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Time Changes in Na^+,K^+-ATPase, Mg^{++}-ATPase, and Acetylcholinesterase Activities in the Rat Cerebrum and Cerebellum Caused by Stress

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TSAKIRIS, S. AND A. KONTOPOULOS. *Time changes in Na^+,K^+-ATPase, Mg^{++}-ATPase, and acetylcholinesterase activities in the rat cerebrum and cerebellum caused by stress.* PHARMACOL BIOCHEM BEHAV **44**(2) 339–342, 1993.— Na^+,K^+-ATPase, Mg^{++}-ATPase, and acetylcholinesterase activities were determined in homogenated rat cerebrum and cerebellum in unstressed animals (control) and exposed to cold and immobilization for 45–180 min. Na^+,K^+-ATPase and Mg^{++}-ATPase activities were not affected within the first 80 min of stress, while they were increased about 50–70% after 120–180 min, where the maximum enzyme stimulation was observed. However, acetylcholinesterase activity was increased considerably by less than 45 min of stress and reached a plateau in 80–180 min to a higher value in the cerebrum ($\cong$100%) than in the cerebellum ($\cong$40%) related to the control. Our results suggest that: a) The stress used can stimulate acetylcholinesterase in a different way and more quickly than Na^+,K^+-ATPase and Mg^{++}-ATPase; b) acetylcholinesterase in the cerebellum is stimulated to a lower level than in the cerebrum by stress, probably because of the presence of a relatively small cerebellar cholinergic innervation.

Na^+,K^+-ATPase Mg^{++}-ATPase Acetylcholinesterase Rat cerebrum and cerebellum
Cold and immobilization stress Time-related changes

ACUTE stress activates a multitude of neuroendocrine and neurochemical responses (17). The turnover of acetylcholine (ACh), norepinephrine (NE), dopamine, and serotonin is increased in some brain regions following stress (1,2,7–9, 20,22,28,29). Pituitary cyclic adenosine monophosphate (cAMP) and plasma corticosterone levels in rats are significantly elevated within 15 min of immobilization stress (12,28). Immobilization also induces increases in NE release in some brain regions and they reach a maximum value during 60–180 min of stress (29). However, in this study experiments were not performed in the cerebellum.

After the simultaneous exposure of mice to cold and immobilization for 120 min, the whole-brain Na^+,K^+-ATPase activity significantly increased (5). This effect is discussed parallel to the brain NE neuronal activity. However, the responsiveness of Na^+,K^+-ATPase to this acute stress was not studied in the cerebellum.

It is yet unknown how stress induces different functions in the cerebrum and cerebellum and emergency responses such as emotion, arousal level, and endocrine and autonomic nervous system responses. The purpose of this study was to determinate in the rat cerebrum and cerebellum time-related changes in the activities of Na^+,K^+-ATPase, Mg^{++}-ATPase, and acetylcholinesterase (AChE) in the responsiveness of adrenergic or cholinergic neurons to cold and immobilization stress. The results are discussed in correlation to possible responses of the corticosterone, NE, and cAMP in the brain during stress.

METHOD

Animals

Male, albino Wistar rats, 100–150 g, were used in all experiments. Rats were housed four to a cage at constant room temperature (22 ± 1°C), under a 12 L : 12 D (light 0800–2000 h) cycle and acclimated 1 week before use. Food and water were provided ad lib. Animals were cared for in accordance with the principles of the *Guide to the Care and Use of Experimental Animals*.

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Stress Procedure

Immobilization stress was employed by enclosing rats in a flexible wire mesh (2 × 2 mm) initially formed into a cone and then bent to conform to the size of individual animals. This cone permitted only restricted movements of the rat and was placed in a dark and cold room (4 ± 1°C) for periods of 45, 80, 120, and 180 min. Behavioral changes occurred during stress such as vocalization and defecation, which were observed too in immobilization stress by others (11). Unstressed (control) rats were kept in their home cages at constant room temperature (22 ± 1°C) during stress treatment and used at the corresponding time for tissue preparation and biochemical determinations.

