mals [5], and in human thyroid cancer [6]. There is no doubt that Mn moves and functions dynamically in the body. To understand the real roles of Mn in the body it is very important to know the controlling mechanisms of its transport from the circulation. In the present study it has been possible to bring to light at least one of the regulatory mechanisms by which Mn-uptake by the thyroid is controlled.

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1 Mena, I., in: Disorders of Mineral Metabolism, vol. 1, p. 233. Eds F. Bronner and J. W. Coburn. Academic Press, New York 1981.
2 Sakurai, H., Nishida, M., Yoshimura, T., Takada, J., and Koyama, M., Biochim. biophys. Acta *841* (1985) 208.
3 Kawada, J., Nishida, M., Yoshimura, Y., and Yamashita, K., Endocr. Japn. *32* (1985) 635.
4 Nishida, M., Kawada, J., Ishizuka, H., and Katsura, S., Zool. Sci. *5* (1988) 1043.
5 Nishida, M., Sakurai, H., and Kawada, J., Naturwissenschaften *76* (1989) 220.
6 Nishida, M., Sakurai, H., the late Tezuka, U., Kawada, J., Koyama, M., and Takada, J., Clin. chim. Acta *187* (1990) 181.
7 Halmi, N. S., Spirtos, B. N., Bogdanove, E. M., and Lipner, H. J., Endocrinology *52* (1953) 19.
8 Gafni, M., and Gross, J., Endocrinology *97* (1975) 1486.
9 Männistö, P. T., Ranta, T., and Leppäluoto, J., Acta endocr. *91* (1979) 271.
10 Greenspan, F. S., and Forsham, P. H., Basic and Clinical Endocrinology, pp. 138–139. Lange Medical Publications, Los Altos, USA 1983.
11 Chiraseveenuprapund, P., Buergi, U., Goswami, A., and Rosenberg, I. N., Endocrinology *102* (1978) 612.
12 Teraoka, K., Minakuchi, K., Takasugi, M., Akamatsu, S., Nishida, M., and Kawada, J., J. Trace Elem. Electrolytes Health Dis. *4* (1990) 169.
13 Harpern, R., Cooper, D. S., Kieffer, J. D., Saxe, V., Mover, H., Maloof, F., and Ridgway, E. C., Endocrinology *113* (1983) 915.
14 Sterling, K., Lashof, J. C., and Man, E. B., J. clin. Invest. *33* (1954) 1031.
15 Gibbons, R. A., Dixon, S. N., Hallis, K., Russell, A. M., Sansom, B. F., and Symonds, H. W., Biochim. biophys. Acta *444* (1976) 1.
16 Scheuhammer, A. M., and Cherian, M. G., Biochim. biophys. Acta *840* (1985) 163.
17 Buthieau, A.-M., and Autissier, N., C. r. Séanc. Soc. Biol. *171* (1977) 1024.
18 Umazume, K., Nishida, M., Sakurai, H., Yoshimura, Y., and Kawada, J., Biochem. Inter. *21* (1990) 85.
19 Sakurai, H., and Nishida, M., Trace Nutr. Res. *4* (1988) 139.

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Effect of stress on choline acetyltransferase activity of the brain and the adrenal of the rat [1]

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Abstract. Choline acetyltransferase (ChAT) activity was determined in cerebral cortex, hypothalamus, hippocampus, cerebellum, medulla oblongata, midbrain and adrenal gland of rats exposed to acute or chronic stress. The exposure of animals to acute immobilization and cold stress (4 °C) for one hour resulted in a significant decline of ChAT activity in all brain regions examined except for the medulla oblongata. Moreover, the exposure to acute stress resulted in significant increase of the same enzyme in the adrenal gland. However, chronic exposure of animals to cold stress (4 °C) for 7 days resulted in no significant changes of ChAT activity in all tissues examined except for a decline in the midbrain and an increase in the medulla oblongata. The administration of corticosterone (2.0 mg/kg) 1 h prior to sacrificing caused an effect similar to that of acute stress on ChAT activity in all brain regions except for the hypothalamus and the cerebellum. It was concluded from this experiment that stress-induced changes in the ChAT activity of specific brain regions might be mediated by the adrenal steroids.
Key words. Choline acetyltransferase; cerebral cortex; hypothalamus; hippocampus; cerebellum; medulla oblongata; midbrain; adrenal gland.

