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Growth Hormone Releasing Factor of a Guinea-Pig Hypothalamic Extract: Its Activity in Guinea Pig and Rat.* (30411)

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The involvement of central nervous system in the secretion of pituitary trophic hormones has obtained wide experimental support particularly for corticotropin, thyrotropin and gonadotropins(1-3).

Only recently evidence of the existence of a nervous regulation also for growth hormone secretion has been accumulated. Growth is poorly supported in hypophysectomized animals by pituitaries grafted in the kidney capsule(4,5); conversely it can continue almost normally if the pituitary is transplanted in the hypophysiotropic area of the hypothalamus(6). Rats with experimentally produced hypothalamic lesions fail to grow(7). A growth hormone releasing activity of hypothalamic extracts has been demonstrated in the rat by the experiments *in vitro* of Deuben and Meites(8) and by the *in vivo* studies of Pecile *et al*(9).

The existence of a nervous control of growth hormone secretion in the rat has prompted us to investigate whether growth hormone releasing activity is present also in other animals. Our aim was to provide not only new and larger evidence of the physiological role of nervous regulation in growth processes, but also to evaluate if and to what extent, growth hormone releasing activity of the hypothalamus of an animal might reproduce its biological effect in animals of different species. Thus in the present work growth hormone releasing activity of guinea-pig hypothalamic extracts was investigated by injecting the extracts in guinea pigs as well as in rats.

Materials and methods. Thirty-day-old female guinea pigs were used. The stalk median eminence region (SME) and the cerebral cortex (CC) of 80 guinea pigs were removed, weighed, pooled and homogenized with 0.1 N HCl. The diluted extract was centrifuged at 3,000 rpm for 15 minutes at 4°C. The pH of the supernatant was adjusted to 7.4 by addition of $NaHCO_3$. The final volume was made up so that the acid extract from 5 mg of guinea-pig SME or CC was contained in 0.2 ml of medium. As recipient animals, groups of 15 (30-day-old) female guinea pigs and (30-day-old) Sprague-Dawley female rats were used. All recipient animals were injected in the carotid with 5 mg/100 g bw of SME or CC. Fifteen minutes after injection recipient guinea pigs and rats were killed by decapitation; the pituitaries of each experimental group of ani-

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TABLE I. Growth Hormone Activity of Pituitaries of Guinea Pigs and Rats Treated with Saline or Cerebral Cortical (CC) Extracts or Stalk Median Eminence (SME) Extracts.

Groups	Material inj	Treated animals	Evaluation by "tibia test" of growth hormone activity of pituitaries from treated animals: No. of hypophysectomized assay animals, Dose 1*	No. of hypophysectomized assay animals, Dose 2*	Epiphysial width, μ† (mean ± S.E.), Dose 1*	Epiphysial width, μ† (mean ± S.E.), Dose 2*	Significance of differences from saline treated animals‡
A	Saline	Guinea-pig	8	10	234 ± 4.8	311 ± 4.6	—
B	Guinea-pig CC (5 mg/100 g b.w.)	"	8	10	229 ± 3.9	298 ± 6.3	
C	Guinea-pig SME (5 mg/100 g b.w.)	"	8	10	213 ± 2.6	228 ± 7.0	A–C $p < .001$
D	Saline	Rat	8	10	221 ± 8.5	286 ± 3.5	
E	Guinea-pig CC (5 mg/100 g b.w.)	"	8	10	231 ± 6.5	290 ± 5.7	
F	Guinea-pig SME (5 mg/100 g b.w.)	"	8	10	209 ± 5.2	237 ± 4.9	D–F $p < .05$
	Standard growth hormone		8	10	235 ±10.7	288 ± 6.4	

* Dose 1: Guinea-pig pituitary (groups A–B–C) 1.5 mg/die/4 days; rat pituitary (groups D–E–F) 0.312 mg/die/4 days; GH standard 25 μg/die/4 days. Dose 2: Guinea-pig pituitary (groups A–B–C) 3.0 mg/die/4 days; rat pituitary (groups D–E–F) 0.625 mg/die/4 days; GH standard 50 μg/die/4 days.
† Mean value of epiphysial width of hypophysectomized animals was 147 ± 7.2.
‡ Evaluated by means analysis of variance.

mals were removed, weighed on a torsion balance, pooled and kept at −10°C for subsequent bioassay of growth hormone content which was performed following the method of Greenspan(10) as modified by Reichlin (11). Eight to ten hypophysectomized rats were used to assay each dose of the samples. NIH preparation of growth hormone (GH-B 8) was used as the reference standard.

Pituitaries from each experimental group were prepared by homogenizing the pooled glands with saline. Dilutions were made in order to provide 2 dosage levels in the same volume of injected fluid (0.5 ml). The same procedure was followed in preparing the homogenates with control pituitaries taken from groups of 15 female guinea pigs or rats (30-day-old) treated only with 0.2 ml of saline. The potencies of the pituitary homogenates were calculated on the basis of the results of the 4-point assay according to Finney(12).

Results. From the results of our experiments (Tables I and II) it appears that pituitary homogenates from guinea pigs treated with guinea-pig SME extract, when given to hypophysectomized assay animals, induced a widening of the epiphysial cartilage markedly lower than that obtained with pituitary homogenates from saline treated control guinea pigs. The growth hormone decrease induced by guinea pig SME extract corresponded to

TABLE II. Growth Hormone Content of Pituitaries of Guinea Pigs and Rats Treated with Saline or with Stalk Median Eminence (SME) Extracts.

