Effect of Aging on the Activities of Acetylcholinesterase, Na^+, K^+-ATPase and Mg^{2+}-ATPase in Rat Pituitary and Hypothalamus

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Z. Naturforsch. **53c,** 168–172 (1998); received October 13/October 31, 1997

Aging, Rat Pituitary, Rat Hypothalamus, Acetylcholinesterase, Na^+,K^+-ATPase, Mg^{2+}-ATPase

Acetylcholinesterase (AChE), Na^+, K^+-ATPase and Mg^{2+}-ATPase activities were estimated in homogenised rat pituitary and hypothalamus of 4- and 22-month-old rats. AChE activity was not altered in the pituitary of aged compared to adult rats, while it was found decreased by about 40% in the hypothalamus. Na^+,K^+-ATPase activity remained stable in the hypothalamus, while it was decreased by about 38% in the pituitary. Mg^{2+}-ATPase activity remained unchanged in the hypothalamus, but was increased by about 83% in the pituitary. This pituitary Na^+, K^+-ATPase inactivation may result in pathological mood and decreased neural excitability and metabolic energy production in aged animals.The age-related alterations of AChE, Na^+, K^+-ATPase and Mg^{2+}-ATPase activities may reflect changes in secretion and responses of some hormones of pituitary and hypothalamus.

Introduction

Normal brain aging in the rat is accompanied by histopathological changes (Feldman, 1976; Geinisman *et al.*, 1978) such as decreases in the level of proteins, loss of neurons in several subcortical nuclei, reductions in the enzymes that synthesize dopamine, norepinephrine, serotonin and acetylcholine, degradation of their receptors and degeneration of synapses (Goldman and Côté, 1991). These age-related alterations could explain some of the characteristics of senescence: alterations in neuroendocrine functions, sleep pattern, mood, appetite, motor activity, memory and learning (Goldman and Côté, 1991). Some of these functions are related to the pituitary and hypothalamus. It has already been shown (Tsakiris *et al.*, 1996) that whole brain acetylcholinesterase (AChE) activity was decreased, Na^+,K^+-ATPase was stimulated significantly, while Mg^{2+}-ATPase activity remained unchanged in aged compared to adult rats. Moreover, *in vivo* and *in vitro* acetylcholine synthesis, release, and choline uptake are decreased by aging (Cotman and Peterson, 1989).

It is known that the role of AChE is very important in ACh cycle, including ACh release (Kouniniotou-Krontiri and Tsakiris, 1989) and cholinergic mechanisms are implicated in neuroendocrine functions. Moreover, Na^+, K^+-ATPase is implicated in neural excitability (Sastry and Phillis, 1977), activity-dependent metabolism of energy (Mata *et al.*, 1980), Na^+-dependent tryptophan uptake system (Herrero *et al.*, 1983) and its activity can be regulated by noradrenergic and/or serotoninergic mechanisms (Swann, 1984; Hernàndez, 1987). Furthermore, the Mg^{2+}-ATPase activity can regulate intracellular Mg^{2+} concentration and modulate activities of other enzymes (e.g. adenylate cyclase) (Bockqert *et al.*, 1984) and hormonal function of the pituitary gland (Gyevai *et al.*, 1988). The purpose of this study was to investigate the effect of aging on the activities of AChE, Na^+,K^+-ATPase and Mg^{2+}-ATPase in rat pituitary and hypothalamus. Moreover, the possible age-related alterations of cholinergic, noradrenergic and serotoninergic mechanisms are discussed in relation to hormonal function.

