



Effects of delayed treatment with nafronyl oxalate on microsphere embolism-induced changes in monoamine levels of rat brain regions

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1 The present study was undertaken to examine the effects of delayed treatment with nafronyl oxalate (nafronyl), a cerebral vasodilator, on monoamine neurotransmitters of brain regions in the microsphere-embolized rat.

2 Microsphere embolism was induced by injecting 900 microspheres with a diameter of 48 μm into the right internal carotid artery of rats. Microsphere-embolized rats were treated with nafronyl, 15 mg kg⁻¹, i.p., twice daily from the first to the 5th day. Levels of monoamines and their metabolites in the cerebral cortex, striatum, and hippocampus were measured on days 3 and 5 after the operation by a high-performance liquid chromatograph with electrochemical detection. *In vivo* tyrosine or tryptophan hydroxylation was estimated by measurement of the accumulation of 3,4-dihydroxyphenylalanine or 5-hydroxy-1-tryptophan after administration of 3-hydroxybenzylhydrazine dihydrochloride, an inhibitor of aromatic L-amino acid decarboxylase.

3 Microsphere embolism induced decreases in dopamine, noradrenaline and 5-hydroxytryptamine in three brain regions of the right hemisphere on days 3 and 5. In the left hemisphere, the monoamines were reduced, but to a lesser degree than in the right hemisphere. On days 3 and 5, the decrease in the monoamines of the right hemisphere was attenuated by nafronyl treatment except for noradrenaline on day 3. The decrease in the monoamines levels in the left hemisphere was almost completely prevented by nafronyl treatment.

4 On day 3 after microsphere embolism, *in vivo* tyrosine and tryptophan hydroxylation was lower than the pre-embolic value in all three brain regions. Treatment of the embolized rats with nafronyl significantly attenuated the decrease in *in vivo* tyrosine and tryptophan hydroxylation in the ipsilateral hemisphere, but not hippocampal tryptophan hydroxylation.

5 The results suggested that treatment with nafronyl improves or attenuates changes in monoamine neurotransmitter metabolism of the brain regions impaired by microsphere embolism. The mechanisms underlying this effect may be attributed to preservation of the ability to synthesize monoamines when the brain is ischaemic or oligoemic.

Keywords: Cerebral ischaemia; microsphere embolism; monoamine neurotransmitters; nafronyl oxalate

Introduction

Nafronyl oxalate, [2-(diethylamino)ethyltetrahydro- α -(1-naphthylmethyl)-2-furanpropionate ester oxalate](nafronyl), has been shown to increase cerebral blood flow in normal animals (Young *et al.*, 1983; Hiramatsu *et al.*, 1988) and in human subjects (Yamada, 1984; Ohtomo *et al.*, 1986), and thus, has been used clinically to improve cerebral circulation in several European countries (Admani, 1978).

Previous studies from our laboratory demonstrated that administration of microspheres into the internal carotid artery induces prolonged cerebral ischaemia and infarction in rats. In these studies, we showed that cerebral embolism with microspheres induced a marked decrease in cerebral blood flow (Miyake *et al.*, 1993), a pronounced disturbance in brain glucose metabolism (Takeo *et al.*, 1991) and high-energy phosphate production (Takeo *et al.*, 1992), and a significant reduction in acetylcholine and neurotransmitter amino acids (Taguchi *et al.*, 1993) in the cortex, striatum and hippocampus of the microsphere-injected hemisphere. We also found that delayed treatment with nafronyl has beneficial effects on the microsphere-induced pathophysiological alteration (Miyake *et al.*, 1989, 1994; Takeo *et al.*, 1991; Taguchi *et al.*, 1994).

Microsphere-induced brain ischaemia, characterized as

above, is considered to resemble clinical cerebrovascular disease because multiple infarction is a common cause of cerebrovascular disease or vascular dementia (Naritomi, 1991). However, little is known concerning the pathophysiological alteration of prolonged stroke and its therapy by drugs in experimental models.

The purpose of the present study was to determine whether microsphere-embolism induces changes in monoaminergic neurotransmitter metabolism and, if so, to examine the effects of delayed treatment with nafronyl, a cerebral vasodilator, on monoamine neurotransmitters of brain regions in the microsphere-embolized rat. For this purpose, we examined changes in the content of monoamine and its metabolite and *in vivo* synthesis of monoamines in the cerebral cortex, striatum and hippocampus of the microsphere-embolized rat. These brain regions are recognized as vulnerable to ischaemic insult (Kirino, 1982; Pulsinelli *et al.*, 1982; Smith *et al.*, 1984).

Methods

Surgical procedure

Male Wistar rats weighing 180 to 220 g (Charles River Japan, Inc., Atsugi, Japan) were used in the present study. The animals were maintained under artificial conditions at 23 \pm 1°C

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with a constant humidity of $55 \pm 5\%$ with a cycle of 12 h light and 12 h dark, and had free access to food and tap water according to the Guidelines of Experimental Animal Care issued by the Prime Minister's Office of Japan. Microsphere-induced cerebral embolism was performed by the method previously described (Miyake *et al.*, 1989). The rat was anaesthetized i.p. with sodium pentobarbitone, 35 mg kg^{-1} , and fixed in the supine position on 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 (3 Fr. Atom Co., Tokyo) was inserted into the right common carotid artery. Nine hundred microspheres ($47.5 \pm 0.5 \mu\text{m}$ in diameter; NEN-005, New England Nuclear Inc., Boston, MA, U.S.A.) suspended in 20% dextran solution were injected into the right internal carotid artery through this cannula. The rats which underwent sham operation were injected with the same volume of vehicle without microspheres.

Neurological deficits

Fifteen hours after the operation, the behaviour of the operated rats was scored on the basis of paucity of movement, truncal curvature and forced circling during locomotion, which are considered to be typical symptoms of stroke in rats (Furlow & Bass, 1976; McGraw, 1977). The score of each feature was ranked from 3 to 0 (3, very severe; 2, severe; 1, moderate; 0, little or no symptoms). The rats which had more than 7 points were considered to be type A, 6 to 4 type B and less than 4 type C. In the present study, we used only type A animals for the studies on monoaminergic neurotransmitter metabolism.

