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EFFECT OF CHRONIC TREATMENT WITH SOME DRUGS ON THE ENZYMATIC ACTIVITIES OF THE RAT BRAIN

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Abstract—In untreated and treated rats, age-dependent changes of some cerebral enzymatic activities (lactate dehydrogenase; citrate synthase and malate dehydrogenase; total NADH–cytocrome *c* reductase and cytochrome oxidase) were studied in the homogenate *in toto* and in the crude mitochondrial fraction of the brain from the 16th to the 28th week of age, at 4-week intervals. All the activities studied exhibited a natural peak around the 20th week of life, and subsequently they decreased to lower values. The tested drugs (medibazine, trimetazidine, (−)eburnamonine, papaverine, suloctidil, bamethan, inositol niacinate, and UDP-glucose) were administered daily for periods of 4, 8 or 12 weeks each (16–20, 16–24, 16–28 or 24–28 weeks of life) by intraperitoneal route and at one dose level (1 or 5 mg/kg). The drugs tested exerted different effects in the various administration periods, thus enabling us to differentiate drug action on some important cerebral enzymatic activities after chronic treatment.

Pharmacological investigations performed with acute models on the energy and redox states of the brain have shown that effects on vessels should be dissociated from effects on the biochemical pattern [1]. Furthermore, no drug-induced activation can be found on energy charge, either normal or modified by ischemia or acute hypoxia, whereas this may be present during the recovery period [1–4]. This fact increases the questionability of evaluation of the action on cerebral energy metabolism based on drug ability to affect cerebral blood flow, both in acute and in chronic studies. Furthermore, cerebral deficiencies of maturity and senescence are related more to a failure of metabolic functions to adapt to age, than to a primary blood flow insufficiency [5–7]. Acute investigations on the effect of drugs on cerebral metabolism use hypoxia models (hypoxic, anemic or ischemic hypoxia) and evaluate at brain level the modifications undergone by substrates, glycolytic intermediates, intermediates of the carboxylic acid cycle, energy mediators, etc. [1–4, 8–14]. To complement and improve this approach, chronic models can be developed [6, 15] with regard to natural changes dependent on maturation of brain metabolism, both in terms of function and of regulation, in connection with the behavior of enzymatic activities (for a review see [16]) as an expression of genomic [17], hormonal and pharmacologic regulation [18].

As for the choice of the biochemical parameters related to cerebral metabolism, tissular enzymatic activities prove reliable for interpretation purposes, since they condition the rate of cerebral biochemical reactions in which modifications in substrates, intermediates and end-products represent the effect of their actions.

In the present research, the enzymatic activities studied were: lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27) for the glycolytic pathway; citrate synthase (citrate oxaloacetate-lyase, EC 4.1.3.7) and malate dehydrogenase (L-malate: NAD^+ oxidoreductase, EC 1.1.1.37) for the Krebs' cycle; total NADH-cytochrome *c* reductase (NADH–cytochrome *c*: oxygen oxidoreductase, EC 1.6.99.3) and cytochrome oxidase (ferrocytochrome *c*: oxygen oxidoreductase, EC 1.9.3.1) for the electron transport chain. Some of the enzymatic activities were evaluated in both the homogenate *in toto* and the mitochondrial fraction, since many of them are variously located in the cytoplasm, and since the metabolic behavior of a cell-free system might respond to substrates and cofactors differently in comparison to the homogenate *in toto* [19]. The tested drugs (medibazine, trimetazidine, (−)eburnamonine, papaverine, suloctidil, bamethan, inositol niacinate, UDP-glucose) were chosen because of their vascular and/or metabolic action on the brain and/or other tissues.

