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Effects of cerebral ischemia on dopamine receptors in the gerbil striatum

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Abstract

Dopamine D_1 and D_2 receptors and uptake sites were studied in the gerbil striatum and frontal cortex 1 h to 7 days after 10 min of cerebral ischemia caused by occlusion of the bilateral common carotid arteries. [^{3}H]SCH23390 ([*N*-methyl-^{3}H]*R*[+]-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-7-*ol*-benzazepine), [^{3}H]nemonapride and [^{3}H]mazindol were used as markers of dopamine D_1 receptors, D_2 receptors and uptake sites, respectively. A significant reduction in [^{3}H]SCH23390 binding was found in the striatum from 48 h after ischemia. In contrast, during the recirculation periods, [^{3}H]nemonapride and [^{3}H]mazindol binding was mostly unaffected in this region which was the most vulnerable to ischemia. The frontal cortex, where ischemic neuronal damage was mild, also showed no significant changes in [^{3}H]SCH23390, [^{3}H]nemonapride and [^{3}H]mazindol binding after ischemia. Thus, cerebral ischemia that was associated with cell loss in the striatum resulted in a selective reduction of dopamine D_1 receptors and not D_2 receptors. No changes in dopamine D_1 or D_2 receptors were observed in frontal cortex. If massive dopamine release occurs with cerebral ischemia, it is not reflected by modification in the number of uptake sites located on dopamine terminals.

Keywords: Dopamine D_1 receptor; Dopamine D_2 receptor; Dopamine uptake site; Cerebral ischemia; Receptor autoradiography

1. Introduction

The selective vulnerability of certain neurons to cerebral ischemia is well established morphologically. Neurons known to be susceptible to ischemia include CA1 pyramidal cells of the hippocampus, the small- to medium-sized cells of the dorsolateral striatum and certain cells of the neocortex (Kirino, 1982; Pulsinelli et al., 1982; Smith et al., 1984). There is considerable evidence that supports the role of excitatory amino acid toxicity in hippocampal CA1 neuronal cell death (Benveniste et al., 1989; Rothman and Olney, 1987; Wieloch et al., 1985). However, whether a similar mechanism is implicated in the pathogenesis of post-ischemic striatal neuronal damage is as yet unknown.

It is well known that the *N*-methyl-D-aspartate (NMDA) receptor/channel activates the release of dopamine from the striatum (Roberts and Anderson, 1979; Snell and Johnson, 1986; Werling et al., 1990). The NMDA receptors are normally activated by endogenous glutamate and its coagonist glycine, acting at its own receptor within the NMDA receptor/channel complex (Kleckner and Dingledine, 1988). Several lines of evidence suggest that a massive release of dopamine occurs in the striatum as a result of cerebral ischemia (Globus et al., 1988; Slivka et al., 1988). A previous study also indicated that substantia nigra lesion can protect striatal neurons from ischemic damage despite excessive release of an excitatory amino acid such as glutamate during and after transient ischemia (Globus et al., 1987). From these observations, the changes in dopamine neurotransmission seem to play a key role in the development of ischemic striatal damage. Therefore, to elucidate further the mechanisms of regional ischemic vulnerability, we investigated whether cerebral ischemia is associated with changes in dopaminergic receptors in the striatum or frontal cortex. For this purpose, we examined changes in dopamine D_1 and D_2 receptors and uptake sites in the striatum and neocortex after transient cerebral ischemia in gerbils.

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2. Materials and methods

2.1. Ischemic insult

Male adult Mongolian gerbils weighing 60 to 80 g were anesthetized with 2% halothane in a mixture of 30% oxygen and 70% nitrous oxide. Bilateral common carotid arteries were gently exposed and the arteries were occluded with aneurysm clips for 10 min, and then the gerbils were allowed to survive for 1, 5, 24 and 48 h and 7 days after transient ischemia. Sham-operated animals were treated in the same manner, except for the clipping of the bilateral carotid arteries. Body temperature was maintained at 37–38°C, using a heating pad with a thermostat, throughout the experiments.

2.2. Tissue preparation

The gerbils were decapitated at different reperfusion times as described above, and the brains were removed quickly, frozen in powdered dry-ice and stored at −80°C until assay. Coronal sections 12 μm in thickness were cut on a cryostat and thaw-mounted onto gelatin-coated slides. Adjacent sections were stained with cresyl violet and used for histopathology.

