Since the secretory products of the adrenal cortex are nonprotein substances, the hyperpolarization of PM of ACC long after the injection of ACTH due to activation of protein synthesis in ACC may be thought to be related to an activated synthesis of the enzymes of steroidogenesis. This may be what provides for the enhanced secretion of corticosteroids by "hyperpolarized" AG during *in vitro* stimulation of steroidogenesis in the adrenal cortex by the tropic hormone.

At the same time, other mechanisms underlying the enhanced reactivity of the "hyperpolarized" AG to ACTH cannot be ruled out. In particular, it has been shown that the development of hyperpolarization of cell PM resulting from the activated protein synthesis in tissues is accompanied by activation of Na,K-ATPase of the PM. In addition, the appearance of peptide compounds, so-called invertors, has been demonstrated in hyperpolarized tissues, which are capable of activating Na,K-ATPase of cell PM in intact tissues and thereby affect their functional activity [4]. From this point of view, the hyperpolarization of PM of ACC which develops against the background of activated protein synthesis may serve as an invertor-mediated interface in the chains of direct and feedback regulatory influences, thus modulating the reactivity of ACC to the tropic hormone.

REFERENCES

1. P. G. Kostyuk, *Microelectrode Technique* [in Russian], Kiev (1960).
2. A. G. Reznikov, *Methods of Hormone Assay* [in Russian], Kiev (1980).
3. V. V. Frol'kis, *Aging. Neurohumoral Mechanisms* [in Russian], Kiev (1981).
4. V. V. Frol'kis, *Dokl. Akad. Nauk SSSR*, **324**, 229 (1992).
5. N. A. Yudaev, C. A. Afinogenova, A. A. Bulatov, *et al.*, *Biochemistry of Hormones and Hormonal Regulation* [in Russian], Moscow (1976).
6. J. R. Lymangrover, E. K. Matthews, and M. Saffran, *Endocrinology*, **110**, 462 (1982).
7. J. R. Lymangrover, N. K. Valego, and R. D. Shilling, *Mech. Age. Dev.*, **43**, 269 (1988).
8. E. K. Matthews and M. Saffran, *J. Physiol. (Lond.)*, **189**, 149 (1967).

Age-Specific Effects of Insulin on the Secretion of Somatotropic Hormone

V. P. Fedotov, T. V. Mamaeva, and V. I. Gudoshnikov

UDC 612.433.65.06:612.349.7.018.2].06:612.66

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 3, pp. 304-306, March, 1994
Original article submitted September 12, 1993

It is shown that insulin is able to alter the secretion of somatotropic hormone directly at the level of the pituitary. The direction of the regulatory effect of insulin depends on the age of the animals donating the pituitary cells, while the intensity of the effect of insulin is largely modulated by glucocorticoid and thyroid hormone.

Key Words: *somatotropic hormone; cell culture; pituitary; insulin; postnatal development*

It is now undisputed doubt that there are direct and feedback relationships between the somatotropic hormone (STH) of the pituitary and insulin. As is well known, STH directly stimulates insulin secretion and biosynthesis, as well as the proliferation of β-cells of the pancreatic islets (islets of Langerhans) [7,14]. On the other hand, insulin has been found to directly inhibit the secretion of STH in cultures of normal and tumor cells of the

Laboratory of Biological Research of Hormonal Compounds, Institute of Experimental Endocrinology, Scientific Center of Endocrinology, Russian Academy of Medical Sciences, Moscow. (Presented by Yu. A. Pankov, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0003-0307$12.50