Treatment with nafronyl oxalate

After ensuring stroke-like symptoms of the microsphere-injected rats, nafronyl treatment started (15–16 h after the operation). Type A rats were treated twice a day with 15 mg kg^{-1} nafronyl i.p.. This treatment was performed up to the end of experimental sequences, that is, until the 3rd or 5th days after operation. Treatment with nafronyl employed in the present study was the same as that which exerted beneficial effects on cerebral glucose metabolism, high-energy phosphate production and acetylcholine content of brain regions following microsphere embolism (Miyake *et al.*, 1992; Taguchi *et al.*, 1994).

Measurements of monoamines and their metabolites

At an appropriate time in the experimental sequence, the microsphere-injected, sham-operated and non-operated (control) rats were killed with a focal microwave irradiation of the head with a strength of 5 kW for 0.85 s by a microwave applicator (TMW-6402C, Muromachi Kikai Co., Tokyo). After decapitation, the head of the animal was immersed in liquid nitrogen and left for 10 s (near freezing). The cerebral hemispheres were isolated and separated into three brain regions: cerebral cortex, striatum and hippocampus. Each brain region was homogenized in 0.2 M HClO_4 and 0.01% ethylenediaminetetraacetic acid (EDTA) with a Polytron homogenizer (PT-10, Kinematica, Switzerland). The homogenate was centrifuged at $10,000 g$ for 15 min at 4°C . The supernatant fluid was filtered through a membrane filter ($0.45 \mu\text{m}$). A $5 \mu\text{l}$ aliquot of the supernatant fluid was injected into a high-performance liquid chromatograph with electrochemical detection (h.p.l.c.-e.c.d) to determine the concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), noradrenaline (NA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA).

The h.p.l.c.-e.c.d system consisted of a reverse-phase column (MA-5 ODS, $150 \times 4.6 \text{ mm}$ inside diameter, Eicom, Japan), a model L-6000 pump (Hitachi, Japan) and an electrochemical detector (ECD-100, Eicom). The mobile phase contained 0.1 M citric acid–0.1 M sodium acetate, disodium

EDTA (5 mg l^{-1}), sodium octane sulphonate (230 mg l^{-1}) and 5% methanol in deionized and distilled water, and the pH was adjusted to 3.5.

Determination of *in vivo* tyrosine and tryptophan hydroxylation in the brain regions

To examine changes in *in vivo* synthesis of monoamines, tyrosine or tryptophan hydroxylation, a rate limiting step for synthesis of monoamines, was estimated by measuring the *ex vivo* accumulation of DOPA or 5-HTP, respectively, after inhibition of aromatic L-amino acid decarboxylase with 3-hydroxybenzylhydrazine dihydrochloride (NSD-1015). The microsphere-embolized rat was given 100 mg kg^{-1} i.p. NSD-1015 on day 3 after the operation. The rats which received no surgery were taken as controls. Thirty minutes later the rats were killed with focal microwave irradiation followed by the near freezing in liquid nitrogen as described above, the cerebral hemispheres were isolated and the cortex, striatum, and hippocampus were separated. In the experiment on the effect of nafronyl on *in vivo* tyrosine and tryptophan hydroxylation in the brain regions, nafronyl was administered 35 min before the rats were killed. Tissue content of DOPA and 5-HTP in the brain regions were determined by h.p.l.c.-e.c.d as described above.

Statistics

The results are expressed as the mean \pm s.e.mean. Statistical significance for comparison of monoamines, their metabolites

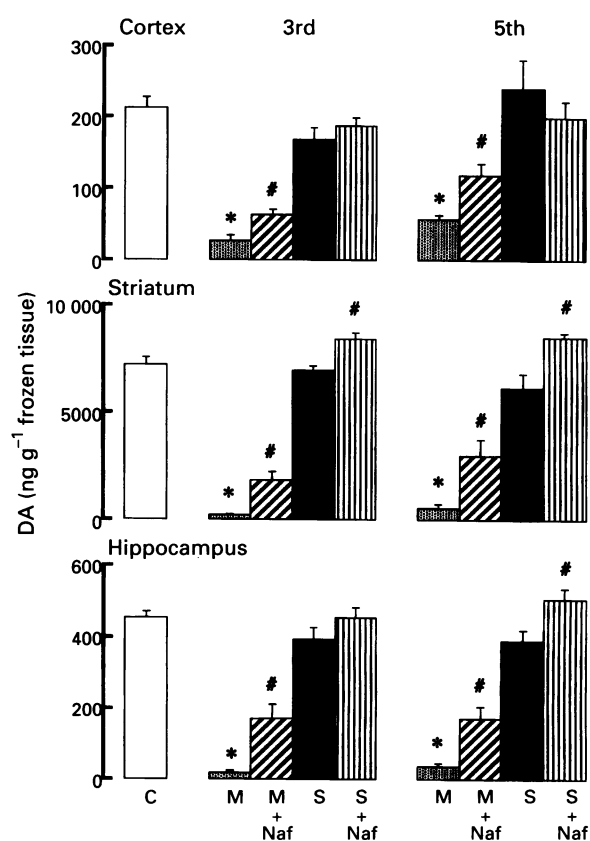


Figure 1 Dopamine (DA) content of the cerebral cortex, striatum and hippocampus of the right hemisphere on days 3 and 5 of microsphere-injected (M and M+Naf) and sham-operated (S and S+Naf) rats with (+Naf) and without nafronyl oxalate treatment, and of control rats (C). Each value represents the mean \pm s.e.mean of 7 experiments. *Significantly different from the corresponding sham-operated group and #significantly different from the untreated group ($P < 0.05$).

and DOPA and 5-HTP accumulation with and without microsphere injection or those with and without nafronyl treatment was evaluated by the *F* test, followed by either Student's or Welch's *t* test. *P* values of less than 0.05 were considered to be statistically significant.

