Long-term changes in gerbil brain neurotransmitter receptors following transient cerebral ischaemia

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- 1 Receptor autoradiographic and histological techniques were used to investigate long-term changes in the gerbil brain following transient cerebral ischaemia.
- 2 Transient ischaemia was induced for 3 min and 10 min, and the animals were allowed to survive for 8 months.
- 3 Histological examination revealed that 3 min ischaemia caused neuronal damage and mild shrinkage only in the hippocampal CA1 sector. Ten minutes of ischaemia produced severe neuronal damage in the striatum and the hippocampal CA1 and CA3 sectors. Considerable shrinkage was seen in the hippocampus; the dentate gyrus, however, was not damaged.
- 4 Three minutes of ischaemia produced changes in the binding of [³H]-quinuclidinylbenzilate ([³H]-QNB), [³H]-muscimol, and [³H]-MK-801 in various brain regions, as determined autoradiographically. In contrast, [³H]-cyclohexyladenosine ([³H]-CHA) and [³H]-PN200-110 ([³H]-isradipine) binding in the brain was unaltered.
- 5 Ten minutes of ischaemia resulted in a major loss of neurotransmitter receptors, especially in the hippocampus. The substantia nigra showed a significant reduction in [³H]-CHA binding, whereas the striatum, which was morphologically damaged, showed no significant changes in any of the neurotransmitter receptors examined.
- 6 The results demonstrated that long-term survival after transient cerebral ischaemia produced alterations in neurotransmitter receptors, especially in the hippocampal formation, where considerable shrinkage was noted. These results also suggest that the hippocampal damage was not static, but progressive.

Keywords: Cerebral ischaemia; neurotransmitter receptor; autoradiography; long-term changes; tissue shrinkage

Introduction

It is now well established that transient cerebral ischaemia can easily be produced in the Mongolian gerbil by bilaterally occluding the common carotid arteries (Crockard *et al.*, 1980; Kirino, 1982). The hippocampal CA1 sector has been shown to be the region of the brain most vulnerable to damage following brief transient ischaemia (Kirino, 1982; Araki *et al.*, 1989; 1990a), although populations of neurones in the neocortex, striatum, and thalamus are also selectively vulnerable to transient ischaemia (Wieloch, 1985). A large number of studies have explored the mechanisms underlying selective neuronal vulnerability (Jorgensen & Diemer, 1982; Pulsinelli *et al.*, 1982; Kogure *et al.*, 1988; Crain *et al.*, 1988; Siesjö & Bengtsson, 1989).

Many hypotheses have been proposed to explain the mechanisms underlying ischaemic brain damage: these include the neurotoxic action of synaptically released excitatory amino acids, such as glutamate, (Benveniste *et al.*, 1984; 1989); the pathological accumulation of intracellular calcium ions, thought to be a major factor leading to neuronal damage (Siesjö, 1981; Simon *et al.*, 1984; Benveniste *et al.*, 1988); activation of protein kinase C (Onodera *et al.*, 1988); impairment of protein synthesis (Xie *et al.*, 1989; Araki *et al.*, 1990b); lipid peroxidation (Kitagawa *et al.*, 1990); imbalance between excitatory and inhibitory inputs (Wieloch, 1985; Sternau *et al.*, 1989; Araki *et al.*, 1990c); metabolic disturbances (Munekata & Hossmann, 1987); and the alteration of intracellular signal transduction processes (Jorgensen *et al.*, 1989; Araki *et al.*, 1992a). Each of these factors has been suggested as participating in the development of ischaemic neuronal damage.

Although many experimental investigations have addressed the short-term pathological and biochemical events that are triggered by transient ischaemia, little is known about the long-term pathological and biochemical changes following transient ischaemia. In the present study, we have utilized autoradiographs to focus on post-ischaemic alterations in the receptors for muscarinic acetylcholine, γ -aminobutyric acid_A (GABA_A), adenosine A₁, and N-methyl-D-aspartate (NMDA), and in voltage-dependent calcium channels in the gerbil brain 8 months after transient cerebral ischaemia.