Tissue Preparation and Biochemical Determinations

Immediately after each treatment, rats were sacrificed by decapitation. The rat cerebrum and cerebellum were quickly removed and homogenized separately in 10 vol ice-cold (0–4°C) medium containing 50 mM Tris HCl, pH 7.4, and 300 mM sucrose, using an ice-chilled glass homogenizing vessel. Then, the homogenate was centrifuged at 1,000× *g* for 10 min to remove nuclei and debris. In the resulting supernatant, the protein content was determined according to Lowry et al. (16) and then the enzyme activities were measured. The enzyme incubation temperature mixture was kept at 37°C.

Na^+,K^+-ATPase was calculated from the difference between total ATPase activity (Na^+, K^+, Mg^{++} dependent) minus Mg^{++}-dependent ATPase activity incubated in a mixture without NaCl and KCl (5,30). The values of Mg^{++}-dependent ATPase activity were similar in the presence or absence in the reaction mixture 1 mM Ouabain and 0.1 mM ethylene glycol bis (2-amino-ethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA). Enzyme activity was not found without $MgCl_2$, NaCl, and KCl in the incubation mixture. Total ATPase activity was assayed in an incubation medium consisting of 50 mM Tris HCl, pH 7.4, 4 mM $MgCl_2$, 7 mM KCl, 120 mM NaCl, 240 mM sucrose, 3 mM disodium ATP, and 80–100 μg protein of the homogenate in a final volume of 1 ml. Incubations were performed in the reaction medium for 20 min under continuous magnetic stirring. The reaction was started by the addition of ATP and stopped with 0.2 ml 50% trichloroacetic acid. The liberated Pi was measured by the method of Fiske and Subbarow (6).

AChE activity was determined according to Ellmann et al.'s method (4). The reaction mixture (1 ml) contained 50 mM Tris HCl, pH 8.0, and 240 mM sucrose in the presence of 120 mM NaCl. Protein concentration was 80–100 μg/1 ml incubation mixture. Then, 0.030 ml 5-5′-dithionitrobenzoic acid (DTNB) and 0.017 ml acetylthiocholine iodide, used as substrate, were added and the reaction was started. The final concentrations of DTNB and substrate were 0.125 and 0.5 mM, respectively. The reaction was followed spectrophotometrically by the increase in absorbance (difference in optical density, $\Delta\overline{OD}$) at 412 nm by using a Beckman Acta MVI spectrophotometer (Beckman Instruments, Fullerton, CA).

Statistical Analysis

The data were analyzed by using two-tailed Student's *t*-test.

RESULTS

Changes of Na^+,K^+-dependent ATPase activity in homogenated rat cerebrum and cerebellum during exposure to cold and immobilization for 180 min are illustrated in Fig. 1. As

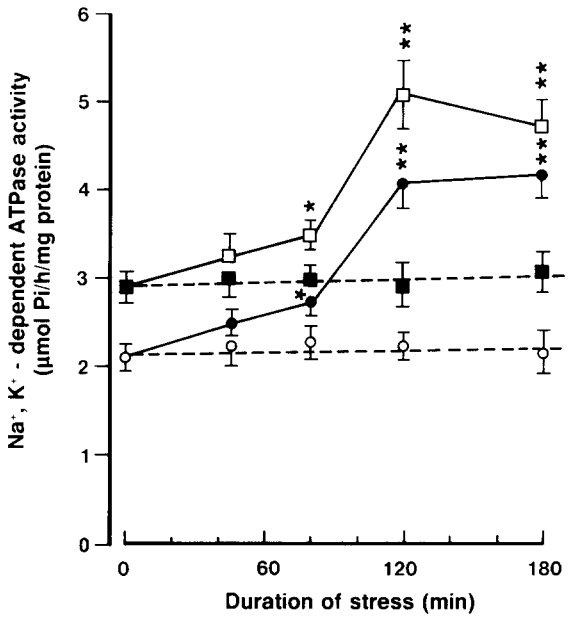

FIG. 1. Changes in the Na^+,K^+-ATPase activities in homogenated cerebrum (□——□) and cerebellum (●——●) of rats exposed to stress for various periods. Enzyme activities of controls at the corresponding time in the cerebrum (■---■) and cerebellum (○---○). Each value indicates the mean ± SD of five independent experiments (five rats). The average value of each experiment came from eight determinations in the homogenated cerebrum and cerebellum of each animal. Significantly different from controls; $0.01 < *p < 0.05$, $0.001 < **p < 0.01$.

can be seen, the enzyme activity in both regions was not affected within the first 80 min of stress, while it was increased about 70–80% after 120–180 min of stress, where the maximum enzyme activation was found. Enzymatic activity of the control was about 35% higher in the cerebrum than in the cerebellum, while it was only 18–20% higher after 120–180 min of stress.

