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EFFECT OF SOME DRUGS ON CEREBRAL ENERGY STATE DURING AND AFTER HYPOXIA AND COMPLETE OR INCOMPLETE ISCHEMIA

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Abstract—The effect of caffeine, nicergoline and medibazine was evaluated in the brain of beagle dogs during various experimental conditions of cerebral damage (hypoxia, hypoxia plus incomplete ischemia, hypoxia plus complete ischemia), and during post-hypoxic recovery and restoration of circulation. The behavior of fuels (glycogen, glucose), of glycolytic pathway intermediates (glucose-6-phosphate, pyruvate) and end-product (lactate), as well as the pool of labile phosphates (ATP, ADP, AMP, creatine phosphate) and the energy charge potential were evaluated in the motor area of the cerebral cortex.

The drugs tested proved unable to improve the deranged brain metabolism and the energy charge potential under these various hypoxemic conditions. On the contrary, a certain pharmacological effect of nicergoline and medibazine could be observed both in the post-hypoxic recovery and in the recovery following hypoxia plus complete ischemia, caffeine being totally ineffective. As for the recovery subsequent to hypoxia plus incomplete ischemia, none of the drugs tested was able to trigger restoration.

INTRODUCTION

If marked hypoxemia in the rat is complicated by unilateral ligation of the common carotid artery, the cerebral hemisphere on the clamped side shows a high lactic acidosis and a derangement in its energy state [1]. In this condition, neuronal cells develop changes of ischemic type demonstrating an irreversible damage, probably due to tissue hypoxia complicated by incomplete ischemia [2]. The resulting acidosis is more pronounced than that observed in total ischemia and adversely affects the tissue via its effect on autolytic processes. The detailed mechanisms of such an hypothetical effect are entirely unknown, and the proposed coupling between acidosis and cell damage is speculative [3, 4]. On the other hand, neurophysiological research in the cat has shown that, if cerebral circulation is only incompletely interrupted, recovery following ischemia is impaired [5, 6]. It was postulated that this is due to the presence of aggregates in cerebral vessels, but this hypothesis has also remained unproved and contradictory results have been reported [7].

A comparative study in the dog on hypoxemia, hypoxemia plus incomplete ischemia, and hypoxemia plus complete ischemia revealed different biochemical modifications at brain level, the only common element being glycogen depletion. Particularly, restoration of air ventilation and circulation after hypoxia plus incomplete ischemia does not allow the partial reversion, which on the contrary takes place following pure hypoxia, or hypoxia plus complete ischemia. It was hypothesized that the derangement primarily induced by physiopathological conditions concerns the function of energy metabolism feed-back enzymes, leading to the formation of abnormal amounts of intermediates or end-products [8].

In spite of differences in the interpretation of the pathogenesis of these phenomena, all the cerebral biochemical findings reported in the papers quoted are in quite good agreement. In this study, we have evaluated the effect of some drugs (caffeine, nicergoline, and medibazine, as examples of xanthine, lysergic and piperazine substances, respectively) in beagle dogs during various experimental conditions of cerebral damage, such as hypoxia, hypoxia plus incomplete ischemia, hypoxia plus complete ischemia, post-hypoxic recovery and restoration of circulation. The behavior of fuels (glycogen, glucose), of glycolytic pathway intermediates (glucose-6-phosphate, pyruvate) and end-product (lactate), as well as the pool of labile phosphates (ATP, ADP, AMP, creatine phosphate) and the energy charge potential were studied in the motor area of the cerebral cortex.

MATERIALS AND METHODS

Animals. The experiments were carried out on female beagle dogs aged 1.5 yr and weighing 10.6 to 14.6 kg. Before the experiments the dogs were maintained under standard environmental conditions (temperature = 22 ± 1°; relative humidity = 60 ± 5%) and fed only a standard diet as pellets (Altromin, Rieper) with water *ad libitum*.

