days of clofibrate treatment the rats were killed by decapitation, without prior fasting, between 8:00 and 11:00 a.m. Blood was collected from the wound and analyzed for thyroxine [9]. All of the thyroidectomized rats had serum thyroxine concentrations that were below detectable levels (i.e. < 1 μg/ml). The normal control rats had levels of approximately 6 μg/ml. In addition, the thyroidectomized rats had markedly decreased growth rates, and low basal metabolic rates that were not increased by intraperitoneal injections of thyrotropin-releasing factor [9].

Kidneys were rapidly excised, decapsulated, sliced in half longitudinally with a scalpel, and demedullated. The remaining cortex was then weighed and 0.5-mm slices were prepared with a Stadie-Riggs tissue slicer. Slices were blotted, weighed, trimmed to between 65 and 85 mg, and placed in cold (4°) 0.154 M NaCl for 5–10 min. Slices were then incubated in the presence of 10 mM NaCl, or the substrates pyruvate and glycerol, for 1 hr at 37° under 95% O_2–5% CO_2 in 5 ml of Krebs–Ringer bicarbonate solution containing 2.54 mM Ca^{2+} [10]. Incubations were carried out in stoppered 50-ml flasks with continuous shaking (120 oscillations/min). Reactions were stopped by adding 1 ml of 2 N $HClO_4$, slices were discarded, and samples were removed for glucose analysis [11]. Net glucose synthesis was calculated by substracting glucose formed in the absence of substrate from that formed in the presence of substrate.

The effect of clofibrate treatment on gluconeogenesis is shown in Fig. 1. Clofibrate markedly increased the rate of glucose synthesis from glycerol and pyruvate by kidney cortex slices from both normal and thyroidectomized rats and, therefore, it may be concluded that thyroxine is not involved in mediating this effect. Previous work has also eliminated metabolic acidosis as a factor [1]. It is possible that renal gluconeogensis is enhanced by a reduction of the plasma insulin/glucagon ratio. Savolainen *et al.* [12] have recently shown that clofibrate causes a fall in the rat plasma insulin level and a decreased glucose tolerance.

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Magnification of some enzymatic activities of brain cortex subfractions

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An *in vivo* investigation on cerebral energy metabolism has demonstrated that a catecholamine-like agent, 1-*p*-hydroxyphenyl-2-butylamino-ethanol or bamethan, favors post-hypoxic recovery at the level of the brain cortex motor area in the dog [1]. This interference affects the adenyl cyclase receptor, the presence of a β-blocking agent inhibiting the adenyl cyclase stimulation induced by bamethan during post-hypoxic recovery [2]. This finding confirms that cerebral adenyl cyclase behaves like a receptor with β-adrenergic characteristics [3]. The study [4], of the subcellular localization of adenyl cyclase has shown that the highest activity is found in the fractions containing nerve endings and synaptic complexes, elsewhere indicated as synaptosomal fractions [5], the myelinic and mitochondrial fractions exhibiting a lower activity. It has therefore proved interesting to examine the effect of bamethan at subcellular level on some cerebral enzymatic activities, evaluated on the mitochondrial and synaptosomal fractions of the rat brain cortex. The following enzymes were investigated: 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 oxidoreductase, EC 1.6.99.3*) and cytochrome oxidase (*Ferrocytochrome c: oxygen oxidoreductase, EC 1.9.3.1*) for the electron transport chain.

MATERIALS AND METHODS

Sprague–Dawley female rats (weight 300 ± 10 g) were used. 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 ± 5%; lighting cycle, 12 hr light and 12 hr darkness; low noise-disturbance) and fed a standard pellet diet. The results indicated in this paper

Table 1. Specific enzymatic activities (μmoles. min^{-1}. mg protein^{-1}) of the purified mitochondrial fraction of brain cortex of control rats and of rats treated with bamethan *in vivo* (120 mg/kg, i.p.)

