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Possible therapeutic effect of naftidrofuryl oxalate on brain energy metabolism after microsphere-induced cerebral embolism

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1 The present study was designed to determine whether naftidrofuryl oxalate exerts a possible therapeutic effect on brain energy metabolism impaired by microsphere-induced cerebral embolism *in vitro*.

2 Injection of microspheres into the right carotid canal resulted in a decrease in tissue high-energy phosphates both in the right and left hemispheres, and an increase in tissue lactate in the right hemisphere, on the 3rd and the 5th day after the embolism. The embolism also induced a marked reduction in mitochondrial oxidative phosphorylation ability and succinate dehydrogenase activity. The results suggest that severe ischaemia was induced in the brain by the microsphere administration.

3 Treatment of microsphere-injected rats with naftidrofuryl oxalate ($15\,mg\,kg^{-1}$) for 3 or 5 days elicited a significant recovery of tissue high-energy phosphate and lactate levels. The recovery was associated with a significant restoration of mitochondrial succinate dehydrogenase activity on the both days and of mitochondrial oxidative phosphorylation rate on the 5th day.

4 The results suggest that naftidrofuryl oxalate is beneficial in the recovery of cerebral energy metabolism impaired by microsphere-induced cerebral ischaemia, presumably through a mechanism involving its direct effect on the cerebral mitochondrial enzyme activities.

Introduction

Cerebral ischaemia or oligemia primarily induces various degrees of disturbances in brain energy metabolism. After a certain period of interrupted blood flow, tissue high-energy phosphate levels markedly decrease and tissue lactate levels increase (Lowry *et al.*, 1964; Ljunggren *et al.*, 1974; Siesjö *et al.*, 1976; Welsh *et al.*, 1977). In such a way, lack of energy and accumulation of this metabolite are considered to produce disturbance in the transmembrane fluxes of ions (Crowe *et al.*, 1981) and cellular acidosis (Welsh *et al.*, 1987). Ischaemic insults have also been shown to induce mitochondrial impairment such as a decrease in calcium accumulating ability (Mela, 1979) and oxidative phosphorylation ability (Lazarewicz *et al.*, 1972; Mela, 1979). In a previous study (Takeo *et al.*, 1989), we have shown that the rat brain is ischaemic over a period of at least 5 days after microsphere-induced cerebral embolism. During these periods mitochondrial oxidative phosphorylation ability and succinate dehydrogenase activity of the microsphere-injected cerebral hemisphere remained markedly decreased. Under these experimental conditions, naftidrofuryl oxalate, 2-(diethylamino)ethyltetrahydro-alpha-(1-naphthyl-methyl)-2-furanpropionate ester oxalate (naftidrofuryl), improved the decreased mitochondrial activities of microsphere-injected rats *in vitro*. The results suggested a direct and beneficial effect of naftidrofuryl on brain mitochondrial activity impaired by ischaemia. Furthermore, since naftidrofuryl has been shown to exert other pharmacological actions such as an improvement in the brain blood flow under normal or pathological conditions (Hagiwara *et al.*, 1973; Kobayashi *et al.*, 1984; Yamada, 1984; Hiramatsu *et al.*, 1988), therapeutic effects of the agent on impaired brain energy metabolism could be expected, even when it is administered after the onset of ischaemia-induced

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derangements. The present study was designed to elucidate a possible therapeutic action of naftidrofuryl on the ischaemic brain energy metabolism *in vivo*.

