Anesthesiology
1997; 87:371–7

Neuroprotective Effects of Dexmedetomidine in the Gerbil Hippocampus after Transient Global Ischemia

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***Background*: Cerebral ischemia induces a massive release of norepinephrine associated with neuronal death in the brain. It has been demonstrated that α_2-adrenoceptor agonists decrease the release and turnover of noradrenaline, and this might prove advantageous in counteracting the neurodegeneration in ischemic brain. Therefore, in the present study, the authors tested whether dexmedetomidine, a selective α_2-receptor agonist, has neuroprotective effects in a gerbil transient global ischemia model.**

***Methods*: Ischemia was induced by bilateral carotid occlusion for 5 min in diethylether-anesthetized normothermic gerbils. Dexmedetomidine was administered subcutaneously in four different treatment paradigms (6–8 animals/group): 3 or 30 μg/kg 30 min before and thereafter at 3, 12, 24, and 48 h after the occlusion, or 3 or 30 μg/kg at 3, 12, 24, and 48 h after the occlusion. Control animals were subjected to forebrain ischemia but received only saline injections. One week after occlusion, animals were transcardially perfused for histochemistry. Neuronal death in the CA1 and CA3 regions of the hippocampus and in the hilus of the dentate gyrus was evaluated in silver-stained 60-μm coronal sections.**

***Results*: Compared with saline-treated ischemic animals, dexmedetomidine at a dose of 3 μg/kg given before and continued after the induction of ischemia reduced the number of damaged neurons in the CA3 area (2 ± 3 *vs.* 17 ± 20 degenerated neurons/mm^2; $P < 0.05$). Also in the dentate hilus, the number of damaged neurons was reduced by dexmedetomidine (3 μg/kg) given before and continued after ischemia (5 ± 7 *vs.* 56 ± 42 degenerated neurons/mm^2; $P < 0.01$).**

***Conclusions:* The present data demonstrate that dexmedetomidine effectively prevents delayed neuronal death in CA3 area and in the dentate hilus in gerbil hippocampus when the management is started before the onset of ischemia and continued for 48 h after reperfusion. Inhibition of ischemia-induced norepinephrine release may be associated with neuroprotection by dexmedetomidine. (Key words: Cerebral ischemia. Dexmedetomidine. Neuronal damage. Neuroprotection.)**

ONE of the initial responses in the central nervous system after cerebral ischemia is the massive release of norepinephrine and various other neurotransmitters in the hippocampus and striatum.[1,2] The importance of increased noradrenergic activity remains unclear because norepinephrine has been suggested to play a protective and a detrimental role in ischemia-induced neuronal injury. It has been demonstrated that α_2-adrenoceptor agonists decrease the release and turnover of norepinephrine in the brain.[3,4] Thus, treatment with such an agent might prevent the release of norepinephrine induced by ischemia. If the increase in norepinephrine release is a causative component of injury, α_2-adrenergic agonists may provide protection against the damaging effects of cerebral ischemia. In support of this, clonidine has been shown to improve neuronal survival from incomplete cerebral ischemia in rats.[5] Further, dexmedetomidine, a more selective α_2-adrenoceptor agonist, prevented ischemic injury in the rat forebrain[6] and decreased neuronal damage in a rabbit focal model of ischemia.[7] Dexmedetomidine also suppressed kainic acid-induced convulsions and prevented hippocampal cell death in rats.[8]

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Received from the Department of Neurology, University of Kuopio, Kuopio, Finland. Submitted for publication: January 20, 1997. Accepted for publication March 27, 1997.

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In the present study, we evaluated the neuroprotective effect of dexemedetomidine against the global forebrain ischemia in gerbils. The efficacy of two different dosages given before and after the ischemic insult were compared.

Methods

These experiments were approved by the Ethics Committee of the University of Kuopio and by the Provincial Government of Kuopio. Female Mongolian gerbils (weight, 60–80 g), obtained from Møllegaard Breeding Centre Ltd. (Copenhagen, Denmark), were used in this study. The gerbils were housed individually, maintained on a 12-/12-h light/dark cycle, and allowed free access to food and water before and after surgical intervention.