Results

Neurological deficits after microsphere embolism

In the present study, 24 rats (18%) out of 136 rats that were injected with microspheres died within 24 h of surgery. After inspection of animal behaviour, it was found that 85 rats (63%) showed typical type A symptoms and of these, 8 rats died within 3 days of the operation. Thirteen rats showed B-type symptoms (10%), and 11 rats (8%) type C. The results were similar to those reported previously (Miyake *et al.*, 1994; Taguchi *et al.*, 1994). The sham-operated rats showed no stroke-like symptoms and survived throughout the experiment period.

Changes in monoamine content

Dopamine content The right cortical, striatal, and hippocampal dopamine contents of control rats were 212.4 ± 15.1 , 7224.4 ± 308.8 and 452.3 ± 17.5 ng g⁻¹ frozen tissue, respectively. In microsphere-embolized rats, a significant decrease in dopamine content in three brain regions of the right hemisphere was seen on day 3 after the embolism: the dopamine contents of cerebral cortex, striatum and hippocampus were 13, 3 and 4% of the corresponding dopamine content of control animals (Figure 1). Treatment of microsphere-embolized rats with nafronyl resulted in a significant restoration of dopamine content in the right hemisphere on day 3. The cortical, striatal and hippocampal dopamine contents of the right hemisphere on day 5 after microsphere embolism were 26, 8 and 8% of those of sham-operated animals. The decrease in dopamine content of the right hemisphere of microsphere-embolized rats on day 5 was attenuated by nafronyl treatment, similar to that on day 3. In the left hemisphere, the hippocampal dopamine content was reduced on day 3, but to a lesser degree than in the right hemisphere (Table 1). On days 3 and 5, the decreased dopamine content of the left hippocampus was completely restored to control

by nafronyl treatment (Table 1). In sham-operated animals treated with nafronyl, there was an increase in dopamine

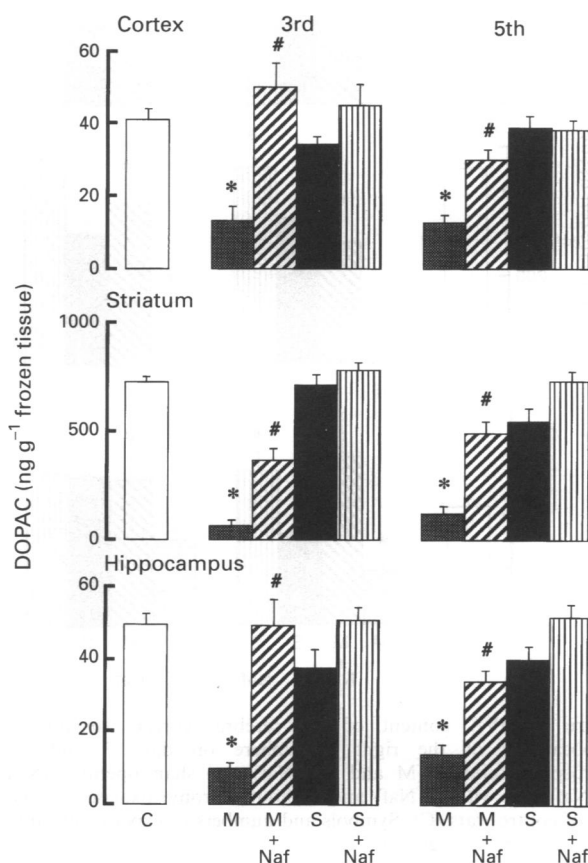


Figure 2 DOPAC content of the cerebral cortex, striatum and hippocampus of the right hemisphere on days 3 and 5 of microsphere-injected (M and M+Naf) and sham-operated (S and S+Naf) rats with (+Naf) and without nafronyl oxalate treatment, and of control rats (C). Symbols and numbers of experiments are the same as those in Figure 1.

Table 1 Dopamine and its metabolites and noradrenaline (NA) contents of the left hemisphere of microsphere-embolized and sham-operated rats with and without nafronyl oxalate (Naf) treatment

	Experimental group	Day 3			Day 5		
		Cortex	Striatum	Hippocampus	Cortex	Striatum	Hippocampus
Dopamine	Sham	180.7 ± 18.3	7705.8 ± 289.6	394.5 ± 25.0	247.9 ± 35.7	7133.2 ± 353.6	391.4 ± 26.5
	Sham + Naf	216.1 ± 9.5	7998.8 ± 447.2	434.5 ± 22.6	210.5 ± 14.7	7977.0 ± 477.2	443.3 ± 18.0
	Microsphere	154.4 ± 18.8	6211.5 ± 453.4*	230.7 ± 22.4*	147.0 ± 10.1	6688.7 ± 464.7	353.4 ± 37.4
	Microsphere + Naf	207.5 ± 24.9	7399.7 ± 398.7	426.6 ± 19.0†	172.3 ± 11.4	7247.5 ± 524.5	428.0 ± 35.4
DOPAC	Sham	34.5 ± 2.6	744.5 ± 69.3	49.2 ± 4.1	35.5 ± 4.4	581.9 ± 49.8	37.3 ± 4.4
	Sham + Naf	41.1 ± 4.1	736.9 ± 38.4	49.2 ± 2.0	44.1 ± 5.9	713.9 ± 55.6	52.5 ± 4.7†
	Microsphere	26.4 ± 3.3*	590.7 ± 57.5	31.2 ± 3.2*	25.6 ± 2.9*	625.9 ± 66.0	42.8 ± 4.3
	Microsphere + Naf	37.0 ± 4.3†	562.8 ± 34.8	42.4 ± 3.2†	25.7 ± 1.9	616.8 ± 49.2	42.2 ± 3.9
HVA	Sham	26.8 ± 3.1	431.4 ± 21.5	24.3 ± 2.4	36.5 ± 3.9	368.8 ± 24.0	23.4 ± 7.1
	Sham + Naf	33.2 ± 2.2	452.8 ± 15.7	30.8 ± 3.1	34.5 ± 1.6	483.7 ± 29.0†	31.5 ± 1.0
	Microsphere	23.4 ± 1.9	313.2 ± 24.4	18.9 ± 0.8	26.7 ± 1.8	470.2 ± 43.8	25.8 ± 3.9
	Microsphere + Naf	30.3 ± 4.9	420.1 ± 28.2†	25.8 ± 3.0	25.6 ± 2.9	480.2 ± 27.4	21.2 ± 3.4
NA	Sham	258.8 ± 5.3	279.8 ± 19.6	363.1 ± 15.9	276.3 ± 9.3	248.9 ± 9.9	360.3 ± 12.7
	Sham + Naf	257.7 ± 29.1	241.0 ± 11.8	349.1 ± 39.0	325.5 ± 14.1	243.6 ± 50.7	422.8 ± 15.8
	Microsphere	228.3 ± 10.1	196.8 ± 7.7	288.1 ± 22.1*	230.0 ± 16.2	204.0 ± 11.1	264.2 ± 11.3*
	Microsphere + Naf	255.9 ± 13.0	251.8 ± 18.2†	342.9 ± 35.3	262.7 ± 18.3	254.8 ± 14.6	371.4 ± 30.7†