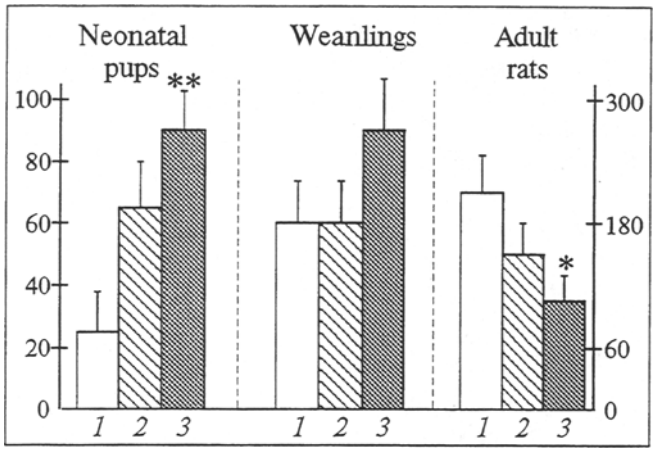

Fig. 1. Effect of insulin on basal secretion of STH in cultures of pituitary cells of rats of different age. Here and in Fig. 2: ordinate: concentration of STH in medium, ng/ml; at left: for neonatal rat pups and weanlings; at right: for adult animals. Groups ($n=5-6$): *1*) control; *2* and *3*) insulin in concentrations of 10^{-8} and 10^{-6} M, respectively. Incubation lasted for 4–5 h. One and two asterisks denote $p<0.05$ and $p<0.01$, respectively, as compared with the corresponding control.

pituitary [9,13]. At the same time, the metabolic effect of insulin in the adenohypophysis is markedly weaker than that in the muscle and adipose tissue [11]. It has been demonstrated by us and other scientists that the somatotropic function of the pituitary is somewhat increased in rats with acute alloxan- or streptozotocin-induced experimental diabetes [3,15], which indicates that negative feedback underlies the regulatory effect of insulin on the pituitary somatotrophs.

During early postnatal development, when the rate of growth and proliferation is high, STH is of primary importance. There is evidence that a combined effect of STH and insulin is required for maintaining the growth of peripheral tissues [10]. We have previously established that pituitary cells derived from neonatal rat pups exhibit an altered hormonal sensitivity which differs from that in adult animals [4,8]. This raises the question as to possible age-specific regulatory effects of insulin in somatotrophs. In the present study we investigated this problem with regard to the establishment of endocrine regulation during postnatal ontogenesis.

TABLE 1. Effect of Insulin on STH Secretion in Pituitary Cells of Rat Weanlings after a Long–Term Premedication with DM and T_3 ($M\pm m$, $n=6$)

Group	STH in medium, ng/ml
Control	59.7±7.44
T_3, 20 nM	42.8±3.06
T_3 + DM, 50 nM	228.2±13.3*
T_3 + DM + insulin, 10 nM	120.6±13.8**
T_3 + DM + insulin, 1 μM	151.6±12.1**

Note. Preincubation with DM and T_3 lasted for 72 h, incubation with these hormones and insulin for 5 h. One and two asterisks, respectively, denote the reliability ($p<0.001$) of differences from the group with T_3 and from the group with T_3 + DM.

MATERIALS AND METHODS

The following age groups of Wistar rats were used in the study: a) neonatal pups (6 to 7 days) of both sexes; b) weanlings (11 to 14 days) of both sexes; c) adult rats (70 to 100 days), female. The preparation of primary cultures of pituitary cells [4,5] and homologous radioimmunoassay of rat STH [1] were described previously. A long-term premedication with dexamethasone (DM) was performed in the cell cultures in a nutrient medium containing 1% fetal calf serum, whereas a long-term treatment of pituitary cells with thyroid hormone and glucocorticoid (GC) was performed in a medium containing 0.1% human serum albumin and 0.1% gelatin. A short-term incubation with insulin and other hormones was carried out in an albumin-containing serum-free medium. The following hormonal preparations were used: swine insulin (Calbiochem, Switzerland), DM (Serva, Germany), and L-3,3',5-triiodothyronine (Fluka, Switzerland). The results were statistically processed using Student's *t* test.

RESULTS

As is shown in Fig. 1, in a concentration of 10^{-8} M insulin showed a tendency to stimulate and in a dose of 10^{-6} M reliably raised the basal secretion of STH in the primary culture of pituitary cells from neonatal rat pups. In the culture of pituitary cells of weanlings insulin in concentrations of 10^{-8} and 10^{-6} M exerted no marked effect on the basal secretion of STH. On the contrary, in a concentration of 10^{-8} M insulin tended to suppress the basal secretion of STH in the primary culture of pituitary cells of adult rats and in a dose of 10^{-6} M reliably inhibited it.