Methods

Animals and operative procedures

Male Mongolian gerbils weighing 70–95 g were maintained on a 12 h light: dark cycle and fed *ad libitum* before experimentation. Animals were anaesthetized with 2% halothane in a mixture of 70% N₂O and 30% O₂. The bilateral common carotid arteries were exposed, anaesthesia was discontinued, and the arteries were clamped with aneurysm clips for either 3 or 10 min. After occlusion, the clips were removed and ischaemic animals were allowed to survive for 8 months. Sham-operated animals were treated in the same manner, except for the clipping of the bilateral common carotid arteries. Body temperature was maintained at 37–39°C with a heating pad equipped with a thermostat until the animals began to move. For receptor autoradiography, the animals were decapitated 8 months after ischaemia. The brains were removed quickly and frozen in powdered dry-ice; sagittal sections (12 μ m) were cut on a cryostat at –20°C and thaw-mounted onto gelatin-coated slides. The sections were stored at –80°C until assay.

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Histopathology

The gerbils were anaesthetized with pentobarbitone (50 mg kg⁻¹) intraperitoneally (i.p.) 8 months after ischaemia. They were briefly subjected to transcardiac perfusion with heparinized saline, followed by perfusion-fixation with 3.5% formaldehyde in 0.1 M phosphate buffer (pH 7.4) for 30 min. The fixed animals were then left at 4°C for 120 min, after which the brains were removed and immersed in the same fixative before being embedded in paraffin. Sections (5 µm) were stained with cresyl violet and haematoxylin-eosin. Adjacent sections prepared for receptor autoradiography were also stained with cresyl violet and haematoxylin-eosin. The sections were examined with a light microscope. Each group contained 4–7 animals.

Receptor autoradiography

[³H]-quinuclidinyl benzilate ([³H]-QNB) binding Autoradiographic localization of muscarinic acetylcholine receptors was performed as described previously (Araki *et al.*, 1991; Kato *et al.*, 1991). Sections were incubated with 1 nM [³H]-QNB (sp. act. 41.5 Ci mmol⁻¹; Amersham) in phosphate buffer (pH 7.4) for 90 min at room temperature. The sections were then washed in buffer for 5 min at 4°C. Nonspecific binding was determined using 1 µM atropine (Sigma).

[³H]-muscimol binding [³H]-muscimol (γ-aminobutyric acid_A, GABA_A) receptor autoradiography was performed according to the method of Onodera *et al.* (1987). To remove endogenous GABA, sections were subjected to a 20 min prewash, at 4°C, in 50 mM Tris-citrate buffer (pH 7.1); they were then incubated for 40 min at 4°C in buffer containing 30 nM [³H]-muscimol (sp. act. 17.1 Ci mmol⁻¹; New England Nuclear). The sections were then washed in buffer for 1 min at 4°C. Nonspecific binding was determined in the presence of 10 µM GABA (Sigma).

[³H]-cyclohexyladenosine binding The method used for autoradiographic localization of adenosine A₁ receptors has been described previously (Araki *et al.*, 1991; Kato *et al.*, 1991). Sections were incubated with 5 nM [³H]-cyclohexyladenosine (CHA, sp. act. 34.4 Ci mmol⁻¹; New England Nuclear) and 2 units ml⁻¹ adenosine deaminase (Boehringer-Mannheim) in 50 mM Tris-HCl buffer (pH 7.4) for 90 min at room temperature, after which they were washed in buffer for 1 min at 4°C. Nonspecific binding was determined with 10 µM L-phenyl-isopropyladenosine (Boehringer-Mannheim).

[³H]-MK-801 binding Autoradiographic distribution of the binding of the N-methyl-D-aspartate (NMDA) antagonist, MK-801 (dizocilpine; (±)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) was determined according to the method of Bowery *et al.* (1988), with minor modifications (Araki *et al.*, 1992b). Sections were rinsed in 50 mM Tris-HCl buffer (pH 7.4) containing 190 mM sucrose and dried in a cold air stream. They were then incubated for 20 min at room temperature in buffer containing 30 nM [³H]-MK-801 (sp. act. 28.8 Ci mmol⁻¹; New England Nuclear), after which they were washed in the buffer twice for 20 s at room temperature. Nonspecific binding was determined with 100 µM MK-801 (Research Biochemicals Inc., U.S.A.).

