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Prevention of Hippocampus Neuronal Damage in Ischemic Gerbils by a Novel Lipid Peroxidation Inhibitor (Quinazoline Derivative)

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ABSTRACT
We investigated the effect of a novel quinazoline derivative (KB-5666), a lipid peroxidation inhibitor, on ischemic neuronal damage using Mongolian gerbils. The animals were sacrificed 7 or 30 days after 5 min of forebrain ischemia induced by bilateral common carotid artery occlusion. Morphologic changes, a microtuble-associated protein 2 (MAP2) immunohistochemical study and quantitative autoradiographic study using [^{3}H]phorbol 12,13-dibutyrate ([^{3}H]PDBu) were evaluated in the hippocampus after ischemia. KB-5666 (3–50 mg/kg, i.v.) showed protective effects against neuronal death of the CA1 subfield 5 min before ischemia, immediately or 1 hr after ischemia, but not 4 hr after ischemia. KB-5666 (i.p.) also showed protective effects in a dose-dependent manner immediately after ischemia. Furthermore, KB-5666 dose-dependently prevented a marked decrease in microtubule-associated protein 2 immunoreactivity in the dendritic fields of the CA1 pyramidal cells after ischemia. The [^{3}H]PDBu binding activity in the stratum oriens and the stratum lacunosum-moleculare of the CA1 subfield was reduced by 19 and 30%, respectively, 7 days after ischemia. [^{3}H]PDBu binding sites were unchanged in the stratum oriens in the CA3 subfield. By contrast, in the molecular layer of the dentate gyrus, the [^{3}H]PDBu binding activity increased by 15%. KB-5666 (i.v.) prevented a decrease in the [^{3}H]PDBu binding activity in the stratum oriens and stratum lacunosum-moleculare of the CA1 subfield and an increase in the molecular layer of the dentate gyrus. These histologic, immunohistochemical and receptor-autoradiographic data indicate that KB-5666 protects the brain from both cellular and functional consequences of ischemia.

Pyramidal neurons in the hippocampus CA1 subfield are selectively vulnerable to brief and transient cerebral ischemia in both animals and humans (Pulsinelli *et al.*, 1982; Zola-Morgan *et al.*, 1986). Brief bilateral common carotid artery occlusion in gerbils has been shown to produce delayed neuronal death in the hippocampal CA1 subfield (Kirino, 1982; Kirino and Sano, 1984). Recent experiments have provided definitive evidence supporting the hypothesis that extracellular accumulation of synaptically released neurotransmitters, such as glutamate and aspartate, are responsible for neuronal death in the hippocampus (Rothman and Olney, 1986). Some other mechanisms involving protein kinase C (PKC) (Hara *et al.*, 1990b), γ-aminobutyric acid (Sternau *et al.*, 1989) and serotonin (Fujikura *et al.*, 1989) have also been thought to participate in the development of delayed neuronal death.

Clinical and experimental data have suggested that ischemic neuronal damage is at least partly induced by free radicals and/or lipid peroxidation, which is produced during ischemia or following reperfusion (Flamm *et al.*, 1978; Siesjö, 1981; Kogure *et al.*, 1982; Chan *et al.*, 1984; Kogure *et al.*, 1985). Recent studies have revealed an increase in lipid peroxide levels in the hippocampus CA1 24 hr following cerebral ischemia in rats (Arai *et al.*, 1987; Bromont *et al.*, 1989). Also, we reported that α-tocopherol (vitamin E), a lipid peroxidation inhibitor and/or free radical scavenger, prevented CA1 pyramidal cell death following ischemia in the gerbil (Hara *et al.*, 1990a).

PKC is a Ca^{2+}- and phospholipid-dependent enzyme highly concentrated in the brain (Kikkawa *et al.*, 1982). The activation of PKC plays a critical role in neurotransmitter release and synaptic plasticity (Malenka *et al.*, 1986; Nishizuka, 1986). The tumor promoter phorbol ester binding to PKC obtained by *in vitro* [^{3}H]PDBu autoradiography is identical to the distribution of PKC. Immunohistochemical localization of PKC (Saito *et al.*, 1988) and phorbol ester autoradiography (Worley *et al.*, 1986) revealed that the hippocampus contains a high concentration of PKC molecules in the brain, with the highest density in the CA1 subfield, where selective pyramidal cell death is observed after ischemia. Hydrolysis of phosphoinositides into diacylglycerol and inositol phosphates, which was observed after ischemia (Abe *et al.*, 1987), may modulate protein phosphorylation *via* PKC (Gonzales *et al.*, 1987). Further, several lines of evidence have indicated the involvement of PKC in the

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ABBREVIATIONS: MAP2, microtuble-associated protein 2; PKC, protein kinase C; PDBu, phorbol 12,13-dibutyrate.

alteration of synaptic efficacy, such as long-term potentiation (Akers *et al.*, 1986; Hu *et al.*, 1987; Malenka *et al.*, 1986; Routtenberg *et al.*, 1986). Thus, PKC plays an important role in the hippocampal CA1 subfield.