Anesthesia and operative procedure. Operative technique, pre-anesthesia (urethane 0.4 g/kg i.p.), anesthesia during the operative procedure (chloralose 20–40 mg/kg i.v. plus nitrous oxide) and curarization (gallamine triethiodide 2–3 mg/kg i.v.) were described and discussed in a previous work [8]. An accurate surgical and anesthetic procedure was followed to protect the animals from any kind of suffering, both for ethical reasons and because the pain stress would

Table 1. Cerebral cortex of motor area of beagle dog (in hypovolemic hypotension: mean arterial blood pressure = 55 ± 4 mm Hg). Behaviour of some biochemical parameters in control condition and at the end of a 15 min period of intracarotid perfusion (0.1 ml min^{-1} kg^{-1}) with caffeine (1×10^{-3} M), nicergoline (5×10^{-5} M) or medibazine (5×10^{-4} M)

Biochemical parameters ($\mu mol.g^{-1}$)	Control condition (a)	Perfusion with caffeine (b)	Perfusion with nicergoline (c)	Perfusion with medibazine (d)
Glycogen	3.39 ± 0.30	2.95 ± 0.07	3.11 ± 0.12	3.17 ± 0.18
Glucose	5.12 ± 0.10	4.37 ± 0.05[1]	5.08 ± 0.07	4.85 ± 0.20
Glucose-6-phosphate	0.129 ± 0.007	0.151 ± 0.008	0.138 ± 0.003	0.131 ± 0.011
Pyruvate	0.113 ± 0.007	0.117 ± 0.001	0.121 ± 0.002	0.114 ± 0.004
Lactate	2.09 ± 0.34	2.66 ± 0.29	2.51 ± 0.29	2.05 ± 0.23
ATP	2.35 ± 0.04	2.41 ± 0.03	2.41 ± 0.09	2.45 ± 0.03
ADP	0.39 ± 0.02	0.43 ± 0.02	0.42 ± 0.03	0.36 ± 0.02
AMP	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Creatine phosphate	4.55 ± 0.09	4.80 ± 0.19	4.43 ± 0.17	4.57 ± 0.07
Energy charge potential	0.904 ± 0.006	0.899 ± 0.004	0.894 ± 0.008	0.906 ± 0.004

Mean ± standard error of three preparations (four assays per preparation). Statistical difference: 1 = (b) or (c) or (d) versus (a); 2 = (c) or (d) versus (b); 3 = (d) versus (c).

have upset the animals' cerebral biochemical conditions. On the other side, it was necessary to avoid the uncontrolled use of general anesthetics since, during the actual experiments, they would have brought about the well-known actions on the cerebral biochemical pattern.

Experimental plan. We evaluated the cerebral biochemical parameters in dogs before, during, and after hypoxemia or hypoxemia plus incomplete or complete ischemia. The three experimental periods were: (1) steady-state period of hypovolemic hypotension (mean arterial blood pressure = 55 ± 4 mm Hg) of 15 min, during air ventilation. At the end of this period, samples for control determinations were obtained; (2) 15 min of acute hypoxia induced by altering the inspired oxygen concentration (5% oxygen–nitrogen mixture) to induce and maintain a PaO_2 ranging from 17 to 19 mmHg. During the last six min of acute hypoxia, in some animals a condition of incomplete or complete ischemia was induced. Incomplete ischemia was induced by bilateral carotid (below the cannulated superior thyroid arteries) and vertebral arteries clamping or ligature. This procedure reduced the blood flow in the cortical region to less than 20 per cent of the control value (range = 12 to 18 per cent). Complete ischemia was induced by increasing the cerebrospinal fluid pressure to values above the experimental arterial blood pressure; (3) three minutes of resumption of respiration with air (after pure hypoxemia) and restoration of circulation by removing the artery clamps (after hypoxemia plus incomplete ischemia) or by reducing spinal fluid pressure to normal values (after hypoxemia plus complete ischemia). Saline solution or drugs were perfused through the superior thyroid arteries, at the rate of 0.1 ml min^{-1} kg^{-1} (at the molar concentration indicated below), during 15 min (hypoxemia with or without ischemia) or 18 min (*idem* plus 3 min of restoration).

Analytical techniques. At the set time, a portion of the motor area of the cortex was cut *in situ* by means of a rotating hollow tube. The isolated cerebral material was quickly (< 0.5 sec) removed by means of a clamp cooled in liquid nitrogen. The removed portion of cerebral tissue was then immediately immersed into liquid nitrogen for 10 to 15 min. The cortical portion of the frozen tissue was isolated and quickly (3–4 sec) powdered in a precooled automatic apparatus (Microdismembrator, Braun) using frozen 1.23 M perchloric acid. The subsequent steps were carried out in a precooled box at 0–5° until a neutral perchlorate-free extract was obtained, which was then used for immediate enzymatic analyses of: glycogen [9]; glucose [10]; glucose-6-phosphate [11]; pyruvate [12]; lactate [13]; ATP [14]; ADP, AMP [15]; creatine phosphate [16], using a double-beam recording spectrophotometer (Beckman 25) and a recording fluorescence spectrophotometer (Perkin-Elmer 204-A). Each value was calculated from the mean of at least four determinations performed blindly on the same sample, and allowing a maximum variation of 3 per cent.