[³H]-PN200-110 binding The method used for autoradiographic visualization of the binding of the voltage-dependent L-type calcium channel blocker, [³H]-PN200-110 (isradipine; isopropyl methyl (±)-4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate), has been described previously (Araki *et al.*, 1991; Kato *et al.*, 1991). Sections were incubated with 0.1 nM [³H]-PN200-110 (sp. act. 71.5 Ci mmol⁻¹; New England Nuclear) in 170 mM Tris-HCl buffer (pH 7.7) for 60 min at room temperature, after which they were washed in buffer for 20 min at 4°C. Nonspecific binding

was determined with 1 µM nitrendipine (Sigma).

All procedures were performed under subdued lighting conditions. The sections were dried under a cold air stream and were apposed to Hyperfilm-³H (Amersham) for 2–4 weeks in X-ray cassettes with a set of tritium standards. The optical density of the brain regions was measured with a computer-assisted image analyzer. The relationship between optical density and radioactivity was obtained with reference to the ³H microscale (Amersham) co-exposed with the tissue sections, using a third-order polynomial function. Anatomical structures were verified by examination of cresyl violet-stained sections, and comparison with the gerbil brain atlas of Loskota *et al.* (1974). Binding assays were performed in duplicate. Statistical comparisons were made by Duncan's multiple range test. Each group contained 4–7 animals.

Results

Histopathology

Sham-operated gerbils showed no neuronal damage in any brain region. Eight months after 3 min ischaemia, severe neuronal damage was seen only in the hippocampal CA1 sector, and mild tissue atrophy was also found in this field. Gerbils subjected to 10 min ischaemia displayed severe neuronal damage in the hippocampal CA1 and CA3 sectors (Figure 1). Further, marked tissue shrinkage was observed in the hippocampus in these animals. The dentate gyrus, however, was intact. In the striatum, most of the small- and medium-sized neurones were destroyed following ischaemia, whereas large-sized neurones, which were sparsely scattered in this region, were relatively well preserved, although they exhibited some mild degeneration (Figure 2).

Autoradiography

Quinuclidinyl benzilate binding In sham-operated gerbils, the distribution of [³H]-QNB binding in the brain was strikingly heterogeneous, being greatest in the hippocampal CA1 sector and dentate gyrus, followed by the striatum, neocortex, and hippocampal CA3 sector. Other regions had a low density of [³H]-QNB binding sites (Figure 3, Table 1). Eight months

Figure 1 Representative photographs of gerbil hippocampus. Left half: whole hippocampal area; bar = 100 µm. Right half: hippocampal CA1 neurones; bar = 50 µm. (a) Sham-operated group, hippocampal CA1 sector is intact (arrow). (b) Eight months after 3 min ischaemia. Severe neuronal damage is seen in the hippocampal CA1 sector (arrow). (c) Eight months after 10 min ischaemia. Severe neuronal damage and tissue shrinkage is seen in the hippocampal CA1 sector (arrow) and hippocampal CA3 sector. Gliosis is also seen in the hippocampal CA1 sector. However, there is no neuronal damage in the dentate gyrus. Cresyl violet staining.

Figure 2 Representative photomicrographs of the gerbil striatum: (a) sham-operated group; (b) eight months after 3 min ischaemia; the striatal neurones are intact; (c) eight months after 10 min ischaemia; small- and medium-sized neurones are almost all destroyed, while the large-sized neurones are relatively well preserved (arrow). Cresyl violet staining. Bar = 50 μ m.

Table 1 Regional distribution of [3 H]-quinuclidinyl benzilate ([3 H]-QNB) binding sites in gerbil brain 8 months after transient cerebral ischaemia

	Sham-operated (6)	Cerebral ischaemia	
		3 min (7)	10 min (4)
Frontal cortex	302 $\pm$ 42	310 $\pm$ 66	356 $\pm$ 34
Striatum	389 $\pm$ 23	371 $\pm$ 48	402 $\pm$ 112
Hippocampus			
CA1 sector	439 $\pm$ 29	248 $\pm$ 53**	115 $\pm$ 48**
CA3 sector	284 $\pm$ 17	259 $\pm$ 53	159 $\pm$ 122*
Dentate gyrus	411 $\pm$ 38	385 $\pm$ 55	451 $\pm$ 44
Thalamus	116 $\pm$ 17	92 $\pm$ 17	129 $\pm$ 59
Hypothalamus	104 $\pm$ 40	84 $\pm$ 28	145 $\pm$ 28
Substantia nigra	84 $\pm$ 21	76 $\pm$ 16	104 $\pm$ 19
Cerebellum	20 $\pm$ 4	18 $\pm$ 4	16 $\pm$ 6

Optical density was converted to fmol mg $^{-1}$ tissue with 3 H microscans.

