# MORPHOLOGY AND PATHOMORPHOLOGY

# Effect of Insulin and Adrenocorticotropic Hormone on Rat Brain Cortex in the Early Postnatal Period

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Administration of insulin and adrenocorticotropic hormone to 1-, 2-, and 3-day-old rats leads to an increase in the RNA and total protein contents of cortical neurons, while administration of adrenocorticotropic hormone alone increases the number of dying neurons in layer V of the cortex.

**Key Words:** *brain; early postnatal period; hormones*

In the early postnatal period the brain grows intensively [5], and its neurons are capable of dividing [6,12]. Its development is influenced by many hormones, whose levels vary in the embryonal and perinatal periods depending on the state of the maternal organism [4]. For example, mothers suffering from diabetes mellitus often give birth to children with disorders of the central nervous system [3]. In such cases hyperinsulinism of the fetus and hypoglycemia of the newborn have been observed [2]. Insulin is known to cross the blood-brain barrier [7,8] and to directly affect the metabolism and the differentiation of neurons and glial cells [1,10,11]. On the other hand, hyperinsulinism and hypoglycemia in 1.5-day-old rats raise the blood concentration of adrenocorticotropic hormone (ACTH) [9]. Hyperproduction of this hormone typically occurs during stress, including stress occurring during the embryonal period [4]. An increase in the ACTH concentration is interesting from the viewpoint of brain development, since this hormone has a profound effect on the brain and higher nervous activity [6].

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In this study we examined the effects of insulin and ACTH on rat brain in the early postnatal period.

## MATERIALS AND METHODS

Two series of experiments were performed. In the first series insulin (Lente) was administered subcutaneously to rats three times (at the age of 1, 2, and 3 days) in a dose of 3 (group I), 10 (group II), and 50 (group III) U/kg. In the second series corticotropin (H. P. Acthar Gel, VAN'S Discount Pharmacy) was administered subcutaneously once a day at the same ages in a dose of 5 (group IV) and 25 (group V) U/kg. In both series rat pups of the same litters were used as controls. In the first series, the control group consisted of intact pups and pups given subcutaneous injections of 0.9% NaCl. In the second series, only intact animals served as controls. The results obtained on the intact controls in both series were not statistically different and were therefore pooled. The first series included 14 litters (133 pups), and the second series included 7 litters (66 pups). In both series the animals were decapitated at the age of 5 days and weighed. The brain was fixed in Carnoy's fluid and weighed on a torsion balance.