Figure 2 presents the changes of Mg^{++}-dependent ATPase activity in homogenated cerebrum and cerebellum during stress. It was found that the enzyme activity was not affected within the first 80 min of stress, while it was increased about 50–70% after 120–180 min of stress, where the maximum enzyme stimulation was observed. Enzymatic activity of the control was about 25% higher in the cerebrum than in the cerebellum, while it was about 40% higher after 120–180 min of stress.

Changes of AChE activity in homogenated cerebrum and cerebellum during stress are illustrated in Fig. 3. As can be observed, the enzyme activity was increased considerably by less than 45 min of stress and reached to a plateau in 80–180 min to a higher value in the cerebrum (≅100%) than in the cerebellum (≅40%) related to the control. Enzymatic activity of the control was about 100% higher in the cerebrum than in the cerebellum, while it was about 200% higher after 80–180 min of stress.

DISCUSSION

Na^+,Ka^+-ATPase, the enzymatic basis of univalent cation transport (27) and of activity-dependent energy utilization in nerve (18), appears to be stimulated by catecholamines in vitro

and in vivo (21,25); this effect may reflect an adaptation of adrenergic synaptic mechanisms to periods of neuronal activity induced by stress. It is known that plasma corticosterone levels and brain NE release are significantly elevated during stress (28,29). Moreover, it has been reported that glucocorticoids (cortisol) and NE can stimulate brain Na^+,K^+-ATPase, by increasing the fluidity of brain synaptosomal plasma membranes (3,26). Therefore, the observed Na^+,K^+-ATPase stimulation could be caused by increased release of NE and/or corticosterone during stress. The differences in the enzyme activity between the cerebrum and cerebellum may be induced by different responsiveness of adrenergic neurons to cold and immobilization stress.

Mg^{++}-ATPase and Na^+,K^+-ATPase are different enzymes in brain nerve endings (15,24). Brain intracellular Mg^{++} concentration ($\cong$ 1 mM) is maintained at high level because of Mg^{++} pump activity. Therefore, the observed Mg^{++}-ATPase stimulation by the used stress can increase brain intracellular Mg^{++} concentration. Moreover, the differences in enzyme activity between the cerebrum and cerebellum during stress may indicate different Mg^{++} concentrations in these regions. Changes of intracellular Mg^{++} can control rates of protein synthesis and growth of the cell and may be caused by catecholamines and glucocorticoids (cortisol) action (13,24). Therefore, Mg^{++} pump stimulation may be the result of increased release of NA and/or corticosterone; this may possibly be for helping the adaptation of the adrenergic synaptic