2.3. [^{3}H]SCH23390 binding assay

Autoradiographic distribution of dopamine D_1 receptors was measured using [^{3}H]SCH23390 ([*N*-methyl-^{3}H]*R*[+]-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-7-*ol*-benzazepine) by the method of Dawson et al. (1985, 1986) with minor modifications (Araki et al., 1992a). Sections were incubated with 1 nM [^{3}H]SCH23390 (New England Nuclear; 71.1 Ci/mmol) in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ for 30 min at room temperature. The sections were then dipped in the buffer at 4°C, followed by 25-min rinses in fresh buffer at 4°C. Non-specific binding was determined using 1 μM non-labeled SCH23390 (Research Biochemicals Int.).

2.4. [^{3}H]Nemonapride (YM-09151-2) binding assay

Autoradiographic localization of dopamine D_2 receptors was performed according to the method of Unis et al. (1990) with slight modifications. Briefly, the sections were incubated with 0.4 nM [^{3}H]nemonapride (New England Nuclear; 86.1 Ci/mmol) for 60 min at room temperature in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 10 μM paraglycine hydrochloride and 0.1% ascorbic acid. After incubation, the sections were rinsed in fresh buffer for 2 min at 4°C and dipped in distilled water at 4°C. Non-specific binding was determined using 1 μM haloperidol (Sigma).

2.5. [^{3}H]Spiperone binding assay

Autoradiographic localization of dopamine D_2 receptors was performed according to the method reported by Palacios et al. (1981) with minor modifications. Sections were pre-incubated for 10 min at 25°C in 50 mM Tris-HCl buffer (pH 7.6) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.001% ascorbic acid. The sections were then incubated with 0.6 nM [^{3}H]spiperone (New England Nuclear, 23.0 Ci/mmol) for 60 min at 25°C in the same buffer including 100 mM mianserin (Sigma) to block the binding of [^{3}H]spiperone to the serotonin receptor (Altar et al., 1985; Richfield et al., 1987). After incubation, the sections were washed 2 times in fresh ice-cold buffer for 5 min, and dipped in ice-cold distilled water. Non-specific binding was determined using 10 μM sulpiride (Sigma).

2.6. [^{3}H]Mazindol binding assay

Autoradiographic distribution of dopamine uptake sites was performed according to the method of Przedborski et al. (1991) with minor modifications. Briefly, the sections were pre-incubated for 15 min at 4°C in 50 mM Tris-HCl buffer (pH 7.9) containing 120 mM NaCl and 5 mM KCl. The sections were then incubated with 15 nM [^{3}H]mazindol (New England Nuclear; 24.0 Ci/mmol) for 60 min at 4°C in 50 mM Tris-HCl buffer (pH 7.9) containing 300 nM NaCl, 5 mM KCl and 0.3 μM desmethylimipramine (DMI, Sigma). DMI was used to block the binding of [^{3}H]mazindol to norepinephrine uptake sites, as described previously (Kujirai et al., 1990). After incubation, the sections were washed 2 times in fresh ice-cold buffer for 3 min, and dipped in ice-cold distilled water. Non-specific binding was determined using 30 μM Benztropine (Sigma).

The sections were quickly dried under a cold air stream and were exposed to Hyperfilm-^{3}H (Amersham) for two to four weeks in X-ray cassettes with a set of ^{3}H microscales (Amersham). The optical density of the brain regions was measured with a computer-associated image analyzer, as described previously (Araki et al., 1992a). Binding assays were performed in duplicate. Values were expressed as the mean ± S.D. Statistical comparisons were made using an analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Each group contained five to eight animals.

2.7. Histopathology

Adjacent sections prepared for receptor autoradiography were also stained with cresyl violet and hematoxylin-eosin. Stained sections were examined with a light microscope, and ischemic neuronal damage was graded on a semiquantitative scale: 0, normal; 1, a few neurons damaged: 2, many neurons damaged; 3, majority of neurons damaged, as described previously (Araki et al., 1992b). Each group contained five to eight animals.

3. Results

3.1. Receptor autoradiography

Representative autoradiographs of dopamine D_1 and D_2 receptors and uptake sites are shown in Fig. 1. Post-ischemic changes in dopamine D_1 and D_2 receptors and uptake sites are summarized in Tables 1 and 2.