Stress is known to lead to a series of biochemical, physiological, and behavioral changes mediated through the neuroendocrine system that alter normal homeostasis [3–7]. Factors such as stress, drugs, anesthesia and hormones have been shown to have significant effects on the nervous system performance [7–13]. It has been proposed that the effects of an acute and chronic stress are partially mediated by the central muscarinic system with capacities to activate the adrenergic nervous system [3, 14]. The cholinergic regions of the central nervous system play an essential role in the capability of living organisms to cope with external or internal demands, particularly when the limits of tolerance tend to be exceeded [8, 15, 16]. For example, it was shown that an acute swimming stress in the rat produces changes in cholinergic muscarinic receptors selective for certain regions of the central nervous system [15]. A neurotransmitter, such as acetylcholine (ACh) is known to produce behavioral, neuroendocrine, cardio-

vascular, and noradrenergic effects typifying stress [17]. In this regard the biosynthetic enzyme for ACh, choline acetyltransferase (ChAT, EC 2.3.1.6) is currently the most reliable biomarker for cholinergic neuron activity [18–21].

The extensive literature on stress includes very few investigations concerned with the cholinergic system in the brain [4, 7, 22]. Most investigations on stress have dealt with the study of choline uptake, synthesis and release of ACh, while few have dealt with the cholinergic enzymes [3, 4, 7, 17]. Moreover, specific brain regions as well as the adrenal glands are also known to be involved, either directly or indirectly, in the neuroendocrine regulatory processes of the nervous system when subjected to various stressors [15, 19]. Therefore, the purpose of this investigation was to study the effect of stress on ChAT activity in various brain regions and the adrenal gland of the rat.

Materials and methods

Male Sprague-Dawley rats, weighing 120–150 g obtained from Southern Animals Farms (Prattville, AL), were used in this study. All animals were kept under controlled environmental conditions for two weeks prior to any experimentation. They were adapted to a temperature of 21 ± 1 °C and 12:12 h of light-dark cycle, lights were on at 08:00 h. Standard pellet diet (Purina, St. Louis, MO) and water were provided ad libitum.

In the first experiment, thirty rats were divided equally into three groups. The acute stress group was immobilized and exposed to cold at 4 ± 1 °C for 1 h. The chronic stress group was allowed free movement but was subjected to the same cold stress for seven days. The control group was allowed free movement at normal environmental temperature.

In the second experiment, twenty rats were divided into two equal groups, a control group injected with the drug vehicle, and a corticosterone-treated group which was treated with a single injection (2.0 mg/kg, i.p.) of corticosterone (Sigma Co., St. Louis, MO), one hour before they were sacrificed.

At the end of each experiment, animals were sacrificed by decapitation at 09.00 h, and the brain was rapidly removed and dissected on ice into : cerebral cortex, cerebellum, hippocampus, hypothalamus, midbrain, and medulla oblongata. Both adrenal glands were also removed. Each tissue was then individually homogenized (1% W/V) in an ice-cold 0.5 M sodium phosphate buffer (pH 7.0). ChAT activity in the homogenates was then assayed according to the spectrophotometric method of Chao and Wolfgram [23], and expressed as nmoles Co-enzyme A Sulfhydril (COASH) formed/min/g of tissue.

Overall comparisons between the different groups were performed by one-way analysis of variance [24]. Multiple-comparisons for the separation of the means of various regions were determined using the LSD test [24]. $P \leq 0.05$ was considered significant in all cases.

Results

The effects of acute stress (immobilization and cold 4 °C for 1 h) or chronic stress (cold environment at 4 °C for 7 days) on ChAT activity in various brain regions and adrenal gland are presented in figures 1 and 2. The exposure of animals to acute immobilization and cold stress, resulted in a significant decline of ChAT activity in the cerebral cortex (59%), hypothalamus (48%), hippocampus (30%) and midbrain (38%) and an increase in ChAT activity in the medulla oblongata (50%) and adrenal gland (46%). No change in ChAT activity in the cerebellum was noted with acute stress.

Chronic exposure of animals to cold stress did not result in altered ChAT activity in the brain regions examined with the exception of a decline in midbrain (69%) and a significant increase in medulla oblongata (184%).