Since the blood level of GC and thyroid hormones is markedly increased during the early postnatal development of rats [12], we attempted to assess possible changes of the responsiveness of somatotrophs to the regulatory effect of insulin following a long-term premedication with GC per se (Fig. 2) and in combination with thyroid hormone (Table 1). As is seen from Fig. 2, DM sharply raised the rate of STH secretion in all three age groups; however, the degree of stimulation was slightly higher in the group of neonates.

Against the background of stimulation with DM, insulin tended to further raise the secretory activity of somatotrophs in the culture derived from neonatal pups. However, in the group of weanlings insulin in a concentration of 10^{-6} M markedly inhibited the DM-induced secretion of STH, while in the group of adults a lower dose (10^{-8} M) of insulin markedly inhibited the GC-stimulated secretion of STH.

As follows from Table 1, triiodothyronine (T_3) per se did not reliably change the STH release in the culture of pituitary cells of weanlings. However, in the presence of T_3 DM raised the secretory activity of somatotrophs more than 5-fold. Against the background of stimulation with DM and T_3 in combination, both test concentrations of insulin (10^{-8} and 10^{-6} M) reliably inhibited STH secretion.

Our findings show that in somatotrophs of neonatal and adult rats the regulatory effects of insulin are diametrically opposite. In our view, such age-specific differences may partly explain the excessive body weight of children born of mothers with diabetes mellitus. It is well known that in a number of cases the somatotropic activity of the pituitary may be increased in such children [6]. Another characteristic feature is that the regulation of STH release by insulin and DM in combination is also age-specific. It is noteworthy that in weanlings the response to insulin and DM in combination is much closer to the pattern of regulation in adults.

It is of special interest that after a long-term premedication with GC and thyroid hormone in combination, somatotrophs of weanlings become sensitive to the inhibitory effect of a low dose of insulin. Evidently, we managed to simulate the formation of the "adult pattern" of insulin-mediated regulation in the cell culture. In our opinion, this result is important for understanding the physiological significance of the definite sequence of hormonal events in postnatal ontogenesis. In addition, as follows from our results, in order to avoid serious disturbances of the natural course of events during the hormone-dictated postnatal maturation, extreme care and responsibility are required when hormone treatment is administered during the early postnatal ontogenesis. Incidentally, this conclusion may serve to show how model experiments enable clinicians and, notably, pediatricians-endocrinologists, to focus on particular age-related specificities of hormonal regulation at the level of the pituitary.

It should be taken into account that the insulin test for stimulated secretion of STH has long been used in clinical practice [2]. However, in this

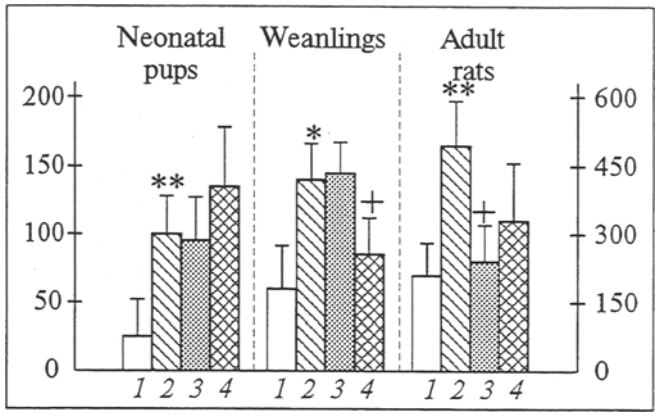

Fig. 2. Effect of insulin on secretion of STH in cultures of pituitary cells of rats of different age after a long-term premedication with DM in a dose of 10 nM for neonate and adult rats and in a dose of 50 nM for weanlings. Groups ($n=5-11$): *1*) control; *2*) DM; *3* and *4*) DM in combination with insulin in doses of 10^{-8} and 10^{-6} M, respectively. Incubation with DM lasted for 48–72 h, and incubation with DM and insulin for 4–5 h. One and two asterisks denote $p<0.001$ and $p<0.01$, respectively, as compared with the control; a cross denotes $p<0.05$ vis-a-vis DM.

case insulin-induced hypoglycemia is probably the active principle, and changes in the secretory activity of somatotrophs are caused not directly but via the central glucoregulation mechanisms [2]. Our experiments were performed under conditions simulating normoglycemia, and their results reflect the relationships between the pituitary and β-cells of the pancreatic islets for physiological and metabolic homeostasis. Nevertheless, elucidation of the specific features of the combined effect of glucose and insulin on the somatotrophic function of the pituitary represents an important task in further studies.