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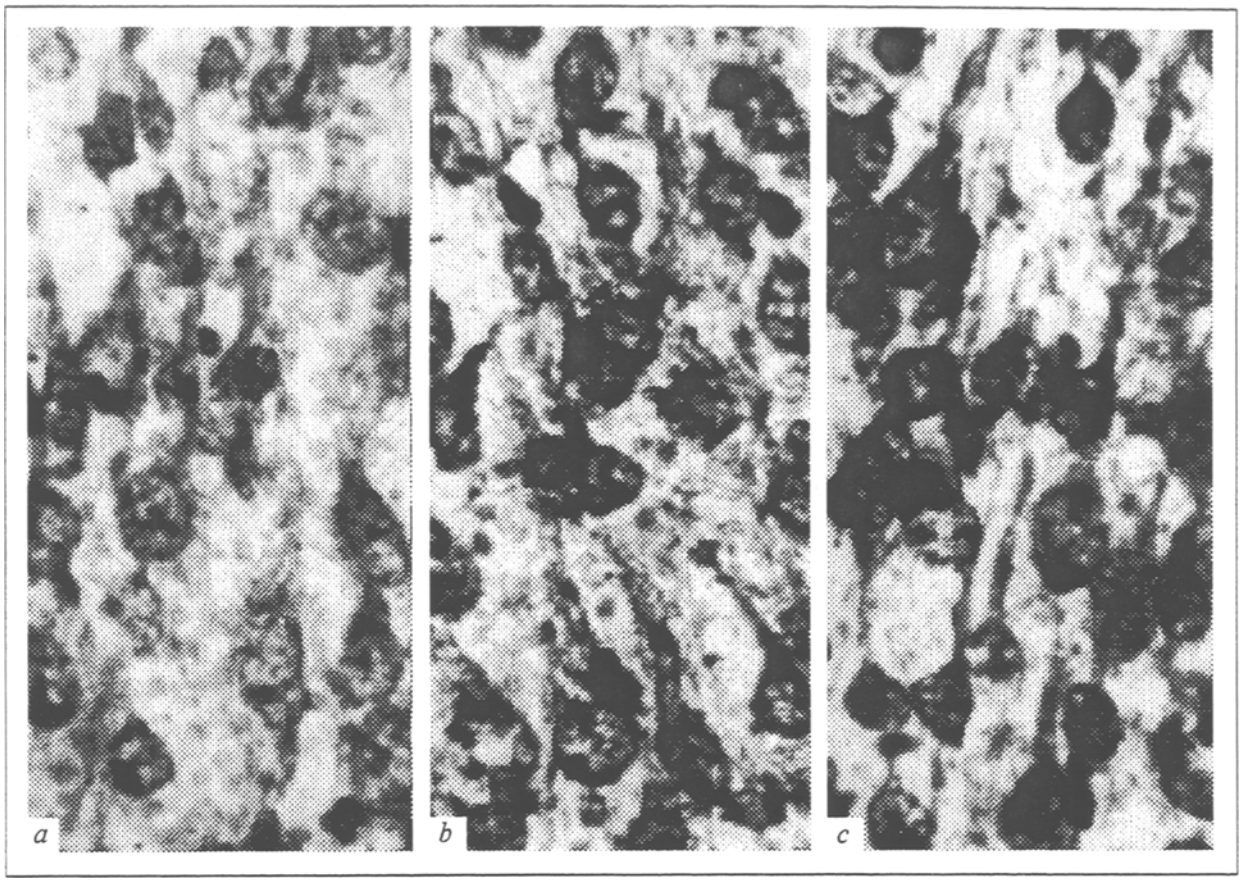


**Fig. 1.** Layer V of the brain cortex of 5-day-old rats. Staining for nucleic acids. ×500. *a*) control; *b*) administration of 50 U/kg insulin ; *c*) administration of 25 U/kg ACTH.

The anterior parietal region [5] was excised and embedded in paraffin. Seven-micron-thick sections were cut and stained with 1% methylene blue, with gallocyanin for nucleic acids, and with bromphenyl blue for the total protein. The preparations were examined visually, and the width of the cortex was measured [5]. Dead neurons were counted in 16 standard fields of view of the forming layer V. The concentrations of RNA and protein in the cytoplasm of pyramidal neurons in layer V were measured cytophotometrically(25 cells were viewed in each case at wavelength 550 nm, Fig. 1). The difference was considered to be significant at $p<0.05$.

## RESULTS

In 5-day-old male pups, the absolute brain mass was 412±7.3 mg, its relative mass was 45.4±0.8 mg/g, and the width if the brain cortex was 907±18 μ. In females these parameters were 412±9.0 g, 47.3± ±1.0 mg/g, and 840±26 μ, respectively. Administration of NaCl solution or insulin induced no significant changes in them. The RNA concentration in the cytoplasm of neurons in layer V of intact rats was 137±7.2 arb. units in males and 127±7.2 arb. units in females. It was somewhat lowered after administration of normal saline: 124±4.7 and 104±7 arb. units, respectively. Insulin induced a dose-dependent increase in the RNA concentration, which was 148±14.2 arb. units in males and 174± ±16.6 arb. units in females after administration of 3 U/kg insulin, 178±20.3 and 194±14.4 arb. units, respectively, after 10 U/kg, and 283±8.2 and 287±24 arb. units, respectively, after 50 U/kg (Table 1). There were no statistically significant changes in the total protein concentration in the cytoplasm of neurons after administration of saline: in intact males and females it was 86.6±14.4. and 83±9.6 arb. units, respectively, and it was 101±8.6 and 97±6.1 arb. units, respectively, after administration of saline. After administration of 3 U/kg insulin, these parameters were 89±11.6 and 110±6.6 arb. units in males and females, respectively, after 10 U/kg insulin 112±8.7 and 138±13.3 arb. units, and after 50 U/kg insulin 186±17 and 148±17.1 arb. units, respectively. The differences with the control (0.9% NaCl) were statistically significant in females given 10 U/kg insulin and in males and females given 50 U/kg insulin (Table 1). They were significantly higher compared with the intact control. The number of