The effect of corticosterone administration (2.0 mg/kg) on the activity of ChAT in the same brain regions and

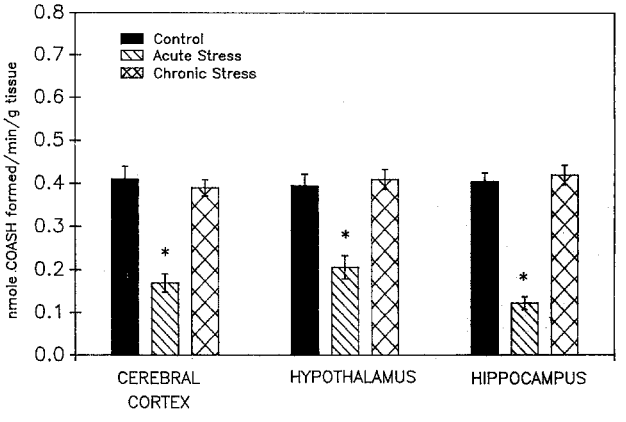

Figure 1. Effect of acute stress (immobilization at 4 °C for 1 h) or chronic stress (cold at 4 °C for 7 days) on ChAT activity in the cerebral cortex, hypothalamus, and hippocampus. Each bar represents the mean ± SEM for 10 rats.
*Significantly ($p \leq 0.05$) different from the control group.

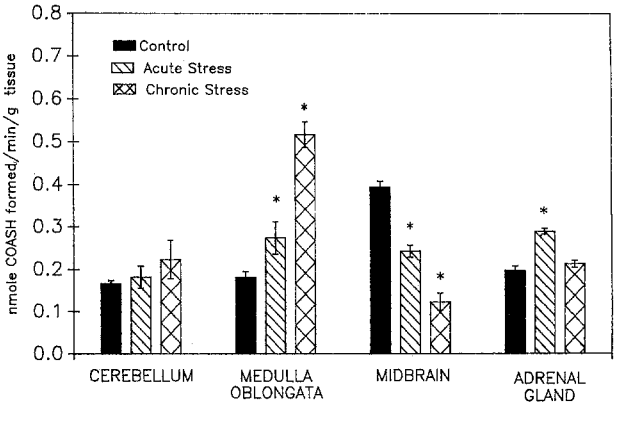

Figure 2. Effect of acute stress (immobilization at 4 °C for 1 h or chronic stress (cold at 4 °C for 7 days) on ChAT activity in the cerebellum, medulla oblongata, midbrain, and adrenal gland. Each bar represents the mean ± SEM for 10 rats.
*Significantly ($p \leq 0.05$) different from the control group.

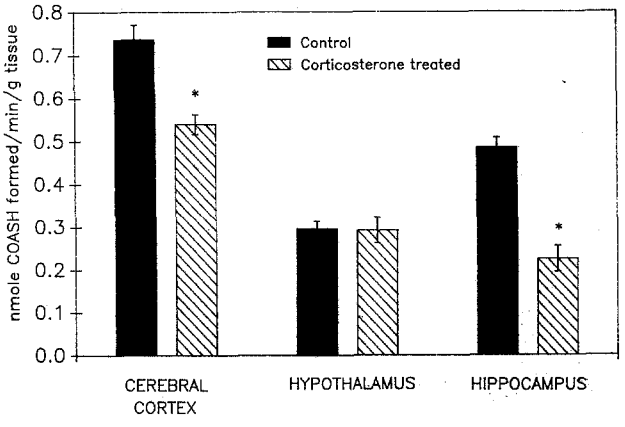

Figure 3. Effect of corticosterone treatment (2.0 mg/kg) for 1 h before sacrificing on ChAT activity in the cerebral cortex, hypothalamus and hippocampus. Each bar represents the mean ± SEM for 10 rats.
*Significantly ($p \leq 0.05$) different from the control group.

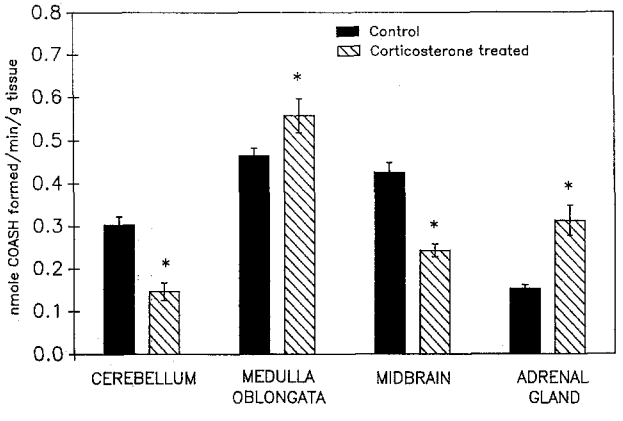

Figure 4. Effect of corticosterone treatment (2.0 mg/kg) for 1 h before sacrificing on the activity of ChAT in the cerebellum, medulla oblongata, midbrain, and adrenal gland. Each bar represents the mean ± SEM for 10 rats.
*Significantly ($p \leq 0.05$) different from the control group.

adrenal gland is presented in figures 3 and 4. Corticosterone treatment resulted in a significant decline of ChAT activity in cerebral cortex (27%), hippocampus (54%), cerebellum (52%) and midbrain (43%) but caused significant increases ($p < 0.05$) in medulla oblongata (20%) and adrenal gland (106%). No change in ChAT activity in the hypothalamus was noted.