Values are expressed as ng g⁻¹ frozen tissue. Each value represents the mean ± s.e.mean of 7 experiments.

*Significantly different from the corresponding sham-operated group (*P* < 0.05).

†Significantly different from the untreated group (*P* < 0.05).

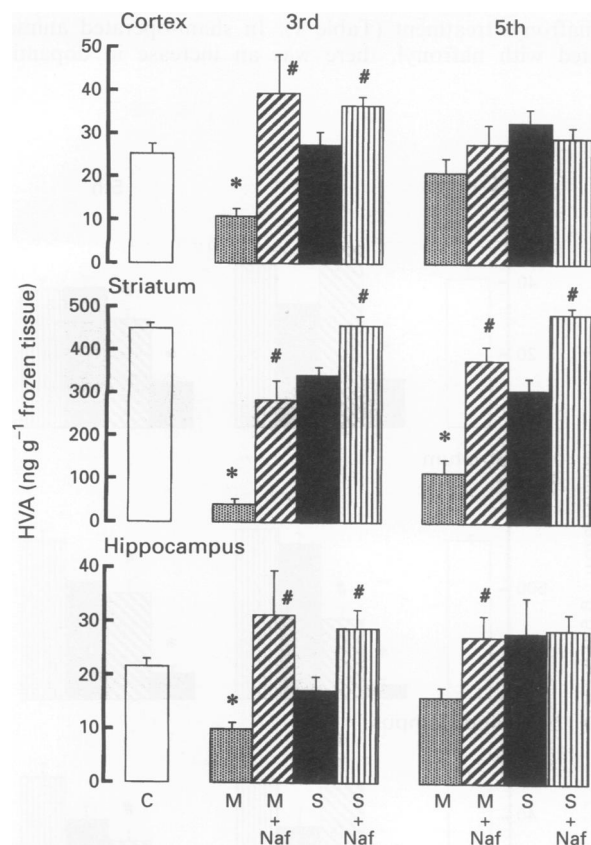


Figure 3 HVA content of the cerebral cortex, striatum and hippocampus of the right hemisphere on days 3 and 5 of microsphere-injected (M and M+Naf) and sham-operated (S and S+Naf) rats with (+Naf) and without nafronyl oxalate treatment, and of control rats (C). Symbols and numbers of experiments are the same as those in Figure 1.

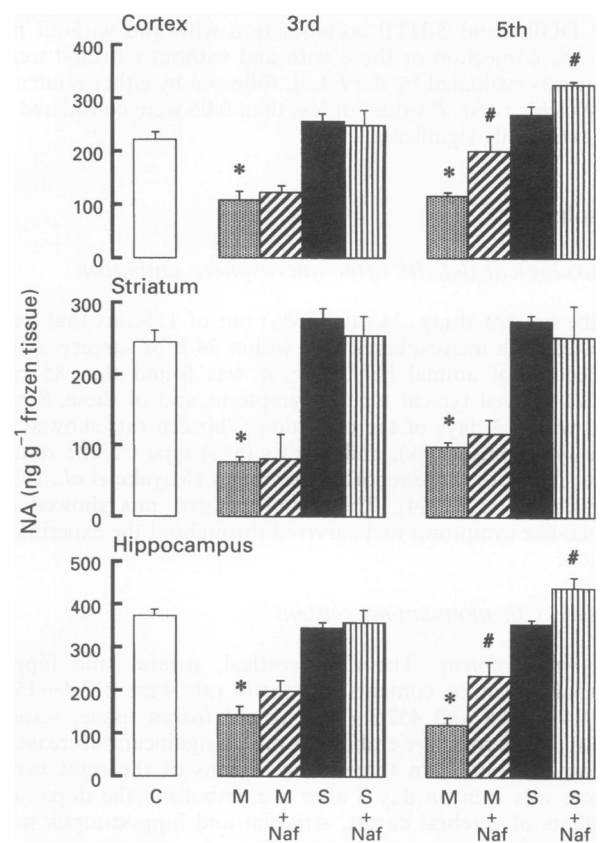


Figure 4 Noradrenaline (NA) content of the cerebral cortex, striatum and hippocampus of the right hemisphere on days 3 and 5 of microsphere-injected (M and M+Naf) and sham-operated (S and S+Naf) rats with (+Naf) and without nafronyl oxalate treatment, and of control rats (C). Symbols and numbers of experiments are the same as those in Figure 1.

content of the right striatum on days 3 and 5, and of the right hippocampus on day 5.

