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Adenosine triphosphatase potassium
cerebral cortex sodium
corticosteroid hormones

Effects of Adrenocortical Steroids and of Adrenocorticotrophic Hormone on (Na^+-K^+)-ATPase in Immature Cerebral Cortex

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Summary

The effect of cortisol, methylprednisolone, and ACTH on (Na^+-K^+)-ATPase activity in developing cerebral cortex has been measured. Stimulation of (Na^+-K^+)-ATPase by these agents has been found in whole brain homogenates of kittens as early as age 8 days, and in whole homogenates and light microsomal fractions of young rats at 14 and 28 days. (Na^+-K^+)-ATPase activity in animals treated with corticosteroids or ACTH for 4 days was found to be 15–30% higher than activity in littermate controls. Brain potassium concentration was increased in 14-day-old rats treated with methylprednisolone.

Speculation

Corticosteroid hormones and ACTH are effective in the treatment of seizures in infancy, especially in patients with infantile spasms and hypsarrhythmia. Stimulation of (Na^+-K^+)-ATPase activity by these hormones provides a plausible mechanism for the anticonvulsant effect.

Corticosteroid hormones and ACTH have an anticonvulsant effect in infants and children which is not observed in the adult. The effect is most marked in infants with massive myoclonic seizures (infantile spasms) (15, 28), but it has also been described in older children with intractable convulsions (18). Especially in the young infant, there frequently is persistence of seizure control after hormone treatment is withdrawn, suggesting that corticosteroids may induce long lasting changes in the immature brain, perhaps by influencing developmental events in cerebral cortex. A possibility that needs to be considered is that these agents stimulate the activity of enzyme systems in developing brain that play a role in the regulation of neuronal excitability.

The present study is concerned with effects of corticosteroid hormones and of ACTH on activity of sodium- and potassium-activated adenosine triphosphatase ((Na^+-K^+)-ATPase) in developing cerebral cortex. (Na^+-K^+)-ATPase has an important role in the maintenance of concentration gradients of sodium and potassium across neuronal membranes, which in turn influence neuronal excitability (27). Cortisol stimulation of (Na^+-

K^+)-ATPase in kidney (1, 17) and in the brain of the chick embryo (29) has previously been described, but the effects of corticosteroids on the enzyme system in developing mammalian brain are unknown.

MATERIALS AND METHODS

(Na^+-K^+)-ATPase assays were performed at ages 14–28 days in rats of Sprague-Dawley strain and at ages 8–29 days in kittens. The following substances were given intramuscularly for 4 days prior to the chemical assays. Methylprednisolone acetate suspension 0.01 mg/g body weight/day; cortisol, 0.02 mg/g body weight/day; ACTH gel, 0.015 units/g/day. Control animals were injected with an equal volume (about 0.02 ml/day) of normal saline. The immature rats ranged in weight from 35–100 g, the kittens from 150–300 g. Control animals were from the same litters as experimental animals. The animals were killed rapidly by decapitation. In the rats the cerebral hemispheres were dissected from the rest of the brain; cerebral cortex plus subcortical white matter were used for analysis. In kittens, the gray matter of precruciate gyrus was used. Samples were weighed and were frozen at −20° for at least 1 hr before processing. Analyses were completed within 24 hr after the death of the animals.

Homogenates of whole brain were made by mixing 9 vol ice-cold 0.1% Tris-deoxycholate in 25 mM Tris buffer, pH 7.8, with 1 vol brain in a glass homogenizer with Teflon pestle. In homogenates used for preparations of cell membrane (light microsomal) fractions 0.25 M sucrose was substituted for Tris buffer. The pestle was moved through the homogenate at 1500 rpm at least 70 times. The homogenates were strained through a double layer of gauze to remove meninges and blood vessels. After homogenization the pH of the brain mixture was readjusted to 7.8. The mixture was centrifuged at 480 × *g* for 10 min and aliquots of supernatant, labeled "whole homogenate," were used for ATPase analysis and for protein determination.

To obtain the light microsomal fraction the whole homogenate was centrifuged at 39,100 × *g* for 30 min at 0°. The sediment was discarded, and the supernatant was recentrifuged at 105,000 × *g* for 60 min. The sediment, representing the light microsome fraction, was resuspended in 4 ml Tris buffer and was stored at −20° prior to the ATPase assays.