The experiment consisted of 45 gerbils randomized to sham-operated animals, occluded animals that received 0.9% saline, animals that received 3 or 30 μg/kg dexmedetomidine 30 min before and thereafter at 3, 12, 24, and 48 h after occlusion, or animals that received 3 or 30 μg/kg dexmedetomidine at 3, 12, 24, and 48 h after occlusion. Dexmedetomidine was dissolved in 0.9% saline. All injections were made subcutaneously in a volume of 0.01 ml/g body weight.

Each gerbil was anesthetized with diethyl ether inhalation *via* a mask before surgery. The common carotid arteries were exposed through an anterior midline cervical incision and separated carefully from the surrounding vagus nerves. The gerbils were then placed on an electric heating blanket (Harvard Apparatus, South Natick, MA), and a rectal temperature probe was inserted. The rectal temperature was maintained at 37°C during the ischemic period. The anesthesia was discontinued immediately before the induction of ischemia, which was performed by occluding the carotid arteries with atraumatic miniature aneurysmal clips for 5 min. Cessation of circulation and beginning of recirculation were visually confirmed. Sham-operated animals were only anesthetized, and their carotid arteries exposed. The animals were followed systematically after the ischemia period. The behavior of animals that received 3 μg/kg dexmedetomidine was not different from control animals. However, the animals that received 30 μg/kg of demedetomidine were drowsy. None of the animals experienced epileptic seizures.

One week after occlusion, the animals were anesthetized (chlornembutal 0.3 ml/100 g, intraperitoneally) and perfused transcardially with 0.9% saline for 10 min and then for 60 min with fixative (4% paraformaldehyde, 0.05% glutaraldehyde, 0.2% picric acid in 0.1 M phosphate buffer pH 7.4). The brains were removed from the skull, postfixed in the same fixative if necessary, and coronal sections through the dorsal hippocampus (bregma −1.40 to −2.0 mm)[9] were cut with a vibratome at 60 μm. Sections were stained using the silver impregnation technique.[10]

Microscopic evaluation of delayed neuronal death was done in a blind manner on coded sections. Both hippocampi were analyzed, and several sections (4–8) were evaluated from each animal. The CA1 and CA3 pyramidal cells were counted using an ocular micrometer grid limiting a circular area of 0.08 mm^2 under the microscope. In the hilus of the dentate gyrus, the entire area confined by the dorsal and ventral blades of the granule cell layer was analyzed. Only the shrunken argyrophilic neurons were considered to be irreversibly degenerated. The mean number of damaged cells was calculated for each of the three hippocampal regions.

To study the possible effect of dexmedetomidine on various physiologic variables, the left external iliac artery was cannulated in additional animals during halothane anesthesia (2% halothane, 62% N_2O, and 35% O_2).[11] Mean arterial blood pressure (MABP; CardioCap II, Datex, Finland), blood pH, p_{O2}, and p_{CO2} (ABL5, Radiometer, Denmark) and blood glucose concentrations (OneTouch II, LifeScan, Milpitas, CA) were measured at baseline, 30 min after saline or dexmedetomidine (3 (g/kg) administration, during 5 min carotid occlusion, and 30 min after reperfusion. To monitor skull temperature of the animals (Omega, Model 680, Stamford, CT), a probe was placed in the temporalis muscle. In addition, rectal temperature was measured 30 min, 90 min, and 150 min after reperfusion.

The neuroprotective effect of different dexmedetomidine doses and management regimens was evaluated by Kruskall-Wallis test followed by the Mann-Whitney U-test. Physiologic data were compared using analysis of variance (ANOVA). If a significant effect was found, Student's *t* test was performed.

Results

Severity of Neuronal Damage in the CA1 Region

Complete forebrain ischemia over a period of 5 min resulted in a consistent neuronal degeneration in the hippocampal CA1 area in saline-treated animals with a mean of 1125 ± 305 degenerated neurons/mm^2 (fig.

