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SHORT COMMUNICATIONS

Improvement of postischemic rat brain energy metabolism and function by naftidrofuryl

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Animal models have been little used to define the effects of naftidrofuryl in cerebral ischemia or hypoxia. Meynaud *et al.* [1] reported increased ATP and creatine-phosphate levels and decreased lactate levels after 10 sec of decapitation ischemia in mice. Larsen *et al.* [2], who embolized rats with microspheres via the carotid artery, were able to demonstrate a moderate reduction in lethality. After 5 min of hypoxia, ATP levels in naftidrofuryl-treated rats were higher than in the corresponding controls [3]. The same workers also found a protective action of naftidrofuryl against cyanide-induced reduction of EEG-voltage in anaesthetized rats. On exposure of mice to lethal hypoxia Krieglstein and Heuer [4] were able to demonstrate an increase in survival time in drug-treated animals.

The aim of this study was to obtain information about possible effects of naftidrofuryl on brain energy metabolism and function as affected by complete cerebral ischemia. Furthermore, we were interested to examine the effects of repetitive ischemic periods on brain energy metabolism since it has been shown that naftidrofuryl enhanced the quality of the EEG as measured by EEG-power-spectra after repetitive ischemic periods in a similar experimental design (Wiernsperger, personal communication). The isolated perfused rat brain was used since ischemia can be clearly defined in this model. Furthermore, this model offers suitable experimental conditions to analyse brain energy metabolism, global cerebral blood flow and EEG during complete ischemia and the early recovery period [5–8].

Materials and methods

The preparation of the isolated brain was performed according to Andjus *et al.* [9]. The perfusion technique has been described in detail [10, 11]. Male Sprague–Dawley rats (IWV, Geretrsried, F.R.G.) were anaesthetized by intraperitoneal injection of 1.2 g/kg urethane. Closed-circuit perfusion was performed with 100 ml of a fluorocarbon perfused medium (Green Cross Corporation, Osaka, Japan) which was gassed throughout the experiment with 95% O_2 + 5% CO_2. Naftidrofuryl oxalate (10 μmol/l; Lipha, Lyon, France) was added to the perfusion medium before perfusion was started. Medium containing an equivalent amount of oxalic acid served as control.

Complete ischemia was achieved by interrupting the supply of medium to the brain. Bipolar electroencephalograms (EEG preamplifier BioAC, Hellige, Freiburg, F.R.G.) were recorded from the parietal areas of each cerebral hemisphere. Decrease in EEG amplitude below and increase above 10 μV was defined as disappearance and reappearance of spontaneous electrical activity, respectively. After a perfusion period of 40 min two ischemic periods were induced (40th to 42nd min and 50th to 52nd min) and perfusion was then continued for up to 30 min. After various time periods metabolism was stopped by immersing the brains into liquid nitrogen. The frozen brains were then stored at −80°. For analysis most of the cortical tissue was removed from the frozen brain at −20°, weighed and extracted according to procedures given by Folbergrova *et al.* [12]. ATP, ADP, AMP, glucose and lactate were determined enzymatically [13].

Results

Preischemic EEGs were found to be altered by naftidrofuryl with a tendency towards slower waves of higher amplitude. During both ischemic periods EEG disappeared within 20–30 sec. Naftidrofuryl did not influence the time of EEG disappearance. However, EEG reappeared considerably faster in naftidrofuryl-treated brains than in controls after both ischemic periods (Table 1) and was comparable to the preischemic EEG after only 3 min of reperfusion. In control brains EEG did not normalize after the first ischemic period and was found to be similar to preischemic EEG only 20–30 min after the second ischemia.

Naftidrofuryl caused a small but statistically significant decrease in flow rate in the preischemic perfusion period. During the first minute of recovery after each ischemic period perfusion rate was a little higher in naftidrofuryl-treated brains (i.e. after a second ischemic period: 3.69 ± 0.75 and 4.23 ± 0.78 ml/min, respectively; $N = 16$, $P < 0.05$).

