Biochemical Pharmacology, Vol. 30, No. 17, pp. 2399–2408, 1981.
Printed in Great Britain.

0006-2952/81/172399-10 $02.00/0

THE EFFECT OF ISCHEMIA AND PHARMACOLOGICAL TREATMENT EVALUATED ON SYNAPTOSOMES AND PURIFIED MITOCHONDRIA FROM RAT CEREBRAL CORTEX

R. F. VILLA, G. BENZI and D. CURTI
Department of Science, Institute of Pharmacology, University of Pavia, Italy

(*Received* 9 *October* 1980; *accepted* 17 *February* 1981)

Abstract—Changes in the maximal rate of some cerebral enzymatic activities related to energy transduction (lactate dehydrogenase; citrate synthase and malate dehydrogenase; total NADH-cytochrome *c* reductase and cytochrome oxidase) as well as both glutamate dehydrogenase and acetylcholine esterase were assayed in the purified mitochondrial fraction or in crude synaptosomal fraction from cerebral cortex. The evaluations were performed in rats before and after a postdecapitative normothermic ischemia of 5, 10, 20 and 40 min duration. The ischemic damage resulted in a decrease in the activity of mitochondrial malate dehydrogenase and total NADH-cytochrome *c* reductase, and of synaptosomal acetylcholine esterase.

The biochemical evaluations were performed also after an i.p. pretreatment with vincamine, trimetazidine and suloctidil (50 mg/kg). The drugs induced different changes in enzyme activities as a function of the ischemia duration. These various interferences are discussed with regard to the possible drugs mode of action.

Whether the cerebral blood flow is stopped (e.g. vascular occlusion, increased intracranial pressure, asystole or severe arterial hypotension) or cut off (e.g. decapitation), the brain becomes a closed thermodynamic system in which the energy transduction is limited to the degradation of endogenous substrates. The concept of irreversible damage that occurs in normothermic animals after a period of complete ischemia for more than 5–10 min has been previously postulated [1–6]. Nevertheless, more recent experimental data show that, under particular conditions, the neuronal activity may be restablished after longer ischemic periods [7–12]. In any case, many interrelated factors have been implicated in the vulnerability of the brain to ischemic damage, including: (a) changes in cerebral circulation; (b) brain edema; (c) oxygen and substrates deprivation; (d) tissular lactate or other end-products accumulation; (e) modifications of chemico-physical conditions; (f) alterations in membrane phospholipid composition; and (g) changes in cerebral enzymatic activities.

Although the precise nature of the processes bringing about cerebral damage is largely unknown, in the present study we sought to determine the response to postdecapitative ischemia and pharmacological treatment of some brain cortex enzymatic activities related to the energy transduction. The rationale of this approach is the following: when the cerebral circulation is totally and suddenly interrupted, the brain can obtain energy from the rearrangement of the enzymatic activities related to both carbohydrates and amino acids. For example, the cerebral ischemia of the adult rats resulted in a decrease in the NADP-dependent isocitrate dehydrogenase activity in mitochondrial external membranes by about 40 per cent, without significant changes in enzyme specific activity in the internal membranes and matrix [13]. In addition the proportion of the active form of pyruvate dehydrogenase was found to increase in ischemia, known to lead to an elevation of ADP content and decrease of the ATP:ADP ratio in the brain [14]. On the other hand, ischemia resulted in the diminution of both Na^+-K^+-ATPase activity in the brain cortex microsomal fraction [15] and of glucose 6-phosphatase activity in microsomal fraction, with concomitant increase in enzyme activity in the cytosol [16].

In any case, as regards the energy transduction, data concerning the behaviour of enzymatic activities of brain cortex during ischemia were recorded at different periods of ischemic damage. Therefore this work is intended to overcome it, besides being aimed at detecting the changes in the maximal rate of some enzymatic activities: (a) at short and regular intervals of time (after 5, 10, 20 and 40 min of postdecapitative ischemia); (b) in two cortical subcellular fractions (purified mitochondria and crude synaptosomes); (c) evaluating some enzyme activities of glycolytic pathway (lactate dehydrogenase), Krebs' cycle (citrate synthase; malate dehydrogenase), electron transport chain (total NADH-cytochrome *c* reductase; cytochrome oxidase), amino acid metabolism (glutamate dehydrogenase) and acetylcholine metabolism (acetylcholine esterase); and (d) utilizing drugs used in the medical management of cerebral ischemia (vincamine, trimetazidine, suloctidil). At any rate, this paper does not intend to tackle the problem of describing or predicting therapeutic measures with regard to acute cerebrovascular insuf-

ficiency in humans. Rather, drugs are used as tools able or unable to affect the cerebral enzymatic reactivities in a biological model of total complete and irreversible ischemia.