Values are expressed as means $\pm$ s.d.

* P < 0.05; ** P < 0.01 vs. sham-operated group (Duncan's multiple-range test). The number of animals in parentheses.

after 3 min ischaemia, a significant reduction in [3 H]-QNB binding sites was observed only in the hippocampal CA1 sector. Gerbils subjected to 10 min ischaemia showed a significant reduction in [3 H]-QNB binding sites in both the hippocampal CA1 and CA3 sectors. Other regions exhibited no significant alterations in [3 H]-QNB binding sites.

Muscimol binding In sham-operated gerbils, [3 H]-muscimol binding was greatest in the thalamus, followed by the dentate gyrus, cerebellum, neocortex, hippocampal CA1 sector, substantia nigra, and striatum. The hippocampal CA3 sector exhibited a relatively low density of [3 H]-muscimol binding sites and in the hypothalamus these were almost undetectable (Figure 3, Table 2). Eight months after 3 min ischaemia, the hippocampal CA1 sector and CA3 sectors showed a significant reduction in binding. In contrast, the substantia nigra exhibited a significant elevation in [3 H]-muscimol binding. Gerbils subjected to 10 min ischaemia revealed marked reduction in [3 H]-muscimol binding in the hippocampal CA1 sector, hippocampal CA3 sector, and dentate gyrus. Other regions showed no significant alterations in [3 H]-muscimol binding.

Figure 3 Representative autoradiograms of [3 H]-quinuclidinyl benzilate ([3 H]-QNB), [3 H]-muscimol, [3 H]-cyclohexyladenosine ([3 H]-CHA), [3 H]-MK-801 and [3 H]-isradipine ([3 H]-PN200-110) binding sites in gerbil brain. (a) Sham-operated group; (b) eight months after 3 min ischaemia; (c) eight months after 10 min ischaemia. A conspicuous reduction in [3 H]-QNB, [3 H]-muscimol and [3 H]-MK-801 binding sites is seen in the hippocampal CA1 sector, whereas [3 H]-CHA and [3 H]-PN200-110 binding show no significant reduction in this field. Further, after 10 min ischaemia, there was marked reduction in neurotransmitter receptors in the whole hippocampus; this feature, however, was not found in the striatum.

Table 2 Regional distribution of [³H]-muscimol binding sites in gerbil brain 8 months after transient cerebral ischaemia

	Sham-operated (6)	Cerebral ischaemia	
		3 min (7)	10 min (4)
Frontal cortex	328 ± 32	356 ± 49	312 ± 51
Striatum	170 ± 18	186 ± 26	131 ± 40
Hippocampus			
CA1 sector	293 ± 36	224 ± 46*	42 ± 37**
CA3 sector	100 ± 30	144 ± 23	51 ± 34**
Dentate gyrus	373 ± 41	413 ± 33	313 ± 56*
Thalamus	417 ± 55	379 ± 54	337 ± 113
Hypothalamus	30 ± 17	51 ± 22	44 ± 7
Substantia nigra	186 ± 48	281 ± 76*	185 ± 34
Cerebellum	361 ± 38	370 ± 53	372 ± 37

Optical density was converted to fmol mg⁻¹ tissue with ³H microscans.

Values are expressed as means ± s.d.

P* < 0.05; *P* < 0.01 vs. sham-operated group (Duncan's multiple-range test). The number of animals in parentheses.

Cyclohexyladenosine binding In sham-operated gerbils, [³H]-CHA binding was greatest in the hippocampal CA1 sector and the molecular layer of the cerebellum, with significant binding also being detected in the dentate gyrus, hippocampal CA3 sector, neocortex, striatum, thalamus, and substantia nigra. Other regions exhibited a low density of [³H]-CHA binding sites (Figure 3, Table 3). Eight months after 3 min ischaemia, [³H]-CHA binding showed no significant alteration in any brain region. Gerbils subjected to 10 min ischaemia, in contrast, showed a significant decrease in [³H]-CHA binding in the hippocampus and substantia nigra. Other regions showed no significant alterations in [³H]-CHA binding.