Total ATPase activity was estimated in reaction mixtures containing 100 mM NaCl, 30 mM KCl, 3 mM $MgCl_2$, 3 mM Tris-ATP (Sigma Chemical Company, St Louis, MO), and 0.36 ml brain homogenate or membrane fraction in a total volume of 2 ml. Mg^{++}-ATPase activity was measured in the same reaction mixture, except for omission of NaCl and KCl. The pH of the reaction mixtures was adjusted to 7.8, and the mixtures were then incubated at 40° for 10 min. The reaction was stopped by addition of 1 ml ice-cold 5% trichloroacetic acid. Inorganic phosphate was measured in 1 ml of the supernatant by the method of Fiske and SubbaRow (6). All determinations were carried out in duplicate. Variations were less than 5%. Protein content of the homogenates and of the microsomal fractions was determined by the method of Lowry *et al.* (20). ATPase activity was expressed in terms of micromoles of inorganic phosphate liberated per mg protein per 10 min. (Na^+-K^+)-ATPase activity was calculated by subtracting Mg^{++}-ATPase from total ATPase.

Sodium and potassium concentrations were measured in whole brain homogenates in which protein had been digested by addition of concentrated nitric acid. A flame photometer with internal standards was used.

RESULTS

(Na^+-K^+)-ATPase assays in control animals resembled those reported in the literature (14, 19, 22, 25). In the rat, activity of the enzyme was slightly over 50% of adult values at age 15 days, and then rose rapidly to 90% of adult activity by age 28 days. In agreement with previous reports, corticosteroid hormones were found to produce marked toxicity in newborn rats (5, 10, 12, 21). In these animals, there was growth failure and high mortality. The majority of studies in the rat therefore were performed in weanlings, at ages 24–28 days. In these animals, no significant effects of 4-day courses of cortisol, methylprednisolone, or ACTH on brain or body weight were observed.

The effects of hormone administration on (Na^+-K^+)-ATPase activity in weanling rats are summarized in Table 1. All three hormone-treated groups showed significantly higher enzyme activities in whole brain homogenates than did matched controls. The effect of methylprednisolone on (Na^+-K^+)-ATPase was assayed in adult rats for comparison. In these animals a mean increase in activity of 13% was observed, when compared with adult controls. The difference between the two adult groups did not reach statistical significance.

Assays of (Na^+-K^+)-ATPase in the light microsomal fraction which concentrates membrane bound ATPase confirmed the findings in whole homogenates. Effects of methylprednisolone on ATPase activity in the light microsomal fraction of 28-day-old and adult rats are summarized in Table 2.

Several experiments on the effect of cortisol on cerebral (Na^+-K^+)-ATPase were carried out in rats less than 28 days old. An increase in (Na^+-K^+)-ATPase activity comparable to that in the 28-day-old animals was found at ages 14 and 18 days, when ATPase activity was expressed per mg brain protein. However, the data in the 14- to 18-day-old rats were complicated by the fact that corticosteroid hormones had a significant effect on brain growth. Mean weight of the cerebral hemispheres after removal of cerebellum and brain stem was 0.55 g ± 0.02 SEM in 14-day-old animals that had received methylprednisolone for 4 days, vs. 0.61 g ± 0.02 SEM in littermate controls ($n = 17$, $P < 0.05$). Body weight also was significantly less in the methylprednisolone-treated group.

The effect of corticosteroid hormones on (Na^+-K^+)-ATPase during early stages of postnatal brain development was therefore further assessed in young kittens, which were found to tolerate

Table 1. *Activity of (Na^+-K^+)-ATPase in whole brain homogenates of 28-day and adult rats treated with adrenocortical hormones or ACTH*[1]

	No. of exp	Control	Exp	% Diff.
Methylprednisolone, 28 days	8	2.63 (0.14)	3.22 (0.19)	+22
		($P < 0.01$)		
Cortisol, 28 days	10	2.62 (0.16)	3.28 (0.22)	+25
		($P < 0.01$)		
ACTH, 28 days	8	2.67 (0.19)	3.08 (0.18)	+15
		($P < 0.05$)		
Methylprednisolone, adult	4	2.83 (0.36)	3.20 (0.37)	+13
		(NS)		

[1] Enzyme activity is expressed as μMP′ per mg protein per 10 min. The numbers in parentheses represent the SEM.