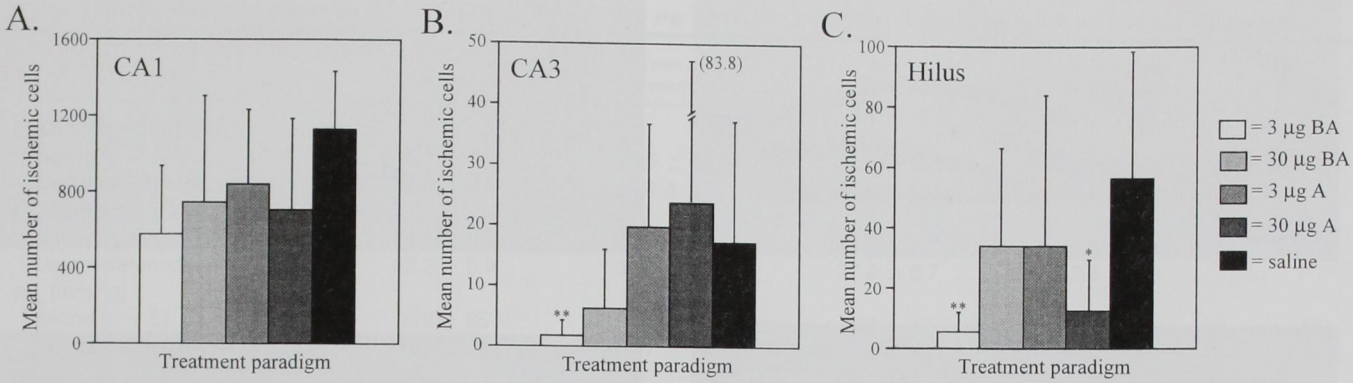

Fig. 1. Bar graphs show the effects of dexmedetomidine on pyramidal cells in hippocampal CA1 (*A*) and CA3 subfields (*B*) and on neurons in the dentate hilus (*C*) after 5 min of global forebrain ischemia in gerbils. The animals were perfused 7 days after the induction of ischemia. Cell counts were made from 4–8 silver-stained hippocampal sections through both hemispheres. The bars represent the mean ± SD of degenerated cells in each treatment paradigm. The number of animals in each group was 6–8. BA = the drug (3 μg/kg or 30 μg/kg) given 30 min before the induction of ischemia and thereafter at 3, 12, 24, and 48 h after the induction of ischemia; A = the drug given at 3, 12, 24, and 48 h after the induction of ischemia. Statistical significance: *$P < 0.05$; **$P < 0.01$ (compared with saline-treated ischemic gerbils, Kruskall-Wallis test followed by Mann-Whitney U-test).

1A and fig. 2B). Although intergroup analysis failed to demonstrate a difference among the treatment groups in the CA1 area ($P = 0.125$), dexmedetomidine at a dose of 3 μg/kg given before and continued after ischemia induction seemed to decrease cell damage to 571 ± 362 degenerated neurons/mm^2. The higher dose of dexmedetomidine (30 μg/kg) or dexmedetomidine given only after ischemia did not protect from neuronal damage.

Severity of Neuronal Damage in the CA3 Region

In the CA3 region, the saline-treated ischemic animals had an average of 17 ± 20 degenerated neurons/mm^2 (fig. 1B). Dexmedetomidine (3 μg/kg) given before and continued after the induction of ischemia reduced damage to 2 ± 3 degenerated neurons/mm^2 ($P < 0.01$). The 30 μg/kg dose given similarly did not reduce damage. In addition, neither dexmedetomidine dose given only after the induction of ischemia was effective in reducing cell damage in the CA3 region.

Severity of Neuronal Damage in the Hilus of the Dentate Gyrus

In the dentate hilus, the saline-treated ischemic animals had 52 ± 42 degenerated neurons/mm^2 (fig. 1C). The lower dose of dexmedetomidine (3 μg/kg) given before and continued after the ischemic period decreased the ischemic damage to 5 ± 7 degenerated neurons/mm^2 ($P < 0.01$). The dose of 30 μg/kg given only after the ischemia also provided statistically significant neuroprotection ($P < 0.05$). Interestingly, the group that received the 30 μg/kg dose before and dosing maintained after ischemia induction did not differ significantly from the saline-treated control animals.

Physiologic Variables in Ischemic Animals after Dexmedetomidine

Additional animals were included to study the effect of dexmedetomidine (3 μg/kg) on various physiologic variables. There were no differences in blood gases (p_{O2}, p_{CO2}), blood pH, or blood pressure between saline and dexmedetomidine groups (table 1). Blood glucose concentrations increased by 68–83% after dexmedetomidine administration (analysis of variance [ANOVA], $F(1,32) = 47.01$; $P < 0.001$). Skull temperature as measured from the temporalis muscle or rectal temperature did not differ between saline and dexmedetomidine group during the observation period. Rectal temperatures measured at 90 and 150 min after reperfusion did not differ between groups.