Methods

Introduction of microsphere-induced cerebral embolism in rats

Male Wistar rats, weighing 210–290 g (CRJ; Japan Charles River Co.), were used in the present study. Microsphere-induced cerebral embolism was performed by methods described previously (Takeo *et al.*, 1989). Briefly, rats were anaesthetized by intraperitoneal administration of 30 mg kg^{-1} sodium pentobarbitone, and fixed in the supine position to an operation plate. After cervical incision, the right common carotid artery was isolated. The right external carotid and the right pterygopalatine arteries were ligated with strings. A polyethylene catheter (2 FR, Atom Co., Tokyo) was inserted into the common carotid artery. Six hundred and eighty microspheres (47.5 ± 0.5 μm in diameter), suspended in 150 μl of 20% dextran solution, were injected into the right carotid canal through this cannula. In the present study we have used slightly more microspheres (about 15% more than those used in the previous study). This could induce more definite changes in the cerebral energy metabolism and the behaviour of the animals operated on. After the injection, the right common carotid artery was ligated and the wound was closed by sutures. The rats which underwent sham operation were injected with the same volume of 20% dextran solution without microspheres. Fifteen hours after the surgery, the behaviour of operated rats was monitored. The behaviour of microsphere-injected rats was scored on the basis of paucity of movement, truncal curvature and forced circling towards the right during locomotion, which are considered to be typical symptoms of stroke (Furlow & Bass, 1976; McGraw, 1977). The score for each item was ranked from 0 to 2. The rats which had 5 to 6 points of the score were considered to be A type, 3 to 4 to be B type and less than 2 to be C type. In the present biochemical study, the A type rats were used.

Treatment with naftidrofuryl

After inspection of the symptoms of the animals on the first day after surgery, the rats which showed typical symptoms (A type) were treated twice daily with naftidrofuryl (15 mg kg^{-1}, i.p., 10 h 00 min and 18 h 00 min). The agent was dissolved in distilled water at a concentration of 45 mg ml^{-1}. The vehicle was similarly administered to the control group. The rats killed on the 3rd day received 5 injections, and those on the 5th day, 9. The dose employed in the present study has been shown to be effective in preventing ischaemia-induced damage to brain energy metabolism of bilateral carotid artery-ligated mice (Takeo *et al.*, 1988).

Determination of tissue high-energy phosphates, lactate and pyruvate

Adenosine triphosphate (ATP), creatine phosphate (CP), lactate and pyruvate in the brain hemispheres were determined by methods described previously (Takeo *et al.*, 1987; 1988; 1989). Briefly, the rats were decapitated in an appropriate experimental sequence and the heads were immersed in liquid nitrogen within 0.5 s after decapitation. Both hemispheres were isolated and their frozen weights were determined. Each hemisphere was pulverized separately in a stainless centrifuge tube with a stainless steel plunger under liquid nitrogen-cooling. The brain tissue powder was extracted with 0.3 M $HClO_4$ + 0.25 mM EDTA. After centrifugation of the extract at 1,000 *g*, the supernatant fluid was neutralized with 2.5 M K_2CO_3 and sampled for determination of tissue high-energy phosphates, lactate and pyruvate. The ATP content was determined enzymatically (Bücher, 1947) and CP, according to the methods of Lowry & Passonneau (1972). The tissue lactate and pyruvate were determined by the methods of Gutman & Wahlefeld (1974) and Czok & Lamprecht (1974), respectively. The content of tissue metabolites was expressed as μmol g^{-1} dry tissue. The ratio of dry tissue weight to the frozen tissue weight was estimated in a preliminary study and found to be 0.200 ± 0.002 (n = 10).

Isolation of cerebral mitochondria

Isolation of cerebral mitochondria was carried out according to the methods of Sordahl *et al.* (1971). The brain was removed within 15 s after decapitation, and immersed in cold 0.3 M mannitol + 0.1 mM EDTA solution, pH 7.4 (ME buffer). The cerebral hemispheres were separated in the cold ME buffer and employed for isolation of the mitochondria from both sides. After determining the wet weight, the brain was homogenized with a loose fitting glass teflon homogenizer. The homogenate was centrifuged at 600 *g* for 8 min at 0–4°C. The supernatant fluid was centrifuged at 10,000 *g* for 10 min at 0–4°C. The pellet was rehomogenized in ME buffer, then centrifuged at 5,000 *g* for 10 min. After decantation of the supernatant fluid, the pellet was washed with 20 ml of ME buffer. The washing solution and the supernatant fluid described above

