The Dynamics of Choline Acetyltransferase and Acetylcholinesterase Changes in Dog Spinal Cord during Ischemia

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Abstract. Activities of choline acetyltransferase (CAT) and acetylcholinesterase (AChE) were measured in several regions of the spinal cord, spinal ganglia and the sciatic nerve following ligation of the abdominal aorta just below the renal arteries for 20; 40; 80 and 120 min. Aortic ligation for 40 min produced a significant increase in the activities of both enzymes in the lumbar spinal cord. After ligation lasting 80 min the activity of CAT dropped under the control level and that of AChE remained elevated and returned to the control level in animals sacrificed 120 after the ligation. Similar but smaller AChE elevation was also found in the lower thoracic and the sacral spinal cord, respectively. In ischemic spinal ganglia and the sciatic nerve a decrease in AChE activity was found.

Key words: Choline acetyltransferase — Acetylcholinesterase — Incomplete ischemia — Spinal cord

Introduction

The effect of complete ischemia on acetylcholinesterase (AChE, EC 3.1.1.7) has been reported by Arsenio-Nunes et al. (1973), Mršulja et al. (1978) and Villa (1981). They showed a decrease in AChE activity in normothermical postdecapitative ischemic brain of different species; they however did not assay choline acetyltransferase (CAT, EC 2.3.1.6) activity.

Under the conditions of incomplete ischemia produced by the occlusion of arteries or of the aorta changes of cholinergic enzymes occurring in the brain differ from those occurring in the spinal cord; these changes have not yet been described in detail (Ott et al. 1975; Kása 1978; Gadamski et al. 1978). Ott et al. (1975) observed accumulation of AChE in the partially ischemic brain cortex and the basal ganglia of baboons. An increase in AChE activity was also found in our previous work (Malatová and Maršala 1980) in the spinal cord of dogs after clamping of the abdominal aorta for 80 min.

On the other hand, Kása (1978) reported that clamping of the thoracic aorta in

rat for 22 min followed by two weeks survival reduced the activities of both CAT and AChE, particularly in the Rexed lamina VII containing interneurons. Ischemia produced by the same technique by Gadamski et. al. (1978) in the dog resulted in histochemical changes in both the intensity and distribution of AChE which became most pronounced after 24 h survival, with subsequent normalisation of the enzymatic activity and its return to the control level within 6 days.

In view of the incompleteness of the information available on the effects of ischemia on the activities of CAT and AChE we have decided to investigate systemically changes of these two enzymes in different segments of the spinal cord, in the spinal ganglia and in the sciatic nerve of dogs in time intervals ranging between 20 and 120 min after the ligation of the abdominal aorta.

Materials and Methods

Experiments were performed on dogs of either sex, aged 1—5 years, weighing 9—20 kg; the animals were anesthetized with intravenous thiopental (Thiopental Spofa 30 mg per kilogram b.w.). Abdominal incision was made under deep anaesthesia and the aorta was ligated just below the origin of the renal arteries. The aorta remained occluded for 20; 40; 80; or 120 minutes. Sham-operated controls were treated exactly like the experimental animals, except that their aorta was not ligated. The experimental groups consisted of five dogs each and 3 sham-operated dogs served as controls for each of the time intervals. After the ischemic period, the spinal cords were removed together with the spinal ganglia and the sciatic nerves. The tissue was packed in an aluminium foil and stored in liquid nitrogen untill processed. On the second day at the latest the nervous tissue was sampled, weighed and homogenized as follows.

After identification of the segments the membranes were stripped off and cross section samples of the segments C 3—5, Th 10—12, L 3—5 and S 1—2 respectively, were taken. The spinal ganglia from both the lumbar and sacral parts were taken as one sample. The sciatic nerve (8—10 cm) was stripped of the epineurium. The tissue samples were homogenised (1:20) in a cooled solution (2 °C) containing (in mmol.l^{-1}): 200 NaCl; 40 sodium phosphate buffer (pH 7.4); 10 $MgCl_2$ and 0.5 % Triton X-100. Aliquots of the homogenates were used to determine CAT activity and protein content. The remaining homogenates were centrifuged at 14,000 g for 15 min and the supernatants were used to determine AChE activity.