Materials and Methods

Male Albino Wistar rats (Saint Sabbas Hospital, Athens, Greece) were used in all experiments. Body weight was 206 (SD ± 15) g in adult (4 mo)

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and 319 (SD±50) g in aged (22 mo) rats. Rats were housed four in a cage, at a constant room temperature (22±1 °C), under a 12L:12D (light 08.00–20.00 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.* Rats were sacrificed by decapitation. Pituitary and hypothalamus were removed, weighed and thoroughly perfused with isotonic saline. Individual hypothalami or pools of three pituitaries were homogenized in 10 vol ice-cold (0–4 °C) medium containing 50 mM Tris(hydroxymethyl)aminomethane–HCl (Tris-HCl), pH 7.4 and 300 mM sucrose using an ice-chilled glass homogenizing vessel at 900 rpm (4–5 strokes). Then, the homogenate was centrifuged at 1,000x*g* for 10 min to remove nuclei and debris. In the resulting supernatant, the protein content was determined according to Lowry *et al.* (1951) and then the enzyme activities were measured. The enzyme incubation temperature mixture was kept at 37 °C.

AChE activity was determined according to the method of Ellman *et al.* (1961). 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 0.050 ml acetylthiocholine iodide, used as a 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 ($\Delta\overline{OD}$) at 412 nm.

Na^+,K^+-ATPase was calculated as the difference between total ATPase activity (Na^+,K^+,Mg^{2+}-dependent) and Mg^{2+}-dependent ATPase activity. Total ATPase activity was assayed in an incubation medium consisting of 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 20 mM KCl, 4 mM $MgCL_2$, 240 mM sucrose, 1 mM ethylenediamine tetraacetic acid K_2-salt (K^+-EDTA), 3 mM disodium ATP, and 80–100 μg protein of the homogenate, in a final volume of 1 ml. Ouabain (1 mM) was added in order to determine the activity of the Mg^{2+}-dependent ATPase. The values of Mg^{2+}-dependent ATPase were similar in the presence of ouabain in the reaction mixture and in its absence and without NaCl and KCl. The reaction was started by adding ATP and was stopped after a 20 min incubation period by the addition of 2 ml of a mixture of 1% lubrol and 1% ammonium molybdate in 0.9 M H_2SO_4 (Atkinson *et al.*, 1971; Bowler and Tirri, 1974). The yellow colour which developed was read at 390 nm.

All chemicals were analytical grade and purchased from Sigma Chemical Company, St. Louis, MO, USA. The data were analysed by two-tailed Student's *t*-test.

Results and Discussion

Table I presents body, pituitary and hypothalamic weights and protein concentration in homogenized tissues in adult and aged rats. Although body and whole rat brain weight increases by about 50% in aged animals (Tsakiris *et al.*, 1996), pituitary and hypothalamic weight remains constant ($p>0.05$). We found a decrease of about 20% in the protein concentration of homogenized pituitary and an increase of about 40% in homogenized hypothalamus of aged rats. Therefore, aging decreases the level of pituitary proteins (Goldman and Côté, 1991), but may not affect the protein synthesis in the hypothalamus.

Table I. Body, pituitary and hypothalamic weights and protein concentration in homogenized tissues of adult and aged rats.

Rats	Weight [g]	Pituitary [mg]	Pituitary [mg protein/ml]	Hypothalamus [mg]	Hypothalamus [mg protein/ml]
4 mo old rats	206±15	9.08±1.08	6.07±0.35	40.05±4.09	3.17±0.22
22 mo old rats	319±50***	10.25±1.20	4.85±0.45**	43.90±4.90	4.49±0.30***

Values represent means±SD of eight independent experiments (eight rats) for 4-month-old rats and six experiments (six rats) for 22-month-old rats. Values of pituitary protein concentration represent means±SD of five independent experiments (five pools of three animals each) for adult or aged rats.
** $p<0.01$; *** $p<0.001$; compared to 4-month-old rats.

Table II. Effect of aging on the activities of acetylcholinesterase, Na^+,K^+-ATPase and Mg^{2+}-ATPase determined in homogenized rat pituitary and hypothalamus.

Age	Activity Acetylcholinesterase ($\Delta\overline{OD}$/min×mg protein)	Na^+,K^+-ATPase (μmol Pi/h×mg protein)	Mg^{2+}-ATPase (μmol Pi/h×mg protein)
Pituitary			
4 mo old rats	0.096±0.008	2.280±0.220	5.120±0.410
22 mo old rats	0.108±0.012	1.420±0.130***	9.380±0.940***
Hypothalamus			
4 mo old rats	0.499±0.035	7.460±0.690	5.920±0.360
22 mo old rats	0.299±0.030***	7.520±0.920	6.440±0.650

Values represent means±SD of five independent experiments (five pools of three animals each) for adult or aged rats for the pituitary and eight independent experiments (eight rats) for 4-month-old rats and six experiments (six rats) for 22-month-old rats for the hypothalamus. The average value of each experiment came from three determinations.
*** $p<0.001$; compared to 4-month-old rats.