MK-801 binding In sham-operated gerbils, [³H]-MK-801 binding was greatest in the hippocampal CA1 sector and dentate gyrus, followed by the thalamus, striatum, neocortex, and hippocampal CA3 sector. The hypothalamus, substantia nigra, and cerebellum also had a relatively high density of [³H]-MK-801 binding sites (Figure 3, Table 4). Eight months after 3 min ischaemia, the striatum, hippocampal CA1 sector, dentate gyrus, and cerebellum showed a significant reduction in [³H]-MK-801 binding sites. In gerbils subjected to 10 min ischaemia, in contrast, there was a significant reduction in [³H]-MK-801 binding only in the hippocampal CA1 sector.

Isradipine binding In sham-operated gerbils, [³H]-PN200-110 binding was greatest in the dentate gyrus and hippocampal CA3 sector, followed by the neocortex, striatum, hippocampal CA1 sector, thalamus, and cerebellum. Other regions exhibited a low density of [³H]-PN200-110 binding sites (Figure 3, Table 5). Eight months after 3 min ischaemia, no alterations in [³H]-PN200-110 binding sites were seen in any region. In gerbils subjected to 10 min ischaemia there was a significant reduction in [³H]-PN200-110 binding sites in the hippocampal CA1 and CA3 sectors, while, in contrast, the substantia nigra showed a significant elevation in [³H]-PN200-110 binding sites.

Discussion

The present study clearly demonstrated that long-term survival after transient cerebral ischaemia in the gerbil produced alterations in various neurotransmitter receptors, particularly in the hippocampal formation, where considerable shrinkage was noted. Three minutes of ischaemia produced neuronal damage and mild shrinkage only in the hippocampal CA1 sector. Ten minutes of ischaemia, in contrast, caused severe

Table 3 Regional distribution of [³H]-cyclohexyladenosine ([³H]-CHA) binding sites in gerbil brain 8 months after transient cerebral ischaemia

	Sham-operated (6)	Cerebral ischaemia	
		3 min (7)	10 min (4)
Frontal cortex	174 ± 39	164 ± 23	155 ± 30
Striatum	142 ± 23	131 ± 11	138 ± 16
Hippocampus			
CA1 sector	270 ± 54	267 ± 37	79 ± 44**
CA3 sector	139 ± 31	137 ± 18	62 ± 60**
Dentate gyrus	170 ± 32	163 ± 18	125 ± 48*
Thalamus	111 ± 14	107 ± 12	111 ± 5
Hypothalamus	46 ± 11	48 ± 22	61 ± 8
Substantia nigra	116 ± 27	110 ± 20	82 ± 18*
Cerebellum			
Average	141 ± 15	136 ± 16	129 ± 20
Molecular layer	306 ± 19	288 ± 28	280 ± 44

Optical density was converted to fmol mg⁻¹ tissue with ³H microscans.

Values are expressed as means ± s.d.

P* < 0.05; *P* < 0.01 vs. sham-operated group (Duncan's multiple-range test). The number of animals in parentheses.

Table 4 Regional distribution of [³H]-MK-801 binding sites in gerbil brain 8 months after transient cerebral ischaemia

	Sham-operated (6)	Cerebral ischaemia	
		3 min (7)	10 min (4)
Frontal cortex	244 ± 74	196 ± 48	222 ± 62
Striatum	249 ± 74	164 ± 28*	181 ± 30
Hippocampus			
CA1 sector	326 ± 60	134 ± 60**	76 ± 67**
CA3 sector	211 ± 63	150 ± 40	147 ± 22
Dentate gyrus	308 ± 51	198 ± 53**	267 ± 9
Thalamus	257 ± 51	187 ± 33*	216 ± 55
Hypothalamus	196 ± 52	155 ± 38	138 ± 40
Substantia nigra	186 ± 62	142 ± 41	158 ± 89
Cerebellum	177 ± 31	115 ± 22**	146 ± 48

Optical density was converted to fmol mg⁻¹ tissue with ³H microscans.