Animals	Citrate synthase	Malate dehydrogenase	Total NADH-cytochrome c reductase	Cytochrome oxidase
Controls	0.27 ± 0.02 (7)	2.44 ± 0.06 (7)	0.18 ± 0.008 (7)	1.67 ± 0.05 (7)
Treated with bamethan	0.58 ± 0.06 (6)	4.60 ± 0.36 (7)	0.36 ± 0.04 (7)	2.47 ± 0.28 (6)
P	< 0.01	< 0.01	< 0.01	< 0.02

Results expressed as the mean ± S.E.M.; number of animals in parenthesis. P = significance level of the difference between treated and control rats.

Table 2. Brain cortex of control rats and of rats treated with bamethan *in vivo* (120 mg/kg, i.p.). Specific enzymatic activities (μmoles. min^{-1}. mg protein^{-1}) evaluated on the synaptosomal fraction

Animals	Lactate dehydrogenase	Citrate synthase	Malate dehydrogenase	Total NADH-cytochrome c reductase	Cytochrome oxidase
Controls	0.16 ± 0.02 (6)	0.047 ± 0.005 (6)	0.48 ± 0.03 (7)	0.028 ± 0.002 (7)	0.060 ± 0.001 (7)
Treated with bamethan	ND	0.031 ± 0.004 (6)	0.58 ± 0.04 (7)	0.039 ± 0.005 (7)	0.079 ± 0.007 (6)
P	—	< 0.05	< 0.05	< 0.05	< 0.02

Results expressed as the mean ± S.E.M.; number of animals in parenthesis; P = significance level; ND = Not determined.

are related only to the animals treated with bamethan injected i.p. with a dose of 120 mg.kg^{-1}. Preliminary studies were made also with lower doses (13.3 and 40 mg. kg^{-1}) at various times after drug administration (30, 60 and 120 min). Significative effects were observed only at 30 min with 120 mg.kg^{-1}. In others cases, bamethan induced changes were not significative, effective dose–response and time–effect relationships were therefore impossible to depict. The control animals were i.p. treated with vehicle. The treatment was always made at 10:00 hr, because of diurnal changes in some enzymatic activities (e.g. phosphodiesterase, adenylcyclase). Consequently, the pharmacological treatments inducing changes in enzymatic activities may have different effects depending upon the time of administration of the drug. Always after 30 min the animals were sacrificed by decapitation, their brain removed within 15 sec and immersed in a cold solution of sucrose 0.32 M. The isolated brain cortex was weighed and homogenized in sucrose 0.32 M for 10 sec in a Potter–Elvehjem homogenizer with two strokes up and down (total clearance, 0.25 mm). All these manipulations were performed in a cooled box at −5°. The homogenate was then submitted to fractioning [6] for isolating the crude mitochondrial fraction and obtaining the cellular subfractions. Three centrifugations at 900 *g* were performed to remove nuclei and contaminating materials. The crude mitochondrial fraction was obtained with two centrifugations at 11.500 *g* (Sorvall RC-5 supercentrifuge), and subsequently layered on a discontinuous sucrose gradient (1.4; 1.2; 1.0; 0.8 M) and centrifuged 2 hr at 50.000 *g* (Spinco–Beckman L5-50 ultracentrifuge). The synaptosomal fraction was removed by aspiration, while the mitochondrial fraction was resuspended in a sucrose solution 0.32 M (protein concentration, 10 mg. ml^{-1}). Proteins were dosed [7]. The following enzymatic activities were evaluated on samples of the pure mitochondrial preparation and/or of the synaptosomal fraction; malate dehydrogenase [8], total NADH-cytochrome *c* reductase [9], cytochrome oxidase [10, 11], citrate synthase [12], lactate dehydrogenase [13]. The synaptosomal fraction was treated also with Triton-X-100 (0.5%). Enzymatic activities were measured by graphic recordings of at least 3 min (Beckman model 25 spectrophotometer), and expressed as μmoles. min^{-1}. mg protein^{-1}. The data obtained were submitted to the Student '*t*' test.