Discussion

The present study demonstrates that the α_2-adrenoceptor agonist, dexmedetomidine, has neuroprotective properties against delayed neuronal death in a transient global ischemia model in gerbils. Dexmedetomidine given subcutaneously at a dose of 3 μg/kg, but not at a dose of 30 μg/kg, significantly protected against

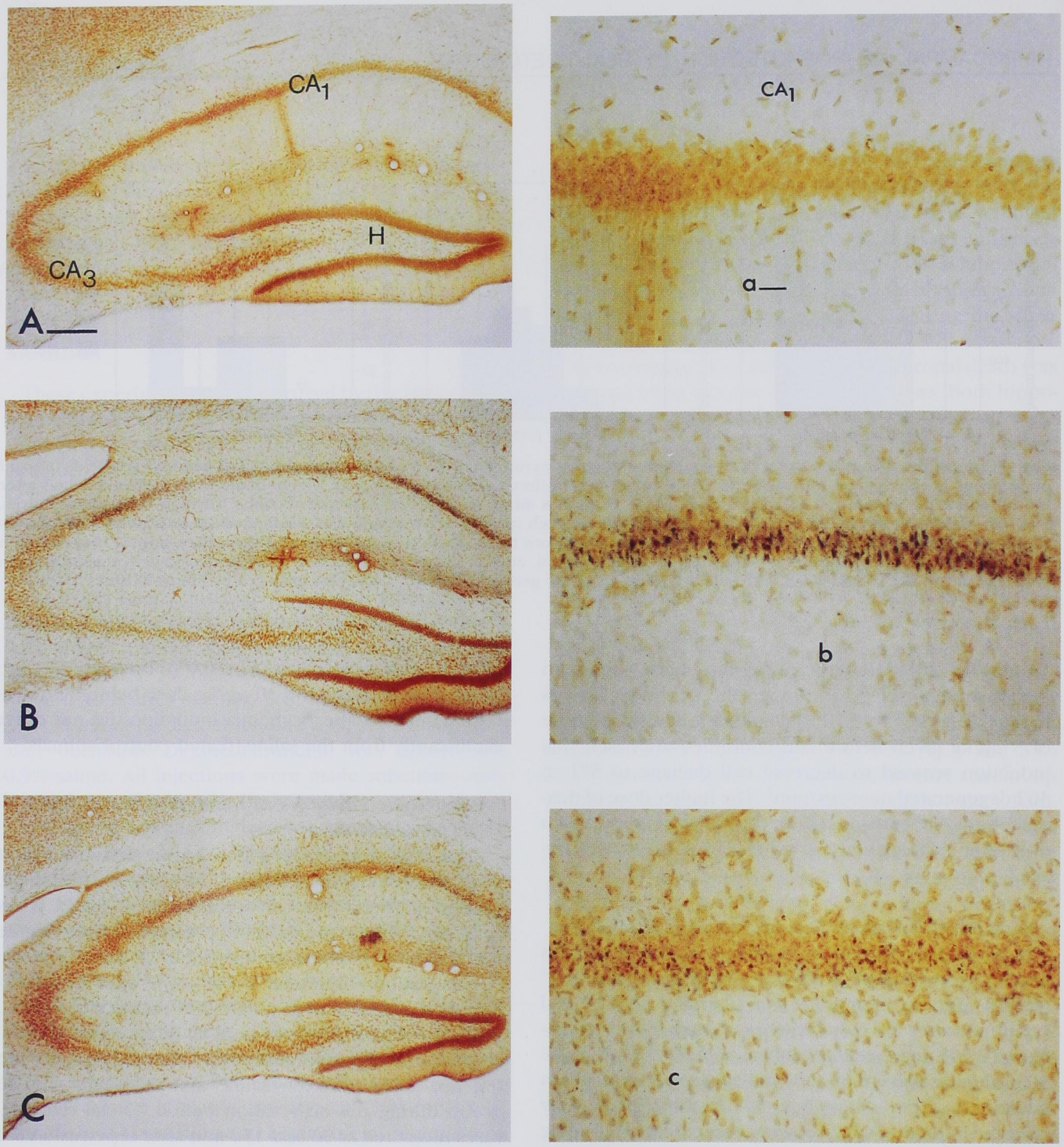

Fig. 2. Light micrographs of silver-impregnated hippocampal sections from a control gerbil (*A,a*), from an ischemic gerbil treated with 0.9% saline (*B,b*), and from an ischemic gerbil given 3 μg/kg dexmedetomidine before and subsequently after occlusion (*C,c*). At higher magnification, the pyramidal cell layer of the CA1 is shown in detail (*A–C*). *A,* After silver impregnation, the nucleus was stained yellow in normal healthy cells and no accumulation of silver granules can be observed. *B,* Occlusion of carotid arteries resulted in degeneration of the pyramidal cells in the CA1 and CA3, where dying cells can be seen as shrunken, argyrophilic profiles. In addition, an accumulation of glial cells is visible. *C,* Dexmedetomidine at a dose of 3 μg/kg given before and continued after the occlusion prevented cell damage in the CA3 area and in the dentate hilus. Note that although there were degenerating cells in the CA1 pyramidal cell layer, there was also a large number of healthy cells in this subfield. Scales: *A–C,* 250 nm; *a–c,* 25 μm.

Table 1. Physiologic Variables in Additional Gerbils Given 3 μg/kg of Dexmedetomidine 30 min before Carotid Occlusion

	Baseline	30 min after Treatment	During Occlusion	30 min after Reperfusion	*P*
