Pituitary Growth Hormone Content and Hypothalamic Growth Hormone Releasing Activity in Neonatal Rats after Stress* (33571)

ANTONIO PECILE, GABRIELLA FERRARIO, GIANNI FALCONI AND EUGENIO E. MÜLLER
(Introduced by G. Pozza)

Department of Pharmacology, School of Medicine, University of Milan; and Department of Pharmacology, School of Pharmacy, University of Pavia, Italy

The presence of growth hormone in the pituitary of fetuses or newborn animals of different species has been ascertained by many authors (1–4). The growth hormone content in fetal human pituitaries was studied by Gershberg (5). He found that its concentration per milligram of gland was similar in fetuses and in adults, the total amount of growth hormone being higher simply as a reflection of the larger pituitaries present in the adults. Although the ability of fetal pituitary to synthesize growth hormone during fetal life and also in the early postnatal period seems established, there is no investigation on the degree of maturity reached at these stages of development by the hypothalamic releasing mechanism(s). Thus, we thought it would be interesting to ascertain first of all, whether growth hormone releasing (GRF) activity would be present in the hypothalami of neonatal rats. Then we tested whether the application of the electric shock stimuli, which are potent releasers of growth hormone in the adult rats, could be effective also in the neonatal animals.

Materials and Methods. Sprague-Dawley newborn rats (10 days old) were used in the first series of experiments. The stalk median eminence (SME) region of these animals was accurately removed, pooled, and homogenized with 0.1 *N* HCl. The diluted extract was centrifuged at 3000 rpm for 15 min at 4°. The supernatant was kept frozen (—20°) until before use.

For evaluating growth hormone releasing activity, SME extracts were adjusted to pH 7.4 by addition of $NaHCO_3$ solution. The final volume was made up so that the extract from one newborn rat SME was contained in 0.2 ml. The extracts were injected into the carotid artery of 30-day-old recipient rats (6) and the animals were killed by decapitation 15 min later. Pituitaries were removed, weighed on a microtorsion balance to the nearest 0.05 mg, and homogenized in saline. The diluted material was kept frozen until immediately before injection. The GH activity of the samples was measured by the "tibia test" method of Greenspan *et al.* (7). Six to eight hypophysectomized rats (8) were used to assay each sample. The results are expressed directly in terms of the width of the epiphysial cartilage. Significance of differences in epiphysial cartilage width was determined by Student's *t* test.

In the second series of experiments neonatal rats of 1 or 5 or 10 days of age were submitted to electric shock stimuli (see Table II) and 15 min later were killed by decapitation. The growth hormone content of each group of pooled pituitaries was evaluated as indicated above. The hypothalamic SME of 5-day-old rats submitted to electric shock were tested for their growth hormone releasing activity.

Results and Discussion. When injected into the carotid artery, stalk median eminence extracts of newborn rats were active in inducing release of growth hormone from the pituitary of recipient animals (Table I). This indicates that in this early stage of develop-

* Supported by PHS Research Grant HD 01109-03 from the National Institute of Child Health and Human Development, Public Health Service, USA.

TABLE I. Growth Hormone (GH) Releasing Activity of Hypothalamic Extracts of Neonatal Rats.[a]

Groups (8 recipient animals/group)	Material injected into the carotid artery of recipient animals	Evaluation by tibia test of GH activity of pituitaries from treated animals[c] (epiphysial width in μ)[d] (mean ± SE)
A	—	291 ± 4.9
B	Saline	287 ± 7.7
C	3 SME of 10-day-old rats[b]	238 ± 8.9
D	3 SME of 10-day-old rats[b]	243 ± 6.7

[a] Significance among groups: B vs C, $p < 0.001$; B vs D, $p < 0.001$.

[b] A different pool of SME was used for groups C and D.

[c] 6–8 hypophysectomized assay rats per group were used.

[d] The mean value of epiphysial width of hypophysectomized animals was 159 ± 7.2.

ment, not only growth hormone is present in the pituitary (4, 9) but also a remarkable growth hormone releasing activity exists in the hypothalamus. The availability of the hormone and of its hypothalamic releaser seems thus clear. From the results of the second series of our experiments, summarized in Table II, it appears that electric shock stimuli applied to neonatal animals of different ages induced a clear-cut depletion of pituitary growth hormone content. No significant differences were noticed in the response at the various ages tested. These results point to the ability of the brain centers to elicit growth hormone release in response to stressful stimuli.

The similarity between the behavior of neonatal and adult animals is apparent also from data on growth hormone releasing activity in rats submitted to electric stimuli (Table III). The GRF activity in the hypothalami of animals submitted to shocking stimuli was markedly reduced, thus indicating the involvement of GRF in the mechanism of GH release evoked by electric shock. From the reported data it may be concluded that in the very early stages of development all mechanism(s) which bring about growth hormone release are available and may be put at work.

Considering these observations it seems improbable that there exists a so-called stress nonresponsive period for GH in young animals as postulated for ACTH secretion by Schapiro *et al.* (11). On the other hand, Zarrow *et al.* (12) demonstrated that stress like heat or electric shock in infancy may produce an immediate measurable effect upon the adrenal gland in infant rats.