Discussion

The results of this study show that ChAT activity varies in the different brain regions studied. Regional variation in ChAT activity has also been reported[25]. Thus alterations of ChAT activity in different brain regions reported here might be a reflection of regional differences in the response of cholinergic activity under such stressful conditions.

However, the exposure of animals to chronic cold stress for seven days resulted in recovery of ChAT activity to normal levels in all tissues examined except for the medulla oblongata and midbrain. This recovery phenomenon of ChAT activity in animals exposed to chronic stress may represent a type of adaptation. It has been suggested that after chronic stress the hippocampal cholinergic system undergoes major changes[26]. These changes may play an important role in a neuronal network in the brain which provides for this stress-induced plasticity[26]. Several investigations have shown that changes in cholinergic muscarinic receptors in response to stress are selective and reversible for certain regions of the nervous system[15]. Moreover, rapid decrease of muscarinic receptors density in the cerebral cortex and basal ganglia after rats were submitted to forced swimming stress, have been reported. This decline in the muscarinic receptors density returned to normal levels after a period of time[15].

Although stress is difficult to quantify, these findings are in agreement with Selye's classical concept of stress, in that there is an initial period of alarm, followed by a period of adaptation[27]. This may suggest that the activity of the cholinergic system undergoes regulation resulting from the action of exogenous forms of stress to maintain the body's homeostasis. This action may lead to changes in the ACh turnover rate (i.e. synthesis and/or degradation) which may result in alteration in the cholinergic enzyme activity. In addition, the existence of variety of other non-cholinergic functions may contribute to alteration of activities of the cholinergic enzymes[3, 17].

The administration of corticosterone to animals caused similar effects to an acute stress on ChAT activity in all brain regions studied, except the hypothalamus and a decrease in the cerebellum. Although we have not measured the plasma levels of corticosterone, it has been repeatedly reported that following an acute or chronic exposure of rats to cold stress, a rise in the circulating levels of corticosterone was noted[28]. Moreover, the exposure of rats to three days of stress showed an inhibition in body weight and an induced adrenal enlargement[29]. An increase in plasma corticosterone levels was also noticed following immobilization stress[30].

The effect of cold stress on ChAT activity of the brain has not received much attention in the past. However, under other stressful conditions, significant changes in ChAT activity were reported. For example, after electroshock, ACh concentration was found to be reduced in specific brain regions of the rat[31]. The findings of the present experiment indirectly confirm these results as they relate to ChAT activity of the majority of brain regions examined after acute stress. The present results also confirm the data obtained using ChAT inhibitor (4-1-naphthylvinylpyridine) on brain ACh concentration of the rat[32]. It was indicated that the reduction of ACh level by this drug can be potentiated by the concurrent exposure of the rat to stress in the form of forced swimming[32].

In previous work from this laboratory using a similar animal model for acute and chronic stress, we have found that the brain acetylcholinesterase (AChE) activity was

inhibited following stress or glucocorticoids administration [7]. The results obtained here and in previous work with AChE activity might indicate that both ChAT and AChE activities are affected by stress and glucocorticoids might mediate this effect.

