitary FSH stores were depleted only by the first injection, while the second induced, on the contrary, an increase[17]. These results indicate the existence of some differences in the control of synthesis and release of the two gonadotropins and of GH.

Résumé. Rats mâles adultes, anesthésiés avec l'uréthane, reçurent deux injections intracarotidiennes successives d'un extrait d'éminence médiane de rat, séparées par un interval de temps d'une heure. Seulement après la seconde injection on observa une augmentation hautement significative de la quantité circulante d'hormone somatotrope.

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