The Involvement of Cyclic 3′, 5′-Adenosine Monophosphate in the Growth Hormone Release Mechanism (s)[1]

It has recently been shown by SCHOFIELD[2] that the addition of theophylline to anterior pituitaries incubated in vitro significantly enhances the output of growth hormone (GH) in the incubation medium. These results, which are interpreted to mean that the release of growth hormone is stimulated by an increase in the pituitary level of cyclic 3′, 5′-adenosine monophosphate (3′, 5′-AMP), prompted us to investigate whether: (1) the action of theophylline, evident in vitro, was still present in vivo; (2) 3′, 5′-AMP was capable by itself of stimulating release of growth hormone. Thus theophylline and/or 3′, 5′-AMP dibutyrate were injected into brain lateral ventricle of the rat and their effect on GH release from the pituitary was evaluated by the 'tibia test' method of GREENSPAN et al.[3]. In a second series of experiments it was studied whether an enhanced availability of 3′, 5′-AMP, induced by a pretreatment with theophylline, might allow GH release in response to a stimulus as formalin administration. Such a stimulus was previously found inactive in the rat in depleting the hormone from the pituitary, at variance with other stressful procedures[4]. In our experiments, Sprague-Dawley female rats of 120–140 g body weight maintained in group cages, at constant temperature (22 ± 2 °C), were used. All animals were fasted overnight before the treatment. The following day they were injected with test drugs dissolved in saline. (For other details of the experimental procedure see Table I and II).

Animals were killed by decapitation 30 min or 1 h before the last injection. The pituitaries were removed, pooled by groups and their GH activity was measured by the 'tibia test' method of GREENSPAN et al.[3]. Since in the GREENSPAN assay the width of epiphyseal cartilage is directly related to the log-dose of GH over the linear portion of the curve, the results are expressed directly in terms of the width of epiphyseal cartilage. The significance of differences between groups was determined by Student's *t*-test. A sample of venous blood was withdrawn from experimental rats at the time of sacrifice for blood glucose determinations (Glucostat, Worthington, Biochemicals).

Results. From the results reported in Table I it appears that while cyclic AMP was inactive in untreated animals (group 3 vs. 1), in rats pretreated s.c. with theophylline, a clear effect on GH release was noticed (group 5 vs. 1 and 3). This is apparent from the narrower epiphyseal cartilage in hypophysectomized rats receiving pituitary homogenates from animals injected with theophylline and 3′, 5′-AMP. On the other hand, theophylline, which, when injected s.c. was ineffective on GH release (group 4 vs. 1), when given intraventricularly induced a marked

[1] Supported by USPHS Research Grant No. HD 01109-03.
[2] J. G. SCHOFIELD, Nature *215*, 1383 (1967).
[3] F. S. GREENSPAN, C. H. LI, M. E. SIMPSON and H. M. EVANS, Endocrinology *45*, 455 (1949).
[4] E. E. MÜLLER, T. SAITO, A. ARIMURA and A. V. SCHALLY, Endocrinology *80*, 109 (1967).

Table I. Effect of cyclic 3′,5′ adenosine monophosphate (3′,5′-AMP) given by intraventricular injection on pituitary growth hormone activity in animals pretreated or not with theophylline

Group	Treatment and dose	No. of animals	Epiphyseal width (μ)	*P*	Blood glucose (mg/100 ml)
1	Saline (0.02 ml e.v.)	6	255 ± 7.7	–	84.7 ± 6.7
2	Theophylline (5 μg/0.02 ml e.v.)	6	213 ± 11.0	0.01	103.3 ± 1.7
3	3′,5′-AMP (5 μg/0.02 ml e.v.)	6	255 ± 8.2	n.s.	94.4 ± 4.4
4	Theophylline (50 mg/kg s.c.)	5	245 ± 8.2	n.s.	100.0 ± 3.9
5	Theophylline (50 mg/kg s.c.) + 3′,5′-AMP (5 μg/0.02 ml e.v.)	6	217 ± 9.7	0.01	108.6 ± 12.0

Animals were injected with theophylline or 3′,5′-AMP by intraventricular injection (e.v.)[a] or with theophylline by s.c. and killed 30 min after the last injection. In the combined treatment theophylline was given s.c. 2 h before 3′,5′-AMP. In the 'tibia test' 6–8 hypophysectomized assay animals per group were used. Values are means and standard errors. n.s., not significant.