Skull temperature (°C)					
Saline (n = 5)	35.8 ± 0.4	36.5 ± 0.2	36.1 ± 0.3	36.1 ± 0.4	NS
Dexmedetomidine (n = 5)	36.0 ± 0.5	36.4 ± 0.3	35.9 ± 0.4	36.2 ± 0.2	
p_{CO_2} (mmHg)					
Saline (n = 5)	34.8 ± 6.5	27.0 ± 6.1	26.2 ± 7.7	23.8 ± 8.5	NS
Dexmedetomidine (n = 5)	32.2 ± 5.9	33.0 ± 5.9	27.2 ± 6.7	25.2 ± 3.0	
p_{O_2} (mmHg)					
Saline (n = 5)	128 ± 25	151 ± 17	107 ± 47	156 ± 21	NS
Dexmedetomidine (n = 5)	132 ± 15	127 ± 18	114 ± 14	153 ± 15	
*p*H					
Saline (n = 5)	7.33 ± 0.03	7.32 ± 0.03	7.31 ± 0.04	7.29 ± 0.06	NS
Dexmedetomidine (n = 5)	7.35 ± 0.03	7.30 ± 0.04	7.29 ± 0.03	7.26 ± 0.07	
B-Glu (mM)					
Saline (n = 5)	4.9 ± 0.6	4.2 ± 0.4	4.9 ± 1.4	4.8 ± 0.6	<0.001
Dexmedetomidine (n = 5)	5.3 ± 0.7	7.7 ± 1.6*	8.4 ± 1.9†	8.1 ± 1.5*	
MABP (mmHg)					
Saline (n = 5)	69 ± 5	62 ± 14	86 ± 8	56 ± 13	NS
Dexmedetomidine (n = 5)	66 ± 7	54 ± 7	76 ± 18	55 ± 15	

NS = not significant.

Values are mean ± SD. The number of animals is in parentheses. Statistical significance between saline and dexmedetomidine groups (ANOVA).

* $P < 0.01$ *versus* saline (Student's *t* test).

† $P < 0.05$ *versus* saline (Student's *t* test).

neuronal death in hippocampal layer CA3 and in the hilus of the dentate gyrus. This neuroprotective effect against ischemia-induced neuronal death only occurred when dexmedetomidine was administered 30 min before induction of ischemia and when dosing continued for 48 h after reperfusion. The one exception to this was the dentate hilus, where the 30 μg/kg dose given after occlusion effectively salvaged neurons from degeneration.