RESULTS AND DISCUSSION

Table 1 shows the data relative to the enzymatic activities tested (citrate synthase, malate dehydrogenase, total NADH-cytochrome *c* reductase, cytochrome oxidase) of pure mitochondria extracted from the brain cortex of control rats and of rats treated *in vivo* with bamethan. All the enzymatic activities tested showed significantly higher values in the pure mitochondria of rats treated with bamethan *in vivo*. The substance increased to various degrees the activity of some enzymes of the Krebs' cycle and of electron transport chain.

The treatment with bamethan *in vivo* magnified in the synaptosomal fraction some enzymatic activities, as compared to the corresponding control values, but not citrate synthase (Table 2). The use of the detergent during the preparatory stage could make the increase of enzymatic activities sometimes more evident (Tables 2 and 3), the effect being consistent with the peculiar subcellular localization of the enzymes. This explains why the activity of the total NADH-cytochrome *c* reductase, a membrane-bound enzyme [14], appears negligible after submitting the synaptosomal fraction to the detergent, acting at membrane level. Furthermore, lactate dehydrogenase was highly sensitive to the effect of the detergent *in vitro*, its activity being magnified from 0.16 ± 0.02 (Table 2) to 0.73 ± 0.10 μmoles. min^{-1}. mg protein^{-1} (Table 3). This confirms the effectiveness of the concentration of Triton-

Table 3. Brain cortex of control rats and of rats treated *in vivo* with bamethan (120 mg/kg, i.p.). Specific enzymatic activities (μmoles. min^{-1}. mg protein^{-1}) evaluated on the synaptosomal fraction following the addition of Triton-X-100.

Animals	Lactate dehydrogenase	Citrate synthase	Malate dehydrogenase	Total NADH-cytochrome c reductase	Cytochrome oxidase
Controls	0.73 ± 0.10 (6)	0.079 ± 0.004 (7)	1.34 ± 0.04 (6)	n.a. (7)	0.17 ± 0.02 (6)
Treated with bamethan	0.78 ± 0.19 (6)	0.20 ± 0.05 (7)	1.65 ± 0.14 (6)	n.a. (7)	n.a. (6)
P	> 0.05	< 0.05	< 0.05	—	—

Results expressed as the mean ± S.E.M.; number of animals in parenthesis; P = significant level; n.a. = negligible activity.

X-100 employed. In an overall evaluation of these results, it should be pointed out that malate dehydrogenase activity is enhanced by the treatment with bamethan *in vivo*, both in the pure mitochondrial fraction and in the synaptosomal fraction, either treated with detergent or not. This finding, together with the failure of the drug to affect lactate dehydrogenase (Table 3), is a further proof of the ability of bamethan to magnify the enzymatic activities connected with the aerobic mechanism, as also shown by its action on total NADH-cytochrome *c* reductase and cytochrome oxidase (Tables 1 and 2) in the mitochondrial and synaptosomal fractions.

The treatment with bamethan *in vivo* increased in the mitochondrial fraction the activity of citrate synthase (Table 1), whose changes may be interpreted as in the case of malate dehydrogenase. However, this event does not take place at synaptosomal level, where the activity of citrate synthase in the animals treated with bamethan is even lower than in controls (Table 2). Nevertheless, the bamethan effect at synaptosomal level can be more evident after Triton-X-100 treatment action on subcellular membranes (Table 3). This finding might be accounted for by the difficult penetration of substances and substrates into the membranous structures of synaptosomes. This event is concomitant with the peculiar localization of the citrate synthase and does not occur, or occurs to a much lesser extent, in the case of malate dehydrogenase, which is more variously distributed. Although not studied in the present work, it would be very interesting to show the effect of bamethan also on the adenyl cyclase activity in order to confirm in the present experimental model the effectiveness previously observed by us [2] *in vivo* in the beagle dog.

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