DOPAC content The right cortical, striatal, and hippocampal DOPAC contents of control rats were 41.0 ± 3.0 , 726.1 ± 23.1 and 49.5 ± 2.9 ng g⁻¹ frozen tissue, respectively. In microsphere-embolized rats, significant decreases in DOPAC content of the three brain regions of the right hemisphere were detected on days 3 and 5 after the microsphere embolism (Figure 2). Treatment with nafronyl attenuated the reduction in DOPAC content of the three brain regions on days 3 and 5 after microsphere embolism. The decrease in DOPAC content of the left cortex and hippocampus on day 3 was reversed by treatment with nafronyl (Table 1).

HVA content The right cortical, striatal, and hippocampal HVA contents of control rats were 25.2 ± 2.2 , 450.0 ± 12.4 and 21.6 ± 1.5 ng g⁻¹ frozen tissue, respectively. In the right hemisphere, a significant decrease in HVA content was seen in the three brain regions on day 3 and in the striatum on day 5 (Figure 3) when the values were compared with those of sham-operated animals. Treatment of microsphere-embolized rats with nafronyl resulted in a significant restoration of HVA content in the right hemisphere on days 3 and 5. The level of HVA content in the left striatum was completely restored to its control level by nafronyl treatment (Table 1).

NA content The right cortical, striatal and hippocampal NA contents of control rats were 221.2 ± 13.6 , 244.1 ± 17.3 and 372.1 ± 15.5 ng g⁻¹ frozen tissue, respectively. Microsphere

embolism induced a significant reduction of NA content of the three brain regions in the right hemisphere on days 3 and 5 (Figure 4). Treatment of microsphere-embolized rats with nafronyl did not attenuate the decrease in NA content of the three brain regions except for that of the cortex and hippocampus on day 5. In the left hemisphere of microsphere-embolized rats, there were no significant alterations in NA content except for the hippocampal NA content on days 3 and 5 (Table 1). In the sham-operated rat, the cortical and hippocampal NA contents were increased on day 5 by nafronyl treatment.

5-HT content The right cortical, striatal, and hippocampal 5-HT contents of control rats were 484.8 ± 32.8 , 703.8 ± 19.8 and 501.0 ± 13.7 ng g⁻¹ frozen tissue, respectively. In microsphere-embolized rats, a marked decrease in 5-HT content of the three brain regions of the right hemisphere was observed on days 3 and 5. The 5-HT content of the left hemisphere of the microsphere-embolized and sham-operated rats were not different from the control values. Nafronyl treatment significantly attenuated the decrease in 5-HT content of the three brain regions, except for the striatal content on day 5 (Figure 5). Significant decreases in the cortical, striatal and hippocampal 5-HT contents were seen in the left hemisphere of the microsphere-embolized rats on days 3 and 5 (Table 2). The levels of 5-HT in the left hemisphere were completely restored to control by nafronyl treatment (Table 2). In the sham-operated rat, the hippocampal 5-HT content on day 3 was increased by nafronyl treatment.

5-HIAA content Right cortical, striatal, and hippocampal 5-HIAA contents of control rats were 116.0 ± 6.9 , 388.7 ± 14.2 , and 276.1 ± 8.9 ng g⁻¹ frozen tissue, respectively. In the mi-

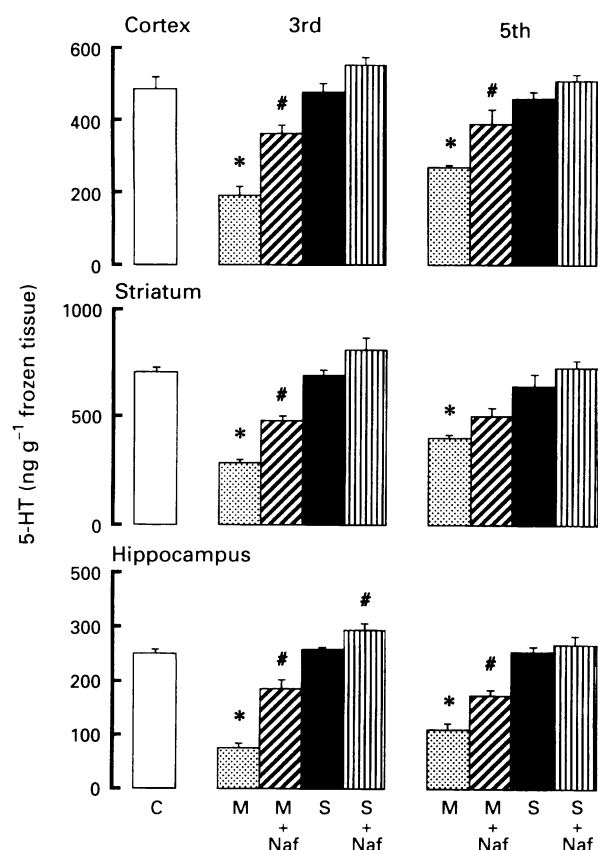


Figure 5 5-HT content of the cerebral cortex, striatum and hippocampus of the right hemisphere on days 3 and 5 of microsphere-injected (M and M+Naf) and sham-operated (S and S+Naf) rats with (+Naf) and without nafronyl oxalate treatment, and of control rats (C), respectively. Symbols and numbers of experiments are the same as those in Figure 1.