MATERIALS AND METHODS

The present investigation was carried out in male rats (Sprague–Dawley strain) fed a standard diet as pellets and water *ad libitum*, and housed three, and subsequently two, per cage under optimal environmental conditions (22°, 55–60% relative humidity, 12-hr day cycle) until they reached the age of 16–28 weeks. The rats were housed at first for one month under an automatic fixed dark–light schedule. In fact, the circadian activities of various enzymes are stable only after about 3 weeks in the adult rat at the same time of the nycthemeron. Evaluations were made every 4 weeks in lots of 8 animals each. The initial allocation of animals to the different lots was made by randomization. The time course of the examinations performed in the lots was established by means of permutation tables.

The drugs were administered during four periods as follows: (1) from 16 to 20 weeks of age: the animals of this group were treated between 9:10 and 9:30 a.m.; (2) from 16 to 24 weeks of age: the rats of this group were treated between 10:10 and 10:40 a.m.; (3) from 16 to 28 weeks of age: the rats of this group were treated between 9:30 and 9:50 a.m.; (4) from 24 to 28 weeks of age: the animals of this group were treated between 9:50 and 10:10 a.m. Even though the time interval of daily treatment is the maximum on which to base comparative statistical analysis, this is at least partially

excused by the number of animals utilized in the experiment. Treatment was carried out daily (6 days a weeks) by intraperitoneal administration, using one dose of drug: (a) papaverine and suloctidil = 1 mg/kg; (b) medibazine, trimetazidine, (−)eburnamonine, bamethan, inositol niacinate, and UDP-glucose = 5 mg/kg. Control animals were given the vehicle only by the same route. Blind biochemical evaluations were performed after 4, 8 and 12 weeks of treatment (at 20, 24 or 28 weeks of age). All animals were killed between 9:30 and 10:10 a.m., 48 hours after the last injection. This interval is very important to differentiate the drug interference with basic cellular components or activities from the immediate effect of a sustained treatment with a drug.

At the set time the animals were sacrificed by decapitation and their brains removed from the skull within 15 sec in a precooled box at −5°. The 0.32-M sucrose washed and weighed brains were homogenized in 0.32-M sucrose for 30 sec (precooled Potter–Braun S homogenizer, with a teflon pestle). The homogenate obtained was diluted with 0.32-M sucrose (10% w/v) and an aliquot of each sample was taken for the assay of enzymatic activities. The remaining homogenate was submitted to a series of centrifugations (Sorvall RC-5 Supercentrifuge) for the preparation of the crude mitochondrial fraction [20] obtained at 14,000 *g* for 20 min. On both the homogenate and the mitochondrial preparation samples, protein content was evaluated [21] and the following enzymatic activities were measured: malate dehydrogenase [22]; total NADH–cytochrome *c* reductase [23]; cytochrome oxidase [24, 25]. The activity of lactate dehydrogenase was evaluated only in homogenate samples [26], while that of citrate synthase was measured only in the mitochondrial preparation samples [27]. Enzymatic activities were recorded (Beckman 25 Spectrophotometer Recorder) and calculated using the straight portion of the reaction curves. Results were expressed as specific activities: μmoles · min^{-1} (mg protein)$^{-1}$.

With regard to the statistical analysis of the results, data on eight enzymatic activities were available (4 evaluated in the homogenate *in toto* and 4 evaluated in the crude mitochondrial fraction), concerning 9 treatments for each enzymatic activity, performed in groups of 8 rats each. Each enzymatic activity was measured after 4, 8 and 12 weeks of treatment. Two statistical tests (Anova and Dunnett's tests) were applied to these data, after checking the homogeneity of variance by the Bartlett's test (with regard to both the above mentioned tests). Anova was employed to evaluate the "treatments by times" interactions, i.e. to detect a possible difference in effect of the various drugs in the treatment time. The Dunnett's test was used, at each individual time, to assess differences between the cerebral enzymatic activities of controls and those of the treated rats.