MATERIALS AND METHODS

The experiments were performed on female Sprague–Dawley rats, weighing 250 ± 10 g, that were fasted overnight before the utilization. The animals were selected according to randomized experimental procedures, kept from the birth under standard cycling and caging conditions (temperature: 22 ± 1°; relative humidity: 60 ± 3 per cent; 12-hr day cycle; from 7:00 a.m. to 7:00 p.m.; low noise disturbances), fed a standard pellet diet and water *ad lib.*, housed three and subsequently two per cage. The time course of ischemia performed in the lots was established by permutation tables.

Postdecapitative normothermic ischemia [13] was performed for 5, 10, 20 and 40 min, always starting at 10:00 a.m. After decapitation, the brain was removed within 15 sec from the skull and immersed in a cold 0.32 M sucrose solution. All manipulations were performed in a precooled box (0 to −5°). Cerebral cortex was isolated, then immersed in cold 0.32 M sucrose solution and homogenized (Potter–Braun S homogenizer, with Teflon pestle rotating at 1000 rpm) for 30 sec, with two strokes up and down. The homogenate was submitted to differential centrifugation [17] for isolating the purified mitochondrial fraction and the crude synaptosomal fraction. Three centrifugations at 900 *g* were performed to remove nuclei. The crude mitochondrial fraction was obtained by two centrifugations at 11,500 *g* for 30 min (Beckman J 21 C supercentrifuge; rotor JA 20). The crude mitochondrial fraction was subsequently layered on discontinuous sucrose gradient (1.4; 1.2; 1.0; 0.8 M) and centrifuged for 2 hr at 50,000 *g* (Beckman L5-50 Ultracentrifuge; rotor SW 50.1). The synaptosomal fraction was removed by aspiration and then pelletted at 50,000 *g*, for 30 min. All fractions were suspended in a 0.32 M sucrose solution and proteins were dosed [18]. The maximal rate of the following enzymatic activities were evaluated on samples of purified mitochondrial preparations: *citrate synthase* (citrate oxaloacetate-lyase, EC 4.1.3.7) [19]; *malate dehydrogenase* (L-malate:NAD^+ oxidoreductase, EC 1.1.1.37) [20]; *total NADH cytochrome* c *reductase* (NADH cytochrome *c*: oxygen oxidoreductase, EC 1.6.99.3) [21]; *cytochrome oxidase* (ferrocytochrome *c*: oxygen oxidoreductase, EC 1.9.3.1) [22–23]; *glutamate dehydrogenase* (L-glutamate:NAD^+ oxidoreductase deaminating, EC 1.4.1.3) [19]; on samples of crude synaptosomal preparations, the maximal rate of the following enzymatic activities were evaluated: *malate dehydrogenase* [20]; *lactate dehydrogenase* (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27) [24]; *acetylcholine esterase* (acetylcholine hydrolase, EC 3.1.1.7) [25]. Enzymatic activities were measured by graphic recordings for at least 3 min (Beckman 25 double-beam recording spectrophotometer) and expressed as μmoles.min^{-1}.(mg protein)$^{-1}$. Each value was calculated from three determinations performed blindly on the same sample.

The animals were treated with intraperitoneal injections of: (a) saline solution; (b) vincamine theophyllinil-propane sulfonate (*vincamine TPS*); (c) 1-(4-isopropylthiophenyl)-2*n*-octylaminopropanole (*suloctidil*); (d) 1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride (*trimetazidine DC*). The drugs were injected at 9:00 a.m., at the dose of 50 mg/kg, 60 min before the normothermic postdecapitative cerebral ischemia for 5, 10, 20 and 40 min duration. The dose used was chosen after preliminary tests with dilution 2.5 and 6.25.

Because of seasonal variations in the enzymatic activities, the groups of ischemic rats treated with saline solution or drug solutions were compared with their own group of controls at each individual time of ischemia. For statistical analysis, the Student's *t*-test was applied.

RESULTS

Drug action in control conditions (*Table* 1). Under control conditions, the cerebral cortex responded to the intraperitoneal injection with trimetazidine DC and suloctidil with no changes in the maximal rate of the tested enzymatic activities, both in purified mitochondria and crude synaptosomes. On the contrary, vincamine TPS treatment resulted in a slight increase of malate dehydrogenase activity in the mitochondrial fraction, and a decrease of the acetylcholine esterase activity in crude synaptosomal fraction, by about 36 per cent.