Substances used. The following substances were perfused into the carotid arteries, at the indicated molar concentration: (1) 1,3,7-trimethylxanthine hydrochloride: caffeine = 1×10^{-3} M; (2) 1,6-dimethyl-8β-(5-bromonicotinoyl-hydroxymethyl)-10α-methoxyergoline tartrate: nicergoline = 5×10^{-5} M; (3) 1-(diphenylmethyl)-4-piperonyl-piperazine: medibazine = 5×10^{-4} M. The concentrations used were the most active experimental doses employed intravenously in the animals, and were chosen after preliminary tests with magnification $\times 10$ and $\times 10^{-1}$.

Cerebral energy charge potential. The energy charge potential [17] is defined in terms of actual concentrations of the adenine nucleotides as: ([ATP] + 0.5 [ADP])/([ATP] + [ADP] + [AMP]).

Statistical analysis. The Student's '*t*' test was applied to the differences, at the $P < 0.05$ level. Even though three animals per condition and time are the bare minimum upon which to base statistical analysis, this is excused by the nature of the experiment.

RESULTS

Drug action during normoxia. (Table 1). Under control conditions (hypovolemic hypotension during room air ventilation) the cerebral cortex responded

Table 2. Effect of *hypoxia* on the cerebral cortex of motor area of beagle dog (in hypovolemic hypotension: mean arterial blood pressure = 55 ± 4 mm Hg). Behaviour of some biochemical parameters: (1) in control condition; (2) at the end of 15 min of hypoxemia (PaO_2 = 17 to 19 mm Hg), and intracarotid perfusion (0.1 ml min^{-1} kg^{-1}) with saline solution or drugs; (3) after 3 min of post-hypoxic recovery and intracarotid perfusion with saline solution or drugs: caffeine (1×10^{-3} M), nicergoline (5×10^{-5} M) or medibazine (5×10^{-4} M)

Biochemical parameter (μmol. g^{-1})	Control condition	Hypoxemia and intracarotid perfusion with				Post-hypoxic recovery and intracarotid perfusion with			
	(a)	Saline solution (b)	Caffeine (c)	Nicergoline (d)	Medibazine (e)	Saline solution (f)	Caffeine (g)	Nicergoline (h)	Medibazine (i)
Glycogen	3.39 ± 0.30	0.66 ± 0.13[1]	0.23 ± 0.07[1,2]	0.48 ± 0.07[1]	0.68 ± 0.07[1]	0.58 ± 0.08[1]	0.29 ± 0.05[1,3]	0.52 ± 0.06[1]	0.81 ± 0.04[1]
Glucose	5.12 ± 0.10	6.18 ± 0.46	4.70 ± 0.39[2]	5.62 ± 0.29[2]	5.58 ± 0.38	5.48 ± 0.32	4.45 ± 0.25[1,3]	5.62 ± 0.37	5.84 ± 0.57
Glucose-6-P	0.129 ± 0.007	0.183 ± 0.008[1]	0.213 ± 0.012[1,2]	0.204 ± 0.005[1]	0.183 ± 0.006[1]	0.195 ± 0.027[1]	0.242 ± 0.026[1,3]	0.226 ± 0.027[1]	0.172 ± 0.015[1]
Pyruvate	0.113 ± 0.007	0.269 ± 0.024[1]	0.305 ± 0.016[1,2]	0.274 ± 0.014[1]	0.263 ± 0.025[1]	0.174 ± 0.008[1]	0.183 ± 0.008[1]	0.187 ± 0.011[1]	0.168 ± 0.012[1]
Lactate	2.09 ± 0.34	20.59 ± 1.36[1]	24.13 ± 1.47[1]	21.34 ± 1.19[1]	19.71 ± 1.55[1]	13.02 ± 0.58[1]	15.81 ± 1.03[1]	16.03 ± 0.75[1,3]	12.82 ± 1.46[1]
ATP	2.35 ± 0.04	1.79 ± 0.12[1]	1.55 ± 0.03[1,2]	1.54 ± 0.05[1,2]	1.73 ± 0.07[1]	1.78 ± 0.05[1]	1.93 ± 0.05[1]	2.14 ± 0.05[1,3]	2.17 ± 0.01[1,3]
ADP	0.39 ± 0.02	0.59 ± 0.02[1]	0.57 ± 0.03[1]	0.62 ± 0.08[1]	0.62 ± 0.02[1]	0.37 ± 0.13	0.36 ± 0.08	0.29 ± 0.03[1,3]	0.31 ± 0.03[1,3]
AMP	0.08 ± 0.01	0.53 ± 0.10[1]	0.67 ± 0.03[1]	0.65 ± 0.09[1]	0.55 ± 0.07[1]	0.32 ± 0.03[1]	0.36 ± 0.10[1]	0.14 ± 0.04[3]	0.16 ± 0.03[1,3]
P-Creatine	4.55 ± 0.09	2.11 ± 0.16[1]	2.04 ± 0.18[1]	2.13 ± 0.11[1]	2.14 ± 0.12[1]	2.94 ± 0.08[1]	2.64 ± 0.06[1]	3.77 ± 0.12[1,3]	3.55 ± 0.35[1,3]
Energy charge potential	0.904 ± 0.006	0.715 ± 0.032[1]	0.658 ± 0.011[1]	0.658 ± 0.023[1]	0.703 ± 0.026[1]	0.798 ± 0.009[1]	0.796 ± 0.028[1]	0.891 ± 0.016[3]	0.880 ± 0.006[3]