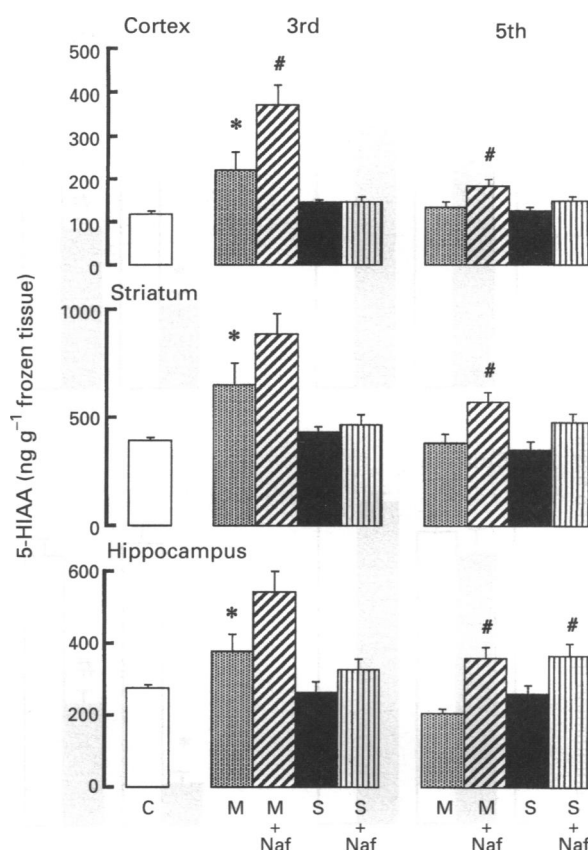


Figure 6 5-HIAA content of the cerebral cortex, striatum and hippocampus of the right hemisphere on days 3 and 5 of microsphere-injected (M and M+Naf) and sham-operated (S and S+Naf) rats with (+Naf) and without nafronyl oxalate treatment, and control rats (C). Symbols and numbers of experiments are the same as those in Figure 1.

Table 2 5-Hydroxytryptamine (5-HT) and its metabolite contents in the left hemisphere of microsphere-embolized and sham-operated rats with and without nafronyl oxalate (Naf) treatment

	Experimental group	Day 3			Day 5		
		Cortex	Striatum	Hippocampus	Cortex	Striatum	Hippocampus
5-HT	Sham	565.7 ± 35.4	707.4 ± 22.5	513.3 ± 10.7	565.7 ± 35.5	749.1 ± 50.2	542.1 ± 9.5
	Sham + Naf	543.6 ± 22.5	856.8 ± 41.0	597.1 ± 8.0	503.1 ± 15.5	752.2 ± 30.9	547.4 ± 28.4
	Microsphere	342.8 ± 30.9*	543.5 ± 37.6*	386.1 ± 10.5*	365.9 ± 7.3*	557.1 ± 32.5*	409.1 ± 13.6*
	Microsphere + Naf	487.2 ± 17.7†	677.4 ± 21.4†	518.6 ± 22.4†	515.3 ± 25.6†	771.7 ± 31.9†	561.7 ± 20.2†
5-HIAA	Sham	145.6 ± 8.2	452.1 ± 25.1	323.3 ± 20.7	126.8 ± 11.0	404.0 ± 30.5	284.7 ± 22.1
	Sham + Naf	149.5 ± 17.8	461.7 ± 39.0	329.2 ± 31.8	147.6 ± 15.0	476.4 ± 44.9	358.9 ± 37.7
	Microsphere	185.2 ± 19.3	528.6 ± 52.9	343.5 ± 30.7	151.4 ± 14.0	440.4 ± 27.8	293.9 ± 27.4
	Microsphere + Naf	164.4 ± 17.9	481.6 ± 44.3	348.5 ± 33.5	160.2 ± 7.8	499.2 ± 21.2	340.1 ± 27.0

Values are expressed as ng g⁻¹ frozen tissue. Each value represents the mean ± s.e. mean of 7 experiments.

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

†Significantly different from the untreated group ($P < 0.05$).

microsphere-embolized rats, a significant increase in 5-HIAA content of the three brain regions of the right hemisphere was detected on day 3. Treatment with nafronyl resulted in a significant increase in 5-HIAA content in the right hemisphere compared with the value of nafronyl-untreated rats (Figure 6). In the left hemisphere of microsphere-embolized rats, there was no significant alteration in 5-HIAA content with or without nafronyl treatment (Table 2).

Monoamine content on day 28 To determine whether microsphere embolism-induced changes in monoamine content are irreversible or not, the monoamines and their metabolites were

measured on day 28 after the embolism. We found that microsphere embolism induced a sustained decrease in monoamine content in all brain region examined 28 days after the embolism. For example, the right cortical, striatal and hippocampal dopamine content on day 28 of the microsphere-embolized rats were 112.2 ± 32.1 , 2675.9 ± 548.3 and 106.3 ± 22.3 ng g⁻¹ frozen tissue; the NA contents were 146.0 ± 25.0 , 144.5 ± 30.0 and 169.4 ± 33.2 ng g⁻¹ frozen tissue, and the 5-HT contents, 387.9 ± 40.8 , 487.8 ± 94.1 and 331.5 ± 17.0 ng g⁻¹ frozen tissue, respectively. These values were lower than those of the corresponding sham-operated group ($P < 0.05$).