Drug action during ischemia (*Tables* 2–5). After 5 min of brain ischemia (Table 2) the cerebral cortex showed a significant decrease both of the total NADH-cytochrome *c* reductase activity in mitochondria and of acetylcholine esterase activity in synaptosomes. Pretreatment with vincamine and trimetazidine did not change this response although trimetazidine either induced a weak ($P < 0.05$) decrease of malate dehydrogenase activity in the mitochondrial fraction and lactate dehydrogenase activity in the crude synaptosomal fraction. Pretreatment with suloctidil did not prevent the ischemia-induced decrease of acetylcholine esterase activity in synaptosomes, but clearly prevented the drop of the total NADH-cytochrome *c* reductase activity in mitochondrial fraction. On the other hand, suloctidil pretreatment resulted in an increase of both citrate synthase and malate dehydrogenase activities in the purified mitochondria, and of the latter activity in synaptosomes, reaching values higher than those of the cerebral cortex in control rats.

After 10 min of brain ischemia (Table 3), the cerebral cortex showed a decrease in the malate dehydrogenase, NADH-cytochrome *c* reductase, and glutamate dehydrogenase activities in the purified mitochondrial fraction. Pretreatment with vincamine, (a) was able to increase the malate dehydrogenase activity in mitochondria and (b) decreased the acetylcholine esterase activity in synaptosomes respect to the control condition, the other enzymatic activities being unchanged in respect to those of the ischemic saline solution-treated rats. Pretreatment with trimetazidine maintained the ischemia-induced decrease of some specific enzymatic activities (malate

Table 1. Rat cerebral cortex. Enzymatic activities related to energy transduction in control condition and after i.p. treatment with drugs (50 mg/kg) or saline solution

		After i.p. treatment with							
		Saline solution		Vincamine		Trimetazidine		Suloctidil	
Subfraction	Enzymatic activities	N	S.A.	N	S.A.	N	S.A.	N	S.A.
Mitochondria	Citrate synthase	11	0.283 ± 0.009	9	0.294 ± 0.016	6	0.275 ± 0.018	8	0.277 ± 0.011
	Malate dehydrogenase	11	3.308 ± 0.077	8	3.614 ± 0.100*	9	3.393 ± 0.100	8	3.382 ± 0.120
	Total NADH-cytochrome *c* reductase	10	0.249 ± 0.015	7	0.289 ± 0.029	8	0.248 ± 0.013	8	0.247 ± 0.019
	Cytochrome oxidase	11	1.999 ± 0.084	8	2.207 ± 0.012	8	2.149 ± 0.051	8	1.971 ± 0.064
	Glutamate dehydrogenase	7	0.280 ± 0.017	4	0.267 ± 0.022	5	0.285 ± 0.048	5	0.280 ± 0.028
Synaptosomes	Lactate dehydrogenase	10	0.577 ± 0.040	8	0.502 ± 0.037	7	0.505 ± 0.045	7	0.660 ± 0.051
	Malate dehydrogenase	9	1.884 ± 0.119	7	1.886 ± 0.123	7	1.818 ± 0.123	7	1.895 ± 0.126
	Acetylcholine esterase	9	0.122 ± 0.005	7	0.087 ± 0.003†	7	0.100 ± 0.008	6	0.101 ± 0.011

The specific enzymatic activities [S.A. = μmoles.min^{-1}.(mg protein)$^{-1}$] were evaluated both in the purified mitochondrial fraction and in the crude synaptosomal fraction, and are expressed as the mean values ± S.E.M. for each group of N animals.

* Differs from saline solution treated rats: $P < 0.05$, Student's *t*-test.

† Differs from saline solution treated rats: $P < 0.01$, Student's *t*-test.

Table 2. Rat cerebral cortex. Enzymatic activities related to energy transduction in control condition and after both an ischemic period of 5 min duration and i.p. pretreatment with drugs (50 mg/kg) or saline solution. Normothermic ischemia was induced 60 min after the drug injection