were combined, and the resulting suspension was centrifuged at 5,000 *g* for 10 min. The collected pellet and the pellet described above were suspended in ME buffer or 30 mM potassium phosphate – 0.1% bovine serum albumin and sampled for determination of oxidative phosphorylation ability or succinate dehydrogenase activity of the cerebral mitochondria, respectively. Mitochondrial protein concentrations were determined by the methods of Lowry *et al.* (1951). The protein yields of the mitochondria from the right and the left cerebral hemisphere ranged from 0.55 ± 0.01 to 0.60 ± 0.01 in each experimental group. There were no significant differences in the protein yield of the isolated mitochondria irrespective of the operation performed or the hemisphere to which microspheres were administered.

Measurements of the oxidative phosphorylation ability

The oxidative phosphorylation ability of the isolated cerebral mitochondria was measured according to the method of Sordahl *et al.* (1971). The oxygen consumption of the mitochondria was continuously monitored at 25°C on an oximeter with Clark type DO/O_2 sensor (Oxygraph-8, Central Sciences Co., Tokyo). The reaction mixture (Total volume: 1 ml) contained 0.3 M mannitol, 10 mM KCl, 10 mM Tris/HCl, 5 mM K_2HPO_4, 0.2 mM EDTA and about 1.0 mg of mitochondrial protein. Either 10 mM potassium glutamate or 10 mM potassium succinate was used as the substrate for the phosphorylation. State 3 respiration was initiated by the addition of 250 nmol ADP. As indicators for the oxidative phosphorylation ability of the mitochondria, the following parameters were measured: respiratory control index (RCI; ratio of oxygen consumption at state 3 to that at state 4), ADP/O (nmol ADP natom^{-1} oxygen consumption at state 3), rate of oxygen consumption at state 3 (QO_3; natoms oxygen min^{-1} mg^{-1} protein), rate of oxygen consumption at state 4 (QO_4; natoms oxygen min^{-1} mg^{-1} protein) and oxidative phosphorylation rate (OPR; nmol ATP produced during state 3 min^{-1} mg^{-1} protein).

Measurements of succinate dehydrogenase activity

The mitochondrial succinate dehydrogenase activity was measured according to the methods of Slator & Bonner (1952). In a 1 ml cuvette, 300 μl of 0.3 M potassium phosphate buffer (pH 7.6), 30 μl of 30 mM EDTA, 90 μl of 0.4 M potassium succinate (pH 7.6), 30 μl of 3% bovine serum albumin, 30 μl of 75 mM $K_3Fe(CN)_6$, 360 μl of distilled water and 25 μl of mitochondrial protein (about 500 μg) were mixed and the absorbance of the mixture was measured at 455 nm for 2 min at 25°C. The initial rate of reduction of absorbance was taken as the succinate dehydrogenase (SDH) activity. The absorbance of the mixture without succinate served as the blank value.

Statistics

Values in the text represent the mean ± s.e.mean. The observed values of activities of rats without operation were used as standard (normal value). As statistical tests, Student's *t* test was used for comparison of the activities of the mitochondria and the amounts of metabolites measured. Differences with a probability of 5% or less were considered to be statistically significant ($P < 0.05$).

Results

Microsphere-induced cerebral embolism

Microsphere-induced embolism was evoked in 310 rats. Numbers of animals with A type symptoms were 168 (54%). Eighty-three rats (27%) died before inspection of the behaviour or determination of metabolites. Thirty-seven rats showed slightly milder symptoms than A type (B type; 12%). There were twenty-two rats which showed very light symptoms of stroke (C type; 7%). Twenty-nine rats with A type symptoms were used for a preliminary study and morphological examination. The rest of the A type animals, 115 rats, were used for biochemical examination. Sham operations were performed with another 42 rats. None of these animals died during the experiment. An additional 19 rats were used for measurement of tissue metabolites and mitochondrial SDH activity as standard values.

