Effect of CDP-choline on Hippocampal Acetylcholinesterase and Na^+,K^+-ATPase in Adult and Aged Rats

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Z. Naturforsch. **58c**, 277–281 (2003); received October 15/November 11, 2002

The aim of this study was to investigate the effect of different cytidine-5′-diphosphocholine (CDP-choline) concentrations (0.1–1 mM) on acetylcholinesterase (AChE), (Na^+,K^+)-ATPase and Mg^{2+}-ATPase activities in homogenates of adult and aged rat hippocampi. Tissues were homogenised, centrifuged at $1000 \times g$ for 10 min and in the supernatant, AChE activity and Na^+,K^+-ATPase and Mg^{2+}-ATPase activities were determined according to Ellman's method and Bowler's and Tirri's method, respectively. After an 1–3 h preincubation of the homogenised tissue with CDP-choline, a maximal AChE stimulation of about 25% for both adult and aged rats ($p < 0.001$) and a Na^+,K^+-ATPase activation of about 50% for adult rats ($p < 0.001$) and about 60% for aged rats ($p < 0.001$) were observed, while hippocampal Mg^{2+}-ATPase activity was not influenced in either adult or aged animals. It is suggested that: CDP-choline can restore hippocampal AChE and Na^+,K^+-ATPase activities in the aged rat and thus it may play a role in improving memory performance which is impaired by aging and some neuronal disturbances.

Key words: Rat Hippocampal Acetylcholinesterase, Rat Hippocampal Na^+,K^+-ATPase, CDP-choline

Introduction

CDP-choline is an essential intermediate in the biosynthetic pathway of the structural phospholipids of cell membranes, especially in that of phosphatidylcholine (PC) (Knapp and Wurtmann, 1999). The enzyme choline phosphotransferase catalyzes the CDP-choline transformation to PC (Dorman *et al.*, 1982). The synthesis of CDP-choline is the limiting step in PC formation (Secades and Frontera, 1995). CDP-choline also participates in the biosynthesis of sphingolipids and in particular in that of sphingomyelin (Secades and Frontera, 1995), an important constituent of neuronal membranes. CDP-choline crosses the blood-brain barrier as cytidine and choline, which reach the brain and synthesize again CDP-choline in the cytoplasm (Secades and Frontera, 1995). CDP-choline is incorporated widely in various brain areas (Romero *et al.*, 1983). CDP-choline, supplied exogenously, has beneficial physiological actions on cellular function that have been extensively studied and characterized in numerous model systems. CDP-choline and its hydrolysis products (cytidine and choline) play important roles in generation of phospholipids involved in membrane formation and repair. They also contribute to such critical metabolic functions as formation of nucleic acids, proteins and acetylcholine (ACh) (Secades and Frontera, 1995). CDP-choline also affects the levels of various neurotransmitters (dopamine, serotonin and noradrenaline) (Martinet *et al.*, 1979; Lopez *et al.*, 1986; Petkov *et al.*, 1990). This substance was shown to increase noradrenaline levels in the cortex and hypothalamus (Petkov *et al.*, 1990). Moreover, it was shown to increase total urinary excretion of 3-methoxy-4-hydroxyphenylglucol, which reflects noradrenergic activity in rats and humans, suggesting that this compound increases noradrenaline release (Lopez *et al.*, 1986).

In animal models of brain aging, CDP-choline can improve learning and memory performance, probably by means of cholinergic action (Secades and Frontera, 1995). Experiments showed that this substance, given in animal models of cerebral edema, restores Na^+,K^+-ATPase activity, possibly through the formation of PC (Cohade *et al.*, 1982).

CDP-choline improves cognitive function in patients with Alzheimer's disease; this is due, in part, to the ability of the drug to stimulate the activity

0939–5075/2003/0300–0277 $ 06.00

of serotoninergic, dopaminergic, and noradrenergic systems (Corona *et al.*, 1983; Secades and Frontera, 1995). Finally, this substance is effective as cotherapy for Parkinson's disease (Acosta *et al.*, 1988).

The aim of this study was to investigate the effect of different CDP-choline concentrations on the activity of three brain enzymes: (a) acetylcholinesterase (AChE, EC 3.1.1.7), the role of which is very important in ACh cycle and ACh release; (b) Na^+,K^+-ATPase (EC 3.6.1.3), an enzyme implicated in neural excitability (Sastry and Phillis, 1977), metabolic energy production (Mata *et al.*, 1980), uptake and release of catecholamines (Bogdanski *et al.*, 1968) and Na^+-dependent tryptophan uptake system (Herrero *et al.*, 1983); and (c) Mg^{2+}-ATPase, the role of which is to maintain high brain intracellular Mg^{2+} concentrations, changes of which can control rates of protein synthesis and cell growth (Sanui and Rubin, 1982). The effect of different CDP-choline concentrations on AChE and Na^+,K^+-ATPase activities was studied in homogenates of adult and aged rat hippocampi.