Subfraction	Enzymatic activities	Control animals		After 5 min of ischemia and i.p. pretreatment with: Saline solution		Vincamine		Trimetazidine		Suloctidil	
		N	S.A.	N	S.A.	N	S.A.	N	S.A.	N	S.A.
Mitochondria	Citrate synthase	15	0.233 ± 0.010	15	0.230 ± 0.008	6	0.238 ± 0.010	4	0.218 ± 0.015	6	0.297 ± 0.024†§
	Malate dehydrogenase	15	3.097 ± 0.060	15	3.058 ± 0.055	6	3.008 ± 0.051	5	2.856 ± 0.048*	6	3.577 ± 0.075†§
	Total NADH-cytochrome *c* reductase	19	0.305 ± 0.011	19	0.256 ± 0.008†	7	0.247 ± 0.009†	8	0.246 ± 0.011†	6	0.297 ± 0.017‡
	Cytochrome oxidase	14	2.072 ± 0.066	14	1.917 ± 0.051	6	2.046 ± 0.070	4	1.900 ± 0.117	6	2.095 ± 0.105
	Glutamate dehydrogenase	13	0.276 ± 0.006	13	0.279 ± 0.008	4	0.287 ± 0.014	6	0.269 ± 0.013	6	0.300 ± 0.012
Synaptosomes	Lactate dehydrogenase	16	0.644 ± 0.028	16	0.590 ± 0.027	6	0.613 ± 0.036	6	0.535 ± 0.023*	6	0.692 ± 0.048
	Malate dehydrogenase	13	1.734 ± 0.113	15	1.635 ± 0.108	6	1.914 ± 0.101	6	1.393 ± 0.074	5	2.237 ± 0.130*§
	Acetylcholine esterase	17	0.137 ± 0.004	17	0.110 ± 0.005†	6	0.104 ± 0.007†	6	0.111 ± 0.005†	7	0.120 ± 0.007*

The specific enzymatic activities [S.A. = μmoles.min^{-1}.(mg protein)$^{-1}$] were evaluated both in the purified mitochondrial fraction and in the crude synaptosomal fraction, and are expressed as the mean values ± S.E.M. for each group of N animals.

Differs from control rats: * $P < 0.05$; † $P < 0.01$.

Differs from ischemic rats treated with saline solution: ‡ $P < 0.05$; § $P < 0.01$; Student's *t*-test.

Table 3. Rat cerebral cortex. Enzymatic activities related to energy transduction in control condition and after both an ischemic period of 10 min duration and i.p. pretreatment with drugs (50 mg/kg) or saline solution. Normothermic ischemia was induced 60 min after the drug injection

Subfraction	Enzymatic activities	Control animals		After 10 min of ischemia and i.p. pretreatment with: Saline solution		Vincamine		Trimetazidine		Suloctidil	
		N	S.A.	N	S.A.	N	S.A.	N	S.A.	N	S.A.
Mitochondria	Citrate synthase	9	0.229 ± 0.015	11	0.186 ± 0.013	9	0.209 ± 0.018	8	0.167 ± 0.012‡	10	0.173 ± 0.014†
	Malate dehydrogenase	9	2.931 ± 0.084	12	2.509 ± 0.055‡	11	2.772 ± 0.065§	10	2.424 ± 0.064‡	9	2.565 ± 0.107†
	Total NADH-cytochrome c reductase	7	0.274 ± 0.017	7	0.195 ± 0.013‡	7	0.204 ± 0.015†	8	0.161 ± 0.011‡	6	0.177 ± 0.015‡
	Cytochrome oxidase	7	1.834 ± 0.137	8	1.488 ± 0.156	6	1.672 ± 0.117	8	1.303 ± 0.094‡	8	1.346 ± 0.113†
	Glutamate dehydrogenase	8	0.349 ± 0.016	13	0.292 ± 0.014*	10	0.332 ± 0.018	9	0.269 ± 0.020‡	10	0.290 ± 0.016*
Synaptosomes	Lactate dehydrogenase	9	0.630 ± 0.037	10	0.570 ± 0.028	10	0.591 ± 0.045	10	0.545 ± 0.039	10	0.553 ± 0.039
	Malate dehydrogenase	9	1.578 ± 0.088	11	1.400 ± 0.086	11	1.431 ± 0.062	11	1.443 ± 0.059	10	1.314 ± 0.072*
	Acetylcholine esterase	11	0.153 ± 0.007	11	0.132 ± 0.007	10	0.118 ± 0.006‡	10	0.123 ± 0.007‡	10	0.115 ± 0.008‡

The specific enzymatic activities [S.A. = μmoles.min^{-1}.(mg protein)$^{-1}$] were evaluated both in the purified mitochondrial fraction and in the crude synaptosomal fraction, and are expressed as the mean values ± S.E.M. for each group of N animals.

Differs from control rats:* $P < 0.05$; † $P < 0.02$; ‡ $P < 0.01$.

Differs from ischemic rats treated with saline solution: § $P < 0.01$; Student's *t*-test.

dehydrogenase, total NADH-cytochrome *c* reductase, glutamate dehydrogenase in mitochondria; acetylcholine esterase in synaptosomes) and resulted in a decrease of other mitochondrial enzymatic activities, such as citrate synthase and cytochrome oxidase. Pretreatment with suloctidil maintained the ischemia-induced decrease of the above-quoted specific enzymatic activities, resulting on the other hand in a decrease of other mitochondrial (citrate synthase; cytochrome oxidase) and synaptosomal ones (malate dehydrogenase; acetylcholine esterase).