KB-5666, 2-(allyl-1-piperazinyl)-4-*n*-amyloxyquinazoline fumarate (fig. 1), has been found to inhibit lipid peroxidation (malondialdehyde method) and brain mitochondria swelling, to prevent both the formation of brain edema and histologic neuronal damage in the middle cerebral artery occlusion of rats (H. Hara *et al.*, submitted for publication), and to improve memory deficits in various hypoxic and ischemic animal models (H. Hara *et al.*, manuscript in preparation). The purpose of the present study is to estimate morphologically and functionally the effects of KB-5666 on ischemia-induced neuronal damage in the gerbil hippocampus by means of histopathology, immunohistochemistry using MAP2, a marker protein of dendrites, and quantitative *in vitro* receptor autoradiography using [^{3}H] PDBu.

Methods

Male adult Mongolian gerbils (Seiwa Experimental Animals, Fukuoka, Japan) weighing 60 to 80 g each were divided at random, namely no selection criteria were used for attributing the animals to the various groups. The animals were anesthetized with a mixture of 2% halothane, 70% N_2O and 30% O_2. Bilateral common carotid arteries were exposed and occluded with Sugita No. 51 temporary aneurysm clips for 5 min. Sham-operated animals were treated in the same manner, except for no occlusion. By i.v. administration, KB-5666 (3, 10, 30 or 50 mg/kg, 5 ml/kg), pentobarbital (30 mg/kg, 5 ml/kg) or the vehicle (saline) was injected during a period of 1 min, 5 min before ischemia, and immediately, 1 hr or 4 hr after ischemia. By i.p. administration, KB-5666 (10, 30 or 50 mg/kg, 5 ml/kg) or the vehicle was injected immediately after ischemia. KB-5666 was kindly donated by Kanebo Ltd. (Osaka, Japan). Pentobarbital was purchased from Dainippon Pharmaceutical Co. Ltd. (Tokyo, Japan). The body temperature of the animals was maintained at 37–38°C using a heating pad with a thermostat and heating lamp during the operation period and until righting reflex reappeared, because hypothermia is shown to protect brain neurons against postischemic damage (Clifton *et al.*, 1989; Buchan and Pulsinelli, 1990; Minamisawa *et al.*, 1990). KB-5666 at 10 mg/kg (i.v.) does not produce either sedation, muscle relaxation, hexobarbital sleep or hypothermia in gerbils and mice (data not shown).

Histology. Seven or 30 days after ischemia, the animals were perfused transcardially with heparinized saline followed by perfusion-fixation with 10% formalin under pentobarbital anesthesia (40 mg/kg, i.p.). The removed brains were postfixed, dehydrated and embedded in paraffin using standard procedures. Coronal sections, 5 μm thick, were stained with Cresyl violet and hematoxylin and eosin. The number of neurons per 1-mm linear length of stratum pyramidale of the hippocampal CA1 subfield (neuronal density) was calculated by counting living neurons using a microscope and measuring the total length of the CA1 cell layer in each section. The average of the neuronal density for both sides was regarded as the neuronal cell density of each gerbil, according to the method of Kirino *et al.* (1986). The linear length of the CA1 subfield was measured with a digitizer (Wacom Co., Japan).

Immunohistochemistry. The immunohistochemical study was performed according to the method of our previous report (Hara *et al.*, 1990b). Coronal sections, 5 μm thick, were immunostained with a monoclonal antibody to MAP2 (1:1000; Sigma) for 2 hr at room temperature. An anti-mouse avidin-biotinylated horseradish peroxidase (ABC) kit was used according to the supplier's recommendations (Vector Laboratories, Burlingame, CA), and the sections reacted with 3,3-diaminobenzidine tetrahydrochloride. KB-5666 (10, 50 mg/kg, i.v.) was administered immediately after ischemia. The intensity of MAP2 immunostaining in the hippocampal CA1 subfield's dendrites was graded on a 4-point scale (0 = extensive staining, 1 = moderate staining, 2 = slight staining, 3 = no staining).

Autoradiography. The animals were decapitated 7 days after ischemic insult, and the brains were removed quickly, frozen in pow-

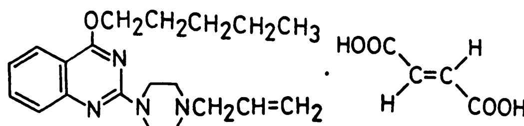

Fig. 1. Chemical structure of 2-(allyl-1-piperazinyl)-4-*n*-amyloxyquinazoline fumarate (KB-5666).