Materials and Methods

Animals

Albino adult (4 months old) and aged (22 months old) Wistar rats of both sexes (Saint Savvas Hospital, Athens, Greece) were used in all experiments. Body weight was 200 ± 15 g (mean ± SD) for adult and 305 + 30 g for aged rats. The rats were housed four in a cage, at a constant room temperature (22° ± 1 °C) under a 12 h L:12 h D (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*.

Tissue preparation

Rats were sacrificed by decapitation. The hippocampus of individual adult (73 ± 11 mg) or aged rats (79 ± 11 mg) was rapidly removed, weighed and thoroughly washed with isotonic saline. Tissues from sixteen adult or aged animals 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 $1000 \times g$ 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. Three or four experiments were carried out using the same brain supernatant. The enzyme incubation mixture was kept at 37 °C.

Determination of enzyme activities

AChE activity was determined according to Ellman's method (1961) and Na^+,K^+-ATPase, Mg^{2+}-ATPase activities according to Bowler and Tirri (1974). The enzyme reaction mixture and assay conditions of these enzyme activities were described in detail previously (Tsakiris, 2001).

Statistical analysis

Data were analyzed by two-tailed Student's *t*-test. P values of < 0.05 were considered statistically significant.

Results

The enzymatic activity measurements were carried out on homogenised adult and aged rat hippocampi. In the experiments in which enzyme preincubation with CDP-choline was needed, the determination of the activity was carried out *in vitro* after preincubation with 0.1, 0.5, 0.8 or 1 mM of CDP-choline at 37 °C. It has been estimated that the concentration of CDP-choline in rat plasma is approximately 1 mM, when a maximum dose of 30 mg per kg rat body weight is administered intravenously (Martinet *et al.*, 1979). An estimate of the intracytoplasmic concentration of CDP-choline in rat brain (*in vivo*) has not been reported (Secades and Frontera, 1995). The enzyme activities were investigated as a function of time of CDP-choline action on the enzyme activity and as a function of CDP-choline concentration.

Effect of CDP-choline on AChE activities

In the absence of CDP-choline, AChE activity in the homogenate of adult rat hippocampi remained at a steady level during the 3 h duration of the experiment. In the presence of this substance

(1 mM), the enzyme activity increased progressively reaching a maximum value of 20–25% ($p < 0.01$) after 1 h of CDP-choline action. Additionally, the effect of different concentrations of this compound on AChE activity was investigated in hippocampal homogenate of adult and aged rats. The results, illustrated in Fig. 1, showed that 1 h of CDP-choline action on AChE in the hippocampus of adult rats resulted in a statistically significant stimulation of the enzyme activity by about 7% ($p < 0.05$) for a concentration of 0.1 mM, 17% ($p < 0.01$) for 0.5 mM and finally 23% ($p < 0.001$) for 0.8 mM and 1 mM. Fig. 1 also shows that 1 h of CDP-choline action on AChE in the hippocampus of aged rats resulted in a statistically significant stimulation of the enzyme activity by about 8% ($p < 0.01$) for a concentration of 0.1 mM, 18% ($p < 0.001$) for 0.5 mM and finally 25% ($p < 0.001$) for 0.8 mM and 1 mM.

Effect of CDP-choline on Na^+,K^+-ATPase and Mg^{2+}-ATPase activities

In the absence of CDP-choline, (Na^+,K^+)-ATPase activity in the homogenate of adult rat hippocampi remained at a steady level during the 3 h duration of the experiment. In the presence of this substance (1 mM), the enzyme activity increased progressively reaching a maximum value of about 45% ($p < 0.001$) after 1 h of CDP-choline action. Additionally, the effect of different concentrations of this compound on Na^+,K^+-ATPase activity was investigated in hippocampal homogenate of adult and aged rats. The results, illustrated in Fig. 2, showed that 1 h of CDP-choline action on Na^+,K^+-ATPase of hippocampus of adult rats resulted in a stimulation of the enzyme activity by about 19% ($p > 0.05$) for a concentration of 0.1 mM, 35% ($p < 0.01$) for 0.5 mM, 45% ($p < 0.001$) for 0.8 mM and 50% ($p < 0.001$) for 1 mM. Fig. 2 also shows that 1 h of CDP-choline action on Na^+,K^+-ATPase in the hippocampus of aged rats resulted in a statistically significant stimulation of the enzyme activity by about 21% ($p < 0.05$) for a concentration of 0.1 mM, 43% ($p < 0.01$) for 0.5 mM, 53% ($p < 0.01$) for 0.8 mM and finally 58% ($p < 0.001$) for 1 mM.