Mean ± standard error of three preparations (four assays per preparation). Statistical difference ($P < 0.05$): 1 = from (b) to (i) versus (a); 2 = (c), (d) or (e) versus (b); 3 = (g), (h) or (i) versus (f).

Glucose-6-P = Glucose-6-phosphate; P-Creatine = Creatine phosphate.

to intracarotid perfusion with caffeine, nicergoline, medibazine with no changes in the cerebral biochemical parameters tested. The energy charge potential remained unchanged indicating that during drug perfusion the rate of ATP synthesis matched the rate of ATP utilization.

Drug action during and after hypoxemia (Table 2). In the hypoxic brain, the cerebral cortex showed a decrease in glycogen and an increase in both glucose-6-phosphate and pyruvate. The increase in lactate can be related to the activation of both glycogenolysis and glycolysis. This activation induced only a partial recharge of the cerebral energy utilized during hypoxia, because stored energy decreased. Caffeine, nicergoline or medibazine intracarotid perfusion (during the 15 min-period of hypoxemia) induced no favourable changes in the cerebral biochemical pattern. In spite of the decrease in ATP concentration, both caffeine and nicergoline induced no significant change in the energy charge potential. Caffeine intracarotid perfusion increased glucose-6-phosphate and pyruvate, and decreased glycogen.

Three minutes after the resumption of room-air ventilation there was an incomplete restoration of biochemical parameters and the energy charge. The intracarotid perfusion with nicergoline or medibazine (during the 15 min-period of hypoxemia and the 3 min-period of resumption of room air ventilation) increased the energy charge potential to values near that of the control. Both drugs increased ATP and creatine phosphate, while they decreased ADP and AMP cerebral concentrations. The intracarotid perfusion with caffeine induced no changes in the energy charge, in spite of the glycogen and glucose depletion.

Drug action during and after hypoxemia plus incomplete ischemia (Table 3). After 15 min of brain hypoxia

Table 3. Effect of *hypoxia plus incomplete ischemia* on the cerebral cortex of motor area of beagle dog (in hypovolemic hypotension: mean arterial blood pressure = 55 ± 4 mm Hg). Behaviour of some biochemical parameters: (1) in control condition; (2) at the end of 15 min of hypoxemia (PaO_2 = 17 to 19 mm Hg) plus 6 min of incomplete ischemia, and intracarotid perfusion (0.1 ml min^{-1} kg^{-1}) with saline solution or drugs; (3) after 3 min of both post-hypoxic recovery and complete recirculation, and intracarotid perfusion with saline solution or drugs: caffeine (1×10^{-3} M), nicergoline (5×10^{-5} M) or medibazine (5×10^{-4} M)