After 20 min of brain ischemia (Table 4), the cerebral cortex showed a decrease in malate dehydrogenase and total NADH-cytochrome *c* reductase activities in the purified mitochondrial fraction and a slight but significant decrease of acetylcholine esterase activity in the crude synaptosomal fraction. The pretreatment with the tested drugs did not change this ischemia-induced decrease of the above-quoted enzymatic activities, but resulted also in the decrease of glutamate dehydrogenase activity for suloctidil.

Also after 40 min of brain ischemia (Table 5), it was observed the decrease of total NADH cytochrome *c* reductase activity in mitochondria and of acetylcholine esterase activity in synaptosomes. At this time the pretreatment with the tested drugs maintained this ischemia-induced decrease of the above-quoted enzymatic activities, but resulted also of: (a) glutamate dehydrogenase and synaptosomal malate dehydrogenase activities, for trimetazidine; (b) malate dehydrogenase and cytochrome oxidase activities, for suloctidil; (c) mitochondrial malate dehydrogenase, cytochrome oxidase and glutamate dehydrogenase activities for vincamine.

DISCUSSION

As stated in the introduction, one of the objectives of the present research was to study the changes in the maximal rate of some enzymatic activities related to the energy transduction and evaluated both in the purified mitochondrial preparation and in the crude synaptosomal fraction from rat cerebral cortex, during total normothermic ischemia of 5, 10, 20 and 40 min duration. At mitochondrial level as concerns the enzymes related to the Krebs' cycle, malate dehydrogenase decreased in activity at 10 and 20 min of ischemia, with a partial restitution of the value after 40 min of ischemia. As concerns the enzymes related to the electron transport chain, total NADH-cytochrome *c* reductase decreased in activity from 0 to 40 min of ischemia. Glutamate dehydrogenase exhibited a significant decrease in activity only at time 10 min of the ischemic period. At synaptosomal level, acetylcholine esterase decreased in activity at 5, 20 and 40 min of ischemia, lactate dehydrogenase and malate dehydrogenase being unchanged.

These findings are consistent with the rearrangement of some enzymatic activities observed by other authors during cerebral ischemia. Indeed, the active form of pyruvate dehydrogenase was described to increase by about 40 per cent in forebrain of guinea-pig subjected to 3 min postdecapitative ischemia [26]. In control condition, the active form of the enzyme represents 62 per cent of total enzyme activity [26]. This means that in ischemia the active form of this enzyme makes as much as 90 per cent, indicating that the active form of pyruvate dehydrogenase in the brain strongly depends upon its functional state. In fact, regulation of pyruvate dehydrogenase activity underlies various mechanisms, such as the protection of the enzyme against inactivating action of pyruvate dehydrogenase kinase [27] and the inhibition by acetyl-CoA [28] or NADH [29], of which the level is related both to the functional state of mitochondria and to the rate of glycolysis. In the case of a 5 min normothermic postdecapitative ischemia it was found in guinea-pig brain changes in glucose-6-phosphatase distribution accompanied by a decrease in phosphatidylcholine and phosphatidylethanolamine content, indicating that during postdecapitative ischemia the activation of endogenous membrane-bound phospholipases occurs [16]. Furthermore, 0.5–5 min postdecapitative ischemia [30] resulted in a strong decrease of phospholipids content of guinea-pig cerebral mitochondria, particularly in the ethanolamine phospholipid fraction, consistent with the increase of both the free fatty acids pool and the level of malonyldialdehyde, one of the end products of unsaturated lipid peroxidation. Indeed, during prolonged ischemia a tissular increase of free fatty acids (FFA) [31, 32] and of their oxidation products [30] takes place. These events appear to be the primary cause of a mitochondrial damage such as e.g. the uncoupling of oxidative phosphorylation [13, 31]. In particular, the increase of FFA is an event which occurs during the first 0.5 min of ischemia (about 32 per cent increase) reaches about 34 per cent after 6 min of postdecapitative normothermic ischemia [30] and then gradually but moderately increases up to 50 min since the beginning of ischemia [32]. The accumulation of peroxide oxidation products in mitochondrial membranes causes: uncoupling of oxidative phosphorylation [33, 34], swelling of mitochondria and changes on membrane permeability [35, 36]. In the present research, the ischemia resulted in a decrease in the activity of mitochondrial malate dehydrogenase and total NADH-cytochrome *c* reductase, and of synaptosomal acetylcholine esterase. On the other hand, in the synaptosomal fraction, the cytochrome oxidase activity (0.100 ± 0.010 μmoles.min^{-1}.(mg protein)$^{-1}$, in cerebral cortex of control rats) raised to 0.170 ± 0.014 in 20 min ischemic rats treated with saline solution and to 0.171 ± 0.020, 0.166 ± 0.031 and 0.156 ± 0.016 in 20 min ischemic rats pretreated with vincamine, trimetazidine and suloctidil, respectively. This increase in cytochrome oxidase activity ($P < 0.01$) observed in synaptosomes during ischemia may be ascribed to functional and morphological damages of the phospholipids of mitochondrial membranes [33–36]. Therefore, the analysis of the present results and of the literature data allows to suggest the occurrence in brain tissue of a variety of interrelated factors implicated in the ischemia-induced changes of the maximal rate of the mitochondrial enzymatic activities related to the energy transduction. These include: (a) rearrangement of the enzymatic activities because of the changed metabolic and chemico-