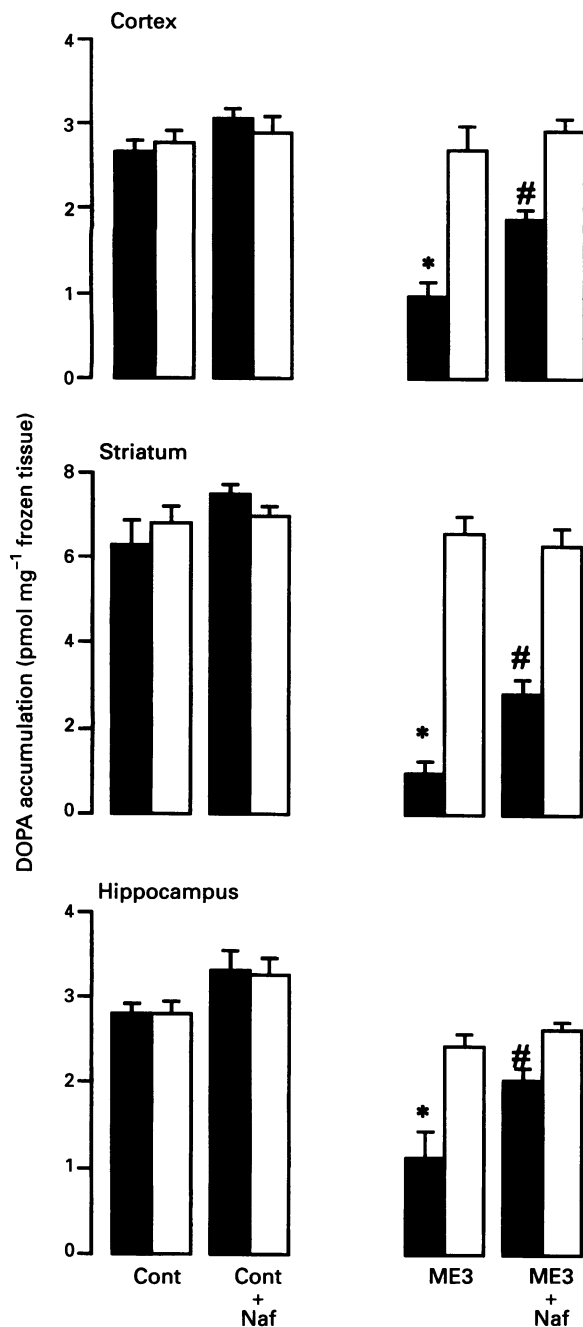


Figure 7 *In vivo* tyrosine hydroxylation of the cerebral cortex, striatum and hippocampus of the right (solid columns) and left (open columns) hemispheres on day 3 of microsphere-injected (M and M + Naf) and control (C and C + Naf) rats with (+Naf) and without nafronyl oxalate treatment. *In vivo* tyrosine hydroxylation was estimated by measurement of the accumulation of DOPA after administration of aromatic L-amino acid decarboxylase inhibitor. Each value represents the mean \pm s.e. mean of 4 to 6 experiments. *Significantly different from the corresponding control group and #significantly different from the untreated group ($P < 0.05$).

In vivo tyrosine hydroxylation in the brain regions

Changes in *in vivo* tyrosine hydroxylation were estimated by measuring the accumulation of DOPA after inhibition of aromatic L-amino acid decarboxylase with NSD-1015 in the brain regions of microsphere-embolized and control rats and the results are shown in Figure 7. Control DOPA contents of the cortex, striatum, and hippocampus in the right hemisphere were 2.65 ± 0.16 , 6.31 ± 0.54 and 2.80 ± 0.11 pmol mg⁻¹ frozen tissue, respectively. Nafronyl had no significant effect on the *in vivo* tyrosine hydroxylation in the brain regions of control rats.

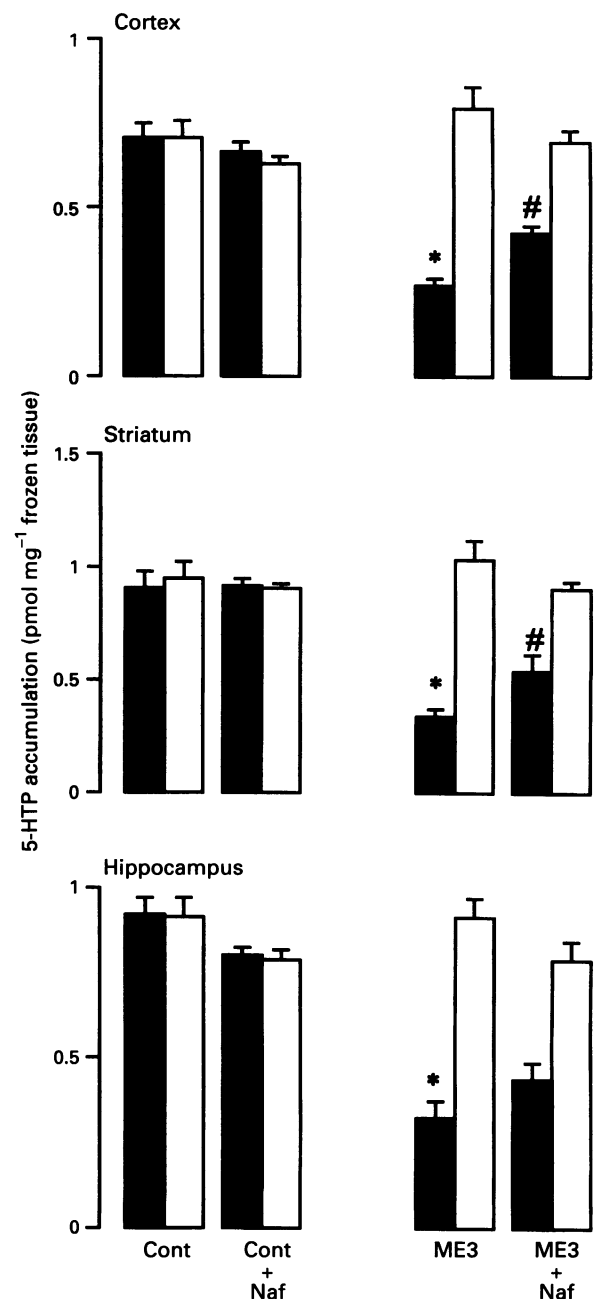


Figure 8 *In vivo* tryptophan hydroxylation of the cerebral cortex, striatum and hippocampus of the right (solid columns) and left (open columns) hemispheres on day 3 of microsphere-injected (M and M + Naf) and control (C and C + Naf) rats with (+Naf) and without nafronyl oxalate treatment. *In vivo* tryptophan hydroxylation was estimated by measurement of the accumulation of 5-HTP after administration of aromatic L-amino acid decarboxylase inhibitor. Each value represents the mean \pm s.e. mean of 4 to 6 experiments. *Significantly different from the corresponding control group and #significantly different from the untreated group ($P < 0.05$).

On day 3 after the operation, cortical, striatal and hippocampal DOPA contents of the right hemisphere were significantly decreased after microsphere embolism. Treatment with nafronyl significantly attenuated the decrease in the *in vivo* tyrosine hydroxylation of the three brain regions of the right hemisphere of microsphere-embolized rats.