TABLE 1

Effects of i.v. administration of KB-5666 and pentobarbital on delayed neuronal death of CA1 pyramidal cells in the gerbil hippocampus

KB-5666 and pentobarbital were administered 5 min before ischemia, 0 min, 1 hr or 4 hr after ischemia. The perfusion was carried out 7 days after ischemia. Values are expressed as mean ± S.E. for the number of neurons per mm of linear length of CA1 subfield.

Treatment	Dose (i.v.)	*N*	Neuronal Density	% of Sham
	mg/kg			
Sham		8	252 ± 8**	
Pre −5 min				
Vehicle		13	85 ± 19	34
KB-5666	3	12	150 ± 32	60
	10	10	205 ± 21**	81
	30	10	221 ± 25**	88
	50	9	263 ± 5**	104
Pentobarbital	30	10	177 ± 37*	70
Post 0 min				
Vehicle		9	23 ± 7	9
KB-5666	3	9	47 ± 26	19
	10	10	120 ± 32**	48
	30	10	130 ± 30**	52
	50	9	180 ± 30**	71
Pentobarbital	30	11	126 ± 29**	50
Post 1 hr				
Vehicle		12	38 ± 20	15
KB-5666	50	8	153 ± 38**	61
Pentobarbital	30	6	74 ± 32	29
Post 4 hr				
Vehicle		12	38 ± 20	15
KB-5666	50	6	78 ± 34	31

* $P < .05$; ** $P < .01$ *vs.* Vehicle (two-tailed Mann-Whitney *U* test).

TABLE 2

Effect of i.p. administration of KB-5666 on delayed neuronal death of CA1 pyramidal cells in the gerbil hippocampus

KB-5666 was administered immediately after ischemia. The perfusion was carried out 7 or 30 days after ischemia. Values are expressed as mean ± S.E. for the number of neurons per mm of linear length of CA1 subfield.

Treatment	Dose (i.p.)	*N*	Neuronal Density	% of Sham
	mg/kg			
Sham		8	252 ± 8**	
7 Days				
Vehicle		15	33 ± 12	13
KB-5666	10	8	69 ± 25	27
	30	8	132 ± 31**	52
	50	8	197 ± 30**	78
30 Days				
Vehicle		7	13 ± 6	5
KB-5666	50	7	160 ± 45**	63