RESULTS

Tables 1 to 5 depict the behavior of the tested enzymatic activities, as measured both in the homogenate *in toto* and in the crude mitochondrial fraction. These activities refer to both controls and animals treated with the different drugs, by the intraperitoneal administration route. In controls (Table 1), all enzymatic activities showed an increase from the 16th to the

Table 1. Rat cerebral enzymatic activities related to the energy transduction evaluated from 16 to 28 weeks of age*

	Homogenate *in toto*				Mitochondrial fraction			
Age	Lactate dehydrogenase	Malate dehydrogenase	Total NADH–cyt.*c* reductase	Cytochrome oxidase	Citrate synthase	Malate dehydrogenase	Total NADH–cyt. *c* reductase	Cytochrome oxidase
16 weeks	0.644 ± 0.020	1.53 ± 0.03	0.032 ± 0.001	0.250 ± 0.011	0.059 ± 0.003	1.21 ± 0.04	0.087 ± 0.003	0.469 ± 0.019
20 weeks	0.784 ± 0.029	1.91 ± 0.07	0.038 ± 0.001	0.351 ± 0.021	0.090 ± 0.004	1.62 ± 0.03	0.132 ± 0.003	0.643 ± 0.028
24 weeks	0.722 ± 0.027	1.65 ± 0.04	0.034 ± 0.001	0.292 ± 0.015	0.063 ± 0.001	1.12 ± 0.04	0.098 ± 0.002	0.412 ± 0.009
28 weeks	0.595 ± 0.017	1.26 ± 0.04	0.028 ± 0.001	0.205 ± 0.009	0.042 ± 0.001	0.78 ± 0.03	0.053 ± 0.002	0.328 ± 0.009

* The enzymatic activities (μmoles · min^{-1}·mg protein^{-1}) were evaluated both in the homogenate *in toto* and in the mitochondrial fraction from the rat brain and are expressed as the mean values ± S.E.M. for each group of 8 animals.

Table 2. Cerebral enzymatic activities related to the energy transduction. Effect of the intraperitoneal pharmacological treatment in rat for 4 weeks (6 days a week) from 16 to 20 weeks of age*

	Homogenate *in toto*				Mitochondrial fraction			
Intraperitoneal treatment with:	Lactate dehydrogenase	Malate dehydrogenase	Total NADH–cyt *c* reductase	Cytochrome oxidase	Citrate synthase	Malate dehydrogenase	Total NADH–cyt *c* reductase	Cytochrome oxidase
Saline solution	0.784 ± 0.029	1.91 ± 0.07	0.038 ± 0.001	0.351 ± 0.021	0.090 ± 0.004	1.62 ± 0.03	0.132 ± 0.003	0.643 ± 0.028
(−)Eburnamonine (5 mg/kg)	0.876 ± 0.029	2.03 ± 0.05	0.040 ± 0.001	0.383 ± 0.023	0.079 ± 0.003	1.57 ± 0.04	0.140 ± 0.003	0.758 ± 0.034‡
Medibazine (5 mg/kg)	0.837 ± 0.017	2.21 ± 0.07‡	0.048 ± 0.002‡	0.424 ± 0.015†	0.103 ± 0.005	1.78 ± 0.03‡	0.141 ± 0.003	0.762 ± 0.005‡
Trimetazidine (5 mg/kg)	0.753 ± 0.046	1.82 ± 0.06	0.041 ± 0.001	0.298 ± 0.011	0.072 ± 0.003‡	1.42 ± 0.03‡	0.112 ± 0.002‡	0.584 ± 0.003
Papaverine (1 mg/kg)	0.934 ± 0.024‡	2.06 ± 0.06	0.040 ± 0.001	0.463 ± 0.019‡	0.096 ± 0.003	1.69 ± 0.03	0.143 ± 0.003†	0.685 ± 0.026
Suloctidil (1 mg/kg)	0.927 ± 0.029‡	2.02 ± 0.05	0.046 ± 0.001‡	0.475 ± 0.012‡	0.105 ± 0.001†	1.84 ± 0.03‡	0.123 ± 0.002	0.661 ± 0.011
Bamethan (5 mg/kg)	0.796 ± 0.016	1.98 ± 0.06	0.036 ± 0.001	0.391 ± 0.017	0.098 ± 0.004	1.69 ± 0.03	0.140 ± 0.002	0.704 ± 0.012
Inositol niacinate (5 mg/kg)	0.816 ± 0.013	1.86 ± 0.04	0.038 ± 0.001	0.334 ± 0.014	0.084 ± 0.003	1.50 ± 0.05	0.125 ± 0.002	0.683 ± 0.011
UDP-glucose (5 mg/kg)	0.763 ± 0.016	1.93 ± 0.04	0.035 ± 0.001	0.340 ± 0.013	0.082 ± 0.002	1.64 ± 0.03	0.127 ± 0.002	0.654 ± 0.012