Biochemical parameter (μmol. g^{-1})	Control condition	Hypoxemia plus incomplete ischemia and perfusion with				Post-hypoxic recovery and complete recirculation and intracarotid perfusion with			
	(a)	Saline solution (b)	Caffeine (c)	Nicergoline (d)	Medibazine (e)	Saline solution (f)	Caffeine (g)	Nicergoline (h)	Medibazine (i)
Glycogen	3.39 ± 0.30	0.54 ± 0.08[1]	0.20 ± 0.01[1,2]	0.27 ± 0.05[1,2]	0.60 ± 0.17[1]	0.48 ± 0.06[1]	0.24 ± 0.02[1,3]	0.36 ± 0.08[1]	0.63 ± 0.14[1]
Glucose	5.12 ± 0.10	4.82 ± 0.40	4.62 ± 0.28	4.52 ± 0.32	4.78 ± 0.41	4.65 ± 0.35	4.31 ± 0.43	4.38 ± 0.49	4.56 ± 0.34
Glucose-6-P	0.129 ± 0.007	0.234 ± 0.024[1]	0.237 ± 0.010[1]	0.202 ± 0.003[1]	0.242 ± 0.038[1]	0.236 ± 0.023[1]	0.240 ± 0.016[1]	0.218 ± 0.027[1]	0.225 ± 0.008[1]
Pyruvate	0.113 ± 0.007	0.084 ± 0.005[1]	0.098 ± 0.002	0.093 ± 0.004	0.085 ± 0.004[1]	0.145 ± 0.024	0.155 ± 0.025[1]	0.130 ± 0.009	0.119 ± 0.012
Lactate	2.09 ± 0.34	34.63 ± 1.95[1]	38.34 ± 1.49[1]	39.49 ± 1.17[1]	33.12 ± 2.13[1]	32.20 ± 2.53[1]	41.05 ± 2.30[1,3]	42.13 ± 2.45[1,3]	36.41 ± 3.53[1]
ATP	2.35 ± 0.04	0.86 ± 0.04[1]	0.92 ± 0.03[1]	0.94 ± 0.05[1]	0.99 ± 0.06[1]	0.87 ± 0.06[1]	0.70 ± 0.03[1]	0.82 ± 0.04[1]	0.89 ± 0.08[1]
ADP	0.39 ± 0.02	0.94 ± 0.06[1]	0.96 ± 0.06[1]	0.90 ± 0.03[1]	0.89 ± 0.03[1]	0.91 ± 0.08[1]	1.06 ± 0.04[1]	0.96 ± 0.06[1]	0.89 ± 0.05[1]
AMP	0.08 ± 0.01	1.20 ± 0.09[1]	1.10 ± 0.07[1]	1.09 ± 0.06[1]	1.05 ± 0.04[1]	1.09 ± 0.08[1]	1.36 ± 0.10[1]	1.27 ± 0.11[1]	1.12 ± 0.04[1]
P-Creatine	4.55 ± 0.09	0.42 ± 0.05[1]	0.61 ± 0.16[1]	0.70 ± 0.12[1]	0.34 ± 0.04[1]	0.58 ± 0.10[1]	0.47 ± 0.10[1]	0.57 ± 0.11[1]	0.59 ± 0.19[1]
Energy charge potential	0.904 ± 0.006	0.444 ± 0.022[1]	0.469 ± 0.014[1]	0.474 ± 0.019[1]	0.490 ± 0.012[1]	0.463 ± 0.019[1]	0.394 ± 0.019[1]	0.428 ± 0.013[1]	0.460 ± 0.019[1]

Mean ± standard error of three preparations (four assays per preparation). Statistical difference ($P < 0.05$): 1 = from (b) to (i) versus (a); 2 = (c), (d) or (e) versus (b); 3 = (g), (h) or (i) versus (f).

Glucose-6-P = Glucose-6-phosphate; P-Creatine = Creatine phosphate.

complicated by incomplete ischemia (during the last 6 min of hypoxemia), the cerebral cortex showed an increase in glucose-6-phosphate and lactate, and a decrease in glycogen and pyruvate. Furthermore, glucose did not decrease below control values, thus ruling out the presence of ischemia at a degree capable of limiting substrate supply. ATP and creatine phosphate decreased while ADP and AMP increased, causing a drop of the energy charge potential. The intracarotid perfusion with caffeine, nicergoline or medibazine induced no changes in the cerebral parameters tested, except for the higher glycogen depletion induced by both caffeine and nicergoline.