CAT activity was determined twice in 35 μl of homogenates according to a modification of the Fonnum radioisotope method (Fonnum 1969). After the addition of 15 μl of basic incubation medium the reaction mixture contained mmol.l^{-1} [1—^{14}C] acetyl-coenzyme A (The Radiochemical Centre Amersham, SRA 74 MBq per mmol) 0.25; choline chloride 10; eserine sulphate 0.2; NaCl 300; Na-phosphate buffer (pH 7.4) 28; $MgCl_2$ 7; serum albumin 0.5 mg per ml and 0.35 % Triton X-100. The mixture was incubated at 37 °C for 15 min and the reaction was stopped by adding 2 ml of ice-cold solution containing 10 mmol.l^{-1} Na phosphate (pH 7.4) and 0.2 mmol.l^{-1} acetylcholine chloride. The synthesized acetylcholine was shaken into 1 ml Na tetraphenylborate (Kalignost) in butyl acetate (15 mg per ml). After centrifugation, the ^{14}C-acetylcholine content in an aliquot amount of the organic top layer was measured in a liquid scintillation counter (Pacard tricarb C2425). Blanks without any tissue were processed in parallel. CAT activity was expressed as the amount of acetylcholine synthesized in katals per gram protein.

AChE activity was assayed by the colorimetric method of Ellman et al. (1961). The incubation mixture contained 50 μl of the 14,000 g supernatant and 5,5-dithio-bis-2-nitrobenzonic acid (DTNB, Sigma) 1 mmol.l^{-1} as chromagen in Tris-HCl buffer 0.2 mmol.l^{-1} (pH 7.6). After 5 min preincubation

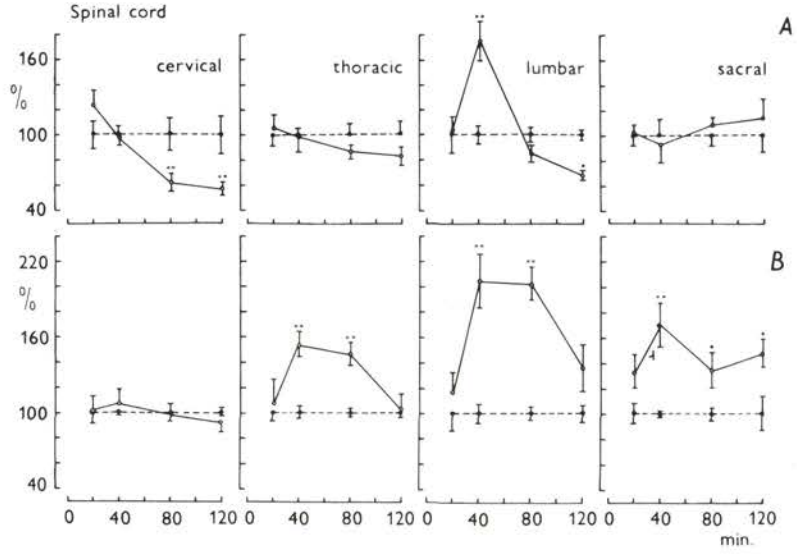

Fig. 1. Time course of changes in CAT and AChE activities in different parts of the spinal cord after abdominal aortic ligation just below the renal arteries (empty circles, full lines), expressed as percentual values of represent control activities (sham-operated animals, full circles, dashed lines). Means ± S.E.M. are given. Statistical significance: xx = $p<0.01$, x = $p<0.05$, *A*: CAT; *B*: AChE

at 25 °C the substrate, acetylthiocholine iodide (Lachema, Brno) 1 mmol.l^{-1} was added and the change in absorbance was recorded for 5 min at 412 nm wavelength on a SPECORD UV VIS spectrophotometer (Carl Zeiss, Jena). AChE activity was read from the calibration curve and expressed as the amount of acetylthiocholine hydrolysed in katals per gram protein.

Protein was determined by the method of Lowry et al. (1951) and read from the standard albumin curve.

Statistical analysis was performed using variance analysis with Duncan's F test (Duncan 1955).

Results

CAT and AChE activities in different parts of the spinal cord, in the spinal ganglia and the sciatic nerve following abdominal aortic ligature just below the renal arteries for 20; 40; 80; or 120 min expressed as percentual values of corresponding sham-operated control animals are illustrated in Figs. 1 and 2. Absolute mean values of CAT and AChE activities in 40 and 120 min following ligation are shown in Table 1.

In the lower thoracic segments of the spinal cord, only insignificant decrease in CAT activity was found. Contrary to this, AChE activity was significantly elevated (by 45—53 %) at the 40th and 80th post ligation min respectively and it returned to the control values at 120 min.