* The enzymatic activities (μmoles · min^{-1} · mg protein^{-1}) were evaluated both in the homogenate *in toto* and in the mitochondrial fraction from the rat brain (age: 20 weeks) and are expressed as the mean values ± S.E.M. for each group of 8 animals.

† Differs from control: $P < 0.05$
‡ Differs from control: $P < 0.01$
} Dunnett's test.

Table 3. Cerebral enzymatic activities related to the energy transduction. Effect of the intraperitoneal pharmacological treatment in rat for 8 weeks (6 days a week) from 16 to 24 weeks of age*

	Homogenate *in toto*				Mitochondrial fraction			
Intraperitoneal treatment with:	Lactate Dehydrogenase	Malate dehydrogenase	Total NADH–cyt *c* reductase	Cytochrome oxidase	Citrate synthase	Malate dehydrogenase	Total NADH–cyt *c* reductase	Cytochrome oxidase
Saline solution	0.722 ± 0.027	1.65 ± 0.04	0.034 ± 0.001	0.292 ± 0.015	0.063 ± 0.001	1.12 ± 0.04	0.098 ± 0.002	0.412 ± 0.009
(−)Eburnamonine (5 mg/kg)	0.801 ± 0.020†	1.78 ± 0.05	0.036 ± 0.001	0.268 ± 0.010	0.054 ± 0.002‡	1.07 ± 0.04	0.106 ± 0.002	0.469 ± 0.009‡
Medibazine (5 mg/kg)	0.796 ± 0.019	1.92 ± 0.03‡	0.041 ± 0.001‡	0.338 ± 0.010	0.069 ± 0.001	1.23 ± 0.04	0.104 ± 0.002	0.451 ± 0.008†
Trimetazidine (5 mg/kg)	0.738 ± 0.020	1.59 ± 0.04	0.038 ± 0.001†	0.245 ± 0.010	0.052 ± 0.001‡	1.01 ± 0.04	0.091 ± 0.002	0.377 ± 0.010
Papaverine (1 mg/kg)	0.823 ± 0.020‡	1.87 ± 0.03‡	0.031 ± 0.001	0.356 ± 0.008‡	0.060 ± 0.001	1.17 ± 0.05	0.102 ± 0.002	0.437 ± 0.010
Suloctidil (1 mg/kg)	0.734 ± 0.021	1.75 ± 0.03	0.032 ± 0.001	0.333 ± 0.012	0.071 ± 0.001†	1.26 ± 0.02†	0.095 ± 0.002	0.426 ± 0.012
Bamethan (5 mg/kg)	0.770 ± 0.020	1.56 ± 0.04	0.034 ± 0.001	0.367 ± 0.016‡	0.077 ± 0.002‡	1.29 ± 0.03‡	0.112 ± 0.002‡	0.481 ± 0.010‡
Inositol niacinate (5 mg/kg)	0.712 ± 0.017	1.80 ± 0.03†	0.038 ± 0.001	0.321 ± 0.014	0.068 ± 0.002	1.04 ± 0.02	0.102 ± 0.002	0.438 ± 0.008
UDP-glucose (5 mg/kg)	0.762 ± 0.017	1.62 ± 0.03	0.033 ± 0.001	0.306 ± 0.013	0.057 ± 0.002	0.96 ± 0.03†	0.103 ± 0.003	0.432 ± 0.009

* The enzymatic activities (μmoles · min^{-1} · mg protein^{-1}) were evaluated both in the homogenate *in toto* and in the mitochondrial fraction from the rat brain (age: 24 weeks) and are expressed as the mean values ± S.E.M. for each group of 8 animals.