3.2. Dopamine D_1 receptor

In sham-operated gerbils, [^{3}H]SCH23390 binding was greatest in the striatum. The frontal cortex had a very low density of binding. Gerbils subjected to ischemia showed no significant alteration in [^{3}H]SCH23390 binding in the striatum and frontal cortex up to 24 h after recirculation. 48 h after ischemia, a significant reduction in [^{3}H]

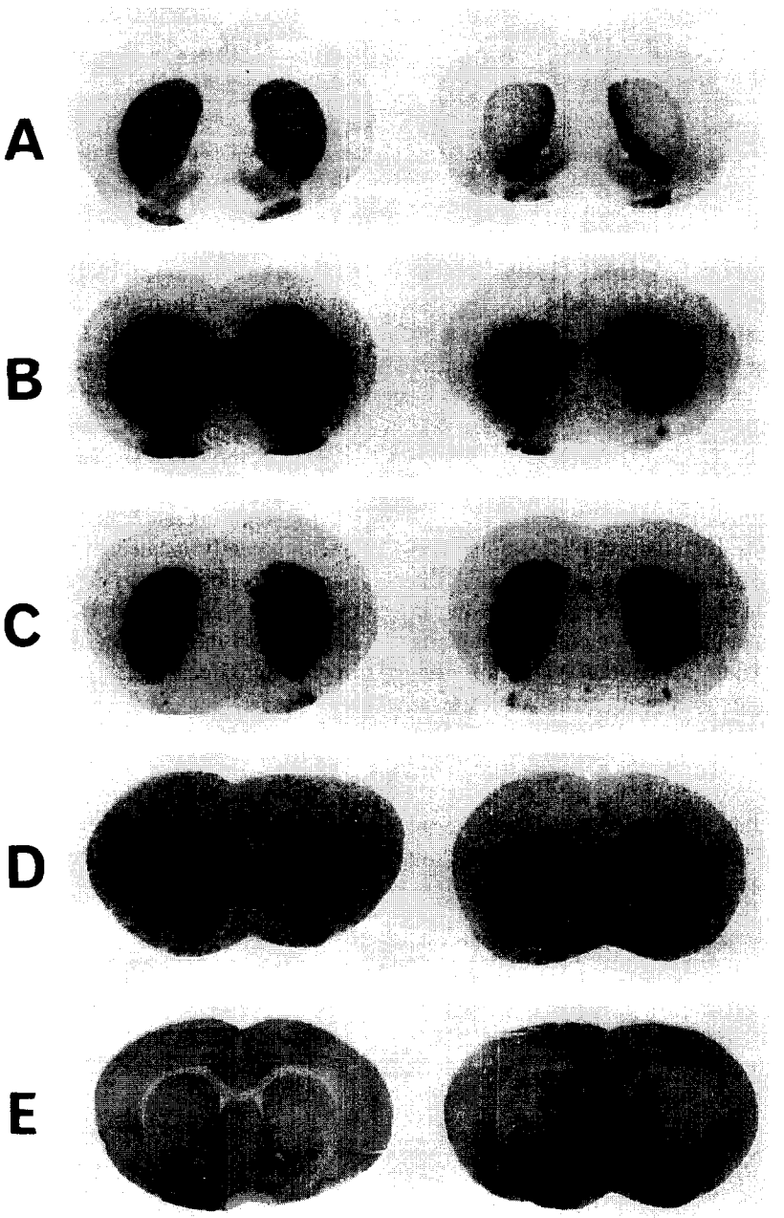

Fig. 1. Representative autoradiograms of [^{3}H]SCH23390 (A), [^{3}H]nemonapride (B), [^{3}H]spiperone (C) and [^{3}H]mazindol (D) binding and photographs of cresyl violet staining (E) in the gerbil striatum and frontal cortex following transient cerebral ischemia. Left half: sham-operated, Right half: 7 days after ischemia. In sham-operated gerbils, [^{3}H]SCH23390, [^{3}H]nemonapride, [^{3}H]spiperone and [^{3}H]mazindol binding was greatest in the striatum, whereas the frontal cortex showed a particularly low density of binding. Seven days after ischemia, a marked reduction in [^{3}H]SCH23390 binding was noted in the striatum, whereas [^{3}H]nemonapride, [^{3}H]spiperone and [^{3}H]mazindol binding was unaffected in this region. No significant changes in binding was found in the frontal cortex (A–D). Sham-operated gerbils showed no neuronal damage in the striatum and frontal cortex. Seven days after ischemia, marked damage was noted in the striatum and mild damage was seen in the frontal cortex (E).

