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Nimodipine reduces postischemic lactate levels in the isolated perfused rat brain

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During recent years, several reports have appeared demonstrating improvements of postischemic brain energy metabolism by calcium antagonists [1–6]. The data include increases in the levels of high-energy phosphates, glucose, glucose-6-phosphate, and decreases in lactate levels. The faster restitution of high-energy phosphates was demonstrated for a variety of calcium antagonists including dihydropyridine, benzothiazepine, and phenylalkylamine derivatives. As shown for emopamil, this effect is due to the vasodilating properties of the calcium antagonists [5]. The faster postischemic degradation of lactate by emopamil, however, was unrelated to flow changes suggesting a different site of action. Besides their properties to block slow inward calcium current, the phenylalkylamines possess a variety of other pharmacological effects unrelated to calcium antagonism [7]. Gallopamil, for instance, is a potent 5-HT2 antagonist in a dose range similar to its ability to bind to the L-type calcium channel [8]. The dihydropyridine derivative nimodipine has not been shown to have such action at 5-HT2 receptors and compared to phenylalkylamine calcium antagonists reveals a somewhat different profile of nonspecific side effects. The present investigation was set up to determine whether nimodipine also faster reduces postischemic brain lactate levels. Such action would provide support to the concept that the previously monitored changes in cortical lactate are related to calcium entry blockade.

Materials and methods

Materials. Nimodipine was a gift from Bayer (Leverkusen, F.R.G.). Enzymes, coenzymes and substrates were obtained from Boehringer (Mannheim, F.R.G.) DL-dithiothreitol. HEPES (4-(2-hydroxyethyl)-piperazine-1-ethane-sulfonic acid), imidazole, 2-mercaptoethanol, and 1-thioglycerol were purchased from Sigma Chemical Co (St Louis, MO). All other chemicals were of reagent grade. Male Sprague–Dawley rats (IWV, Geretsried, F.R.G.) weighing 200–230 g were used. The animals were maintained under controlled environmental conditions (12 hr dark/light cycle, 23 ± 1°, 55 ± 5% rel. humidity) and kept on standard diet (Altromin, Lage, F.R.G.) and tap water *ad lib.* until starting the experiment.

Perfusion technique. The rat brain was isolated [9] and perfused with a fluorocarbon (FC 43) emulsion (Green Cross Corp., Osaka, Japan) following a technique described previously in detail [10]. Perfusion pressure (100 mmHg) was maintained at a constant level. Bipolar electroencephalograms were recorded from the parietal region of each hemisphere (EEG preamplifier BioAC; Hellige, Freiburg, F.R.G.). Nimodipine was added to the perfusion medium at a concentration of 0.5 μmol/l before perfusion was started. After a perfusion period of 30 min, ischemia was induced for 30 min by switching off the medium supply. Following ischemia perfusion was reinstituted for 5, 15, 25, or 40 min. Flow rate was determined by collecting venous perfusate over one min by means of a burette. Metabolism was stopped by immersing brains into liquid nitrogen [11].

Enzymatic methods. Cerebral cortical tissue was removed from the brain, weighed and extracted at −20° [12]. Creatine-phosphate, ATP, ADP, AMP, glucose, pyruvate and lactate were measured spectrophotometrically [13]. Glucose-6-phosphate and fructose-1,6-bisphosphate were measured fluorimetrically [14]. The energy charge of the adenylate pool EC = ATP + 0.5 ADP/ATP + ADP + AMP was calculated according to Atkinson [15].

Statistics. Values are presented as means ± SD. For the analysis of the influence of nimodipine on postischemic lactate levels and on perfusion rate, two-way analysis of variance was used. The rate of postischemic lactate degradation was calculated as the difference between the respective lactate level and the mean value at the end of ischemia divided by the respective reperfusion time. Only data from 5, 15 and 25 min of recirculation were included since lactate degradation seemed to be nearly linear during this time span. Lactate degradation rates of control and nimodipine-treated brains were compared with Student's *t*-test after releasing two values by means of the Dixon test. The influence of mean flow rate on the rate of lactate degradation was determined by linear regression analysis and *F*-test.