[a] The method described by NOBLE et al.[5] was used.

Table II. Effect of formalin on pituitary growth hormone activity in animals pretreated or not with theophylline

Group	Treatment and dose	No. of animals	Epiphyseal width (μ)	*P*	Blood glucose (mg/100 ml)	*P*
1	Saline (0.5 ml/100 g s.c.)	6	250 ± 2.3	–	70.0 ± 9.0	–
2	Formalin 10% (0.5 ml s.c./rat)	6	247 ± 1.6	n.s.	105.0 ± 7.0	0.02
3	Theophylline (50 mg/kg s.c.)	5	248 ± 4.0	n.s.	85.3 ± 8.5	n.s.
4	Theophylline (50 mg/kg s.c.) + Formalin 10% (0.5 ml s.c./rat)	6	218 ± 9.8	0.01	117.3 ± 7.2	0.001

Animals were injected with formalin or with theophylline by s.c. injection and killed 1 h after the injection of formalin or 2 h after the injection of theophylline. In the combined treatment theophylline was given s.c. 1 h before formalin. In the 'tibia test' 6–8 hypophysectomized assay animals per group were used. Values are means and standard errors. n.s., not significant.

depletion of pituitary GH (group 2 vs. 1). No changes of blood glucose were noticed after administration of cyclic AMP or theophylline, or both compounds at the doses used. The second series of experiments are summarized in Table II. The administration of formalin to normal rats did not elicit a significant depletion of pituitary GH (group 2 vs. 1), but was effective in this sense in animals pretreated 1 h before with theophylline (group 4 vs. 1 and 2). Blood glucose levels found in formalin or theophylline plus formalin treated rats were significantly higher than controls.

Discussion. Our results confirm and extend the previous observations of SCHOFIELD[2]. In fact the intraventricular administration of theophylline, which through inhibition of phosphodiesterase presumably enhances endogenous levels of 3′,5′-AMP, brings about a clear GH release. On considering this result, the lack of effect of the intraventricular injection of 3′,5′-AMP appears surprising but is probably due to the low entry into cells of the nucleotide[6], even if by using the dibutyrate derivative a greater rate of entry into cells would be expected[6]. To allow the appearance of an effect sustained by the low amounts of 3′,5′-AMP able to penetrate into cells, a contemporary inhibition of phosphodiesterase seems to be required. This is suggested by the observation that theophylline, although ineffective in releasing GH when given by s.c. injection, favours the appearance of a GH-releasing effect in animals treated intraventricularly with 3′,5′-AMP. It is relevant to this point that GAGLIARDINO and MARTIN[7] observed a sharp rise in the circulating levels of GH in monkeys treated i.v. with 3′,5′-AMP alone. Recently LEVINE[8] has demonstrated in the human clearcut increase of plasma levels of GH after perfusion with dibutyril 3′,5′-AMP. These data fit in well with our results in the rat and in addition point to differences among species with respect to the ability of 3′,5′-AMP to penetrate cell membranes[6]. The action of theophylline in the processes of GH release depends upon the ability of this xanthine to inhibit the cyclic nucleotide phosphodiesterase that hydrolizes cyclic AMP in the pituitary and/or hypothalamus. In the light of the results of VERNIKOS-DANELLIS and HARRIS[9], who have shown that in vivo caffeine and theophylline markedly reduced the phosphodiesterase activity of the anterior pituitary while being quite ineffective on the enzyme activity of the hypothalamic median eminence; and the in vitro work of SCHOFIELD[2], it would appear that theophylline given intraventricularly releases GH, since it is capable of reaching the pituitary by diffusion into the portal vessels. The observation that a stress ineffective in releasing GH in the rat, such as formalin administration[4], induces this effect when animals are pretreated with theophylline, is reminescent of data reported by VERNIKOS-DANELLIS and HARRIS[9], who noticed that pretreatment of rats with theophylline resulted in a potentiation of the stress-induced secretion of ACTH. Apparently the level of 3′,5-AMP in the pituitary mediates the secretion of the gland in response to impulses conveyed by different nervous pathways.