** $P < .01$ *vs.* Vehicle (two-tailed Mann-Whitney *U* test).

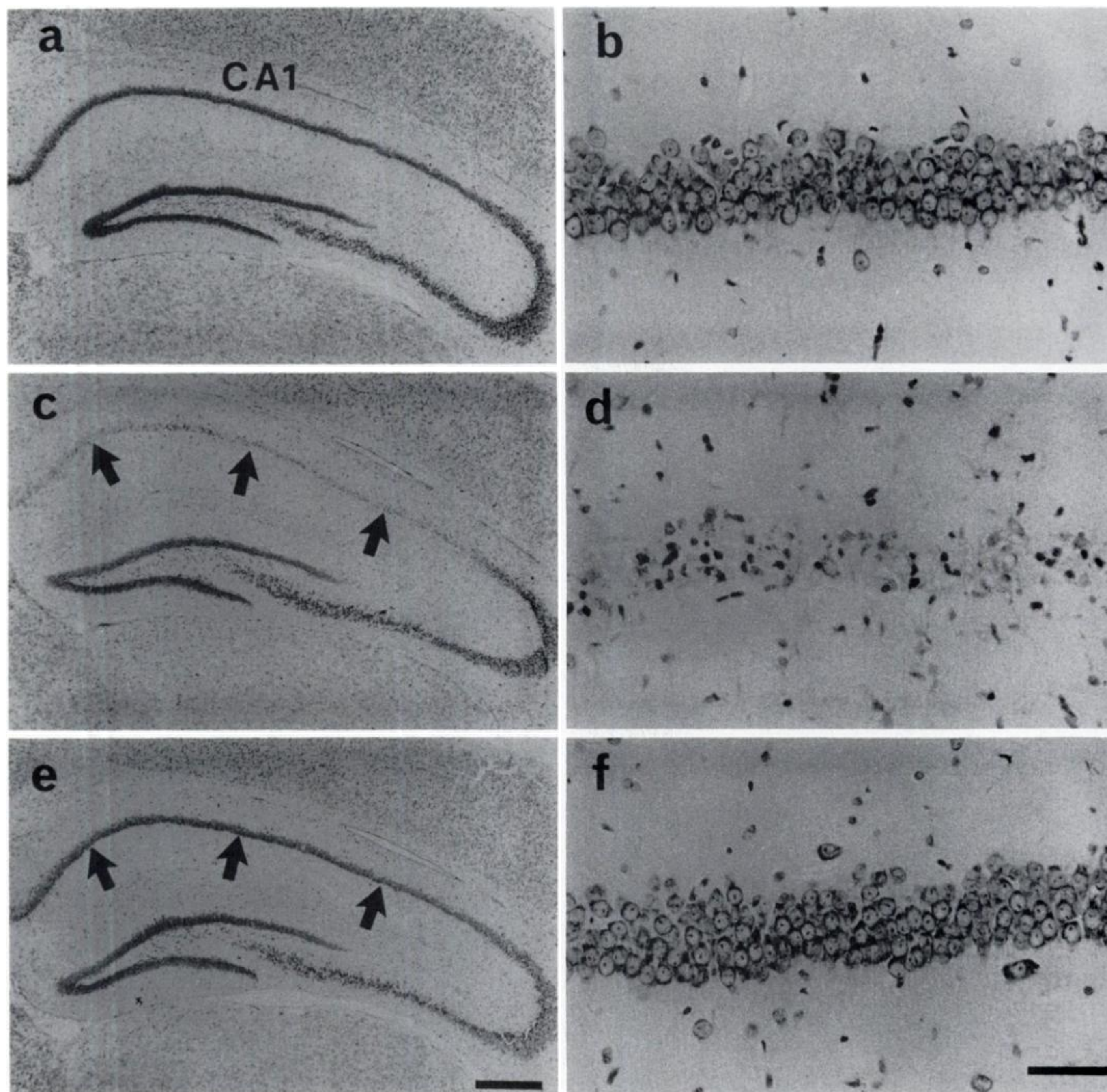

Fig. 2. Representative photomicrographs of hippocampal CA1 subfield 7 days after 5-min bilateral carotid artery occlusion in gerbils. Cresyl violet staining. a and b: Sham operation. The stratum pyramidale in the CA1 subfield. CA1 pyramidal cells were well-preserved under high magnification. c and d: Vehicle-treated ischemia. Note marked damage to the CA1 pyramidal cells (arrows). e and f: KB-5666 treatment. Most of the CA1 neurons were preserved (arrows). KB-5666 (10 mg/kg, i.v.) was administered 5 min before ischemia. Bars for a, c and e = 500 μm; b, d and f = 50 μm.

dered 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. [^{3}H]PDBu autoradiography was carried out as described by Worley *et al.* (1986) and our previous report (Onodera *et al.*, 1989) with minor modifications. The brain sections were incubated for 60 min at 25°C in a solution of 50 mM Tris HCl (pH 7.7), 100 mM NaCl, 1 mM $CaCl_2$ and 2.5 nM [^{3}H]PDBu (specific activity, 13.2 Ci/mmol; New England Nuclear). Nonspecific binding was assessed in the presence of 1 μM PDBu. Following incubation, sections were washed twice with the same solution at 4°C. Sections were dried under a cold stream of air and were apposed to Ultrafilm (Amersham) for 9 days. The optical density of the regions of interest was measured by a computer-assisted image analyzer (Zeiss, the IBAS2 image analyzer system). The relationship between optical density and radioactivity was examined with reference to the tritium standards (Amersham, [^{3}H]microscale) coexposed with the tissue sections (Onodera *et al.*, 1987). The optical density of the brain regions measured in the present study was in the range in which the optical density and the radioactivity of the ^{3}H-microscale showed a near linear relationship. A possible drawback with quantitative autoradiography may be a change in quenching level after ischemia. As discussed in detail in our previous report (Onodera *et al.*, 1987), there was no need to make a quenching correction because of increased gliosis. Quantitative receptor autoradiography has a technical advantage in that the light-microscopic distribution of neurotransmit-

TABLE 3

Effect of KB-5666 on immunohistochemical staining in the gerbil hippocampus 7 days reperfusion

KB-5666 was administered immediately after ischemia. The intensity of staining was graded 0 to 3.

Treatment (i.v.)	N	Grade of Score				Mean ± S.E.
		0	1	2	3	
Sham	8	8	0	0	0	0 ± 0**
Vehicle	6	0	0	1	5	2.8 ± 0.2
KB-5666 (10 mg/kg)	8	3	1	2	2	1.4 ± 0.5*
KB-5666 (50 mg/kg)	6	4	2	0	0	0.3 ± 0.2**

* $P < .05$; ** $P < .01$ *vs.* Vehicle (two-tailed Mann-Whitney *U* test).

ter receptors is obtained without contamination from adjacent structures (Kuhar, 1981). The binding assay was performed in duplicate.