The characteristic behaviour of rats with cerebral embolism on the 3rd and 5th day after the operation was appreciably milder than that observed on the 1st day. The behaviour of microsphere-injected rats on both days tended to be improved by treatment with naftidrofuryl. For example, the score of the rats after treatment with naftidrofuryl ranged from 1 to 3, whereas that without the treatment was 1 to 5. However, the effect could not be clearly defined merely by studying animal behaviour.

Tissue high-energy phosphates

Tissue high-energy phosphate levels of the rat hemispheres isolated on the 3rd day and the 5th day after the onset of microsphere-induced cerebral embolism are shown in Table 1. The tissue ATP and CP levels of the brain hemispheres from the sham-operated rats were slightly lower than the values for non-

Table 1 Tissue high-energy phosphate content of rat hemispheres isolated on the 3rd day and the 5th day after microsphere-induced cerebral embolism

	No. of experiments	*Right hemisphere*		*Left hemisphere*	
		ATP	*CP*	*ATP*	*CP*
No operation	12	12.70 ± 0.11	14.10 ± 0.17	12.61 ± 0.16	14.23 ± 0.13
3rd day					
Sham-operation	7	11.70 ± 0.13	12.26 ± 0.33	12.65 ± 0.10	13.51 ± 0.12
Microsphere-embolism	8	6.43 ± 0.28*	5.53 ± 0.20*	9.26 ± 0.30*	9.16 ± 0.36*
Microsphere-embolism + naftidrofuryl	8	7.62 ± 0.09†	6.96 ± 0.15†	10.67 ± 0.19†	11.13 ± 0.36†
5th day					
Sham-operation	7	11.23 ± 0.27	12.16 ± 0.43	12.16 ± 0.21	13.28 ± 0.16
Microsphere-embolism	8	7.80 ± 0.24*	7.56 ± 0.43*	10.22 ± 0.18*	10.19 ± 0.24*
Microsphere-embolism + naftidrofuryl	8	9.55 ± 0.28†	9.66 ± 0.34†	11.45 ± 0.14†	12.25 ± 0.28†

Values are expressed as $\mu mol\ g^{-1}$ dry wt tissue. Each value represents the mean ± s.e.mean.
* Significantly different from the value for sham-operated rats ($P < 0.05$).
† Significantly different from the value for microsphere-injected rats ($P < 0.05$). CP, creatine phosphate.

operated rats. Microsphere-induced cerebral embolism caused reductions in ATP and CP of about 50 to 40 and 60 to 46% in the right hemisphere on both days monitored, respectively. Similarly, but to a lesser degree, the tissue ATP and CP contents of the left hemisphere were significantly reduced after embolism. Treatment of the rats with naftidrofuryl restored the tissue ATP and CP levels in both hemispheres isolated on the 3rd day after the surgery to a significant degree. Similar results were obtained in the brain hemispheres isolated from rats on the 5th day as shown in Table 1.

Tissue lactate and pyruvate contents

The lactate and pyruvate contents of the rat brain hemispheres isolated on the 3rd and the 5th days after the onset of microsphere-induced cerebral embolism are shown in Figures 1 and 2, respectively. The tissue lactate content of the right cerebral hemisphere of the normal rat was 22.09 ± 1.34 $\mu mol\ g^{-1}$ dry tissue ($n = 12$), whereas the tissue pyruvate content was 1.36 ± 0.16 $\mu mol\ g^{-1}$ dry tissue ($n = 12$). The tissue lactate contents of the right hemisphere of the rat brain on the 3rd and the 5th day (37.97 and 33.96 $\mu mol\ g^{-1}$ dry tissue, respectively; mean value of 8 experiments) were significantly higher than those of the sham-operated rat. The pyruvate content of the right hemisphere of the microsphere-injected rat was also higher than that of the sham-operated rats. When the microsphere-injected rats were treated with naftidrofuryl for 3 or 5 consecutive days, the tissue lactate and pyruvate levels of the right hemisphere returned toward the standard levels to a significant extent (31.81 and 24.20 or 1.56 and 1.11 $\mu mol\ g^{-1}$ dry tissue, respectively; mean value of 8 experiments). Tissue lactate and pyruvate levels of