Table 1
Alteration in [^{3}H]SCH23390 and [^{3}H]nemonapride binding in the gerbil brain after transient cerebral ischemia

	Sham-operated	Recirculation time				
		1 h	5 h	24 h	48 h	7 days
[^{3}H]SCH23390 binding						
Frontal cortex	13 ± 2	14 ± 1	13 ± 3	14 ± 2	13 ± 3	12 ± 3
Striatum						
Lateral	299 ± 21	274 ± 9	258 ± 24	244 ± 79	224 ± 49 [a]	64 ± 11 [b]
Medial	303 ± 31	289 ± 18	283 ± 20	273 ± 40	280 ± 14	173 ± 75 [b]
[^{3}H]Nemonapride binding						
Frontal cortex	14 ± 3	16 ± 2	13 ± 2	13 ± 1	13 ± 2	11 ± 3
Striatum						
Lateral	103 ± 4	99 ± 12	99 ± 6	108 ± 9	119 ± 14 [a]	112 ± 10
Medial	91 ± 2	87 ± 15	84 ± 6	87 ± 11	88 ± 7	77 ± 18

Optical density was converted to fmol/mg tissue using [^{3}H]microscales. Values are expressed as mean ± S.D. [a] $P < 0.05$, [b] $P < 0.01$ vs. sham-operated group (Dunnett's multiple range test). Striatum (Lateral): the dorsolateral part of striatum, Striatum (Medial): the ventromedial part of striatum. $n = 5–8$ animals.

SCH23390 binding was seen in the dorsolateral striatum. Thereafter, a marked reduction in [^{3}H]SCH23390 binding was found in the striatum 7 days after ischemia. In contrast, the frontal cortex exhibited no significant alteration in [^{3}H]SCH23390 binding throughout the recirculation periods.

3.3. Dopamine D_2 receptor

3.3.1. [^{3}H]Nemonapride binding

In sham-operated gerbils, high density [^{3}H]nemonapride binding was found in the striatum. In the frontal cortex, the gray density of [^{3}H]nemonapride binding was very low. In animals subjected to ischemia, [^{3}H]nemonapride binding was unchanged in the striatum and frontal cortex throughout the recirculation periods except for a transient elevation in the dorsolateral striatum after 48 h.

3.3.2. [^{3}H]Spiperone binding

In sham-operated gerbils, [^{3}H]spiperone binding was greatest in the striatum. The frontal cortex had a very low density of binding. In animals subjected to ischemia, a transient reduction in [^{3}H]spiperone binding was found in the striatum. Thereafter, [^{3}H]spiperone binding was unchanged in the striatum up to 7 days after ischemia. The frontal cortex showed no significant alteration in [^{3}H]spiperone binding throughout the recirculation periods.

3.4. Dopamine uptake site

In sham-operated gerbils, [^{3}H]mazindol binding was predominantly located in the striatum. The frontal cortex had a very low density of binding. In animals subjected to ischemia, [^{3}H]mazindol binding was statistically unchanged in the striatum and frontal cortex throughout the recirculation periods.

3.5. Histopathology

Representative photographs of cresyl violet staining in the striatum and neocortex 7 days after ischemia are shown in Fig. 1. Sham-operated gerbils showed no neuronal

Table 2
Alteration in [^{3}H]spiperone and [^{3}H]mazindol binding in the gerbil brain after transient cerebral ischemia

	Sham-operated	Recirculation time				
		1 h	5 h	24 h	48 h	7 days
[^{3}H]Spiperone binding						
Frontal cortex	1.8 ± 1.3	1.3 ± 0.9	1.7 ± 0.5	1.6 ± 1.0	1.0 ± 0.9	1.2 ± 0.2
Striatum						
Lateral	33 ± 4	28 ± 3 [a]	29 ± 2	32 ± 5	32 ± 2	32 ± 1
Medial	29 ± 2	25 ± 3 [a]	25 ± 2	26 ± 5	29 ± 2	28 ± 2
[^{3}H]Mazindol binding						
Frontal cortex	24 ± 4	26 ± 7	25 ± 9	20 ± 6	22 ± 7	22 ± 5
Striatum						
Lateral	88 ± 9	93 ± 6	92 ± 7	103 ± 18	106 ± 17	76 ± 16
Medial	104 ± 9	95 ± 13	91 ± 10	100 ± 20	111 ± 17	119 ± 18