According to our findings, the use of cell cultures of the endocrine gland of animals of different age makes it possible to characterize the stages of formation of the endocrine system. At the same time, we are evidently not talking about "maturation" of interhormonal relationships, culminating in the establishment of a hormonal balance which is peculiar to the adult organism. It is to be assumed that age-specific differences in the sensitivity of the pituitary to hormones of the peripheral endocrine glands are primarily determined by a genetic (and, possibly, epigenetic) program of somatic development during ontogenesis.

It should be emphasized that the cultures of pituitary cells of animals of different age developed by us allow the age-related specificities of the direct regulatory effect of insulin on the secretion of STH to be assessed after a long-term premedication with GC per se and in combination with thyroid hormone. These results cannot be obtained

either *in vivo* (for which many of the endocrine glands have to be removed, this being impractical or even pointless due to the small size of neonate animals and weanlings) or in the incubated tissue fragments of the pituitary, because these are viable only during a limited period, which does not allow the effects of a long-term premedication with hormones to be studied.

Hence, further studies employing our model system for comparison of the hormonal responsiveness of pituitary cells of animals of different age hold great promise, since these studies may help refine or even reinterpret many of the current concepts concerning the maturation of combined hormone-mediated regulation in individual types of hormone-secreting cells of the pituitary.

REFERENCES

1. V. V. Abramov, L. A. Batrameeva, and V. P. Fedotov, *Probl. Endokrinol.*, **32**, No. 1, 56-60 (1986).
2. M. A. Balabolkin, Secretion of Growth Hormone in Health and Pathology [in Russian], Moscow (1978).
3. V. I. Gudoshnikov, I. S. Komolov, G. N. Pluzhnikova, *et al., Byull. Eksp. Biol. Med.*, **88**, No. 11, 536-538 (1979).
4. V. I. Gudoshnikov and V. P. Fedotov, *Probl. Endokrinol.*, **38**, No. 1, 61-64 (1992).
5. I. S. Komolov, L. G. Morozova, I. Fasekash, *et al., Byull. Eksp. Biol. Med.*, **85**, No. 2, 215-217 (1978).
6. J. Pedersen, The Pregnant Diabetic and Her Newborn: Problems and Mangement, (2nd edition, Munksgaard, Copenhagen), The Williams and Wilkins Company, Baltimore (1977).
7. N. V. Sadovnikova and V. P. Fedotov, *Probl. Endokrinol.*, **31**, No. 1, 52-55 (1985).
8. V. P. Fedotov, V. I. Gudoshnikov, I. S. Komolov, and V. V. Abramova, *Byull. Eksp. Biol. Med.*, **113**, No. 4, 402-404 (1992).
9. A. Betteridge and M. Wallis, *J. Endocrinol.*, **80**, 239-248 (1979).
10. D. B. Cheek and J. E. Graystone, *Kidney Int.*, **14**, 317-322 (1978).
11. C. J. Goodner, *Diabetes*, **15**, 115-122 (1966).
12. S. J. Henning, *Amer. J. Physiol.*, **241**, G199-G214 (1981).
13. S. Melmed and S. Slanina, *Endocrinology*, **117**, 532-537 (1985).
14. J. H. Nielsen, S. Linde, B. S. Welinder, *et al., Molec. Cell. Endocr.*, **3**, 165-173 (1989).
15. M. S. Sheppard, B. A. Eatock, and R. M. Bala, *Canad. J. Physiol. Pharmacol.*, **67**, 1315-1320 (1989).