Three minutes after the restoration of both circulation and room air ventilation there was no cerebral biochemical restitution. This was not aided by the intracarotid perfusion (during the 15 min-period of hypoxemia and the subsequent 3 min-period of recovery) with the drugs tested.

Drug action during and after hypoxemia plus complete ischemia (Table 4). After 15 min of brain hypoxia complicated by complete ischemia (during the last 6 min of hypoxemia), the cerebral cortex showed an increase in lactate and a decrease in glycogen, glucose, glucose-6-phosphate and pyruvate. Both creatine phosphate and ATP decreased, while ADP and AMP increased, the energy charge potential falling to a low value. Except for the interference of caffeine and nicergoline on glucose-6-phosphate, the intracarotid perfusion with the drugs tested induced no changes in this biochemical derangement.

Three minutes after the restoration of both circulation and room air ventilation, the cerebral cortex showed a partial restoration with an increase in glucose, glucose-6-phosphate and pyruvate, and a decrease of lactate. The energy charge potential, creatine phosphate and ATP increased, while AMP decreased. The intracarotid perfusion (during the 15 min of hypoxemia and the subsequent 3 min of recovery) with medibazine and nicergoline increased the energy charge potential, medibazine also influencing glycogen recovery. The intracarotid perfusion with caffeine was completely ineffective.

DISCUSSION

Before analyzing the results obtained, we stress that they concern only the motor area of the cerebral cortex. Also, the acute experimental model used envisaged a single contact with the drug during a short period of time such as 15 min (hypoxia) or 18 min (hypoxia plus recovery). Finally, we did not evaluate the pharmacokinetic distribution of the various drugs in the cerebral area examined. Rather, we carried out a comparison between the presence of these agents in cerebral blood and their effects on biochemical pattern at cerebral level.

Under the various hypoxemic conditions studied (hypoxia, hypoxia plus incomplete ischemia, hypoxia plus complete ischemia), caffeine, nicergoline and medibazine failed to improve the deranged brain metabolism, in agreement with the results obtained with other substances in cerebral hypoxia [18–20]. A certain pharmacological effect of nicergoline and medibazine could be observed both in the post-hypoxic recovery and in the restitution following hypoxia plus complete ischemia. On the contrary, none of the drugs tested was able to trigger restoration after hypoxia plus incomplete ischemia. With regard to this physiopathological condition, nicergoline does not trigger restoration though it is able to increase the cerebral blood flow [21–23] and possesses platelet anti-aggregating properties [24–26]. These properties do not appear to be in agreement with the hypothesis according to which the absence of restoration following incomplete ischemia would be of vascular origin [5, 6] due to the presence of blood or platelet aggregates in the vessels, impeding the restoration of circulation. One might therefore think that the hypotheses of an autolytic acidosis

Table 4. Effect of *hypoxia plus complete ischemia* on the cerebral cortex of motor area of beagle dog (in hypovolemic hypotension: mean arterial blood pressure = 55 ± 4 mm Hg). Behaviour of some biochemical parameters: (1) in control condition; (2) at the end of 15 min of hypoxemia plus 6 min of complete ischemia, and intracarotid perfusion (0.1 ml. min^{-1} kg^{-1}) with saline solution or drugs; (3) after 3 min of post-hypoxic recovery and complete recirculation, and intracarotid perfusion with saline solution or drugs: caffeine (1×10^{-3} M), nicergoline (5×10^{-5} M) or medibazine (5×10^{-4} M)

