of twitch type in the early postnatal period in rats is the result not only of the characteristics of cholinergic and GABA-ergic regulation, as was observed in a previous investigation [4], but also of the high activity of the adrenergic (dopaminergic) system, as the present investigation showed. In addition, it was found that chlorpromazine, in the same dose per kilogram body weight but at different age periods, induces varied changes in the reduction of MR frequency: During jumps of growth there is a maximal increase in the blood catecholamine concentration, so that a larger dose of the drug was evidently needed to produce an equal (expressed as a percentage) decrease in the frequency of MR. A high blood level of catecholamines during this period is confirmed by data in the literature [2, 5].

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CONTENT OF ANTIDIURETIC HORMONE IN THE ADULT ALBINO RAT NEUROHYPOPHYSIS AFTER INJECTION OF HYDROCORTISONE IN THE EARLY POSTNATAL PERIOD

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Critical periods in postnatal development of the system for water and electrolyte homeostasis are known to exist in mammals [3]. The writers showed previously that if hydrocortisone is administered in the neonatal period, the osmotic concentration function in the kidney in adult rats can be substantially modified [2]. The period of sensitivity to this procedure is limited to the age interval from 5 to 9 days after birth [10]. Low antidiuretic reactivity in rats induced in early ontogeny is evidently the result of modification of the molecular mechanisms responsible for sensitivity of the renal epithelium to antidiuretic hormone (ADH). Meanwhile the possibility cannot be ruled out that definite changes could also take place at the level of central regulation of water–electrolyte homeostasis, for a transient disturbance of the steroid hormone balance in the early postnatal period leads to changes in function of the hypothalamic centers controlling several other functions [4, 6].

The aim of the present investigation was to study the age dynamics of the content of neurosecretion in the posterior lobe of the pituitary in intact rats and to determine the ADH content in the neurohypophysis of adult rats, induced by hydrocortisone at different times of postnatal ontogeny.

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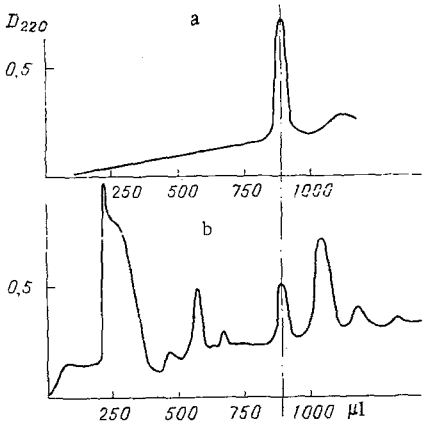

Fig. 1. Chromatographic profiles of synthetic arginine-vasopressin preparation (a) and of neurohypophyseal extract (b).

EXPERIMENTAL METHOD

The ADH content was investigated in a group of intact Wistar rats at the ages of 5, 15, 30, and 60 days, receiving a normal water intake, and also on rats aged 60 days after dehydration for 24 h. Rats of the experimental group received an injection of hydrocortisone acetate (2.5 mg/100 g body weight, intraperitoneally) on the 2nd, 5th, 9th, or 15th day after birth. At the age of 60 days these animals were subjected to dehydration for 24 h and then decapitated. Within a few minutes after decapitation the pituitary was removed, the neurohypophysis was separated on a cold slide, and it was transferred into acetone to remove water and fat (4°C). Neurosecretion was extracted by Nikitin's method [7]. After 3 h the neurohypophysis was transferred into fresh acetone and dehydrated for 12 h at 4°C. To prepare an acetone powder, three to five neurohypophyses were transferred into a glass homogenizer, homogenized in cold redistilled acetone to a thin suspension, and dried in a vacuum exsiccator. The pituitary powder was dissolved in 1 ml of 0.25% acetic acid, placed for 3 min on a boiling water bath, cooled, and centrifuged (6000 rpm, 40 min, 0°C). Aliquots were taken from the supernatant for fractionation by the method of microcolumn liquid chromatography on an Ob'-4 chromatograph, with detection at wavelengths of 220 and 280 nm. Hydrophobic "Nucleosil C_{18}" silica-gel (Macherey-Nagel, West Germany) in a column 170 μl in volume was used as the sorbent. The volume of the samples applied was 15-200 μl. Elution was carried out with a stepwise gradient of 20, 40, 60, and 80% methanol in 0.01 M Tris-HCl buffer, pH 8.6, in volumes of 250 μl, after preliminary rising of the column with 350 μl of 20% methanol. The rate of elution was 50 μl/min. By this method as little as 10^{-8} g of protein in the sample can be determined. Methanol solutions were made up immediately before distillation. An aqueous solution with known concentration of synthetic arginine-vasopressin (the preparation was synthesized in the Department of Natural Compounds, A. A. Zhdanov Leningrad University), was used as the standard for determination of ADH mobility. To estimate ADH quantitatively in the samples, the area of the hormonal peak above the background level of chromatographic distillation was calculated and the relative area of the peak to the ADH standard was determined (Fig. 1). The results of three parallel tests in each group of mice were subjected to statistical analysis.