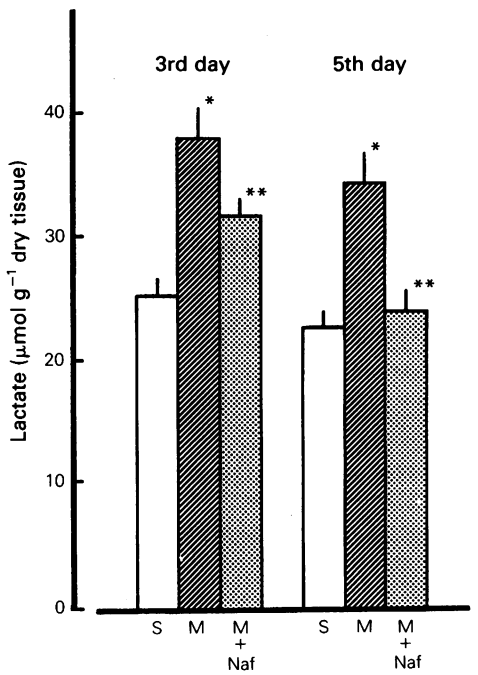

Figure 1 Tissue lactate levels of the right cerebral hemispheres isolated on the 3rd and 5th day after microsphere-induced cerebral embolism in rats. S (open columns): sham-operated group ($n = 7$). M (hatched columns): microsphere-injected group ($n = 8$). M + Naf (stippled columns): microsphere-injected group with naftidrofuryl-treatment ($n = 8$). * Significantly different from the values for the sham-operated group ($P < 0.05$). ** Significantly different from the values for the microsphere-injected group ($P < 0.05$). Vertical bars show s.e.mean.

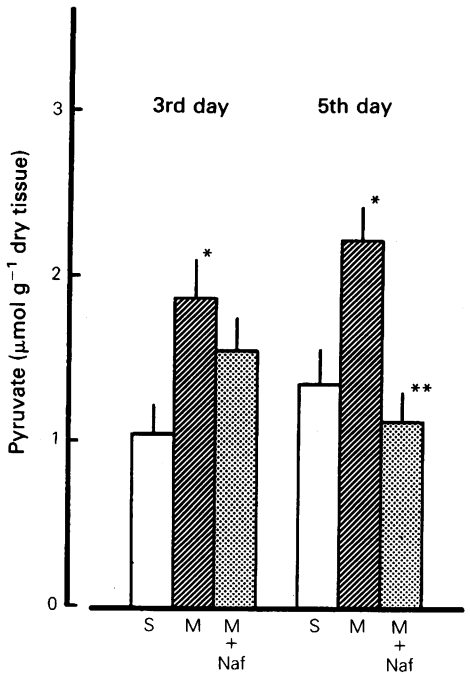

Figure 2 Tissue pyruvate levels of the right cerebral hemispheres isolated on the 3rd and 5th day after microsphere-induced cerebral embolism in rats. S (open columns): sham-operated group ($n = 7$). M (hatched columns): microsphere-injected group ($n = 8$). M + Naf (stippled columns): microsphere-injected group with naftidrofuryl-treatment ($n = 8$). * Significantly different from the values for the sham-operated group ($P < 0.05$). ** Significantly different from the values for the microsphere-injected group ($P < 0.05$). Vertical bars show s.e.mean.

the left hemisphere on both days were not significantly altered by either microsphere-embolism or treatment with naftidrofuryl (data not shown).