Table I shows the effect of high CDP-choline concentration (1 mM) on the activities of AChE, (Na^+,K^+)-ATPase and Mg^{2+}-ATPase in homogen-

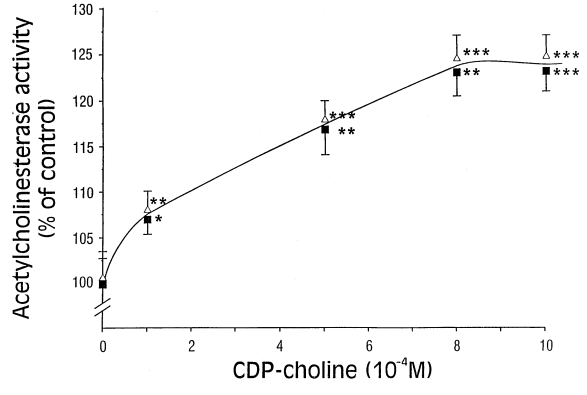

Fig. 1. Effect of different CDP-choline concentrations on AChE activity determined in homogenised hippocampus of adult (■) and aged (△) rats. The control values of the enzyme activity were 0.288 ± 0.012 $\Delta\overline{OD}$/min × mg protein for adult rats and 0.209 ± 0.006 $\Delta\overline{OD}$/min × mg protein for aged rats. Values represent means ± SD of four experiments. The average value of each experiment arises from three determinations of the enzyme activity. In each protocol, control and CDP-choline samples were tested simultaneously after an 1 h preincubation. In all cases, an 1 h preincubation of the homogenate with different concentrations of CDP-choline preceded the substrate addition. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with control.

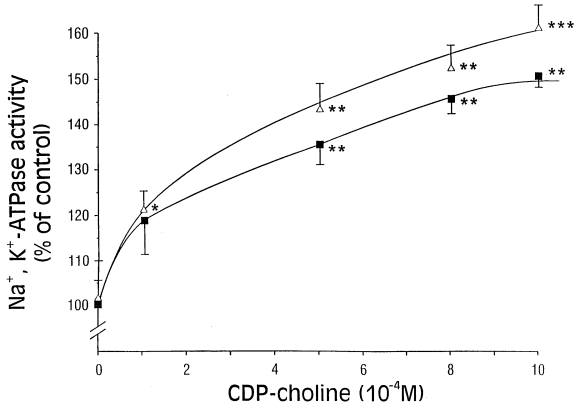

Fig. 2. Effect of different CDP-choline concentrations on Na^+,K^+-ATPase activity determined in homogenised hippocampus of adult (■) and aged (△) rats. The control values of the enzyme activity were 7.62 ± 0.78 μmol Pi/h × mg protein for adult rats and 5.92 ± 0.41 μmol Pi/h × mg protein for aged rats. Values represent means ± SD of four experiments. The average value of each experiment arises from three determinations of the enzyme activity. In each protocol, control and CDP-choline samples were tested simultaneously after an 1 h preincubation. In all cases, an 1 h preincubation of the homogenate with different concentrations of CDP-choline preceded the substrate addition. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with control.

Table I. Effect of CDP-choline on the activities of AChE, (Na^+,K^+)-ATPase and Mg^{2+}-ATPase in homogenised aged rat hippocampi.

	Activities		
	AChE ($\Delta\overline{OD}$/min × mg protein)	Na^+,K^+-ATPase (μmol Pi/h × mg protein)	Mg^{2+}-ATPase
Adult hippocampus	0.288 ± 0.012	7.62 ± 0.78	10.24 ± 1.02
Aged hippocampus	0.209 ± 0.006***	5.92 ± 0.41**	9.02 ± 0.88
+ 1 mM CDP-choline	0.261 ± 0.005*	9.35 ± 0.56**	10.00 ± 1.10

Values represent means ± SD of four experiments. The average value of each experiment arises from three determinations. An 1 h preincubation of the homogenate with 1 mM of CDP-choline preceded the substrate addition. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with enzyme activity values in adult hippocampus.

ised aged rat hippocampi. CDP-choline stimulated AChE by about 25% ($p < 0.001$) in aged hippocampus reaching partly the enzyme activity in adult hippocampus (difference of – 9%, $p < 0.05$). Moreover, this substance stimulated Na^+,K^+-ATPase by about 60% ($p < 0.001$) in aged hippocampus, whereas it increased the enzyme activity in a higher value compared with that in adult hippocampus (difference of + 20%, $p < 0.01$). The enzyme activities were appeared decreased by aging by about 30% ($p < 0.001$) for AChE and 25% ($p < 0.01$) for Na^+,K^+-ATPase.