Statistical analysis. The values were expressed as mean ± S.E. Statistical comparisons were made by one-way analysis of variance, Duncan's multiple-range test and two-tailed Mann-Whitney *U* test.

Results

Histology. Neuronal densities in each group are presented in tables 1 and 2, and representative photomicrographs of the hippocampal CA1 subfield are shown in figure 2. In the sham-operated group, the neuronal density of the CA1 subfield was 252 ± 8 cells/mm (mean ± S.E., $n = 8$). In the vehicle-treated ischemia group, the number of CA1 pyramidal cells was markedly decreased when the vehicle was administered 5 min before ischemia, immediately, 1 hr or 4 hr after ischemia (9–34% of the sham-operated group; $P < .01$, respectively). KB-5666 (3–50 mg/kg, i.v.) administered 5 min before ischemia, or immediately after ischemia, prevented neuronal death in the CA1 subfield in a dose-dependent manner. Further, KB-5666 (50 mg/kg, i.v.) administered 1 hr after ischemia also prevented neuronal death. However, KB-5666 (50 mg/kg, i.v.) administered 4 hr after ischemia did not prevent neuronal death. Pentobarbital (30 mg/kg, i.v.) administered 5 min before ischemia or immediately after ischemia, not 1 hr after ischemia, significantly prevented neuronal death. Further, post-treatment of KB-5666 (10–50 mg/kg, i.p.) immediately after ischemia, dose-dependently prevented neuronal death in the CA1 subfield, which was evaluated in the CA1 subfield 7 days after ischemia (table 2). Post-treatment of KB-5666 (50 mg/kg, i.p.) also prevented neuronal death in the CA1 subfield, which was evaluated 30 days after ischemia.

Immunohistochemistry. The intensities of MAP2 immunostaining in each group are presented in table 3, and representative photomicrographs of the CA1 subfield are shown in figure 3. MAP2 immunoreactivity of the sham group was preserved in the dendritic fields of the CA1 subfield. The vehicle-treated ischemia group showed a marked decrease in MAP2 immunoreactivity in the dendritic fields of CA1 pyramidal cells. In contrast, the KB-5666 (10, 50 mg/kg, i.v.) treatment group prevents dose-dependently the decrease in MAP2 immunoreactivity.

Autoradiography. [^{3}H]PDBu binding activities in each group are presented in table 4, and representative autoradiograms of the hippocampal CA1 subfield are shown in figure 4. As reported by Worley *et al.* (1986) and Tanaka *et al.* (1988), the distribution of [^{3}H]PDBu binding in the rat and gerbil brains was heterogeneous, and hippocampal formation exhibited high binding activity. The stratum oriens, radiatum and stratum lacunosum-moleculare in the CA1 subfield showed the highest grain density among the region examined (fig. 4). The stratum oriens in the CA3 subfield and the molecular layer of the dentate gyrus also had a high grain density. The [^{3}H]PDBu binding activity in the stratum oriens and the stratum lacunosum-moleculare of the CA1 subfield was reduced by 19 and 30%, respectively, 7 days after ischemia. [^{3}H]PDBu binding sites were unchanged in the stratum oriens in the CA3 subfield. By contrast, in the molecular layer of the dentate gyrus, the [^{3}H]PDBu binding activity increased by 15%. Pretreatment of KB-5666 (10, 50 mg/kg, i.v.) prevented a decrease in the [^{3}H]PDBu binding activity in the stratum oriens and stratum lacunosum-moleculare of the CA1 subfield and an increase in the molecular layer of the dentate gyrus.

Discussion

In the present studies, KB-5666, administered i.v. 5 min before ischemia, immediately or 1 hr after ischemia, prevented ischemic neuronal death of CA1 pyramidal cells. However, KB-5666, administrated i.v. 4 hr after ischemia, did not prevent neuronal death. Post-treatment (i.p.) of KB-5666 also prevented ischemic neuronal death of CA1 pyramidal cells, which was evaluated 7 and 30 days after ischemia. Furthermore, KB-5666 prevented the decrease of MAP2 immunoreactivity in the dendritic fields of CA1 pyramidal cells, indicating the preservation of dendrite arborization of the CA1 pyramidal cells (Matus *et al.*, 1981; Caceres *et al.*, 1983; Bernhardt and Matus, 1984). These results indicate that KB-5666 is histologically and immunohistochemically effective in preventing hippocampal CA1 damage induced by transient ischemia when administered either before or up to 1 hr after the ischemic episode, although the magnitude of protection was dependent upon the dose and time schedule of drug administration used.