Mitochondrial oxidative phosphorylation activity

Standard values of the mitochondrial oxidative phosphorylation activity were determined previously (Takeo *et al.*, 1989). All the values in sham-operated rats were similar to those of standard rats except for a slightly higher oxidative phosphorylation rate (OPR) of the right hemisphere in the presence of glutamate and a slight lower OPR in the right hemisphere in the presence of succinate. The OPR of the right hemisphere of the microsphere-injected rats tended to be higher on the 3rd and 5th days after the embolism in the presence of glutamate as a substrate (15 and 25% increases, respectively), whereas the OPR was significantly lower when measured in the presence of succinate as a substrate (30 and 24% decreases, respectively). Trends similar to those mentioned above were seen in the changes of other activities such as RCI, ADP/O and QO_3.

The mitochondrial oxidative phosphorylation activity of microsphere-injected rats on the 5th day was essentially similar to that on the 3rd day, and no significant effects of naftidrofuryl on the mitochondrial oxidative phosphorylation activity were observed in rats on the 3rd day. The results of the rat mitochondria on the 5th day are shown in Table 2. A significant recovery of the OPR was seen only in the right hemisphere isolated on the 5th day from the microsphere-injected rats treated with naftidrofuryl, when the activity was measured in the presence of succinate. This was accompanied by a slight improvement of ADP/O in the right hemisphere.

Table 2 Effects of naftidrofuryl (Naf) on the oxidative phosphorylation activity of the mitochondria isolated from rat brain on the 5th day after microsphere-induced embolism *in vivo*

	Glutamate			*Succinate*		
	Sham	*Microsphere*	*Microsphere + Naf*	*Sham*	*Microsphere*	*Microsphere + Naf*
OPR	83.6 ± 3.5	104.2 ± 5.5*	113.6 ± 4.8	207.6 ± 5.0	158.7 ± 6.1*	182.9 ± 8.7†
RCI	3.10 ± 0.05	3.80 ± 0.21*	3.93 ± 0.13	2.81 ± 0.08	2.44 ± 0.08*	2.55 ± 0.11
ADP/O	2.35 ± 0.05	2.57 ± 0.05*	2.69 ± 0.04	1.92 ± 0.02	1.87 ± 0.04	2.01 ± 0.03†
QO_3	35.6 ± 0.9	40.4 ± 1.7*	42.2 ± 1.7	108.4 ± 3.1	84.9 ± 2.7*	91.0 ± 3.7
QO_4	11.5 ± 0.4	10.8 ± 0.4	10.8 ± 0.3	38.2 ± 1.0	34.9 ± 1.0*	35.9 ± 0.9

* Units: RCI (respiratory control index) = ratio of QO_3/QO_4. ADP/O (ADP consumed during state 3) = nmol ADP natoms^{-1} oxygen. QO_3 (oxygen uptake at state 3) = natoms oxygen mg^{-1} protein min^{-1}. QO_4 (oxygen uptake at state 4) = natoms oxygen mg^{-1} protein min^{-1}. OPR (oxidative phosphorylation rate) = nmol ATP produced during state 3 mg^{-1} protein min^{-1}. Each value represents the mean ± s.e.mean of 7 (sham), 11 (microsphere) and 11 (microsphere plus naftidrofuryl). All parameters seen in the mitochondria isolated from the left cerebral hemisphere were not significantly altered in the present experimental conditions.

* Significantly different from the values for the sham-operated group ($P < 0.05$).

† Significantly different from the values for the microsphere-injected group ($P < 0.05$).