BP 30:17 - B

Table 4. Rat cerebral cortex. Enzymatic activities related to energy transduction in control condition and after both an ischemic period of 20 min duration and i.p. pretreatment with drugs (50 mg/kg) or saline solution. Normothermic ischemia was induced 60 min after the drug injection

Subfraction	Enzymatic activities	Control animals		After 20 min of ischemia and i.p. pretreatment with							
				Saline solution		Vincamine		Trimetazidine		Suloctidil	
		N	S.A.	N	S.A.	N	S.A.	N	S.A.	N	S.A.
Mitochondria	Citrate synthase	10	0.221 ± 0.008	6	0.193 ± 0.018	7	0.171 ± 0.009‡	7	0.179 ± 0.013†	7	0.174 ± 0.009‡
	Malate dehydrogenase	9	3.036 ± 0.069	6	2.495 ± 0.127‡	7	2.532 ± 0.075‡	6	2.637 ± 0.131†	5	2.681 ± 0.105†
	Total NADH-cytochrome *c* reductase	9	0.258 ± 0.005	7	0.155 ± 0.009‡	7	0.174 ± 0.009‡	7	0.148 ± 0.010‡	8	0.160 ± 0.006‡
	Cytochrome oxidase	10	1.645 ± 0.044	7	1.550 ± 0.149	6	1.653 ± 0.107	8	1.613 ± 0.135	6	1.328 ± 0.032‡
	Glutamate dehydrogenase	10	0.325 ± 0.015	7	0.296 ± 0.025	7	0.287 ± 0.018	7	0.290 ± 0.016	8	0.250 ± 0.019‡
Synaptosomes	Lactate dehydrogenase	10	0.527 ± 0.029	6	0.608 ± 0.061	7	0.554 ± 0.038	6	0.534 ± 0.050	7	0.481 ± 0.033
	Malate dehydrogenase	8	1.429 ± 0.075	7	1.298 ± 0.102	6	1.472 ± 0.124	6	1.396 ± 0.096		1.365 ± 0.074
	Acetylcholine esterase	11	0.137 ± 0.005	8	0.111 ± 0.010*	8	0.113 ± 0.004‡	6	0.112 ± 0.010*	8	0.123 ± 0.004

The specific enzymatic activities [S.A. = μmoles.min^{-1}.(mg protein)$^{-1}$] were evaluated both in the purified mitchondrial fraction and in the crude synaptosomal fraction, and are expressed as the mean values ± S.E.M. for each group of N animals.

Differs from control rats: * $P < 0.05$; † $P < 0.02$; ‡ $P < 0.01$.

Table 5. Rat cerebral cortex. Enzymatic activities related to energy transduction in control conditions and after both an ischemic period of 40 min duration and i.p. pretreatment with drugs (50 mg/kg) or saline solution. Normothermic ischemia was induced 60 min after the drug injection

Subfraction	Enzymatic activities	Control animals		After 40 min of ischemia and i.p. pretreatment with: Saline solution		Vincamine		Trimetazidine		Suloctidil	
		N	S.A.	N	S.A.	N	S.A.	N	S.A.	N	S.A.
Mitochondria	Citrate synthase	8	0.275 ± 0.020	6	0.240 ± 0.027	7	0.227 ± 0.020	6	0.248 ± 0.025	5	0.216 ± 0.017
	Malate dehydrogenase	9	2.904 ± 0.088	6	2.784 ± 0.084	6	2.586 ± 0.107*	6	2.710 ± 0.115	7	2.515 ± 0.061‡
	Total NADH-cytochrome *c* reductase	9	0.351 ± 0.025	7	0.226 ± 0.024‡	6	0.209 ± 0.030‡	6	0.226 ± 0.020‡	7	0.203 ± 0.013‡
	Cytochrome oxidase	7	1.844 ± 0.140	7	1.558 ± 0.102	7	1.461 ± 0.106*	6	1.506 ± 0.062	5	1.348 ± 0.158*
	Glutamate dehydrogenase	8	0.395 ± 0.027	7	0.331 ± 0.018	7	0.315 ± 0.020*	6	0.305 ± 0.027*	7	0.345 ± 0.013
Synaptosomes	Lactate dehydrogenase	8	0.525 ± 0.024	7	0.543 ± 0.032	7	0.499 ± 0.036	7	0.468 ± 0.033	7	0.512 ± 0.046
	Malate dehydrogenase	6	1.473 ± 0.079	7	1.347 ± 0.092	6	1.373 ± 0.111	6	1.185 ± 0.087*	5	1.207 ± 0.065*
	Acetylcholine esterase	9	0.122 ± 0.001	8	0.087 ± 0.001†	8	0.088 ± 0.001†	7	0.073 ± 0.001‡	7	0.076 ± 0.001‡