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2 To whom requests for reprints should be addressed.
3 Dilsaver, S. C., Acta psychiatr. scand. *74* (1986) 312.
4 Fatranska, M., Budai, D., Oprsalova, Z., and Kvetnansky, R., Brain Res. *424* (1987) 109.
5 Anisman, H., in: Psychopharmacology of Aversively Motivated Behavior, pp. 119–197. Eds H. Anisman and G. Bignami. Plenum, New York 1978.
6 Kolta, M. G., and Soliman, K. F. A., Endokrinologie *77* (1981) 179.
7 Gabriel, N. N., and Soliman, K. F. A., Hormones Res. *17* (1983) 43.
8 Wahba, Z. Z., and Soliman, K. F. A., Pharmacology *34* (1987) 66.
9 Owasoyo, J. O., and Iramain, C. A., Vet. Sci. Commun. *3* (1979) 243.
10 Vernadakis, A., and Ruthledge, C. O., J. Neurochem. *20* (1973) 1503.
11 Naik, S. R., and Sheth, U. K., Ind. J. Med. Res. *58* (1970) 480.
12 Celesia, G. G., and Jasper, H. H., Neurology, Minneap. *15* (1966) 1053.
13 Russel, R. W., Rev. Pharmac. Toxic. *22* (1982) 435.
14 Janowsky, D. S., Risch, S. C., Hury, L. Y., Kennedy, B., and Zigler, M., Am. J. Psychiat. *142* (1985) 738.
15 Estevez, E. E., Jerusalinsky, D., Medina, J. H., and DeRobertis, E., Neuroscience *12* (1984) 1353.
16 Wahba, Z. Z., and Soliman, K. F. A., Experientia *44* (1988) 742.
17 Fatranska, M., Kiss, A., Opralova, Z., and Kvetnasnky, R., Endocr. Experiment. *33* (1989) 3.
18 Fonnum, F., in: Cholinergic Mechanism, pp. 145–160. Ed. P. G. Waser. Raven, New York 1975.
19 Kuhar, M. J., in: Biology of Cholinergic Function, pp. 3–27. Eds A. M. Goldberg and I. Hanin. Raven, New York 1976.
20 McCaman, R. E., and McCaman, W. M., in: Biology of Cholinergic Function, pp. 485–513. Eds A. M. Goldberg and I. Hanin. Raven, New York 1976.
21 Ichikawa, T., and Hirata, Y., J. Neurosci. *3* (1986) 2286.
22 Janowsky, K. S., and Risch, S. C., Drug Dev. Res. (1984) 125.
23 Chao, L. P., and Wolfgram, F., Analyt. Biochem. *46* 81972) 144.
24 Steel, R. G. D., and Torrie, J. H., Principles and Procedures of Statistics. McGraw-Hill, New York 1960.
25 Hoover, D. B., Meth, E. A., and Jacobowitz, D. M., Brain Res. *153* (1973) 259.
26 Finkelstein, Y., Koffler, B., Rabey, J. M., and Gilad, G. M., Brain Res. *343* (1985) 314.
27 Selye, H., The Physiology and Pathology of Exposure to Stress. Acta Inc., Montréal 1950.
28 Sakellaris, P. C., and Vernikos-Danelis, J., Endocrinology *97* (1975) 597.
29 Taché, Y., Du Russeay, P., Taché, J., Selye, H., and Collu, R., Neuroendocrinology *22* (1976) 325.
30 Mikulaj, L., Kventansky, R., and Murgas, K., Rev. Czch. Med. *20* (1974) 162–169.
31 Longoni, R., Mulas, A., Oderfeld-Novak, B., Pepeu, J. M., and Pepeu, G., Neuropharmacology *15* (1976) 283.
32 Kasa, P., Szepesy, G., Gulya, K., Banasaghy, K., and Rackonczay, Z., Neurochem. Int. *4* (1982) 185.

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Effects of blood sampling, anesthesia and surgery on plasma vasopressin concentration in rats

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Abstract. The influence of blood sampling, anesthesia and surgery on plasma vasopressin concentration was assessed in rats. Mean plasma concentration in conscious, chronically catheterized rats was 1.4 ± 0.1 pg/ml (n = 6). This value remained constant over repeated plasma samplings in the same animals. On the other hand, decapitation increased the plasma vasopressin concentration to 6.0 ± 2.4 (in pg/ml) (n = 6), inactin anesthesia to 2.9 ± 0.6 (n = 6), anesthesia and femoral cannulation to 13.3 ± 5.8 (n = 6) and surgery for renal micropuncture to 81.3 ± 35.0 (n = 6). It is concluded that the level of circulating plasma vasopressin is highly dependent on the sampling technique and is closely related to the extent of surgery.
Key words. Vasopressin; rats; surgery; renal micropuncture; anesthesia.

The plasma concentration of the peptide hormone vasopressin may vary over a wide range, depending on the physiological state of the animal. In the present work, we focused our attention on concentration changes following various experimental procedures, such as anesthesia and the surgery involved in renal physiology studies. It has already been shown that surgical preparation of rats for micropuncture of renal tubules results in a marked change in hematocrit and plasma volume [1]. A sequential study of the different events of such surgery indicated that inactin anesthesia and femoral artery catheterization were not responsible for this increase in hematocrit. However, the stress induced by physically manipulating the animal increases the hematocrit by about 3%. Complete micropuncture surgery involving tracheotomy, cannulation of the jugular vein, a midline abdominal incision