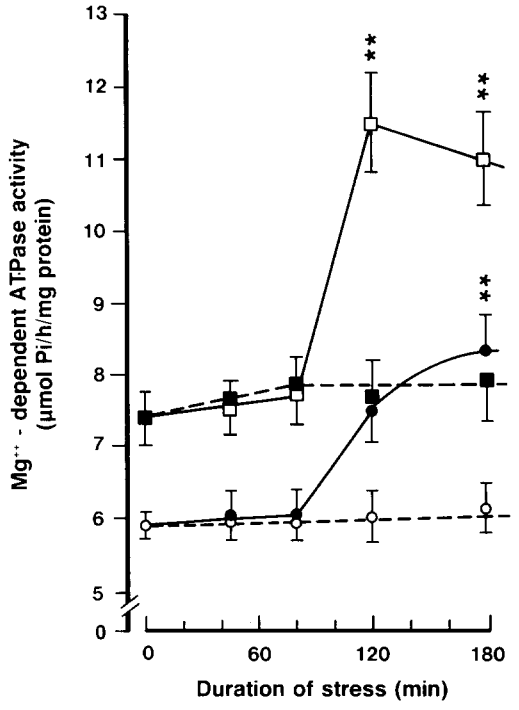

FIG. 2. Changes in the Mg^{++}-ATPase activities in homogenated cerebrum (□——□) and cerebellum (●——●) of rats exposed to stress for various periods. Enzyme activities of controls at the corresponding time in the cerebrum (■---■) and cerebellum (○---○). Each value indicates the mean ± SD of five independent experiments (five rats). The average value of each experiment came from eight determinations in the homogenated cerebrum and cerebellum of each animal. Significantly different from controls; $0.001 < **p < 0.01$.

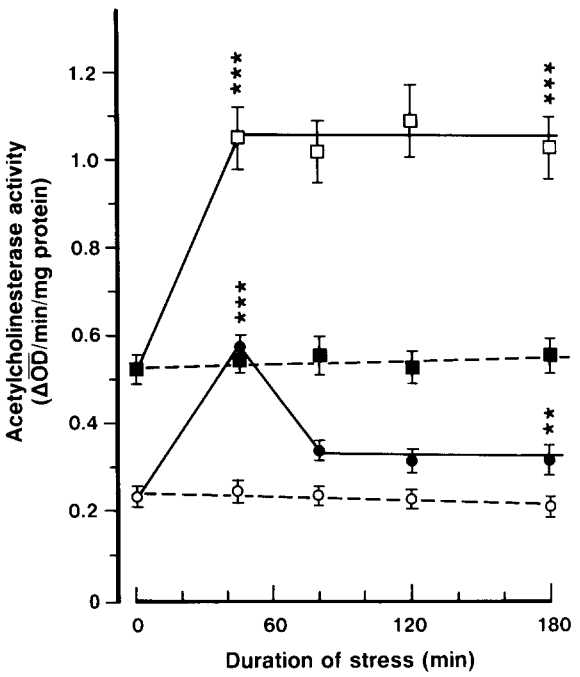

FIG. 3. Changes in the acetylcholinesterase activities in homogenated cerebrum (□——□) and cerebellum (●——●) of rats exposed to stress for various periods. Enzyme activities of the controls at the corresponding time in the cerebrum (■---■) and cerebellum (○---○). Each value indicates the mean ± SD of five independent experiments (five rats). The average value of each experiment came from six determinations in the homogenated cerebrum and cerebellum of each animal. Significantly different from controls; $0.001 < **p < 0.01$, $***p < 0.001$.

mechanisms and/or maintaining the appropriate protein synthesis rates in the cell during stress. The differences in the enzyme activity between the cerebrum and cerebellum may be caused by different responsiveness to catecholamines and/or glucocorticoids, induced by the used stress.

It is known that pituitary cAMP and plasma corticosterone levels in rats are significantly elevated within 15 min of immobilization stress (12,28). Corticosterone may increase AChE activity by interacting with brain synaptosomal plasma membranes and increasing their fluidity (3). Consequently, stress produced an AChE stimulation because the brain cholinergic system in rats may be influenced by corticosterone or intracellularly increased cAMP (12).

In conclusion, comparing the changes of AChE activity to those of Na^+,K^+-ATPase and Mg^{++}-ATPase activities during stress we can suggest that: a) Cold and immobilization stress can stimulate AChE in a different way and more quickly than Na^+,K^+-ATPase and Mg^{++}-ATPase because the brain cholinergic system in rats may undergo a more rapid activation than the adrenergic one after acute stress (7); b) AChE in the cerebellum is stimulated lower than in the cerebrum by the used stress, probably because of the presence of a relatively small cerebellar cholinergic innervation (10,14,19,23). Moreover, the high differences in AChE activity between the cerebrum and cerebellum may indicate different responsiveness of cholinergic neurons in these regions to cold and immobilization stress. These regional differences might be related to serial, physiological, regionally different functions involved in stress-

induced emergency responses such as emotion, arousal level, and endocrine and autonomic nervous system responses.

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