In vivo tryptophan hydroxylation in the brain regions

Changes in *in vivo* tryptophan hydroxylation were estimated by measurement of the accumulation of 5-HTP after inhibition of

aromatic L-amino acid decarboxylase with NSD-1015 and the results are shown in Figure 8. The right cortical, striatal and hippocampal 5-HTP contents of control rats were 0.71 ± 0.05 , 0.90 ± 0.07 and 0.92 ± 0.05 pmol mg⁻¹ frozen tissue, respectively. Nafronyl had no significant effect on the *in vivo* tryptophan hydroxylation in the three brain regions of control rats. On day 3 after microsphere embolism, 5-HTP content was significantly decreased in the three brain regions after microsphere embolism. Treatment with nafronyl significantly attenuated the decrease in the *in vivo* tryptophan hydroxylation in the right cortical and striatal, but not hippocampal, regions of the microsphere-embolized rat.

Discussion

In the present study, a marked decrease in monoamine content of the cerebral cortical, striatal and hippocampal regions following microsphere embolism was seen on days 3 and 5 after the embolism. Since we observed that dopamine, NA and 5-HT decreased almost to a similar extent 28 days after microsphere embolism, the decrease in monoamine levels is severe and irreversible in this model. Several studies have shown a decrease in brain monoamine neurotransmitters in the early stage of cerebral ischaemia in animals (Zervas *et al.*, 1974; Kogure *et al.*, 1975; Welch *et al.*, 1977). In contrast, it has been reported that there were no appreciable changes in monoamine levels during short periods of cerebral ischaemia, and that decreased in the brain monoamines occurred only during prolonged ischaemia (Siesjö, 1978). These observations support our conclusion that microsphere embolism induces severe cerebral ischaemia.

The present study also demonstrated that the dopamine metabolite content of these brain regions decreased after microsphere embolism on days 3 and 5. In a previous study from our laboratory, microsphere embolism induced a sustained decrease in cerebral blood flow (Miyake *et al.*, 1993) and a profound change in cerebral energy metabolism for a period of at least 5 days (Takeo *et al.*, 1992). Since monoaminergic neurotransmitter metabolism are energy-dependent processes, it is considered that the decrease in dopamine metabolites seen after the 3rd day results from significant depletion of dopamine synthesis and storage and severe impairment of dopamine catabolism. In contrast, microsphere embolism did not induce a decrease in the 5-HT metabolite; rather, 5-HIAA increased in the three brain regions on day 3. The increase in 5-HIAA content has also been observed in an ischaemic model with middle cerebral artery occlusion (Uemura *et al.*, 1991). Mrsulja *et al.* (1975) have shown an increase in metabolites in ischaemic gerbils with unilateral carotid artery ligation and suggested that this results from a decrease in transport of metabolites out of the ischaemic brain. Thus, the increase in 5-HIAA is probably due to the disturbance of transport of metabolites out of the microsphere-embolized brain.

The present study showed that treatment with nafronyl restored dopamine and 5-HT content of the right hemisphere on days 3 and 5, and NA content on day 5 to control levels in microsphere-embolized rats. Since dopamine- β -hydroxylase shows a lower affinity for oxygen than tyrosine hydroxylase,

NA synthesis is more susceptible to an ischaemic insult (Iijima *et al.*, 1986). This may explain diverse effects of nafronyl on monoamines levels in various brain regions. Our previous study has shown that nafronyl restored striatal and hippocampal blood flow on day 3 after microsphere embolism (Miyake *et al.*, 1994), which demonstrates that nafronyl is capable of improving cerebral circulation in the 'penumbra' brain regions. In association with the improvement of cerebral circulation, we observed that nafronyl improved energy metabolism of brain regions following microsphere embolism (Miyake *et al.*, 1992). These findings suggest that the restoration of monoamines by nafronyl treatment is related, at least in part, to recovery of energy-dependent cerebral monoamine biosynthesis.

We observed an increase in monoamine contents in some brain regions following nafronyl treatment in the sham-operated rat. This further suggests that nafronyl, in addition to the mechanism of improvement of cerebral circulation, increases cerebral monoamine content through direct stimulation of monoamine biosynthesis. Based on this assumption, we examined *in vivo* tyrosine and tryptophan hydroxylation, as an index of catecholamine and 5-HT biosynthesis, in the embolized brain regions of rats treated with or without nafronyl. We found that microsphere embolism induced severe impairment of tyrosine and tryptophan hydroxylation in the ipsilateral brain regions. It would appear that microsphere-induced decrease in monoamine content of the ipsilateral hemisphere results from impairment of tyrosine and tryptophan hydroxylation, which are the initial and rate-limiting steps of catecholamine and 5-HT biosynthesis, respectively. We also found that treatment with nafronyl attenuated the decrease in the *in vivo* tyrosine and tryptophan hydroxylation in the right brain regions on day 3 after microsphere embolism. The results suggest that nafronyl is capable of preserving, or enhancing dopamine and 5-HT synthesis under ischaemic or oligoemic conditions. Nafronyl increased monoamine contents in some brain regions of the sham-operated rat as described above, despite no effects of treatment with nafronyl on the tyrosine and tryptophan hydroxylation in the left hemisphere or control rat. Therefore, the effect of nafronyl on monoamine contents may, at least, have two aspects. The first is an effect on preserving monoamine synthesis rate that is specific to ischaemic or oligoemic conditions. The second is an effect on monoamine metabolism that occurs irrespective of ischaemic and non-ischaemic conditions.

In conclusion, the results of the present study suggest that delayed treatment with nafronyl improves or attenuates damage to monoamine neurotransmitter metabolism of brain regions impaired by microsphere embolism. The recovery of monoamine content in the cerebral cortex, striatum, and hippocampus of microsphere-embolized rat may be, in part, attributed to restoration or enhancement of tyrosine and tryptophan hydroxylation in the impaired brain regions.

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