The specific enzymatic activities [S.A. = μmoles.min^{-1}.(mg protein)$^{-1}$ were evaluated both in the purified mitochondrial fraction and in the crude synaptosomal fraction, and are expressed as the mean values ± S.E.M. for each group of N animals.

Differs from control rats: * $P < 0.05$; † $P < 0.02$; ‡ $P < 0.01$.

physical conditions in the cerebral cortex; (b) swelling and lysis of mitochondria due to an increase of free fatty acids [31, 37, 38] and of free radical oxidation processes of unsaturated fatty acids [30, 35, 36], with change of membrane permeability and uncoupling of oxidative phosphorylation [31, 37].

Furthermore, another of the objectives of this study was to evaluate the possible effect of some drugs during complete ischemia. As concerns the enzymes tested, the possible changes induced by the drugs studied in the present research should be observed particularly after 5 or 10 min of ischemia; at the subsequent times the physiopathological phenomena prevailed over the pharmacological treatments carried out. Vincamine TPS increased the malate dehydrogenase activity in the purified mitochondrial fraction from cerebral cortex of normal rats. This action occurred in the brain of 10 min ischemic rats, but not at the subsequent times of ischemia. Furthermore, vincamine decreased the activity of acetylcholine esterase in the crude synaptosomal fraction from cerebral cortex of normal rats. Ischemia itself resulted in a decrease in the activity of this enzyme and vincamine pretreatment did not change this behaviour neither magnified the statistical difference from control values. Pretreatment with the drug induced a decrease of citrate synthase after 20 min of ischemia and of both cytochrome oxidase and glutamate dehydrogenase after 40 min of ischemia, these enzymatic activities being unaffected at the previous time of ischemic damage. On the other hand, also the pretreatment with suloctidil showed a biphasic time course of action during ischemia. In fact, the drug prevented and reversed the decrease induced by 5 min ischemia on the cerebral total NADH cytochrome *c* reductase activity. Besides, at this time the drug increased the activity of mitochondrial and synaptosomal malate dehydrogenase and of mitochondrial citrate synthase at values higher than those of normal rats. However, at the subsequent times of ischemia, the trend of suloctidil action resulted in an inhibition of both the above-quoted enzymatic activities and of the cytochrome oxidase and glutamate dehydrogenase activities, the effect being much less evident after 40 min of ischemia. Also the effects induced by trimetazidine DC are quite different according to the time of ischemia duration, although the trend of the drug action is an inhibitory one. In fact, pretreatment with trimetazidine resulted in a decrease of lactate dehydrogenase activity at the early period after ischemia induction, while the inhibition of citrate synthase, cytochrome oxidase and glutamate dehydrogenase activities were delayed and decreased at the last time of ischemia. At present it is impossible to establish whether drugs action is related to a rearrangement of enzymes activities or to changes in mitochondrial membrane structures. In any case it should be stressed that the present data on vincamine, suloctidil and trimetazidine action on cerebral enzymatic activities are consistent with the effects induced on the same enzymes by subchronic treatment performed in normal adult rats [39].

Acknowledgements—We thank Mrs. G. Garlaschi, Mr. L. Maggi and G. Arioli for technical assistance.