**TABLE 1.** Effects of Insulin and ACTH on Morphometric and Cytophotometric Parameters of Rat Brain

| Parameter | Administration of 0.9% NaCl (% of intact animals) | | Administration of insulin (% of 0.9% NaCl administration) | | | | | | Administration of ACTH (% of intact animals) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 U/kg | | 10 U/kg | | 50 U/kg | | 5 U/kg | | 25 U/kg | |
| | M | F | M | F | M | F | M | F | M | F | M | F |
| Body mass | 107.7 | 102.3 | 94.9 | 95.9 | 94.3 | 104.2 | 92.4 | 96.0 | 85.9 | 85 | 90.6 | 92.5 |
| Brain mass | | | | | | | | | | | | |
| absolute | 105.3 | 94.4 | 96.7 | 101.1 | 97.7 | 109.1 | 93.4 | 98.3 | 85.8 | 85 | 93.5 | 93.3 |
| relative | 98.2 | 92.8 | 96.8 | 105.3 | 100.5 | 107.1 | 95.9 | 109.6 | 100.0 | 100 | 102.1 | 101 |
| Width of brain cortex | 94.8 | 95.6 | 92.0 | 101.6 | 105.2 | 106.7 | | 104.3 | 96.6 | 99.2 | 104.2 | 96.8 |
| Number of dead neurons in a field of view of layer V | 97.9 | | 125 | | 130.2 | | 133.3 | | 157.1* | | 215.3* | |
| RNA concentration in neuronal cytoplasm | 90.5 | 81.9* | 121.3 | 164.2* | 152.1 | 213.2* | 216.8* | 258.6* | 149.6* | 157.4* | 159.2* | 169.3* |
| Protein | 117.4 | 116.9 | 88.1 | 110.0 | 114.3 | 148.4* | 146.5* | 192.1* | 140.7 | 160.2 | 151.2* | 145.8* |

**Note.** *F* – females; *M* – males; *$p<0.05$ in comparison with the corresponding control.

dying neurons after administration of insulin in all doses tended to increase (Table 1).

After administration of 5 and 25 U/kg corticotropin, body and brain weights tended to decrease; however, the differences were statistically insignificant. There were no significant changes in the cortex width compared with the control (Table 1). The RNA concentration in layer V neurons was significantly higher compared with the control: 205±26 arb. units in males and 220±11.2 arb. units in females after 5 U/kg and 218±21.6 and 215±10.6 arb. units, respectively, after 25 U/kg. The total protein concentration in cortical neurons after 5 U/kg corticotropin was 121±11 and 138±23.3 arb. units in males and females, respectively. After 25 U/kg corticotropin, these parameters were 130±17.7 and 121±8.7 arb. units, respectively, which was significantly higher than in intact rats. In ACTH-treated animals, the number of dying neurons in layer V was significantly higher than that in the controls (Table 1). This is important for the assessment of the consequences of stress in the early postnatal period, since it is known to be associated with hyperproduction of ACTH.

Our results indicate that ACTH and insulin, agents which have an antagonistic effect on the blood glucose level (an important parameter of neuronal metabolism), nevertheless induce similar changes in the concentration of RNA and total protein (two major components of the neuronal cytoplasm). It is noteworthy that activation of RNA and protein synthesis in neurons is not accompanied by an acceleration of brain and cortex growth if the hormones are administered in that period of ontogenesis when growth is most intensive [5]. The tendency toward a decrease in body weight observed after administration of ACTH went along with unidirectional changes in brain weight. This indicates that the correlation between body and brain weights documented in intact 1-5-day-old rats [5] is retained under the action of corticotropin. Our results can be helpful in the analysis of the factors regulating the growth of the brain and cortex as well as the state of cortical neurons in the early postnatal period in health and in some disorders of perinatal and early postnatal development.

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