A short period of transient cerebral ischemia produces selective pyramidal cell damage within days in the hippocampal CA1 region in Mongolian gerbils[12,13] and rats[14] (delayed neuronal death). Because this is a straightforward and uncomplicated procedure, the gerbil model has gained acceptance as a model of global ischemia, although because of their small size, blood pressure of gerbils and other physiologic variables are usually not controlled. In the present study, physiologic variables were monitored in ischemic animals after administration of dexmedetomidine (3 μg/kg) and indicate that protection from ischemic damage in the hippocampus occurred by a mechanism that is not related to changes in blood gases, blood pH, or brain temperature. In addition, because the blood pressure of the gerbils after 3 μg/kg of dexmedetomidine was similar to that of those in saline control group, the difference in ischemic outcome is not explained by better perfusion caused by hypertension. In contrast, increased blood glucose concentrations were observed after dexmedetomidine possibly associated with α_2-adrenergic inhibition of insulin release.[15] This is unlikely, however, to explain neuroprotection by dexmedetomidine because hyperglycemia (> 10 mM) exacerbates ischemic damage in global ischemia models rather than provides protection.[16]

There is a massive increase in extracellular norepinephrine in the hippocampus[1] and in dopamine in the striatum[2] during ischemic insult. The neuroprotective mechanism of dexmedetomidine in the prevention of cell death could involve these catecholamines. Dexmedetomidine activates presynaptic α_2-adrenoceptors and could thereby reduce ischemic damage by inhibiting catecholamine release.[17,18] Numerous studies have demonstrated that activation of α_2-adrenergic receptors can protect against ischemic injury. For example, Hoffman *et al.*[5] found that clonidine ameliorates the neurologic consequences of incomplete ischemia in rats. They also reported similar but more powerful protective effects with dexmedetomidine.[6] Maier *et al.*[7] described neuroprotection with dexmedetomidine in the rabbit model of focal cerebral ischemia. The effect on

the neurologic consequences in the rat model, however, was scored every 24 h for 3 days.[6] Ischemic damage in the rabbit model was evaluated 6 h after ischemia.[7] Thus, dexmedetomidine clearly provides neuroprotection against immediate necrotic cell death and delays the development of infarction in incomplete ischemia. In the present study, the neuropathologic evaluation was made 7 days after the induction of ischemia, when delayed neuronal death has already occurred in hippocampal neurons. Therefore, our results extend these earlier studies by demonstrating neuroprotective effects of dexmedetomidine against delayed neuronal death in the hippocampus in a global ischemia model.

The fact that the low dose of dexemedetomidine (3 μg/kg) was more effective than the high dose agrees with a previous study, which showed a U-shaped dose-response curve against kainic acid-induced convulsions: dexmedetomidine at doses of 2.5 and 5 μg/kg was more effective than a 30 μg/kg dose.[8] Similarly, higher doses of clonidine reverse the beneficial effects of lower doses in pentylenetrazole-induced epileptic seizures.[19] The effect of the high dose of clonidine was abolished by the α_1-antagonist prazosin,[18] pointing to the involvement of nonspecific α_1 activation with clonidine. In an incomplete ischemia model, clonidine improved the neurologic consequences at a low dose (10 μg/kg, intravenous), but produced prolonged hypertension at a higher dose (50 μg/kg, intravenous), which caused four of five rats to die from pulmonary edema, probably as a result of α_1-agonist.[5] Compared with clonidine, dexmedetomidine is clearly a more selective and potent α_2-agonist.[20] Thus it is not surprising that low doses of dexmedetomidine have a more potent neuroprotective effect against cerebral ischemia and have a wider therapeutic window than the less selective α_2-agonist, clonidine.

Differences in the number of surviving neurons between the various hippocampal subfields after dexmedetomidine treatment may be related to differences in their anatomic connections. The relative distribution and terminal fields of noradrenergic fibers within the different subfields of the hippocampus could explain the regional differences seen in the present study. The noradrenergic afferents arising from the locus ceruleus form a dense network within the hilus of the dentate gyrus and in the stratum lucidum of the CA3 area.[21] However, the noradrenergic input to the CA1 subfield is less extensive and terminates largely in the stratum lacunosum moleculare. Therefore, it is likely that any therapeutic treatment that targets noradrenergic systems would be more potent in the hilus and in the CA3 subfield than in the CA1 region of the hippocampus.

In summary, dexmedetomidine, an α_2-adrenoceptor agonist, prevents delayed neuronal death in the CA3 area and dentate hilus in the gerbil hippocampus when given before, and treatment continued after the induction of ischemia. Low doses of dexmedetomidine (3 μg/kg) provide potent neuroprotective effects against cerebral ischemia.

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