Optical density was converted to fmol/mg tissue using [^{3}H]microscales. Values are expressed as mean ± S.D. [a] $P < 0.05$, [b] $P < 0.01$ vs. sham-operated group (Dunnett's multiple range test). Striatum (lateral): the dorsolateral part of striatum, Striatum (medial): the ventromedial part of striatum. $n = 5–8$ animals.

damage in the striatum and frontal cortex. Gerbils subjected to ischemia also showed no conspicuous neuronal damage in the frontal cortex up to 48 h after ischemia. In contrast, the striatum showed conspicuous neuronal damage from 24 h after ischemia. Seven days after ischemia, severe damage was noted in the striatum. The damage to the frontal cortex was mild.

4. Discussion

Dopamine is a well-documented neurotransmitter in the central nervous system (CNS). The distinction between dopamine receptor subtypes in the CNS has been based on anatomical, biochemical and pharmacological characteristics (Civelli et al., 1991; Dawson et al., 1986; Filloux et al., 1987; Stoof and Kebabian, 1984). The dopamine receptors are predominantly located in the striatum and substantia nigra and are found at least in two main types, D_1 and D_2, coupled via different G-proteins to different second messengers (Vallar and Meldolesi, 1989; Wachtel et al., 1989). Dopamine has been implicated as a possible cause for neuronal damage in experimental animals. A previous study indicated that there is a reduction in dopamine D_1 receptors in the dorsolateral striatum 7 days after transient cerebral ischemia in rats, whereas the dopamine D_2 receptors are not affected in this area (Benfenati et al., 1989). Recent studies also indicated that dopamine and L-DOPA (L-3,4-dihydroxyphenylalanine) cause neuronal death in tissue culture (Tanaka et al., 1991; Mytilineou et al., 1993) and intrastriatal injection of dopamine causes dose-dependent loss of neurons (Filloux and Townsend, 1993). The involvement of the dopamine system in neuronal damage is also supported by the fact that depletion of dopamine by inhibition of synthesis or release prevents striatal neuronal necrosis (Globus et al., 1987; Phebus and Clements, 1989). These observations seem to indicate that the changes in dopamine neurotransmission play a role in the pathogenesis of striatal neuronal damage.

In the present study, transient cerebral ischemia in gerbils caused a severe decrease in [^{3}H]SCH23390-labeled dopamine D_1 receptors in the striatum where severe neuronal damage was noted. This reduction in [^{3}H]SCH23390-labeled dopamine D_1 receptors was seen in the striatum from 48 h after ischemia. In contrast, the frontal cortex, where ischemic neuronal damage was mild, showed no significant change in [^{3}H]SCH23390-labeled dopamine D_1 receptors throughout the recirculation periods. In the present study, however, we did not determine whether the loss of [^{3}H]SCH23390 binding in the striatum indicates a change in B_{max} or a change in K_d. Many studies demonstrate that the B_{max} of various neurotransmitter receptors is reduced in ischemic animal brain, whereas the K_d is unaffected (Chang et al., 1993; Ogawa et al., 1991; Westerberg et al., 1989). Therefore, the present study seems to suggest that the reduction in [^{3}H]SCH23390 binding reflects a change in the B_{max}.

In contrast, [^{3}H]nemonapride-labeled and [^{3}H]-spiperone-labeled dopamine D_2 receptors were unchanged in the striatum and frontal cortex throughout the recirculation periods, except for a transient change in these receptors in the striatum. Thus, the alteration in [^{3}H]-nemonapride-labeled dopamine D_2 receptors was similar to that in [^{3}H]spiperone-labeled dopamine D_2 receptors. A previous study indicated that lesions of the substantia nigra with either 6-hydroxydopamine or ibotenic acid can produce a marked reduction in [^{3}H]sulpiride-labeled dopamine D_2 receptors, but no change in [^{3}H]SCH23390-labeled dopamine D_1 receptors in the substantia nigra (Filloux et al., 1988). In the present study, the area of [^{3}H]SCH23390-labeled dopamine D_1 cell loss was coincident with the extent of histological damage in the striatum. Therefore, the reduction in [^{3}H]SCH23390-labeled dopamine D_1 receptors found in our study may be a reflection of a loss of striatal cells that possess dopamine D_1 receptors, as shown in Fig. 1.