Mg^{2+}-ATPase activity was found to be 10.24 ± 1.02 μmol Pi/h × mg of protein and 9.02 ± 0.88 μmol Pi/h × mg of protein in homogenised hippocampus of adult and aged rats, respectively (Table I). CDP-choline in the concentrations used in our experiments appeared unable to affect the enzyme activity ($p > 0.05$).

Discussion

In this study we observed that 30 min of CDP-choline action in homogenates of hippocampus was able to affect significantly the activity of AChE (about 100% of the maximal effect, $p < 0.01$) and that of Na^+,K^+-ATPase (about 80% of the maximal effect, $p < 0.001$). In a previous study (Plataras *et al.*, 2000) we found that 30 min of CDP-choline action in whole brain homogenates was able to affect significantly the activity of AChE (about 50% of the maximal effect, $p < 0.01$) but not that of Na^+,K^+-ATPase. It seems that the action of CDP-choline is specific for the hippocampus, a fact that could explain many of its pharmacological effects.

High concentrations of CDP-choline (1 mM) stimulated aged hippocampal AChE by about 25% reaching partly the enzyme activity in adult hippocampus. The enzyme activity had been decreased by about 30% because of aging (see Table I). Therefore, CDP-choline might improve inhibited AChE activities in some neuronal disturbances [*e.g.* Alzheimer (Bowen and Dawison, 1986)]. In parallel, CDP-choline has been reported to increase ACh synthesis and/or release (Pinardi *et al.*, 1994; Dixon *et al.*, 1997), which could improve learning and memory performance in neurological disturbances (Secades and Frontera, 1995). This fact is supported by our results that show a faster effect of CDP-choline on AChE in the hippocampus, a major center of memory procedures (Kandel *et al.*, 2000).

Additionally, high concentrations of CDP-choline (1 mM) stimulated aged hippocampal Na^+,K^+-ATPase by 60% and can overcome the enzyme activity compared with that in adult hippocampus. The enzyme activity had been inhibited by about 25% because of aging (see also Table I). Therefore, this stimulating enzymatic effect of CDP-choline could improve neural excitability (Sastry and Phillis, 1977), metabolic energy production (Mata *et al.*, 1980) and influence the catecholaminergic (Bogdanski *et al.*, 1968) and/or serotoninergic mechanisms (Herrero *et al.*, 1983) in some neuronal disturbances of aging. On the other hand hippocampal Mg^{2+}-ATPase activity was not affected either by rat aging or by CDP-choline (Table I).

In conclusion, CDP-choline can stimulate AChE and Na^+,K^+-ATPase in homogenates of adult and aged rat hippocampi. This stimulation is greater and more rapid than the stimulation observed in

whole brain homogenates. Since hippocampus is a major center for memory performance, we believe that the changes in enzyme activities observed in our experiments may in part explain the ameliorating effect of CDP-choline on memory performance especially in cases of old individuals (Secades and Frontera, 1995). Our study supports the findings of previous works (Secades and Frontera, 1995), which suggest that CDP-choline may be a helpful drug in cases of memory impairment.

Acknowledgements

This work was funded by the University of Athens. Many thanks are expressed to Mrs Filia Stratigea for her significant assistance.

Acosta J., Nombela M., Palao A., Pastor M., and Recuero J. (1988), Multicentre trial: treatment of Parkinson's disease with CDP-choline (citicholine). In: New Trends in Clinical Neuropharmacology; Calcium Antagonists, Acute Neurology, Headache and Movement Disorders (Bartko D., Turcani P., and Stern G., eds.). John Libbey & Co Ltd, London, pp. 289–296.

Bogdanski D. F., Tissuri A., and Brodie B. B. (1968), Role of sodium, potassium, ouabain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. Life Sci. **7**, 419–428.

Bowen D. M., and Dawison A. N. (1986), Biochemical studies of nerve cells and energy metabolism in Alzheimer's disease. Br. Med. Bull. **42**, 75–80.

Bowler K., and Tirri R. (1974), The temperature characteristics of synaptic membrane ATPases from immature and adult rat brain. J. Neurochem. **23**, 611–613.