Pentobarbital, administered i.v. 5 min before or immediately after ischemia, prevented ischemia-induced neuronal death of CA1 pyramidal cells at a sedative dose (30 mg/kg, i.v.). However, pentobarbital, given i.v. 1 hr after ischemia, did not prevent neuronal death. This finding is in good agreement with that reported by Kirino *et al.* (1986), who examined the protective effect of pentobarbital by i.p. administration. The therapeutic action of pentobarbital may begin during ischemia by delaying the failure in energy metabolism (Hallmayer *et al.*, 1985). In these respects, the action of KB-5666 was more protective than and different from that of pentobarbital.

In *in vitro* receptor autoradiography, KB-5666 prevented the decrease in [^{3}H]PDBu binding activity in the CA1 subfield and the increase in the molecular layer of the dentate gyrus. Onodera *et al.* (1989) reported that the [^{3}H]PDBu binding in the CA1 subfield was decreased by approximately 40% 7 days after ischemia in a rat four-vessel occlusion model. By contrast, the [^{3}H]PDBu binding in the molecular layer of the dentate gyrus was increased by 33%, resulting in the postischemic alteration of neurotransmission and sprouting from residual terminals following limited lesioning in the dentate gyrus. [^{3}H]PDBu binding activity was reported to be lower in brain tissue from Alzheimer patients (Cole *et al.*, 1988). Thus, the decrease in [^{3}H]PDBu binding activity may reflect a biochemical deficit related to the pathogenesis of ischemic insults and Alzheimer's disease.

Recent evidences suggest that brain edema and/or cellular damage induced by cerebral ischemia might be at least partly due to oxidative stress caused by free radicals and/or lipid