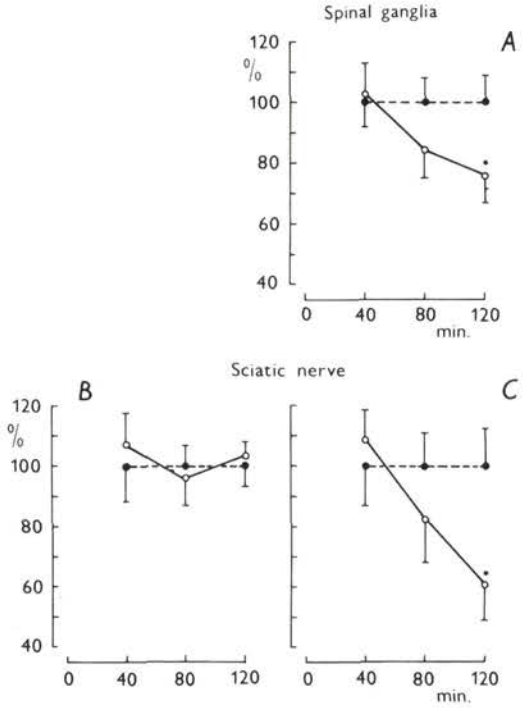

Fig. 2. Time course of changes in AChE activity in the spinal ganglia and the sciatic nerve and of CAT activity in the sciatic nerve after aortic ligation below the renal arteries. For explanation see Fig. 1. *A*: AChE, spinal ganglia; *B*: AChE, sciatic nerve; *C*: CAT, sciatic nerve.

Changes in both CAT and AChE activities were most pronounced in the lumbar spinal cord. No change was apparent twenty minutes after the ligation; at 40 min however CAT and AChE activities were increased by 75 % and 102 %, respectively. In the interval between 40 and 120 min CAT activity dropped below the control level with a high AChE activity persisting to 80 min and returning close to the control value by the last interval only, i.e. at 120 min following the ligation (Fig. 1, Table 1).

In the sacral part of the spinal cord, no changes in CAT activity were observed. AChE activity was increased by 70 % at 40 min following the ligation and, although a subsequent decrease, it remained significantly elevated throughout the period examined (Fig. 1, Table 1).

In the part of the cervical spinal cord analysed (C 3—5; high above the level of ligation) CAT activity decreased during the time period investigated with the AChE activity remaining unchanged (Fig. 1).

In the lumbo-sacral spinal ganglia, CAT activity was very low and oscillated

Table 1. Choline acetyltransferase (CAT) and acetylcholinesterase (AChE) activities in the spinal cord, spinal ganglia and the sciatic nerve after ligation of the abdominal aorta and in control (sham operated) dogs.

Nervous tissue	Experimental group	Duration of the ligation			
		40 min		120 min.	
		CAT	AChE	CAT	AChE
SPINAL CORD	After ligation	12.35 ± 0.72	682 ± 58	6.15 ± 0.46^{++}	619 ± 49
cervical	Control	12.44 ± 0.87	645 ± 14	11.07 ± 1.56	679 ± 11
	After ligation	7.66 ± 0.82	746 ± 43^{xx}	6.07 ± 0.48	520 ± 54
thoracic	Control	7.84 ± 0.40	486 ± 23	7.34 ± 0.76	506 ± 18
	After ligation	14.49 ± 1.34^{xx}	1185 ± 113^{xx}	4.71 ± 0.22^{x}	643 ± 81
lumbar	Control	8.79 ± 0.60	581 ± 48	6.87 ± 0.15	469 ± 32
	After ligation	17.12 ± 2.56	1621 ± 155^{xx}	19.42 ± 2.62	1398 ± 88^{x}
sacral	Control	18.20 ± 2.50	950 ± 16	17.09 ± 2.34	942 ± 119
SPINAL GANGLIA	After ligation	1.56 ± 0.31	1346 ± 116	1.37 ± 0.30	1047 ± 97^{x}
lumbo-sacral	Control	1.28 ± 0.37	1358 ± 86	1.28 ± 0.24	1356 ± 75
	After ligation	17.95 ± 0.09	330 ± 19	16.90 ± 0.84	216 ± 24^{x}
SCIATIC NERVE	Control	16.86 ± 2.06	308 ± 25	16.96 ± 0.92	284 ± 27

CAT and AChE activities (nkat per gram protein) = mean ± S.E.M.
Statistical significance: xx = $p < 0.01$ x = $p < 0.05$

without any significant changes; no results are therefore presented. On the contrary, the relatively high AChE activity was continuously decreasing after the aortic ligation (Fig. 2, Table 1).