† Differs from control: $P < 0.05$
‡ Differs from control: $P < 0.01$
} Dunnett's test.

Table 4. Cerebral enzymatic activities related to the energy transduction. Effect of the intraperitoneal pharmacological treatment in rat for 12 weeks (6 days a week) from 16 to 28 weeks of age*

	Homogenate *in toto*				Mitochondrial fraction			
Intraperitoneal treatment with:	Lactate dehydrogenase	Malate dehydrogenase	Total NADH–cyt *c* reductase	Cytochrome oxidase	Citrate synthase	Malate dehydrogenase	Total NADH–cyt *c* reductase	Cytochrome oxidase
Saline solution	0.595 ± 0.017	1.26 ± 0.04	0.028 ± 0.001	0.205 ± 0.009	0.042 ± 0.001	0.78 ± 0.03	0.053 ± 0.002	0.328 ± 0.009
(−)Eburnamonine (5 mg/kg)	0.635 ± 0.018	1.33 ± 0.03	0.030 ± 0.001	0.193 ± 0.010	0.039 ± 0.001	0.73 ± 0.03	0.057 ± 0.002	0.365 ± 0.008+
Medibazine (5 mg/kg)	0.648 ± 0.021	1.41 ± 0.03+	0.031 ± 0.001+	0.222 ± 0.008	0.045 ± 0.001	0.85 ± 0.03	0.055 ± 0.002	0.356 ± 0.008
Trimetazidine (5 mg/kg)	0.573 ± 0.017	1.14 ± 0.03	0.031 ± 0.001	0.181 ± 0.011	0.037 ± 0.001	0.71 ± 0.03	0.050 ± 0.002	0.312 ± 0.008
Papaverine (1 mg/kg)	0.687 ± 0.020+	1.35 ± 0.03	0.027 ± 0.001	0.232 ± 0.011	0.040 ± 0.001	0.81 ± 0.03	0.054 ± 0.002	0.337 ± 0.013
Suloctidil (1 mg/kg)	0.603 ± 0.021	1.32 ± 0.02	0.030 ± 0.001	0.200 ± 0.008	0.048 ± 0.001+	0.88 ± 0.02	0.052 ± 0.002	0.345 ± 0.010
Bamethan (5 mg/kg)	0.548 ± 0.023	1.23 ± 0.04	0.026 ± 0.001	0.214 ± 0.014	0.046 ± 0.001	0.81 ± 0.02	0.057 ± 0.001	0.342 ± 0.008
Inositol niacinate (5 mg/kg)	0.555 ± 0.022	1.45 ± 0.04‡	0.026 ± 0.001	0.218 ± 0.012	0.043 ± 0.002	0.80 ± 0.02	0.054 ± 0.001	0.330 ± 0.009
UDP-glucose (5 mg/kg)	0.622 ± 0.019	1.21 ± 0.03	0.028 ± 0.001	0.206 ± 0.013	0.040 ± 0.002	0.76 ± 0.03	0.054 ± 0.002	0.339 ± 0.008

* The enzymatic activities (μmoles · min^{-1} · mg protein^{-1}) were evaluated both in the homogenate *in toto* and in the mitochondrial fraction from the rat brain (age: 28 weeks) and are expressed as the mean values ± S.E.M. for each group of 8 animals.

+ Differs from control: $P < 0.05$
‡ Differs from control: $P < 0.01$
} Dunnett's test.