Preischemic levels of high-energy phosphates and glycolytic substrates were found to be in the normal range (Table 2) as reported for rat brain *in vivo* [15, 16]. Two minutes of complete ischemia resulted in substantial decreases in adenylate energy charge (EC) and glucose level with a concomitant rise in lactate levels. These changes were more pronounced following the second ischemic period. After both ischemic periods the decrease in EC was less pronounced in the naftidrofuryl than in control groups (Table 2). Glucose and lactate levels were found to normalize considerably faster in naftidrofuryl-treated than in control brains. In the drug-treated brains the postischemic decline in lactate levels was significantly accelerated. In the control group lactate levels were found to be elevated even

Table 1. Effects of naftidrofuryl on EEG reappearance after ischemia

Reappearance of EEG	Control	Naftidrofuryl (10 μmol/l)	U-test
After 1st ischemic period (sec)	133 ± 121 (N = 34)	32 ± 8 (N = 34)	<0.001
After 2nd ischemic period (sec)	102 ± 47 (N = 16)	26 ± 4 (N = 16)	<0.001

Values are presented as means ± SD in seconds.
N = number of experiments.

Table 2. Effects of naftidrofuryl on energy metabolism in cerebral cortex of the isolated perfused rat brain subjected to ischemia

Substrate (μmol/g)	Naftidrofuryl (μmol/l)	Perfusion (40 min) N = 6	After 1st ischemia (42 min) N = 5)	Perfusion (47 min) N = 6	Perfusion (50 min) N = 6	After 2nd ischemia (52 min) N = 6	Perfusion (57 min) N = 6	Perfusion (62 min) N = 5	Perfusion (82 min) N = 5
EC	0	0.93 ± 0.01	0.60 ± 0.19‡	0.89 ± 0.04	0.89 ± 0.08	0.55 ± 0.25‡	0.90 ± 0.03	0.90 ± 0.05	0.92 ± 0.01
	10	0.95 ± 0.01	0.78 ± 0.06	0.93 ± 0.01	0.93 ± 0.03	0.79 ± 0.03	0.94 ± 0.02	0.93 ± 0.01	0.93 ± 0.01
Glucose	0	1.92 ± 0.34	0.32 ± 0.17	1.33 ± 0.19*	1.64 ± 0.74†	0.11 ± 0.09	1.08 ± 0.36‡	1.87 ± 0.58‡	2.44 ± 0.90
	10	2.20 ± 0.12	0.33 ± 0.19	1.99 ± 0.17	2.50 ± 1.10	0.13 ± 0.05	2.07 ± 0.18	2.99 ± 0.32	1.98 ± 0.33
Lactate	0	0.95 ± 0.38	6.44 ± 0.79	4.91 ± 1.16†	5.20 ± 1.96‡	7.48 ± 2.40	7.23 ± 0.92‡	5.58 ± 1.39‡	2.52 ± 0.63*
	10	1.07 ± 0.22	6.49 ± 0.84	2.81 ± 0.48	2.81 ± 1.64	6.73 ± 1.15	3.21 ± 0.57	1.79 ± 0.26	0.71 ± 0.38

Values are given as means ± SD in μmol/g cortical tissue (except ratios).
The difference between the substrate levels of the drug-treated and the control brains was tested by two-way analysis of variance followed by multiple *t*-tests: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ (*t*-tests). N = number of experiments. To characterize the energy state in cortical tissue the energy-charge of the adenylate pool EC = (ATP + 0.5 ADP)/(ATP + ADP + AMP) according to Atkinson [14] is given.

after 30 min after the second ischemic period whereas in the naftidrofuryl group lactate values in the preischemic range were reached after only 10 min of reperfusion (Table 2).

Discussion

The results obtained in this study clearly show that naftidrofuryl influences EEG, perfusion rate and energy metabolism of the isolated perfused rat brain after complete reversible ischemia. The acceleration by naftidrofuryl of EEG reappearance and lactate degradation were the most striking effects of the present study. Lactate levels were usually found to recover slowly following ischemic periods [8, 15]. Values in the normal range were reached only after about 2 hr of recovery [17, 18]. Our results show that, 30 min after the second ischemic period, lactate levels were still elevated in the control group. Since the rate of disappearance of lactate after ischemia can be taken as an indicator of mitochondrial function [15] the results imply that the drug protects mitochondria against ischemic damage. The earlier reappearance of EEG activity and the improved perfusion rates after each ischemic period in the drug-treated groups provide further evidence that naftidrofuryl could protect brain tissue against damage caused by ischemia.

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