Biochemical parameter (μmol.g^{-1})	Control condition	Hypoxemia plus complete ischemia and perfusion with				Post-hypoxic recovery and complete recirculation and intracarotid perfusion with			
		Saline solution	Caffeine	Nicergoline	Medibazine	Saline solution	Caffeine	Nicergoline	Medibazine
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
Glycogen	3.39 ± 0.30	0.29 ± 0.06[1]	0.22 ± 0.02[1]	0.21 ± 0.03[1]	0.27 ± 0.03[1]	0.36 ± 0.04[1]	0.20 ± 0.04[1]	0.26 ± 0.05[1]	0.56 ± 0.10[1,3]
Glucose	5.12 ± 0.10	0.22 ± 0.05[1]	0.19 ± 0.03[1]	0.24 ± 0.07[1]	0.26 ± 0.08[1]	1.45 ± 0.23[1]	1.34 ± 0.12[1]	1.71 ± 0.27[1]	1.54 ± 0.30[1]
Glucose-6-P	0.129 ± 0.007	0.044 ± 0.006[1]	0.082 ± 0.005[1,2]	0.080 ± 0.012[1,2]	0.042 ± 0.005[1]	0.204 ± 0.017[1]	0.245 ± 0.023[1]	0.265 ± 0.031[1]	0.214 ± 0.010[1]
Pyruvate	0.113 ± 0.007	0.026 ± 0.004[1]	0.021 ± 0.002[1]	0.025 ± 0.009[1]	0.023 ± 0.004[1]	0.193 ± 0.016[1]	0.235 ± 0.009[1]	0.226 ± 0.007[1]	0.190 ± 0.011[1]
Lactate	2·09 ± 0.34	24.14 ± 1.00[1]	24.64 ± 0.79[1]	23.98 ± 2.79[1]	22.95 ± 0.86[1]	17.26 ± 1.33[1]	19.92 ± 0.17[1]	19.85 ± 0.64[1]	16.34 ± 0.62[1]
ATP	2.35 ± 0.04	0.58 ± 0.10[1]	0.54 ± 0.04[1]	0.59 ± 0.10[1]	0.64 ± 0.09[1]	1.42 ± 0.09[1]	1.26 ± 0.07[1]	1.71 ± 0.09[1,3]	1.65 ± 0.12[1]
ADP	0.39 ± 0.02	0.55 ± 0.16	0.64 ± 0.09[1]	0.62 ± 0.10[1]	0.57 ± 0.13[1]	0.81 ± 0.06[1]	0.94 ± 0.04[1]	0.52 ± 0.03[1,3]	0.47 ± 0.05[1,3]
AMP	0.08 ± 0.01	1.86 ± 0.07[1]	1.77 ± 0.09[1]	1.70 ± 0.05[1]	1.62 ± 0.08[1]	0.80 ± 0.20[1]	0.81 ± 0.07[1]	0.28 ± 0.02[1,3]	0.42 ± 0.07[1,3]
P-Creatine	4.55 ± 0.09	< 0.10	< 0.10	< 0.10	< 0.10	1.79 ± 0.08[1]	1.20 ± 0.19[1]	2.12 ± 0.21[1,3]	2.23 ± 0.25[1,3]
Energy charge potential	0.904 ± 0.006	0.285 ± 0.008[1]	0.290 ± 0.006[1]	0.310 ± 0.019[1]	0.325 ± 0.013[1]	0.605 ± 0.065[1]	0.575 ± 0.020[1]	0.785 ± 0.013[1,3]	0.743 ± 0.019[1,3]

Mean ± standard error of three preparations (four assays per preparation). Statistical difference ($P < 0.05$): 1 = from (b) to (i) versus (a); 2 = (c), (d) or (e) versus (b); 3 = (h) or (i) versus (f).

Glucose-6-P = Glucose-6-phosphate; P-Creatine = Creatine phosphate.

process [3, 4] or of an enzymatic derangement [8] are more realistic.

The persistence of a cerebral enzymatic derangement during restitution after incomplete ischemia [8] might perhaps account for the lack of effects by medibazine and nicergoline, which were, on the contrary, active after hypoxic hypoxia and after complete ischemia. At any rate, independently of the actual mechanism of action of nicergoline or medibazine, one might hypothesize that drug action in general on fuels, intermediates and end-products may be mainly due to a direct or indirect interference with the activity of the enzymes catalyzing the substrate degradation sequences. Thus, the ability of ergoline [27] and especially of methylxanthines [28, 29] to interfere, e.g., with the adenyl cyclase system is shown by the activation of glycogenolysis, leading to a reduced concentration of cerebral glycogen (Tables 2 and 3). Medibazine exhibited the opposite trend, even though cerebral glycogen was significantly increased under one experimental condition only (Table 4). On the other hand, this specific role of enzyme interference is demonstrated also by phenobarbital, which causes the storage of glycogen in the brain, since it inhibits phosphorylase while failing to affect glycogen synthase I [30].

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