Results

Cerebral flow rate was unchanged by nimodipine during preischemic perfusion. During postischemic recirculation, however, the calcium antagonist caused an increase in perfusion rate (Fig. 1). Cortical high-energy phosphates and glycolytic intermediates were determined after 30 min of perfusion, 2 and 30 min of complete global ischemia. No change in cortical metabolite levels was induced by nimodipine during control perfusion and ischemia (data not shown; for lactate levels see Fig. 2). After 2 min of ischemia, energy metabolism was not different from the corresponding controls in the nimodipine-treated group (data not shown; for lactate levels see Fig. 2). During recirculation, treatment with the calcium antagonist led to lower lactate levels (Fig. 2). The rate of lactate degradation was significantly higher in the nimodipine-treated groups (0.30 ± 0.04 μmol/g/min in the control group vs 0.36 ± 0.04 μmol/g/min in the nimodipine group; $P < 0.01$) ADP and glucose-6-phosphate levels were also reduced by the calcium antagonist during postischemic reperfusion (data not shown). In Fig. 3 the rates of lactate degradation vs mean postischemic flow rates are depicted for control and nimodipine-treated brains. Linear regression analysis revealed no significant correlation between both parameters.

Discussion

In accordance with our results, nimodipine has been consistently shown to improve the postischemic hypoperfusion state [16–19]. Locally performed flow determinations indicate that this improvement in postischemic blood flow by nimodipine is regionally heterogeneous [19]. In an earlier study, we reported on improved high-energy

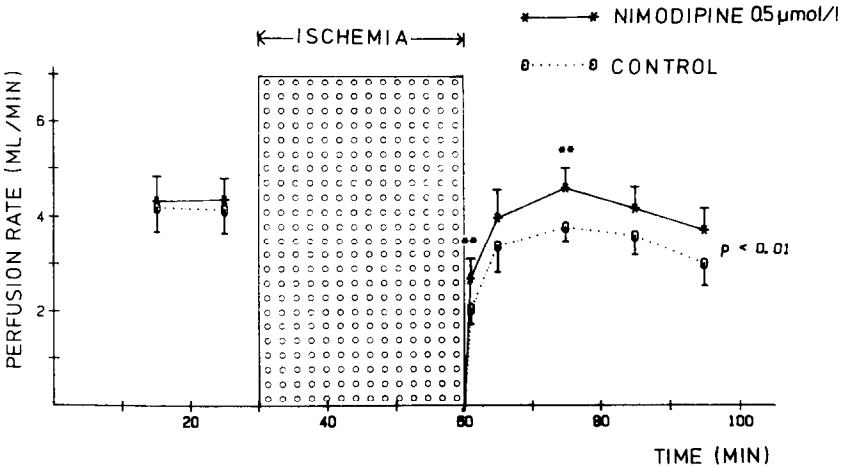

Fig. 1. Effect of nimodipine on the flow rate of the isolated rat brain. Nimodipine (0.5 µmol/l) was added to the perfusate prior to starting perfusion. After 30 min of perfusion and 30 min of complete ischemia, brains were recirculated for up to 40 min. Values are presented as means ± SD. Perfusion rate of drug-treated brains significantly differed from controls during recirculation (two-way analysis of variance; $P < 0.01$).

phosphate levels by nimodipine at 5 min of postischemic recirculation under identical experimental conditions as employed in the present investigation [4]. The lower ADP concentrations observed here are in line with these earlier data. Several phenylalkylamine calcium antagonists have previously been demonstrated to accelerate postischemic lactate degradation [4, 5]. This effect could be attributed to a direct action of the calcium antagonist on cerebral parenchyma devoid of a cerebrovascular contribution. The missing correlation between mean postischemic perfusion rate and lactate degradation rate in the present study lends support to the conclusion that, like emopamil, nimodipine reduces postischemic lactate by a mechanism unrelated to its vasodilating properties. The probable role of lactacidosis as a cause of cell death is well documented [20]. The faster reduction of lactate should cause an improved H^+ homeostasis during recovery. In a recent study, such correction of pH could be demonstrated with nimodipine and verapamil in a model of focal ischemia in the rat [21]. The fact that pH changes were not associated with increases in local cerebral blood flow in that study support the view of a direct action on the brain parenchyma by nimodipine. There is increasing evidence that effects of calcium antagonists unrelated to blood flow make up for the improvements in histological and neurological outcome after an ischemic insult [22–25]. Steen *et al.* [22] report on brain stem lesions falling outside arterial border zones which respond to nimodipine therapy. Flunarizine has been demonstrated to reduce ischemia induced neuronal necrosis without altering blood flow during the period of hypoperfusion [23, 24]. Similar results were obtained with emopamil [25]. Since lactate accumulation during ischemia remains unchanged by the calcium antagonist and the faster postischemic reduction of lactate is unrelated to flow changes, nimodipine seems to improve lactate disappearance by stimulating its oxidative degradation. The action of calcium antagonists on cerebral lactate homeostasis is indicative of a less impaired mitochondrial func-