REFERENCES

1. C. Dennis and H. Kabat, *Proc. Soc. exp. Biol. N.Y.* **40**, 559 (1939).
2. R. C. Grenell, *J. Neuropath. exp. Neurol.* **5**, 131 (1946).
3. H. Hirsch, K. H. Euler and M. Schneider, *Pflügers Arch. ges. Physiol.* **265**, 281 (1957).
4. L. M. Weinberger, M. H. Gibbon and J. H. Gibbon, *Arch. Neurol. Psychiat. (Chic.)* **43**, 961 (1940).
5. C. Heymans, *Physiol. Rev.* **30**, 375 (1950).
6. H. Kabat, C. Dennis and A. B. Baker, *Am. J. Physiol.* **132**, 737 (1941).
7. U. Müller, W. Isselhard, D. H. Hinzen and E. Geppert, *Pflügers Arch. ges. Physiol.* **320**, 181 (1970).
8. D. H. Hinzen, U. Müller, P. Sobotka, E. Gebert, R. Lang and H. Hirsch, *Am. J. Physiol.* **223**, 1158 (1972).
9. K. A. Hossmann and Y. Olsson, *Brain Res.* **22**, 313 (1970).
10. C Benzi, E. Arrigoni, A. Ferrara and P. Mascherpa, *J. pharm. Sci.* **58**, 1161 (1969).
11. B. Ljunggren, R. A. Ratcheson and B. K. Siesjö, *Brain Res.* **73**, 291 (1974).
12. C.-H. Nordström, S. Rehncrona and B. K. Siesjö, *J. Neurochem.* **30**, 479 (1978).
13. A. Gromek and A. Pastuszko, *J. Neurochem.* **28**, 429 (1977).
14. R. Jope and J. P. Blass, *Biochem. J.* **150**, 397 (1975).
15. M. Rossowska, W. Lewandowski and Z. Dabrowiecki, *Bull. Acad. pol. Sci., ser. sci. biol.* **24**, 691 (1976).
16. M. Rossowska and Z. Dabrowiecki, *J. Neurochem.* **30**, 1203 (1978).
17. E. De Robertis, A. Pellegrino De Iraldi, G. Rodriguez de Lores Arnaiz and L. Salganicoff, *J. Neurochem.* **9**, 23 (1962).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
19. P. H. Sugden and E. A. Newsholme, *Biochem. J.* **150**, 105 (1975).
20. S. Ochoa, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan) Vol. 1, pp. 735–739. Academic Press, New York (1955).
21. A. Nason and F. D. Vasington, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan) Vol. 6, pp. 409–415. Academic Press, New York (1963).
22. L. Smith, in *Methods of Biochemical Analysis* (Ed. D. Glik) Vol. 2, pp. 427–434. Wiley Interscience, New York (1955).
23. D. C. Wharton and A. Tzagoloff, in *Methods in Enzymology* (Eds. H. R. V. Estabrook and M. E. Pullman) Vol. 10, pp. 245–250. Academic Press, New York (1967).
24. H. U. Bergmeyer and E. Bernt, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer) Vol. 2, pp. 574–579. Academic Press, New York (1974).
25. G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherstone, *Biochem. Pharmac.* **7**, 88 (1961).
26. H. Ksiezak, *FEBS Lett.* **63**, 149 (1976).
27. T. C. Linn, F. H. Pettit, F. Hucho and L. J. Reed, *Proc. natn. Acad. Sci. U.S.A.* **64**, 227 (1969).
28. E. A. Siess, J. Wittmann and O. H. Wieland, *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 447 (1971).
29. C. S. Tsai, M. W. Burgett and L. J. Reed, *J. biol. Chem.* **248**, 8348 (1973).
30. M. D. Majewska, J. Strosznajder and J. Lazarewicz, *Brain Res.* **158**, 423 (1978).
31. M. D. Majewska, A. Gromek and J. Strosznajder, *Bull. Acad. pol. Sci., ser. sci. biol.* **22**, 267 (1974).
32. N. G. Bazan Jr., *Biochim. biophys. Acta.* **218**, 1 (1970).
33. Z. P. Cheremishina and Yu. A. Vladimirov, *Biochimija* **40**, 242 (1975).
34. W. G. Hanstein and Y. Hatefi, *Arch. Biochem.* **138**, 87 (1970).

35. S. P. Fortney and W. S. Lynn, *Arch. Biochem.* **104**, 241 (1964).
36. F. E. Hunter, J. M. Gebicki, P. E. Hoffstein, I. Weinstein and A. Scott, *J. biol. Chem.* **238**, 828 (1968).
37. A. Gromek, M. D. Majewska, Z. Czernicki, J. Jurkiewicz and A. Kunicki, *Bull. Acad. pol. Sci., ser. sci. biol.* **21**, 701 (1973).
38. M. D. Majewska, J. Lazarewicz and J. Strosznajder, *Bull. Acad. pol. Sci., ser. sci. biol.* **25**, 125 (1977).
39. G. Benzi, E. Arrigoni, F. Dagani, F. Marzatico, D. Curti, S. Raimondo, M. Dossena, M. Polgatti and R. F. Villa, *Exp. Geront.* **15**, 593 (1980).