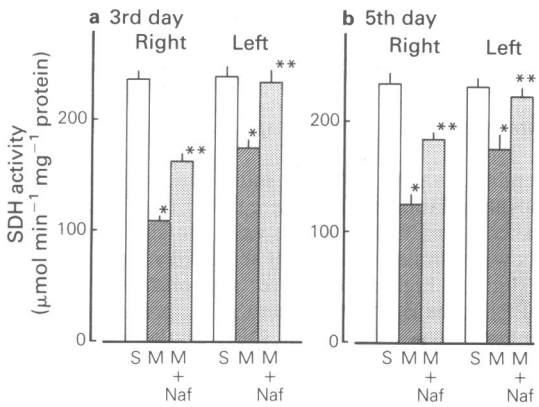

Figure 3 The succinate dehydrogenase activity of the cerebral mitochondria isolated on the 3rd (a) and 5th (b) day after microsphere-induced cerebral embolism in rats. The activity is expressed as μmol NADPH min^{-1} mg^{-1} protein. S (open columns): sham-operated group ($n = 7$). M (hatched columns): microsphere-injected group ($n = 8$). M + Naf (stippled columns): microsphere-injected group with naftidrofuryl-treatment ($n = 8$). * Significantly different from the values of the sham-operated group ($P < 0.05$). ** Significantly different from the values for the microsphere-injected group ($P < 0.05$).

The mitochondrial oxidative phosphorylation activities of the left hemisphere on both days were not affected by either microsphere-embolism or treatment with naftidrofuryl (data not shown).

Mitochondrial succinate dehydrogenase activity

The mitochondrial SDH activity on the 3rd and the 5th days is shown in Figures 3a,b. The mitochondrial SDH activity of the right and the left hemispheres of the normal rat (without operation) was 266.6 ± 5.2 and 272.4 ± 4.1 μmol NADPH min^{-1} mg^{-1} protein respectively ($n = 7$). Microsphere-induced cerebral embolism markedly reduced the mitochondrial SDH activity in the right and left hemispheres regardless of the site of microsphere-administration (125.6 and 177.1 μmol NADPH min^{-1} mg^{-1} protein on the 3rd day, and 110.2 and 175.2 μmol NADPH min^{-1} mg^{-1} protein on the 5th day, respectively; mean value of 8 experiments). Treatment of the microsphere-injected rats with naftidrofuryl markedly restored the decreased SDH activity of both hemispheres on the 3rd and the 5th day after embolism (183.3 and 222.9 μmol NADPH min^{-1} mg^{-1} protein on the 3rd day and 164.3 and 236.5 μmol NADPH min^{-1} mg^{-1} protein on the 5th day, respectively; mean value of 8 experiments). The SDH activity in the left hemisphere recovered almost completely as a result of naftidrofuryl treatment.

Discussion

In a previous study we observed that symptoms of stroke in rats on the 3rd day after the embolism were more severe than in rats on the 5th day, and that the tissue ATP and CP contents were most markedly reduced on the 3rd day after the embolism and, on the 5th day, these levels tended to be slightly restored toward the normal levels (Takeo *et al.*, 1989). These findings allowed us to study extensively the cerebral energy metabolism of microsphere-injected rats, on the 3rd and the 5th days after the embolism as severely injured and recovering stages of impaired brain function and metabolism, respectively.

The high-energy phosphate levels of the cerebral hemispheres were markedly reduced both on the 3rd and the 5th days after microsphere-induced cerebral embolism. The tissue lactate of the right hemisphere also showed higher levels than control on both days. An increase in tissue lactate and a decrease in tissue high-energy phosphate are well accepted to be markers of an ischaemic state in the tissue, as discussed in the Introduction. Thus, the results confirmed that microsphere-induced cerebral embolism in the present study induces cerebral ischaemia, which is sustained for at least 5 days after the surgery. The reduction in tissue high-energy phosphate levels was associated with a significant decrease in oxidative phosphorylation activity of the cerebral mitochondria, the ATP producing ability, and in SDH activity, an activity which plays an important role in the tricarboxylic acid cycle of glucose metabolism in cerebral mitochondria. The changes in these parameters in the right hemisphere are more severe than those in the left hemisphere because the latter site might indirectly receive microspheres through the circle of Willis. It should be mentioned that histological examination of the brain cortex after haematoxylin-eosin staining indicated a spread of a haemorrhage to the forebrain, which was accompanied by necrosis and oedema around the haemorrhagic area. In addition, yellow-brown round substances were seen in the microvessels of the nectrotic area, which can be identified as microspheres.