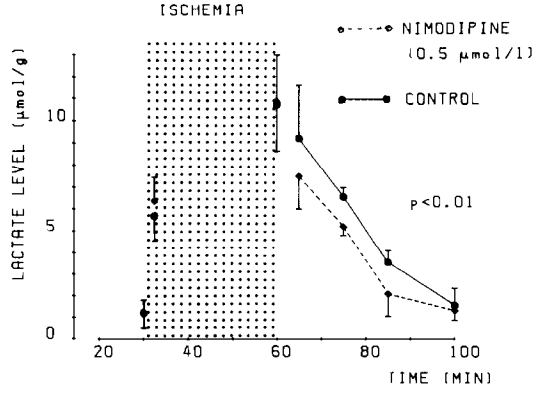

Fig. 2. Effect of nimodipine on cortical lactate levels of the isolated rat brain. After 30 min of perfusion and 30 min of complete ischemia, the brains were reperfused for up to 40 min. Values are given as means ± SD from 3–5 experiments expressed as µmol/g wet wt. Postischemic lactate levels of drug-treated brains significantly differed from controls (two-way analysis of variance; $P < 0.01$).

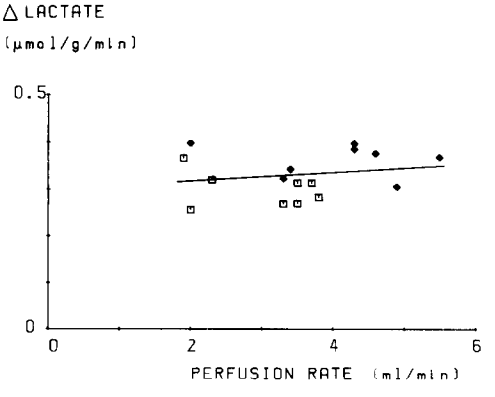

Fig. 3. Relation between mean postischemic perfusion rate and the rate of cortical lactate degradation in the isolated perfused rat brain. □: control animals; ◆: nimodipine-treated animals. No correlation between both parameters was found by linear regression analysis ($y = 0.0086, x + 0.3$; $r = 0.21$; $P = 0.89$).

tion and therefore might have an impact on final cell destruction.

To sum up, during reperfusion of the isolated rat brain ADP, glucose-6-phosphate, and lactate levels were lowered by nimodipine. The calcium antagonist caused no change in preischemic flow rate but increased postischemic perfusion rate considerably. Lactate levels were unrelated to the corresponding flow values. The results suggest nimodipine to improve postischemic mitochondrial function by a direct action on cerebral parenchyma.

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Amrinone potentiates catecholamine-induced lipolysis in fat cells

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Amrinone, a bipyridine derivative, exerts a positive inotropic action *in vitro* [1–3] and *in vivo* [4]. In addition, the drug produces a marked relaxation of vascular [5, 6] and intestinal [5, 7–9] smooth muscle.

In patients with cardiac failure the haemodynamic effects of amrinone are accompanied by an increase in plasma fatty acids and glycerol concentration [10].

The present study was undertaken in order to test the possibility that amrinone may exert a direct stimulatory effect on hormone-sensitive lipolysis in fat cells. This hypothesis is based on the consideration that amrinone inhibits phosphodiesterase activity [3, 11, 12] and hinders the response to endogenous adenosine [2, 9] in various tissues. In fat cells, cyclic AMP formation is an intermediate step in the activation of triglyceride lipase by hormones and neurotransmitters interacting with stimulatory receptors [13]. This activation can be also induced by agents, such as methylxanthines, that act as antagonists at inhibitory membrane receptors coupled to adenylate cyclase and/or increase cyclic AMP levels by inhibiting phosphodiesterase [13–15].

Because of these similarities between the actions of amrinone and of theophylline, a typical lipolytic methylxanthine, the influence of the two drugs on lipolysis was compared and their possible interaction at this level was examined.

Finally, since activation of Ca^{2+} flux in vascular [5], intestinal [8, 16] and cardiac muscle [3, 17] is involved in the final effects of amrinone, and many results indicate an outstanding role of Ca^{2+} in the regulation of lipolysis [18, 20], the influence of external Ca^{2+} concentration on the effects of amrinone was also tested.

Methods

Epididymal fat pads were removed from male Wistar rats (180–240 g b.w.) under light ether anaesthesia and