Table 2. *Effects of methylprednisolone on (Na^+-K^+)-ATPase in light microsomal fraction of 28-day-old and adult rats*[1]

Age	No. of exp	Control	Exp	% Diff.
28 days	8	5.46 (0.53)	7.07 (0.59)	+29
		($P < 0.01$)		
Adult	4	7.88 (1.3)	8.61 (0.90)	+ 9
		(NS)		

[1] Enzyme activity is expressed as μMP′ per mg protein per 10 min.

ACTH at 0.015 u/g/day for 4 days without retardation of brain or body growth. An increase in (Na^+-K^+)-ATPase activity following ACTH administration was demonstrated in cortical samples from 8-day-old kittens, at a time when the activity of the enzyme in brain is still less than 20% of adult levels (14) (Table 3). Stimulation of (Na^+-K^+)-ATPase also occurred in 14- and 29-day-old kittens. The number of experiments at each age is insufficient for statistical analysis. However, when the experiments for all three age groups are combined, the ACTH-treated animals differ significantly from their age-matched controls ($P < 0.01$ by sign test). The magnitude of the effect in kittens is comparable to that observed in immature rat brain.

Stimulation of cerebral (Na^+-K^+)-ATPase activity might be expected to lead to an increase in intracellular potassium and a decrease in intracellular sodium concentration in brain. Published data show no effect of corticosteroid hormones on cerebral sodium or potassium content in adult rat brain (31) and a somewhat equivocal result of ACTH in 28- to 42-day-old rats (30). In the latter study a statistically significant increase in brain potassium was observed in whole brain after ACTH treatment, but there was no significant effect when an attempt was made to calculate potassium concentrations separately for intracellular and extracellular spaces. Therefore, the effect of corticosteroid hormone administration in somewhat younger rats (age 14 days) was investigated at a developmental stage when brain potassium is still significantly below adult concentrations and brain sodium is higher than in the adult. It was hoped that more significant effects of corticosteroids on brain electrolytes might be found at a stage when brain electrolyte concentrations are still undergoing active developmental changes. Data on control and methylprednisolone-treated rats are summarized in Table 4. A statistically significant increase in brain potassium was found. There was no significant change in sodium concentration or in brain water content.

DISCUSSION

The effects of adrenocortical hormones on developing brain clearly are complex, and they differ at different developmental stages and probably also in different species. In rodents, the most striking effect of early postnatal administration is failure of brain growth, with defective myelination and decreased cortical dendritic development (5, 10, 12, 21). This effect seems much less prominent in other species. During later postnatal development we have found a positive effect of adrenocortical steroids and of ACTH on cerebral (Na^+-K^+)-ATPase activity, suggesting that these agents may accelerate certain developmental events in immature brain. Two glucocorticoids as well as ACTH were found to stimulate cerebral (Na^+-K^+)-ATPase activity. The effect of ACTH was somewhat less than that of the adrenocortical hormones themselves, but this may well be related to the particular doses used in these experiments, rather than to true differences in effectiveness.

Table 3. *Effects of ACTH on (Na^+-K^+)-ATPase in whole homogenates of kitten cerebral cortex*[1]

Age	No. of exp	Control	ACTH	% Diff.
8 days	3	0.67	0.86	+28
14 days	2	0.80	0.96	+20
29 days	2	1.35	1.71	+27

[1] Enzyme activity is expressed as μMP' per mg protein per 10 min.

Table 4. *Na^+ and K^+ concentration in brains of 14-day-old rats treated with methylprednisolone*

	No. of animals	mEq K^+/kg wet brain	mEq Na^+/kg wet brain	Brain water %
Control	17	84.1 (1.3)	59.6 (1.2)	87.1 (1.4)
Methylprednisolone-treated	18	89.0 (1.6)	60.9 (1.0)	86.7 (1.5)
		$P < 0.05$	NS	NS

[1] The numbers in parentheses represent the SEM.