Values are expressed as means ± s.d.

P* < 0.05; *P* < 0.01 vs. sham-operated group (Duncan's multiple-range test). The number of animals in parentheses.

Table 5 Regional distribution of [³H]-isradipine ([³H]-PN200-110) binding sites in gerbil brain 8 months after transient cerebral ischaemia

	Sham-operated (6)	Cerebral ischaemia	
		3 min (7)	10 min (4)
Frontal cortex	10.3 ± 3.7	11.0 ± 2.3	12.9 ± 1.5
Striatum	9.8 ± 3.4	9.7 ± 2.2	10.5 ± 2.5
Hippocampus			
CA1 sector	9.1 ± 3.2	6.5 ± 2.5	4.5 ± 2.0*
CA3 sector	12.8 ± 2.2	15.3 ± 2.3	5.6 ± 5.9**
Dentate gyrus	23.3 ± 6.0	25.3 ± 2.9	26.6 ± 3.9
Thalamus	9.1 ± 2.4	9.9 ± 1.3	11.6 ± 1.5
Hypothalamus	1.5 ± 1.0	3.2 ± 2.9	4.4 ± 2.3
Substantia nigra	4.0 ± 1.8	4.3 ± 2.1	7.0 ± 1.6*
Cerebellum	7.7 ± 4.3	8.9 ± 1.3	8.1 ± 2.4

Optical density was converted to fmol mg⁻¹ tissue with ³H microscans.

Values are expressed as means ± s.d.

P* < 0.05; *P* < 0.01 vs. sham-operated group (Duncan's multiple-range test). The number of animals in parentheses.

neuronal damage and marked shrinkage in the hippocampal CA1 and CA3 sectors. The dentate gyrus, however, was not damaged. Thus, the histopathological observations in this study agreed, in part, with previous reports of tissue shrinkage in the hippocampal CA1 region following 5 min ischaemia (Mudrick & Baimbridge, 1989; Kirino *et al.*, 1990).

In the present study, [3 H]-QNB binding sites were significantly reduced only in the hippocampal CA1 sector 8 months after 3 min ischaemia, while 10 min ischaemia caused a marked reduction in these binding sites in the hippocampal CA1 and CA3 sectors. Thus, the post-ischaemic reduction in [3 H]-QNB binding sites that occurred with long-term survival of recirculation was noted only in the hippocampal CA1 and CA3 sectors, i.e., those which were most vulnerable to ischaemia. By contrast, [3 H]-CHA and [3 H]-PN200-110 binding showed no significant alteration in any regions 8 months after 3 min ischaemia. However, 8 months after 10 min ischaemia, [3 H]-CHA binding was significantly reduced in the hippocampal CA1 and CA3 sectors, dentate gyrus, and substantia nigra; [3 H]-PN200-110 binding was also significantly reduced in the hippocampal CA1 and CA3 sectors, although it was significantly elevated in the substantia nigra. That is, there were post-ischaemic alterations in [3 H]-CHA and [3 H]-PN200-110 binding not only in the hippocampal CA1 and CA3 sectors, but also in the dentate gyrus and substantia nigra. Interestingly, the reduction in [3 H]-CHA binding in the dentate gyrus, a region which was resistant to ischaemia, was significant. This finding is essentially in agreement with our previous finding of a reduction in [3 H]-CHA binding in the dentate gyrus just 1 month after 10 min ischaemia (Araki *et al.*, 1991); this phenomenon thus seems to indicate long-lasting post-ischaemic neurotransmitter alterations in the dentate gyrus. We have previously reported in another study (Araki *et al.*, 1989) that transient ischaemia caused neuronal damage not only in selectively vulnerable areas, but also in such brain stem components as the substantia nigra and inferior colliculus; the decrease in [3 H]-CHA binding sites in the substantia nigra thus seems to reflect ischaemic damage. The significantly elevated [3 H]-PN200-110 binding observed in the substantia nigra in this study, however, is difficult to explain. This phenomenon seems to suggest that the elimination of nigral neurones may be involved in the induction of synaptic modifications in the neurotransmitter system, which modifications result in an increase of receptor density on surviving neurones or glial cells. The precise mechanisms responsible for such findings should be investigated in further studies.