Table 5. Cerebral enzymatic activities related to the energy transduction. Effect of the intraperitoneal pharmacological treatment in rat for 4 weeks (6 days a week) from 24 to 28 weeks of age*

	Homogenate *in toto*				Mitochondrial fraction			
Intraperitoneal treatment with:	Lactate dehydrogenase	Malate dehydrogenase	Total NADH–cyt *c* reductase	Cytochrome oxidase	Citrate synthase	Malate dehydrogenase	Total NADH–cyt *c* reductase	Cytochrome oxidase
Saline solution	0.595 ± 0.017	1.26 ± 0.04	0.028 ± 0.001	0.205 ± 0.009	0.042 ± 0.001	0.78 ± 0.03	0.053 ± 0.002	0.328 ± 0.009
(−)Eburnamonine (5 mg/kg)	0.673 ± 0.018+	1.17 ± 0.05	0.030 ± 0.002	0.214 ± 0.009	0.036 ± 0.002+	0.80 ± 0.04	0.053 ± 0.002	0.381 ± 0.011‡
Medibazine (5 mg/kg)	0.640 ± 0.018	1.32 ± 0.05	0.033 ± 0.001+	0.225 ± 0.009	0.045 ± 0.002	0.84 ± 0.03	0.056 ± 0.001	0.383 ± 0.012‡
Trimetazidine (5 mg/kg)	0.602 ± 0.014	1.20 ± 0.04	0.031 ± 0.001	0.179 ± 0.013	0.036 ± 0.003	0.67 ± 0.04	0.048 ± 0.003	0.318 ± 0.012
Papaverine (1 mg/kg)	0.702 ± 0.023‡	1.31 ± 0.05	0.026 ± 0.001	0.242 ± 0.009+	0.044 ± 0.002	0.85 ± 0.04	0.056 ± 0.001	0.332 ± 0.014
Suloctidil (1 mg/kg)	0.681 ± 0.019+	1.35 ± 0.04	0.032 ± 0.001	0.243 ± 0.010+	0.050 ± 0.001‡	0.83 ± 0.04	0.050 ± 0.001	0.329 ± 0.011
Bamethan (5 mg/kg)	0.590 ± 0.018	1.30 ± 0.04	0.029 ± 0.001	0.213 ± 0.009	0.049 ± 0.001+	0.74 ± 0.05	0.060 ± 0.001+	0.356 ± 0.011
Inositol niacinate (5 mg/kg)	0.550 ± 0.016	1.43 ± 0.05+	0.026 ± 0.001	0.194 ± 0.009	0.039 ± 0.002	0.84 ± 0.05	0.051 ± 0.001	0.335 ± 0.010
UDP-glucose (5 mg/kg)	0.655 ± 0.029	1.20 ± 0.06	0.027 ± 0.002	0.208 ± 0.011	0.045 ± 0.001	0.81 ± 0.05	0.049 ± 0.001	0.326 ± 0.009

* The enzymatic activities (μmoles · min^{-1} · mg protein^{-1}) were evaluated both in the homogenate *in toto* and in the mitochondrial fraction from the rat brain (age: 28 weeks) and are expressed as the mean values ± S.E.M. for each group of 8 animals.

+ Differs from control: $P < 0.05$
‡ Differs from control: $P < 0.01$
} Dunnett's test.

20th week and a decrease from the 20th week onward.

As for the "treatments by times" interactions, Anova failed to exhibit any significant difference between the values of the enzymatic activities evaluated in the brain of control rats and of those treated with the various drugs. Therefore, the physiological behavior of cerebral enzymatic activities in the treatment time prevailed over the changes which could be induced by drugs. The results reported below are therefore described with regard to significant differences pointed out by the Dunnett's test at each tested time (4, 8 and 12 weeks of treatment), as shown in Tables 2, 3, 4, 5.