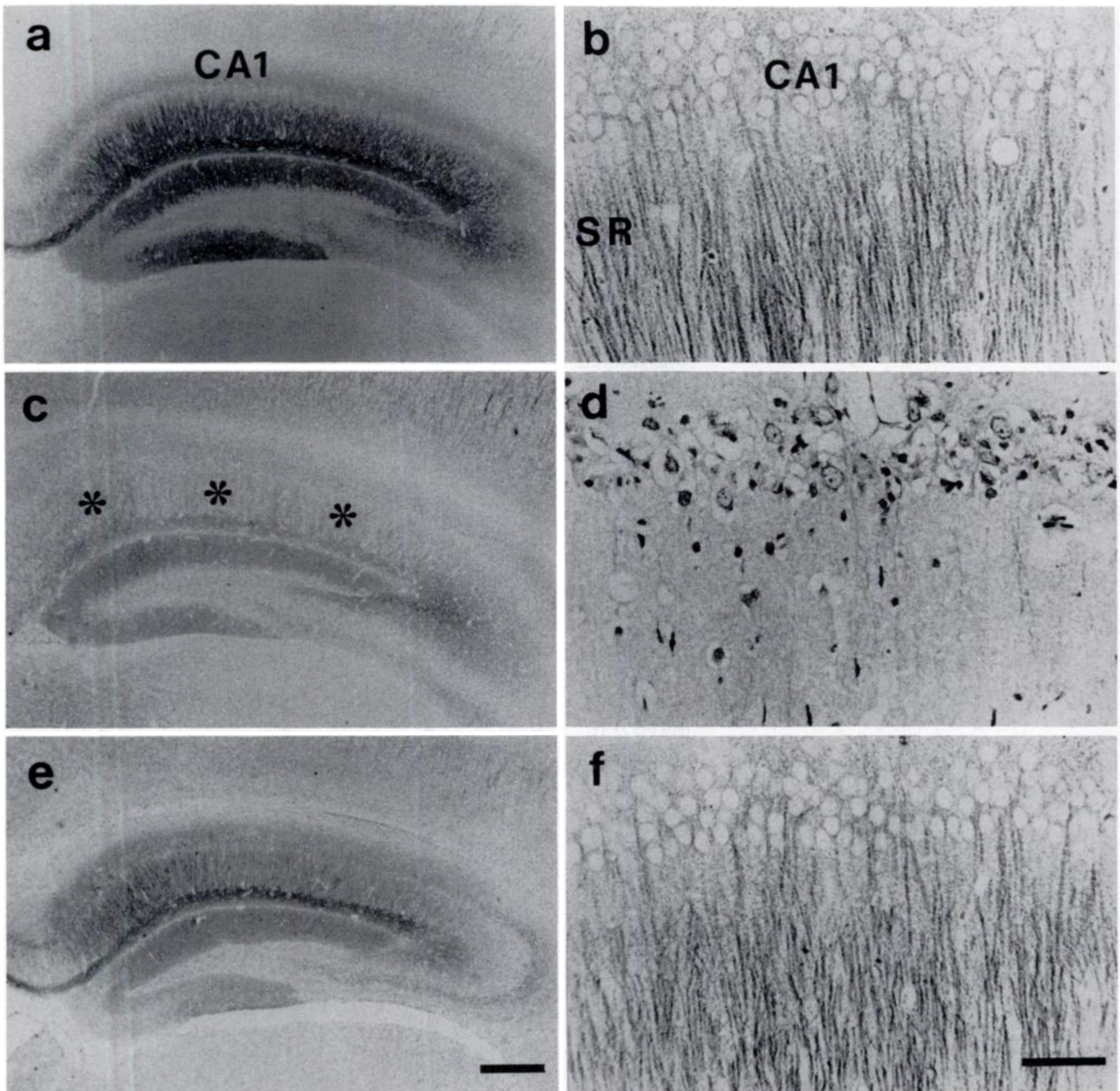

Fig. 3. MAP2 immunostaining. Representative photomicrographs of the hippocampal CA1 subfield 7 days after 5-min bilateral carotid artery occlusion in gerbils. a and b: Sham operation. c and d: Vehicle-treated ischemia. Most of the MAP2 stain disappeared (*). e and f: KB-5666 treatment. MAP2 immunoreactivity was preserved in the dendritic fields of the CA1 subfield. KB-5666 (10 mg/kg, i.v.) was administered immediately after ischemia. SR, stratum radiatum. Bars for a, c and e = 500 μm; b, d and f = 50 μm.

peroxidation. Inhibition of free radical formation during lipid peroxidation prevents the formation of brain edema and neuronal damage (Flamm *et al.*, 1978; Siesjö, 1981; Kogure *et al.*, 1982; Chan *et al.*, 1984; Kogure *et al.*, 1985). α-Tocopherol suppresses the formation of brain edema, promotes the resynthesis of ATP, improves deteriorated neurologic signs and prevents neuronal death in the hippocampal CA1 subfield after ischemia (Yamamoto *et al.*, 1983; Fujimoto *et al.*, 1984; Yoshida *et al.*, 1985; Hara *et al.*, 1990a). Miyamoto *et al.* (1989) have demonstrated that α-tocopherol protected against the glutamate-induced cytotoxicity in a neuronal cell line, and suggested that free radical scavengers may reduce neuronal damage in pathologic conditions associated with excessive glutamate release. Furthermore, a lipid peroxidation inhibitor, U74006F, prolongs the survival time and attenuates morphologic neuronal necrosis following cerebral ischemia in gerbils (Hall *et al.*, 1988).

Recently, it has been reported that a massive influx of calcium and an excessive release of excitatory neurotransmitters occur during and/or after ischemia (Olney *et al.*, 1971; Benveniste *et al.*, 1984; Wieloch *et al.*, 1985; Rothman and Olney, 1986). The latter results in neurofilament degradation,

TABLE 4

Effect of KB-5666 on [^{3}H]PDBu binding in the gerbil hippocampus 7-day reperfusion

Optical densities were converted to fmol/mg tissue using the mean ± S.E. from 7 to 11 animals. Numbers in parenthesis are percentage of sham.

Areas	Sham	Control	KB-5666	
			10 mg/kg, i.v.	50 mg/kg, i.v.
CA1 subfield				
Stratum oriens	1094 ± 52**	888 ± 38 (81)	1022 ± 15 (93)	1067 ± 65* (98)
Stratum radiatum	1175 ± 47	1047 ± 42 (89)	1086 ± 18 (92)	1151 ± 69 (98)
Stratum lacunosum-moleculare	1007 ± 39**	705 ± 64 (70)	802 ± 52 (80)	938 ± 71** (93)
CA3 subfield, stratum oriens	1189 ± 31	1177 ± 38 (99)	1265 ± 38 (106)	1234 ± 38 (104)
Dentate gyrus, molecular layer	980 ± 42*	1127 ± 44 (115)	1143 ± 22 (117)	999 ± 46* (102)

* $P < .05$; ** $P < .01$ *vs.* control (Duncan's multiple-range test).