Under these ischaemic conditions, treatment of the rats which showed typical symptoms of cerebral impairment with naftidrofuryl appreciably influenced these metabolic parameters. That is, treatment with naftidrofuryl significantly restored the tissue high-energy phosphate levels in both hemispheres and suppressed the increase in tissue lactate levels in the right cerebral hemisphere on the 3rd and the 5th days. Welsh and co-workers (1980, 1987) have

emphasized the crucial role of lactic acidosis in the genesis of ischaemic damage to cerebral function and metabolism. Thus, the reduction in cerebral lactate levels by treatment with naftidrofuryl, observed in the present study, would be beneficial to brain function and metabolism after ischaemia with respect to the prevention of brain damage from lactic acidosis.

On the 5th day, the recovery of tissue high-energy phosphate levels was accompanied by a slight but significant recovery of the OPR, assessed in the presence of succinate. As mentioned in the Results section, the OPR after the cerebral embolism was increased when measured in the presence of glutamate, whereas it was decreased when measured in the presence of succinate. It should be noted that embolism-induced increases in the mitochondrial oxidative phosphorylation activities, such as RCI, ADP/O, QO_3 and OPR, in the presence of glutamate still persisted after treatment with naftidrofuryl, whereas the embolism-induced decrease in the mitochondrial oxidative phosphorylation activities in the presence of succinate was significantly restored by the treatment. These findings positively suggest that naftidrofuryl facilitates the cerebral mitochondrial activity and so improves mild or reversible ischaemic damage to brain energy metabolism, particularly during the recovery stage of metabolic function (on the 5th day). Presumably this may be exerted through a direct effect of the agent on mitochondrial oxidative phosphorylation, or through activation of the tricarboxylic acid cycle, because we found, in a previous study, significant improvements in oxidative phosphorylation ability and succinate dehydrogenase activity of cerebral mitochondria of microsphere-injected rats under *in vitro* conditions (Takeo *et al.*, 1989).

A decrease in mitochondrial SDH activity is thought to represent damage to the mitochondrial membrane or an impairment of the tricarboxylic acid cycle. It has been shown that acute hypoxia also induces a significant decrease in SDH activity of rat brain homogenate (Purshottam & Ghosh, 1975). In the present study, mitochondrial SDH activity was greatly diminished on both the 3rd and 5th days to a similar extent, suggesting a sustained impairment of mitochondrial membrane function due to microsphere-induced cerebral embolism. It is obviously beneficial that treatment with naftidrofuryl significantly restored the decreased SDH activity of the rat brain on both days, suggesting a restoration of mitochondrial membrane function by the agent.

In a previous study from our laboratory, we have shown, *in vitro*, that naftidrofuryl restores mitochondrial SDH activity of the cerebral hemispheres from microsphere-injected rats (Takeo *et al.*, 1989). This is comparable with the findings of the current *in vivo* study. It should be noted that treatment of sham-operated rats with naftidrofuryl (in two experiments) did not produce any appreciable changes in tissue high-energy phosphate and lactate levels or in mitochondrial SDH and oxidative phosphorylation activity, suggesting no effect of the agent *per se* on cerebral function and metabolism. The extent of the recovery of SDH activity was more profound and more consistent than that of the oxidative phosphorylation ability in the *in vitro* as well as in the *in vivo* study. Furthermore, the recovery was associated with a slight, but significant restoration of microsphere-induced decreases in brain high-energy phosphate levels. These results alllow us to conclude that restoration of mitochondrial SDH activity or activation of the tricarboxylic acid cycle in the mitochondria may substantially contribute to the therapeutic effect of naftidrofuryl on ischaemic brain energy metabolism.

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