TABLE II. Effect of Electric Shock Treatment on Growth Hormone (GH) Pituitary Activity of Neonatal Rats.[a]

Groups (10 animals/group)	Age of animals (days)	Treatment[b]	Evaluation by tibia test of GH activity of pituitaries from treated animals[c] (epiphysial width in μ)[d] (mean ± SE)
A	1	—	242 ± 6.5
B	1	Shock	155 ± 4.7
C	5	—	269 ± 9.9
D	5	Shock	178 ± 5.5
E	10	—	278 ± 5.4
F	10	Shock	176 ± 4.6

[a] Significance among groups: A vs B, $p < 0.001$; C vs D, $p < 0.001$; E vs F, $p < 0.001$.

[b] The parameters of the shocking treatment were: voltage, 75 V; single shock duration, 0.2 sec; shock frequency, 4/min; number of shocks, 10; treatment time, 2 min 30 sec.

[c] 6–8 hypophysectomized assay rats per group were used.

[d] The mean value of epiphysial width of hypophysectomized animals was 147 ± 6.2.

TABLE III. Growth Hormone (GH) Releasing Activity of Hypothalamic Extracts of Neonatal Rats Submitted or Not to Electric Shock Treatment.[a]

Groups (8 recipient animals/group)	Material injected into the carotid artery of recipient animals	Evaluation by tibia test of GH activity of pituitaries from treated animals[c] (epiphysial width in μ)[d] (mean ± SE)
A	Saline	256 ± 3.4
B	2 SME of 5-day-old rats untreated	193 ± 4.2
C	2 SME of 5-day-old rats shocked[b]	229 ± 9.2

[a] Significance among groups: B vs A, $p < 0.001$; C vs A, $p < 0.001$; and C vs B, $p < 0.01$.
[b] Shock parameters as in Table II.
[c] 6–8 hypophysectomized assay rats per group were used.
[d] The mean value of epiphysial width of hypophysectomized animals was 153 ± 8.3.

It may be relevant to this discussion to recall that brain catecholamines were recently shown to participate in the process of GH release in response to stressful stimuli (13) and that electric shock is one of the most potent releasers of brain catecholamines (14).

The brains of infant rats are more sensitive to the catecholamine-depleting action of drugs like reserpine and tetrabenazine (15). Therefore, the responsiveness to a stressful stimulus might be expected to be higher in infantile animals than in adult ones. Also, stressful stimuli such as formalin administration which are usually found to be inactive as GH releasers in adults might be effective when applied to infant animals.

Summary. A remarkable growth hormone releasing (GRF) activity was found in hypothalamic stalk median eminence (SME) extracts of neonatal rats. The application of electric shock to rats of 1, 5, and 10 days of age induced a clear-cut decrease in pituitary growth hormone content. In the shocked animals GRF activity in the hypothalamic SME extracts was significantly reduced. These results indicate that in the very early stages of development all mechanism(s) which bring about growth hormone release are available, and that a response to stressful stimuli may be expected in infant rats as well as in adult rats.

1. Smith, P. E. and Dortzbach, C., Anat. Record **43**, 277 (1929).
2. Houssay, B. A., Rev. Soc. Arg. Biol. **24**, 24 (1948).
3. Baker, B., Jr., Hollandbeck, R., Norton, H. W., and Nalbandov, A. V., J. Animal Sci. **15**, 407 (1956).
4. Contopoulos, A. N. and Simpson, M. E., Federation Proc. **16**, 24 (1957).
5. Gershberg, H., Endocrinology **61**, 160 (1957).
6. Pecile, A., Müller, E., Falconi, G., and Martini, L., Endocrinology **77**, 241 (1965).
7. Greenspan, F. S., Li, C. H., Simpson, M. E., and Evans, H. M., Endocrinology **45**, 455 (1949).
8. Falconi, G. and Rossi, G. L., Endocrinology **74**, 301 (1964).
9. Contopoulos, A. N., Excerpta Med. Intern. Congr. Ser. **142**, 35 (1967).
10. Müller, E. E., Saito, T., Arimura, A., and Schally, A. V. Endocrinology **80**, 109 (1967).
11. Schapiro, S., Geller, E., and Eiduson, S., Proc. Soc. Exptl. Biol. Med. **109**, 937 (1962).
12. Zarrow, M. X., Haltmeyer, G. C., Denenberg, V. H., and Thatcher, J., Endocrinology **79**, 631 (1966).
13. Müller, E. E. and Pecile, A., Excerpta Med. Intern. Congr. Ser. **158**, 253 (1968).
14. Maynert, E. W. and Levi, R., J. Pharmacol. **143**, 90 (1964).
15. Kulkarni, A. S. and Shideman, F. E., J. Pharmacol. Exptl. Therap. **153**, 428 (1966).

Received July 9, 1968. P.S.E.B.M., 1969, Vol. 130.