Previous attempts to find agents which stimulate the activity of (Na^+-K^+)-ATPase in brain have been largely unsuccessful. The enzyme is unaffected by anticonvulsants, including phenobarbital (7). The effect of diphenylhydantoin appears to be complex. Stimulation of ATPase by diphenylhydantoin was observed *in vitro* at ratios of sodium to potassium in the reaction mixture as high as 25–50:1 (4). However, (Na^+-K^+)-ATPase activity was inhibited by diphenylhydantoin at ratios of sodium to potassium similar to those used in the present experiments (22, 24). Diphenylhydantoin had no effect on (Na^+-K^+)-ATPase activity in *in vivo* experiments. An increase in (Na^+-K^+)-ATPase activity in brain has been found after the administration of pentylenetetrazol (2). This stimulant effect most likely is secondary to the induction of seizure activity. An increase in activity of (Na^+-K^+)-ATPase is seen also in cortical seizure foci produced by freezing and following electroshock-induced convulsions (11, 14, 19).

The finding of a moderate increase in (Na^+-K^+)-ATPase activity after corticosteroid administration does not *per se* imply a functionally significant effect. However, such an effect would appear likely if the increase in enzyme activity were associated with specific changes in ionic concentration of brain. Increased activity of the enzyme would be expected to raise intracellular potassium concentration and to lower intracellular sodium. Measurements of sodium and potassium concentration therefore were carried out in brains of steroid-treated animals, and were compared with controls. A significant increase in cerebral potassium concentration was found in steroid-treated 14-day-old rats. Potassium concentration was measured in whole brain, but one can assume that the increase in potassium was due to concentration of the ion in intracellular spaces, since extracellular potassium contributes only a negligible amount to the total potassium present in brain.

Cerebral sodium concentration showed no significant change in steroid-treated animals. Although one would expect a decrease in intracellular sodium with increased sodium pump activity, such a decrease may not be readily reflected in measurements of total sodium content of brain. A major portion of total brain sodium is contributed by extracellular ion, and a small change in intracellular concentration of sodium may therefore be difficult to detect. No attempt was made to calculate sodium concentrations separately for brain intracellular and extracellular spaces, since there presently is no generally agreed method for accurate determinations of the size of these spaces.

Stimulation of (Na^+-K^+)-ATPase activity, resulting in an increase in intraneuronal potassium, provides a plausible mechanism for the anticonvulsant effect of steroids in infantile seizures. A high intraneuronal sodium to potassium ratio, as well as accumulation of potassium in extracellular spaces of brain, have been proposed as playing a major role in the maintenance of seizure activity (3, 8, 32). Such ionic changes are especially apt to occur in immature brain, both because of immaturity of the sodium pump system, and because of the presence of primarily small neurons in which the ratio of cell membrane surface to cell volume is large. Massive leakage of potassium from cerebral neurons, sufficient to raise the potassium concentration in cerebrospinal fluid, appears to occur in human infants in certain abnormal states. For example, a significant increase in spinal fluid potassium has been found following neonatal asphyxia (16). The hypsarrhythmia EEG pattern seen in infants with myoclonic seizures is best explained as secondary to maintained abnormal electrical discharges in large groups of partially depolarized neurons which are unable to maintain normal concentrations of potassium and sodium. It

is therefore of interest that corticosteroid hormones are especially effective in this specific group of infantile seizures. Recently, defects in growth of cortical dendrites and gross malformations of dendrites have been found in a number of infants with intractable seizures and hypsarrhythmia (13, 23). Such structurally abnormal dendrites may well have membrane properties which predispose to leakage of sodium into nerve cells and of potassium into extracellular fluid.

CONCLUSION

The effects of cortisol, of a synthetic glucocorticoid (methylprednisolone) and of ACTH on (Na^+-K^+)-ATPase activity in developing cerebral cortex were determined. Both in immature rat and kitten brain these agents led to stimulation of (Na^+-K^+)-ATPase activity. Increase in brain potassium concentration was found in 14-day-old rats treated with methylprednisolone. The findings suggest that corticosteroids accelerate the maturation of the sodium pump ((Na^+-K^+)-ATPase) system in developing cerebral neurons. This effect provides a possible explanation for the anticonvulsant action of corticosteroid hormones and of ACTH in infantile seizures.

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