[3 H]-muscimol binding in the hippocampal CA1 sector was reduced 8 months after 3 min ischaemia, while it was significantly elevated in the hippocampal CA3 sector and substantia nigra, where no morphological neuronal damage was seen. Eight months after 10 min ischaemia, however, [3 H]-muscimol binding showed a significant reduction only in the hippocampus. Although the reason for the increased [3 H]-muscimol binding activity in the hippocampal CA3 sector and substantia nigra is unclear at present, this phenomenon suggests possible alteration in [3 H]-muscimol binding in these areas after ischaemia.

Eight months after 3 min ischaemia, [3 H]-MK-801 binding also showed significant reduction not only in the hippocampal CA1 sector, but also in the striatum, dentate gyrus, thalamus, and cerebellum, where no neuronal damage was seen. It is of interest that alterations in [3 H]-MK-801 binding after ischaemia occurred in various brain regions that exhibited no morphological neuronal damage. MK-801 is one

of the most potent non-competitive NMDA antagonists (Wong *et al.*, 1986); the NMDA receptor mediates synaptic transmission and plasticity in widespread regions of the central nervous system (Monaghan *et al.*, 1989; Monaghan, 1991). Further, this receptor contributes to neuronal damage resulting from elevated extracellular glutamate levels in pathological conditions (Choi & Rothmann, 1990). The NMDA receptor may therefore play a crucial part in modifying the efficacy of synaptic transmission and in the degeneration of neuronal cells. Thus, the alteration in [3 H]-MK-801 binding may reflect mild modifications of synaptic transmission or receptor sensitivity in various brain regions that exhibit no cell loss, even 8 months after 3 min ischaemia. Ten minutes of ischaemia, in contrast, caused a reduction in [3 H]-MK-801 binding only in the hippocampal CA1 sector. Westerberg *et al.* (1989) have previously suggested that 10 min ischaemia in rats caused a reduction in NMDA binding sites in the hippocampal CA1 sector and dentate gyrus after 1 week of recirculation. They also suggested that the reduction in NMDA binding decreased progressively in the hippocampal CA1 sector following ischaemia, whereas that in the dentate gyrus was no longer present 2 weeks after ischaemia. Furthermore, they demonstrated that the NMDA receptors were strikingly resistant to degenerative processes after ischaemia. These observations agree, at least in part, with our present findings. Therefore, our results in the present study appear to suggest that severe ischaemia, compared with a brief ischaemic insult, does not always produce synaptic rearrangement or plasticity. However, we cannot rule out the possibility that the lack of reduction in [3 H]-MK-801 binding 8 months after severe ischaemia may reflect a result of tissue shrinkage. Further studies are needed to investigate the precise mechanisms underlying synaptic modification, plasticity, and receptor sensitivity following ischaemia.

Interestingly, there were no alterations in the binding sites of any of the ligands used in the striatum, which was the region most vulnerable to 10 min ischaemia. The reason for this result is unclear. However, our histological study of specimens prepared 8 months after 10 min ischaemia showed that, although the small- and medium-sized neurones in the striatum were almost all destroyed, the large-sized neurones were relatively well preserved and were sparsely scattered in this field. This result is in accord with previous studies (Pulsinelli *et al.*, 1982; Smith *et al.*, 1984). In a previous investigation, it was suggested that GABA receptors were located predominantly on cholinergic neurones (large-sized neurones) in the striatum, which is resistant to ischaemia (Francis & Pulsinelli, 1983). Thus, these large-sized neurones may have a high density of [3 H]-CHA, [3 H]-MK-801, and [3 H]-PN200-110, as well as [3 H]-QNB and [3 H]-muscimol, binding sites. The possible contribution of presynaptic components, glial cells, and blood vessels to these residual binding sites should also be considered. Furthermore, mild tissue shrinkage may occur in the striatum 8 months after ischaemia. Thus, progression of neuronal damage and the alterations that occur in various neurotransmitter receptors in the hippocampus and striatum with long-term survival following ischaemia may have different underlying mechanisms.

In conclusion, the present study demonstrated that long-term survival after transient cerebral ischaemia could modify alterations in neurotransmitter receptors, especially in the hippocampal formation, where considerable shrinkage was noted. These results also suggest that the hippocampal damage, compared with that in other regions, is not static, but progressive.

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