In Table II we observe the effect of aging on the activities of AChE, Na^+, K^+-ATPase and Mg^{2+}-ATPase determined in homogenized pituitary and hypothalamus. AChE activity remained constant in the pituitary of aged compared to adult rats ($p>0.05$), while it was decreased by about 18% in whole brain (Tsakiris *et al.*, 1996) and about 40% in the hypothalamus ($p<0.001$). Some authors have found an age-related decrease of 24% in hypothalamic enzyme activity (Meneguz *et al.*, 1992), possibly because different strain of rats and different centrifugation conditions were used. This AChE inhibition may be related to the decreased acetylcholine synthesis, release, choline uptake and cholinergic receptor density *in vivo* and *in vitro* during aging (Walker *et al.*, 1988; Cotman and Peterson, 1989). However, this enzyme inhibition in the hypothalamus may be induced by an increased corticoid concentration noted in aged rats (Gabriel and Soliman, 1983; Peskind *et al.*, 1995). Moreover, endocrine responses were greater in older subjects than young subjects for CRH, ACTH, beta-endorphin and cortisol (Peskind *et al.*, 1995). This age-related cholinergic activity decrease may result in stimulation of somatostatin, inactivation of growth hormone-releasing hormone (GHRH) and prolactin secretion by acting on the hypothalamus (Hall *et al.*, 1984; Muller *et al.*, 1995) and modulation of neurohypophysial vasopressin secretion (Michels *et al.*, 1991).

Na^+,K^+-ATPase activity remained constant in the hypothalamus during aging ($p>0.05$), while it was decreased by about 38% ($p<0.001$) in the pituitary (Table II). This enzyme inhibition in the pituitary may be due to an age-related increase of endogenous ouabain-like compounds (Illescas *et al.*, 1990) or a decrease in noradrenergic and/or serotoninergic receptor density (Samorajski *et al.*, 1987; Walker *et al.*, 1988) capable of regulating Na^+, K^+-ATPase activity (Swann, 1984; Hernàndez, 1987). This Na^+,K^+-ATPase inactivation can result in pathological mood (Christo and el-Mallakh, 1993) and decreased neural excitability and metabolic energy production in aged animals (Sastry and Phillis, 1977; Mata *et al.*, 1980). It has been demonstrated that oxygen consumption in homogenates of various brain regions (Peng *et al.*, 1977) and oxidation of glucose in slices of cerebral cortex (Patel, 1977) are reduced in sections isolated from aged rats.

Mg^{2+}-ATPase activity remained unchanged in the hypothalamus during aging ($p>0.05$), while it was increased by about 83% ($p<0.001$) in the pituitary (Table II). This enzyme stimulation in the pituitary may be due to an age-related increase of ACTH or corticoid concentration (Peskid *et al.*, 1995). An increase of intracellular Mg^{2+} in the pituitary gland, because of the Mg^{2+}-pump activity, could modulate the activities of other enzymes (e.g. adenylate cyclase) (Bockqert *et al.*, 1984) and secretion of some hormones (e.g. prolactin, growth hormone(GH)) during aging (Gyevai *et al.*, 1988). Therefore, age-related alterations of cholinergic, noradrenergic and serotoninergic mechanisms, which modulate AChE and Na^+,K^+-ATPase activi-

ties, may change the secretion as well as the responses of some pituitary and hypothalamic hormones (e.g. vasopressin, prolactin, GH and GHRH).

Acknowledgements

This work was supported by the University of Athens. Many thanks are extended to Mrs Ar. Doulgeraki and Dr. Ir. Lytrivi for their excellent assistance.

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