EXPERIMENTAL RESULTS

In rats aged 5 and 15 days, substantial quantities of ADH were detected by the chromatographic method in the neurohypophysis (Table 1). The hormone was biologically active, for intraperitoneal injection of the fraction collected after distillation of the neurohypophyseal extract into adult rats in a dose of 0.5 mU/100 g body weight caused an increase in the osmotic concentration index (the ratio of osmolarity of urine to that of plasma) from 1.65 ± 0.09 to 3.47 ± 0.41. In this age period the animals were still on maternal feeding and their blood ADH concentration was extremely low, for secretion of the hormone into the blood stream was not yet regulated [16, 17] and it accumulated in the neurohypophysis [13].

In rats aged 30 days the ADH content in the neurohypophysis was significantly reduced compared both with animals of the younger age groups and also adults aged 2 months (Table 1). At the age of 1 month, young rats are completely independent of their mothers and obtained food and drink for themselves [3]. Enhanced reactivity is characteristic of central regulation of water and electrolyte homeostasis, just as for other regulatory

TABLE 1. Age Dynamics of ADH Content in Neurohypophysis of Wistar Rats (three rats in each group)

Age of rats, days	ADH content, picomoles/ pituitary gland
5	322±24
15	310±53
30	218±11
60	930±68

TABLE 2. ADH Content in Neurohyphophysis of Rats Aged 60 Days after Dehydration for 24 h Followed by Injection of Hydrocortisone on 2nd, 5th, 9th, or 15th Day of Postnatal Ontogeny (n = 3)

Day of injection of hydrocortisone	ADH content, picomoles/ pituitary gland
Intact rats	495±19
2-nd	735±8
5-th	775±31
9-th	655±68
15-th	588±22

systems. Emotional and other types of stress in rats aged 30 days are much stronger than in adult animals, ADH secretion is increased, and the high intensity of release of neurosecretion into the blood stream at this age leads to a state of stable emptying of the neurohypophysis [1, 8]. The decrease in the ADH content in the neurohypophysis of rats aged 30 days reflects the more intensive release of the hormone into the blood stream than in animals of the other age groups.

In adult rats aged 60 days, receiving a normal diet, the ADH concentration in the neurohypophysis was much higher than in rats of the younger groups (Table 1). Compared with animals aged 5 days, the ADH content in their neurohypophysis was 3 times greater.

During postnatal development the quantity of hormone in the neurohypophysis of the intact rats was thus considerably increased. Special features of ontogeny of neurohypophyseal function in young rats include a disproportionately high content of the hormone in the period of material feeding and a reduction in it toward the 30th day of life, evidently as a result of increased sensitivity of the centers before establishment of the definitive level of regulation. A similar age dynamics of the ADH content in the neurohypophysis was described previously in rats [15] and calves [7].

In an investigation of the ADH content determined by a fluorometric method [14], the authors cited found that the vasopressin content in the neurohypophysis of adult intact rats is 879 ± 132 pmole/pituitary gland. Closely similar values were obtained by determination of ADP in the pituitary by bioassay: 1050 ± 62 pmole/pituitary gland [18]. Determination of ADH by radioimmunoassay in albino rats on a normal water intake gave a value for this parameter of 1294 ± 64 pmole/pituitary gland [11]. According to other workers, its value is 1420 ± 68 pmole/pituitary gland [18]. Consequently, the chromatographic method of determination of the ADH content in the neurohypophysis gives results comparable with those obtained by other methods.

During water deprivation a decrease in the quantity of neurosecretion in the pituitary is observed, and the blood concentration of the hormone rises; after a sufficiently long period of dehydration, the content of neurosecretion becomes equal in different rats [18]. In the present experiments dehydration for 24 h was accompanied by a reduction of almost half in the ADH content in the neurohypophysis (Table 2). The same response was observed by other workers, who estimated the quantity of vasopressin in the neurohypophysis by radioimmunoassay (from 1294 ± 64 to 750 ± 65 mmole/pituitary [11] and from 1420 ± 68 to 920 ± 75 pmole/pituitary [18]) and by bioassay (from 1050 ± 62 to 650 ± 68 pmole/pituitary gland [18]). Injection of hydrocortisone in the early postnatal period altered the response of adult rats to dehydration. In rats aged 2 months, receiving

hydrocortisone on the 2nd or 5th day after birth, the ADH content in the neurohypophysis after dehydration for 24 h was not reduced, as it was in intact animals receiving a dry diet throughout this same period of time. Meanwhile in rats receiving an injection of hydrocortisone on the 9th or 15th day after birth, dehydration for 24 h was accompanied by a definite decrease in the ADH content in the posterior lobe of the pituitary gland to values close to the level in dehydrated control animals (see Table 2; $P < 0.05$).

Normal postnatal development of mammals takes place against the background of a definite balance of glucocorticoid hormones, affecting differentiation both of the target organs and of the integrative centers, including hypothalamic nuclei [5, 6]. Deficiency or excess of glucocorticoids at critical periods of development has a significant, often irreversible effect on the definitive functioning of various phsiological systems [6, 9, 19]; changes may take place in both peripheral structures and central mechanisms. The critical period for a change in the effectiveness of osmotic concentration function in the albino rat kidney under the influence of hydrocortisone is the 5th-9th day after birth [2, 10]. Ability of the posterior lobe of the pituitary to release neurosecretion into the blood stream is affected by hydrocortisone at an earlier period – during the first 5 days after birth. Injection of hydrocortisone on the 9th day after birth has a weaker effect, and by the 15th day of life the effect virtually has disappeared.

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