(−)Eburnamonine increased the activity of the mitochondrial cytochrome oxidase at all tested times (after 4, 8 and 12 weeks of treatment, from 16 to 20, from 16 to 24 and from 16 to 28 weeks of age, respectively). This effect was accompanied by a decrease in the activity of citrate synthase and an increase in the activities of lactate dehydrogenase, limited to the first 8 weeks of treatment. These changes were observed also after a four-week treatment, between 24 and 28 weeks of age. After 4 weeks of treatment, medibazine caused an increase in the activities of both cytochrome oxidase and malate dehydrogenase in the mitochondrial fraction. With time, this increase became progressively less evident and disappeared after 12 weeks of treatment. As for the enzymatic activities evaluated in the homogenate *in toto*, medibazine rather steadily increased both the malate dehydrogenase and the total NADH–cytochrome *c* reductase during the various treatment periods. Trimetazidine decreased the enzymatic activities of the mitochondrial fraction (citrate synthase, malate dehydrogenase, total NADH–cytochrome *c* reductase) particularly after 4 weeks of treatment (between 16 and 20 weeks of life). This inhibition became largely less evident and then disappeared after 8 and 12 weeks of treatment. No inhibition was observed when a 4-week treatment was carried out between 24 and 28 weeks of life. Papaverine mainly affected the enzymatic activities evaluated in the brain homogenate *in toto*: the activity of lactate dehydrogenase was steadily increased, while cytochrome oxidase and malate dehydrogenase were enhanced limited to the first treatment periods.

In the first 4 week treatment period (16 to 20 weeks of life) suloctidil caused an increase of several enzymatic activities, both in the homogenate *in toto* (lactate dehydrogenase, total NADH–cytochrome *c* reductase, cytochrome oxidase) and in the mitochondrial fraction (citrate synthase, malate dehydrogenase). In the following 8 and 12 week treatment periods, the increase concerned only the mitochondrial enzymatic activities of the Krebs' cycle and particularly the citrate synthase. The 4 week treatment performed between 24 and 28 weeks of life showed an interference limited to some cerebral enzymatic activities (lactate dehydrogenase and cytochrome oxidase in the homogenate; citrate synthase in the mitochondrial fraction). Bamethan exerted its effect only after 8 weeks of treatment, increasing all the mitochondrial enzymatic activities (cytrate synthase, malate dehydrogenase, total NADH–cytochrome *c* reductase, cytochrome oxidase). This effect disappeared after 12 weeks of treatment (16 to 28 weeks of age). An activation of mitochondrial enzymes (limited to citrate synthase and total NADH–cytochrome *c* reductase) was found after 4 weeks of treatment from 24 to 28 weeks of age.

Treatment with inositol niacinate and UDP-glucose did not cause any changes in the enzymatic activities tested, except for the malate dehydrogenase in the homogenate *in toto* which was stimulated by inositol niacinate.

DISCUSSION

Before discussing the present data, some limitations should be pointed out. In the first place, the present data refer to cerebral tissue as a whole: therefore, some important areas of the brain might undergo different biochemical adjustments, because of the anatomical and functional heterogeneity of the organ. Secondly, the methods used to determine enzymatic activities abolish the autoregulative interactions that maintain the cell as a functionally integrated system. As a consequence, absolute conclusions are not drawn from these experimental data which might be also relatively related to each other.

The data obtained in the untreated adult rat (Table 1) reveal a peak of cerebral enzymatic activities around the 20th week of life, indicating an increased genetic coding as a consequence of the activity of the corresponding genes. The behavior observed in the tested period of life (increase of the enzymatic activities from the 16th to the 20th week and decrease from the 20th week onward) might be attributed also to changes in the hormonal state [18]. In any case, the nature of the enzyme molecule does not change with age, but its induction properties do [28]. This age-dependent change in enzymatic activities related to energy transduction can condition responses to pharmacological stimuli at cerebral level.