Cohado F., Richter E., and Poletto B. (1982), Etude d'un recurseur des phospholipides dans le traitement des comas traumatiqes graves. Neurochirourgie **28**, 287–290.

Corona G. I., Santagostino G., Frattini P., Cucchi M. L., Zerbi F., Toska P., and Savoldi F. (1983), Preliminary data on monoamine metabolite levels in cerebrospinal fluid and in urine during therapy in dementia. IRCS Med. Sci. **11**, 923–924.

Dixon C. E., Ma X., and Marion D. M. (1997), Effects of CDP-choline treatment of neurobehavioral deficits after TBI and on hippocampal and neocortical acetylcholine release. J. Neurotrauma **14**, 161–169.

Dorman R. V., Dabrowiecki Z., DeMedio G. E., Trovarelli G., Porcellati G., and Horrocks L. A. (1982), Control of lipid metabolism in ischemic brain by CDP-amines. In: Phospholipids in the Nervous System (Horrocks L., Ansell B., and Pozcellati G., eds.). Raven Press, New York, pp. 123–135.

Ellman G. L., Courtney D., Andres V., and Featherstone R. M. (1961), A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. **7**, 88–95.

Herrero E., Aragon M. C., Gimenez C., and Valdivieso F. (1983), Inhibition by L-phenylalanine of tryptophan transport by synaptosomal plasma membrane vesicles: implications in the pathogenesis of phenylketonuria. J. Inherit. Metab. Dis. **6**, 32–35.

Kandel E. R., Schwartz J. H., and Jessell T. M. (2000), Principles of Neural Science. Fourth Edition, Learning and Memory. McGraw-Hill, New York, pp. 1227–1277.

Knapp S., and Wurtman R. J. (1999), Enhancement of free fatty acid incorporation into phospholipids by choline plus cytidine. Brain Res. **822**, 52–59.

Lopez G., Coviela I., Agut J., and Wurtman R. J. (1986), Effect of cytidine(5′)-diphosphocholine (CDP-choline) on the total urinary excretion of 3-methoxy-4-hydroxy-phenyl-glycol (MHPG) by rats and humans. J. Neural Transm. **66**, 129–134.

Martinet M., Fonlupt P., and Pacheco H. (1979), Effects of cytidine-5-diphosphocholine on norepinephrine, dopamine and serotonin synthesis in various regions of the rat brain. Arch. Int. Pharmacodyn. **239**, 52–56.

Mata M., Fink D. J., Gainer H., Smith C. B., Davidsen L., Savakis H., Swartz W. J., and Sokoloff L. (1980), Activity-dependent energy metabolism in rat posterior pituitary, primarily reflects sodium pump activity. J. Neurochem. **34**, 213–215.

Petkov V. D., Stancheva S. L., Tocuschieva L., and Petkov V. V. (1990), Changes in brain biogenic monoamines induced by the nootropic drug adafenoxate and meclofenoxate and by citicholine. Gen. Pharmacol. **21**, 71–75.

Pinardi G., Pelissier T., Kramer V., Paeile C., and Miranda H. F. (1994), Effects of CDP-choline on acetylcholine-induced relaxation of the perfused carotid vascular beds of the rat. Gen. Pharmacol. **25**, 635–638.

Plataras C., Tsakiris S., and Angelogianni P. (2000), Effect of CDP-choline on brain acetylcholinesterase and Na^+,K^+-ATPase in adult rats. Clin. Biochem. **33**, 351–357.

Romero A., Serratosa J., Sacristan A., and Ortiz J. A. (1983), High resolution autoradiography in mouse brain 24 h after radiolabelled CDP-choline administration. Arzneim. Forsch./Drug Res. **33**, 1056–1058.

Sanui H., and Rubin H. (1982), The role of magnesium in cell proliferation and transformation. In: Ions, Cell Proliferation and Cancer (Boynton A. L., McKeehan W. L., Whitfield J. P., eds.). Academic Press, New York, pp. 517–537.

Sastry B. S. R., and Phillis J. W. (1977), Antagonism of biogenic amine-induced depression of cerebral cortical neurons by Na^+,K^+-ATPase inhibitors. Can. J. Physiol. Pharmacol. **55**, 170–180.

Secades J., and Frontera G. (1995), CDP-choline: pharmacological and clinical review. Meth. Find. Exp. Clin. Pharmacol. **17** (Suppl. B), 1–54.

Tsakiris S. (2001), Effects of L-phenylalanine on acetylcholinesterase and Na^+,K^+-ATPase activities in adult and aged rat brain. Mech. Ageing Dev. **122**, 491–501.