Material inj	Treated animals	Growth hormone pituitary content: μg GH/mg	μg GH/pituitary	Fiducial limits* P = .95
Saline	Guinea pig	19.10	153.0	16.5–22.9
Guinea-pig SME	"	6.1	48.6	1.6– 9.6
Saline	Rat	74.5	294.0	60.8–90.2
Guinea-pig SME	"	39.5	158.0	21.6–53.9

* Fiducial limits are referred to values expressed as μg GH/mg.

approximately 70% of pituitary growth hormone content (from 19.1 to 6.1 μg GH/mg pituitary) (Table II).

Similarly pituitary homogenates from rats treated with guinea-pig SME extract showed a reduced pituitary growth hormone activity when given to hypophysectomized assay animals. The growth hormone decrease induced by guinea-pig SME extract in rat corresponded to 50% of the control values (from 74.5 to 39.5 μg GH/mg pituitary) (Table II). No depletive effect on growth hormone of rats and guinea pig pituitaries was observed as a consequence of guinea pig CC extract injection.

Discussion. These results provide evidence for the existence of a growth hormone releasing activity in acid extracts of guinea-pig hypothalamus. Since releasing activity points to the presence of a nervous regulation of growth hormone secretion, our finding seems to be of special interest considering that guinea pig is the only vertebrate, with the salamander *Amblystoma tigrinum* Green(13), whose growth is unaffected by hypophysectomy(14) so that it might be considered an "adult fetus"(15) not requiring pituitary stimulation for its growth processes(16).

Our results while suggesting a close similarity between guinea pig and other mammals as far as growth hormone regulation is concerned, strongly suggest the possibility that the unexpected growth of hypophysectomized guinea pigs may be due to the residual secretion of growth hormone maintained by the pars tuberalis-like cells which actually remain after the operation(15).

A second point emphasized by our results is the remarkable growth hormone releasing activity of guinea-pig SME extracts not only on guinea-pig but also on rat pituitary. Although on a quantitative basis this SME releasing effect is not marked in rat as in guinea pig, it is however clear-cut evidence. This may alternatively suggest either that the chemical structure of neurohumoral substances in the different animal species is relatively simple and probably similar, or that hypophyseal tissue of different animal species may be largely responsive to the stimulatory action of neurohumoral substances that, although producing identical biological effects, are chemically different. Lack of selectivity in the response of endocrine organs with respect to species-specificity is a well known phenomenon.

Since growth hormone, unlike other pituitary trophic hormones, is so highly species-specific that the use of non-primate growth hormone in clinical therapy is prevented, the potential importance of neurohumoral substances of animal origin presumably able to stimulate a secretion of endogenous growth hormone in the human is obvious. Such a new pharmacological tool would be valuable for therapeutic purposes.

Summary. Growth hormone releasing activity of a guinea-pig hypothalamic extract has been evaluated in guinea pig and rat. Guinea-pig stalk median eminence (SME) extracts injected in the carotid arteries of both guinea pig and rats induce a marked depletion of pituitary growth hormone content. The potential importance of a relative lack of species-specificity for growth hormone releasing factor(s) is considered.

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Maturation of Rat Megakaryocytes Studied by Microspectrophotometric Measurement of DNA.* (30412)

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The maturation process of the megakaryocyte series of cells is not well understood. It is generally accepted that nuclear division occurs without cytoplasmic division during megakaryocyte maturation, with the result that nuclei of mature megakaryocytes contain several times the normal diploid chromosome complement of the species. There has been no completely objective method of grouping megakaryocytes into maturation stages based on their appearance under the light microscope. It seemed, however, that measurement of the quantity of deoxyribonucleic acid (DNA) in individual megakaryocytes might provide a systematic means of classification. With such a method additional insight into the maturation process of megakaryocytes may be gained, and a clearer assessment of effects of various experimental or disease-related perturbations of the megakaryocyte-platelet system may be possible. We have, therefore, measured microspectrophotometrically the DNA content of megakaryocytes in Feulgen-stained preparations of smears of rat bone marrow. Garcia(1) has recently described the DNA content of megakaryocytes of rabbits measured by essentially the same methods.

Methods. Smears of bone marrow of tibias of 8 male Sprague-Dawley rats, 23 to 107 days old, were made. The smears were air-dried for at least 1 hour, fixed in Carnoy's solution (6 parts absolute alcohol: 3 parts chloroform: 1 part acetic acid) for 5 minutes or 1 hour, and subjected to the Feulgen-staining procedure(2,3). In most cases, smears were hydrolyzed for 6 minutes at 60°C in 1 N HCl, stained in decolorized basic fuchsin for 30 minutes, and rinsed twice with SO_2 for 2-3 minutes, and once with tap water for 5 minutes.

The DNA of individual cells was measured microspectrophotometrically. The 2-wavelength method(4,5) was used since it tends to reduce distributional error introduced by the uneven distribution of chromatin material of megakaryocyte nuclei. A Canalco Ultra-Microspectrophotometer with a digital ratio reporter and a Bausch and Lomb grating monochromator as light source were employed. The image of a nucleus was centered on a mirror (the phototube head mirror) seen by a phototube, and the clear area around the nucleus was minimized by using an iris diaphragm and 2 leaf diaphragms. The light intensities passing through the specimen were measured at 2 wavelengths: (A) near the absorption peak of the Feulgen-stained DNA (565 mμ in our work), and (B) at a wavelength (506 mμ) where the absorption coefficient was half of that at the first wavelength. Background was determined by similar measurements in a clear area of the slide. The instrument automatically presents the results as percent transmission at the 2 wavelengths. The iris and leaf diaphragms were then moved out of the field and a sliding plate having a hole of fixed diameter was moved into the groove formerly occupied by the leaf diaphragms. The ratio between the amount of light transmitted through the fixed diaphragm and the diaphragm employed for the nucleus was used as a measure of the

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