As concerns the behaviour of the curves depicting, as the function of the time, the decrement of the various cerebral enzymatic activities following to the peak value, the tested pharmacological treatments never induced different conditions with respect to those induced by saline solution. In any case, the degree of the enzymatic decrement sometimes varied according to the tested treatment, showing that the pharmacological actions are only superimposed upon the predominant natural change of the enzymatic activities. Furthermore, the pharmacological response tends to be stronger after the first four weeks of treatment (Table 2), while after 12 weeks of treatment it becomes less evident (Table 4). This fact might be related to a reduction in drug power due to prolonged treatment (e.g. because of drug induction), or to an intrinsic inefficiency of the drugs to stop the age-dependent decrement of cerebral enzymatic activities (e.g. taking place from the 20th week of life). Probably, both mechanisms are present, since a one-month treatment performed from the 24th to the 28th week of life (Table 5) caused responses which were: (a) lower than those induced by treatment from the 16th to the 20th week of age (Table 2); (b) higher than those recorded at 28 weeks, after approximately 3 months of treatment (Table 4). In any case, the effect of drugs becomes more specific: for example, after one month of treatment, suloctidil at the 20th week of life increased both the activity of lactate dehydrogenase, total NADH–cytochrome *c* reductase and cytochrome oxidase, as evaluated in the homogenate *in toto*, and the activity of citrate synthase and malate dehydrogenase evaluated in the mitochondrial fraction (Table 2). However, after 8

and 12 weeks of treatment (at 24 and 28 weeks of age) the action of suloctidil was directed towards the enzymatic activities of the Krebs' cycle (Tables 3 and 4).

As regards specifically the dose used for each drug tested, effects were found on some enzymatic activities of the mitochondrial fraction or of the homogenate *in toto*, or of both. In some treatments the enzymatic activities involved were all affected in the same way, all of them showing an increase or a decrease, while in other treatment some activities were increased and some were decreased. This allows a classification (with validity limited to chronic treatment with the dose tested) to be drawn up of the action of the drugs on the pattern of cerebral enzymatic activities: (a) drugs which generically interfere with the tested cerebral enzymatic activities by means of an activating (medibazine) or an inhibitory (trimetazidine) trend of action; (b) drugs which cause a magnification of enzymatic activities evaluated in the homogenate *in toto* (papaverine) or in the mitochondrial fraction (bamethan, suloctidil, (−)eburnamonine); (c) drugs which tend to interfere with the mitochondrial activities related to the Krebs' cycle by an activating (suloctidil) or an inhibitory trend of action ((−)eburnamonine); (d) drugs which tend to interfere with the mitochondrial activities related to the electron transfer chain, by an activating action (bamethan, (−)eburnamonine).

Regarding the treatment time, the bamethan peak of activity was found after 8, rather than after 4 weeks of treatment. This stresses the importance of the factor dose as a function of the factor treatment time, since bamethan at high doses (120 mg/kg i.p.) proves immediately active on the enzymatic activities of the purified mitochondrial fraction [29], while in this experiment a much lower dose (5 mg/kg i.p.), even when administered daily for a long period, exhibited a remarkable latency time. At any rate, the enzymatic response to chronic treatment is qualitatively similar to that of the acute treatment [29].

On the whole, the present study in the rat of the interference *in vivo* of drugs on cerebral enzymatic activities made possible a preliminary definition of the relationships between pharmacological treatment and cerebral metabolism. Indeed, for each drug the trend of interference on the enzymatic activities tested can be established. This provides a first approach to define the possible mode of action at the level of subcellular systems related to intermediary metabolism. Obviously, a definite conclusion regarding energy status from the data obtained with this study is impossible, since, for example, rate limiting enzymatic steps of the glycolysis were not studied. Furthermore, a more detailed description of pharmacological intereference on tissular enzymatic systems also requires their evaluation in more homogeneous subcellular fractions (e.g., in the purified mitochondrial fraction, in synaptosomes, etc.) and also from other fundamental approaches (e.g., enzyme kinetic studies, *in vivo* substrate metabolisation, etc.).

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