In the sciatic nerve, CAT activity remained unchanged and AChE activity decreased by 40 % during the period examined (Fig. 2, Table 1).

Discussion

As follows from the anatomy of the vascular supply in the dog, ligation of the abdominal aorta just below the origin of renal arteries results in partial — incomplete ischemia of the spinal cord. There are regions with low or nearly zero blood flow and also other regions which remain well perfunded. With regard to the uneven blood supply in the spinal cord as well as to the higher metabolic requirements of the gray matter, incomplete ischemia has been reflected by quantitatively and qualitatively uneven morphological and biochemical changes in different anatomic regions of the spinal cord.

In our laboratory the degree of ischemia has previously been evaluated by studying the oxidative metabolism, and by morphological observations (Chavko et al. 1978; Chavko and Danielisová 1980; Badonič 1979; 1981). Metabolic changes were found to be most grave in the lumbar spinal cord. Within two hours following the aortic occlusion, adenosine triphosphate decreased by 70 % and adenosine monophosphate increased several times. Lactate was increased (by 300 %) roughly proportionally to the decrease of available pyruvate (Chavko et al. 1978; Chavko and Danielisová 1980).

Electron microscopic study of Badonič (1979, 1981) revealed the most pronounced changes to occur in motoneurons and synapses of the lumbar spinal cord. Eighty minutes of aortic ligature often resulted in depletion of the synaptic vesicles but vesicular pleomorphism and aggregation also occurred. The presynaptic and postsynaptic parts of the synapses began to separate. After two hours of occlusion osmiphilia increased and the synapses were isolated from the surrounding tissue. The degree of the damage varied from case to case, the alteration being always marked. Ultrastructural alterations in the sacral spinal cord were negligible.

In view of the biochemical and ultrastructural changes described the elevations of CAT and AChE activities observed 40 minutes after the aortic ligature were unexpected and very difficult to interprete. Elevation of AChE activity in the partially ischemic brain cortex 4 hours following middle cerebral artery occlusion in baboons has also been described by Ott et al. (1975). These authors suggested that the accumulation of AChE would reflect an increased hydrolysis of acetylcholine released from the synapses due to ischemic anoxia, they did not however provide any experimental evidence to support such an explanation.

Our explanation is based on the fact that the aortic ligature represents a form

of stress in which the entire neurohumoral apparatus is stimulated to overcome critical situations (Gann et al. 1978). The changes in CAT and AChE activities during two hours of aortic occlusion would then represent a component of the reaction on incomplete ischemia as a stressor. Temporary elevation of enzymatic activities might then be a possible indicator of short term stimulation of the synaptic neurotransmission.

Elevation of both CAT and AChE was found in the spinal cord only; in the spinal ganglia and the sciatic nerves the activities were decreased or remained unchanged. This suggests that only "functional" synapse-associated CAT and AChE were increased in the spinal cord.

The elevation of CAT and AChE activities may be a result of an increase in their synthesis despite a significant decrease in RNA polymerase activity occurring in neuronal and glial cells of the same ischemic spinal cords indicating a reduction in the protein synthesis (Gottlieb et al. 1981). It has been shown that protein synthesis was not completely blocked as ATP was decreased only by 70 % and the activity of ribosomes (*in vitro*) and the polysomal profile remained preserved throughout the period examined (Burda et al. 1980). This results suggested that the synthesis of some proteins related to the reaction on stress and to the activity of synapses might even be increased.

The increase in CAT and AChE activaties might also be the result of enzyme activation by some unknown substance or by changes in the microenvironment; however any experimental evidence to support this idea has not been provided. The reasons for the decrease in CAT activity in the cervical spinal cord, i.e. high above the aortic ligation, are not known. Although CAT and AChE have important unique functions in the mechanism of the cholinergic neurotransmission, changes in CAT are more emphasized as this enzyme is localised only in neurones, while AChE is also contained in glia (Roessman and Friede 1967; Silver and Wolstencroft 1971).

Differences between CAT and AChE changes during the period examined might be due to the different localization of these enzymes in neurons, to their different role in the acetylcholine metabolism, and to different susceptibility to ischemia (Tuček et al. 1978).

The changes in CAT and AChE in ischemic spinal cord reported herein are of interest; their true significance remains however unclear.

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