Riassunto. Il 3′,5′-AMP dibutirrato (5 μg) iniettato nel ventricolo laterale del cervello di ratto, provoca una spiccata liberazione dell'ormone somatotropo soltanto in animali pretrattati con teofillina (50 mg/kg s.c.). La teofillina per via s.c. (50 mg/kg) si dimostra inattiva, ma libera ormone somatotropo quando è iniettata per via endoventricolare (5 μg). Uno stimolo usualmente incapace di liberare somatotropo (formalina) provoca deplezione dell'ormone dall'ipofisi in ratti pretrattati s.c. con teofillina. I risultati sono in favore di una partecipazione del 3′,5-AMP nel meccanismo di liberazione di ormone somatotropo.

E. E. MÜLLER, A. PECILE,
M. KABIR NAIMZADA[10] and GABRIELLA FERRARIO

Department of Pharmacology School of Pharmacy, University of Pavia and Department of Pharmacology School of Medicine, University of Milan, Milano (Italy), 22 January 1969.

[5] E. P. NOBLE, R. J. WURTMAN and J. AXELROD, Life Sci. *6*, 281 (1967).
[6] E. W. SUTHERLAND and G. A. ROBISON, Pharmac. Rev. *18*, 145 (1966).
[7] J. J. GAGLIARDINO and J. M. MARTIN, Acta Endocr. *59*, 390 (1968).
[8] R. A. LEVINE, personal communication.
[9] J. VERNIKOS-DANELLIS and C. G. HARRIS III, Proc. Soc. exp. biol. Med. *128*, 1016 (1968).
[10] Fellow of Italian Foreign Office.

Growth of the Endocrine Organs of Female Hamsters Blinded at 25 Days of Age[1]

Light deprivation from adult female hamsters (*Mesocricetus auratus*) either by exposure to darkness or by blinding leads to gonadal atrophy within 8 weeks; the gonadal inhibitory effects of darkness are prevented if the pineal gland is removed[2,3]. The pineal gland, or epiphysis cerebri, in the hamster lies just beneath the confluence of sinuses between the cerebral hemispheres and is attached to the epithalamus by a tenuous stalk. To test the effect of the absence of light on the neuroendocrine axis of female hamsters blinded before puberty the following experiment was performed.

Materials and methods. Growth of the endocrine and reproductive organs were compared in the following groups of hamsters: (1) normal; (2) blinded and sham-pinealectomized; (3) blinded and pinealectomized. Anesthetized animals were operated on using techniques previously established[4,5]. Since the pineal gland lies directly under the confluence of sinuses it can be extirpated easily after removing a circular piece of bone overlying the area[4]. Animals were maintained under controlled temperature (24 ± 2 °C) and lighting (14 h light per 24 h period) conditions. 8–10 hamsters from each of the 3 groups were killed at 25 day intervals after the operations

[1] Supported by Grant No. HD-02937 United States Public Health Service.
[2] R. A. HOFFMAN and R. J. REITER, Life Sci. *5*, 1147 (1965).
[3] R. J. REITER, R. A. HOFFMAN and R. J. HESTER, J. exp. Zool. *160*, 263 (1966).
[4] R. A. HOFFMAN and R. J. REITER, Anat. Rec. *153*, 19 (1965).
[5] R. J. REITER and R. J. HESTER, Endocrinology *79*, 1168 (1966).