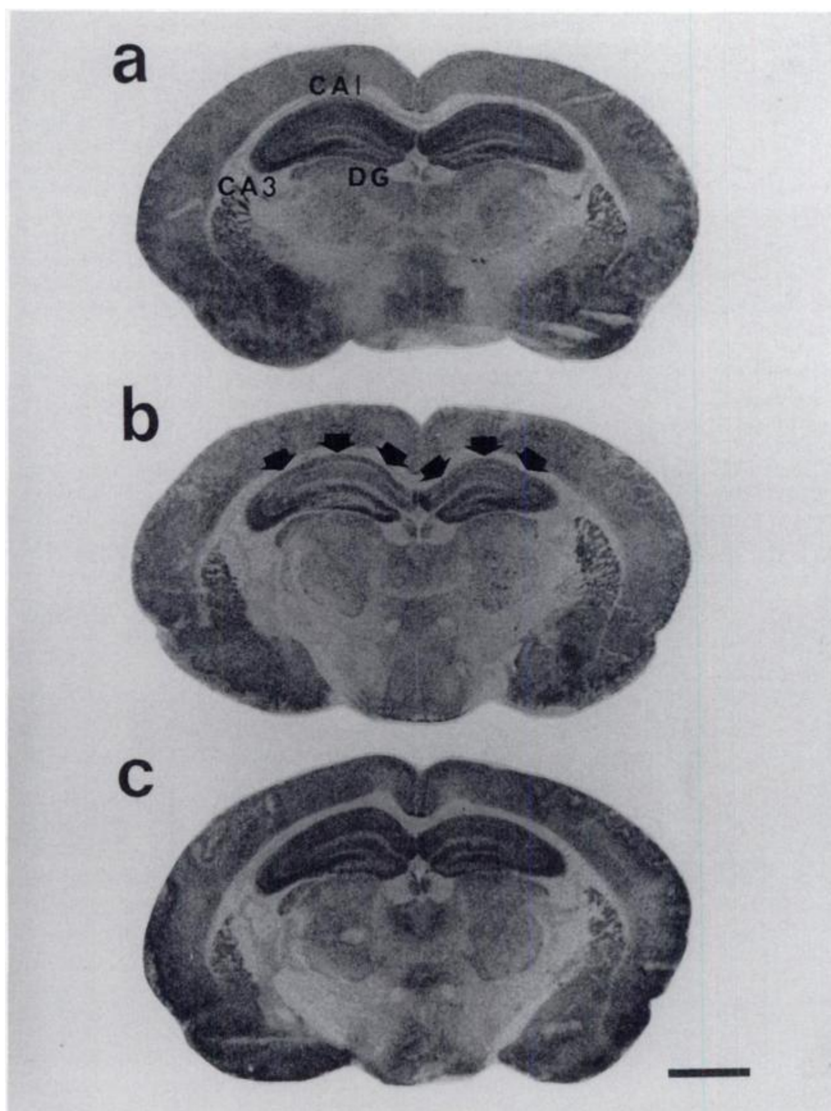

Fig. 4. [^{3}H]PDBu autoradiograms. Representative autoradiograms of the dorsolateral hippocampal coronal sections 7 days after 5-min bilateral carotid artery occlusion in gerbils. a: Sham operation. The CA1 subfield, the stratum oriens of the CA3 subfield and the molecular layer of the dentate gyrus have a high [^{3}H]PDBu binding activity. b: Vehicle-treated ischemia. Note marked decrease to the CA1 subfield (arrows). c: KB-5666 treatment. [^{3}H]PDBu binding of the CA1 subfield were well preserved. KB-5666 (50 mg/kg, i.v.) was administered 5 min before ischemia. DG, dentate gyrus. Bar = 200 μm.

damage to microtubles and lipolysis. In addition, the influx of calcium may occur as a result of lipid peroxidation and acidosis following ischemia (Siesjö, 1981; Uematsu *et al.*, 1988). The mechanism of postischemic protection by KB-5666 is unknown. However, KB-5666 was found to ameliorate brain edema and attenuate histologic neuronal damage induced by focal cerebral ischemia in the rat with middle cerebral artery occlusion and prevent the lipid peroxidative process (H. Hara *et al.*, submitted for publication). Therefore, KB-5666, by inhibiting lipid peroxidation and by stabilizing the state of the membrane (Kogure *et al.*, 1988), may reduce Ca^{2+} overload in neuronal cells and may ameliorate neuronal damage to the hippocampal CA1

pyramidal layers. However, we cannot rule out the possibilities that other actions of KB-5666, such as either the enhancement of γ-aminobutyric acid-ergic inhibitory effect or anticonvulsant action (H. Hara *et al.*, unpublished data), plays a role in the amelioration of hippocampal CA1 damage (Sternau *et al.*, 1989). We are also uncertain as to the extent to which KB-5666 might affect postischemic blood flow and the effect of KB-5666 on ischemic/postischemic release of excitatory amino acids.

In conclusion, pre- and postischemic administration of KB-5666 has a beneficial effect on gerbil hippocampal CA1 pyramidal cell damage up to 1 hr postischemia. Further, the protective effects of KB-5666 were confirmed in immunohistochemical and receptor autoradiography studies. Our finding that KB-5666 is effective even when administered after a period of ischemia could be of particular clinical importance, not only for prophylaxis but also for the treatment of ischemic insult. These results suggest that the lipid peroxidative process is an essential for the pathogenesis of ischemic neuronal damage in the gerbil. Further studies should be performed to ascertain the precise biochemical mechanisms for such effects.

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