In contrast, [^{3}H]nemonapride-labeled dopamine D_2 receptors were mostly unaffected in the striatum after ischemia. A previous study suggested that unilateral perinatal hypoxic-ischemia causes a reduction in both [^{3}H]SCH 23390-labeled dopamine D_1 and [^{3}H]spiperone-labeled D_2 receptors in the rat striatum (Przedborski et al., 1991). Furthermore, Trugman et al. (1986) demonstrated that [^{3}H]spiperone-labeled dopamine D_2 receptors are located on kainic acid-sensitive intrinsic neuronal elements in the rat striatum, whereas D_2 receptors are not located on the terminals of the corticostriatal pathway. These observations seem to suggest that [^{3}H]spiperone-labeled dopamine D_2 receptors are predominantly located on the intrinsic striatal neurons. However, a previous study demonstrated that Ibotenic acid lesion of the striatum produces a marked loss of [^{3}H]SCH 23390-labeled dopamine D_1 receptors in the ipsilateral striatum, whereas [^{3}H]sulpiride-labeled dopamine D_2 receptors are partially removed by this same lesion (Filloux et al., 1988). A recent study using double-label autoradiography also suggested that quinolinic acid lesion of striatum in rats causes a marked reduction in [^{3}H]SCH 23390-labeled dopamine D_1 receptors in striatal area, whereas [^{11}C]nemonapride-labeled dopamine D_2 receptors are relatively well conserved (Ryu et al., 1994). Therefore, the present study is consistent with the evidence that [^{3}H]nemonapride-labeled and [^{3}H]spiperone-labeled dopamine D_2 receptors in the striatum are localized on not only intrinsic striatal neurons, but also on the presynaptic terminals of dopaminergic axons originating from the substantia nigra. Furthermore, it is widely recognized that dopamine D_1 and D_2 receptors are located on phenotypically distinct populations of striatal cells such as striatopallidal and striatonigral neurons (Gerfen et al., 1990; Gerfen, 1992). The striatopallidal neurons express enkephalin and dopamine D_2 receptors, whereas the striatonigral neurons

express substance P, dynorphin and dopamine D_1 receptors. The results of our study, therefore, are also consistent with the possibility that the striatopallidal neurons in the striatum are relatively resistant to transient cerebral ischemia. Further studies are needed to clarify the detailed biochemical mechanisms of our findings.

Neither dopamine D_1 nor D_2 receptors showed significant changes in the frontal cortex after ischemia. For this reason, it is conceivable that we could not observe any significant changes in dopamine D_1 or D_2 receptors in the frontal cortex because the dopaminergic presynaptic structures were preserved after ischemia or the density of these receptors was particularly low in this region. Therefore, our data suggest that changes in both dopamine D_1 and D_2 receptors cannot fully explain the mechanism of neuronal damage in the striatum and neocortex after transient cerebral ischemia. However, [^{3}H]nemonapride is known to label partially dopamine D_3 and D_4 receptors (Cox and Waszczak, 1991; Vile et al., 1995). Therefore, further studies are required to clarify our findings.

[^{3}H]Mazindol is a highly specific and selective ligand for dopamine uptake sites when the binding is performed in the presence of DMI (dismethyimipramine) to block binding to norepinephrine uptake sites (Javitch et al., 1985; Przedborski et al., 1991). Several lesion studies have demonstrated that [^{3}H]mazindol binding is located on the presynaptic terminals of dopaminergic axons originating substantia nigra in rats (Javitch et al., 1985; O'Dell and Marshall, 1988). The present study also showed that [^{3}H]mazindol binding in gerbils was greatest in the striatum, which is innervated by a dopaminergic projection from the substantia nigra. Following ischemia, no significant change in [^{3}H]mazindol binding was found in the striatum and frontal cortex throughout the recirculation periods. Thus, if cerebral ischemia leads to a massive release of dopamine, this is not reflected by changes in the concentration of dopamine uptake sites.

In conclusion, the results obtained here revealed a significant reduction of striatal dopamine D_1 receptors after transient cerebral ischemia. In contrast, dopamine D_2 receptors and uptake sites were mostly unaffected in the striatum after ischemia. Furthermore, the frontal cortex showed no significant changes in the dopamine D_1 and D_2 receptors and uptake sites throughout the recirculation periods.

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