

# Effect of the $\alpha_2$ -Agonist Dexmedetomidine on Cerebral Neurotransmitter Concentrations during Cerebral Ischemia in Rats

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**Background:** This study investigates whether neuroprotection seen with dexmedetomidine is associated with suppression of peripheral or central sympathetic tone.

**Methods:** Thirty fasted male Sprague-Dawley rats were intubated and ventilated with isoflurane and  $N_2O/O_2$  (fraction of inspired oxygen = 0.33). Catheters were inserted into the right femoral artery and vein and into the right jugular vein. Cerebral blood flow was measured using laser Doppler flowmetry. Bilateral microdialysis probes were placed into the cortex and the dorsal hippocampus. At the end of preparation, the administration of isoflurane was replaced by fentanyl (bolus: 10  $\mu\text{g}/\text{kg}$ ; infusion: 25  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). Animals were randomly assigned to one of the following groups: group 1 (n = 10): control animals; group 2 (n = 10): 100  $\mu\text{g}/\text{kg}$  dexmedetomidine administered intraperitoneally 30 min before ischemia; group 3 (n = 10): sham-operated rats. Ischemia (30 min) was produced by unilateral carotid artery occlusion plus hemorrhagic hypotension to a mean arterial blood pressure of 30–35 mmHg to reduce ipsilateral cerebral blood flow by 70%. Pericranial temperature, arterial blood gases, and pH were maintained constant. Cerebral catecholamine and glutamate concentrations and plasma catecholamine concentrations were analyzed using high-performance liquid chromatography.

**Results:** During ischemia, dexmedetomidine suppressed circulating norepinephrine concentrations by 95% compared with control animals. In contrast, brain norepinephrine and glutamate concentrations were increased irrespective of dexmedetomidine infusion before ischemia.

**Conclusions:** The current data show that the increase of circulating catecholamine concentrations during cerebral ischemia was suppressed with dexmedetomidine. In contrast, dexmedetomidine does not suppress elevation in brain norepinephrine and glutamate concentration associated with cerebral ischemia. This suggests that the neuroprotective effects of dexmedetomidine are not related to inhibition of presynaptic norepinephrine or glutamate release in the brain.

STUDIES in rats subjected to incomplete cerebral ischemia have shown that the preischemic administration of agents suppressing the ischemia-induced increase of pe-

ripheral catecholamine concentrations, e.g., ganglionic blocking agents,  $\alpha_2$ -agonists, and anesthetics improve neurologic outcome and reduce histopathologic damage.<sup>1-3</sup> Although these data generally suggest that neuroprotection is related to a reduced sympathetic tone, it is unclear whether this is a function of suppressed plasma catecholamine or brain catecholamine concentrations. Therefore, this study investigates the effect of the  $\alpha_2$ -agonist dexmedetomidine on circulating and cerebral catecholamine concentrations during incomplete cerebral ischemia in rats.

## Materials and Methods

### Preparation

After obtaining approval from the institutional animal care committee (Government of Bavaria), 30 male Sprague-Dawley rats (weighing 300–420 g) were anesthetized in a bell jar saturated with isoflurane. Rats were tracheally intubated and mechanically ventilated with 1.5 vol% isoflurane in nitrous oxide and oxygen (fraction of inspired oxygen = 0.33). Catheters were inserted into the right femoral artery and vein and into the right jugular vein for blood withdrawal, administration of drugs, and blood sampling. A loose ligature was placed around the right common carotid artery for later clamping. The rats were then placed in a stereotactic "U"-frame with nonpenetrating ear bars (Model 962; David Kopf Instruments, Tujunga, CA). After incision of the skull, penetrating burr holes (1 mm in diameter) were drilled into the cranium 4.2 mm posterior and 2.5 mm lateral of the bregma over both hemispheres according to the stereotaxis coordinates of the rat brain.<sup>4</sup> The tip of the drill was continuously flushed with saline to avoid thermal injury. After incision of the dura, the microdialysis probes (CMA12, 4.0 mm length, 0.5 mm diameter; CMA/Microdialysis AB, Solna, Sweden) were carefully inserted into the cortex and the dorsal hippocampus. The probes were then fixed using a zinc polycarboxylate cement (Poly-F Plus; Dentsply, York, PA) and perfused with Ringer's solution (Boehringer Ingelheim Delta Pharm GmbH, Pfullingen, Germany; 147 mM  $\text{Na}^+$ , 2.25 mM  $\text{Ca}^{2+}$ , 4 mM  $\text{K}^+$ , 155.5 mM  $\text{Cl}^-$ ) at a rate of 1.0  $\mu\text{l}/\text{min}$ . Small collector vials were filled with 10  $\mu\text{l}$  of 0.5 M perchloric acid to stabilize catecholamines and placed in a refrigerated fraction collector (CMA 170; CMA/Microdialysis AB). Ninety minutes after implantation of the microdialysis probes, sample fractions of 30 min were

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collected and subsequently stored at  $-70^{\circ}\text{C}$ . Non-penetrating burr holes were drilled 0.5 mm anterior and 1 mm lateral of the bregma into the cranium over both hemispheres for continuous measurement of erythrocyte flow velocity using a laser Doppler flowmeter (Peri-Flux System 4001; Perimed, Järfälla, Sweden). Local cerebral blood flow (CBF) was continuously measured and expressed in arbitrary perfusion units, which were sampled over 0.3 s. The laser Doppler flow probes (Probe 403; Perimed) were placed over both hemispheres and fixed using the stereotactic frame. Care was taken to place the probes over a tissue area devoid of large blood vessel visible through the thinned bone, and correct placement of laser Doppler probes was confirmed by transient hypoventilation. Pericranial temperature was measured with a 22-gauge stainless steel needle thermistor (model 73A, Yellow Springs Instrument Co., YSI Temperature Controller; Yellow Springs, OH) placed beneath the right temporal muscle and was maintained constant at  $37.5^{\circ}\text{C}$  throughout the experiment by a servomechanism using an overhead heating lamp and a heating pad.

#### *Cerebral Ischemia*

At the end of the preparation, all surgical incisions were infiltrated with 0.5% bupivacaine, and the administration of isoflurane was replaced by fentanyl (bolus:  $10\ \mu\text{g}/\text{kg}$ ; infusion:  $25\ \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) while ventilation was continued with nitrous oxide and oxygen (fraction of inspired oxygen = 0.33). Mechanical ventilation was adjusted to maintain arterial carbon dioxide tension at 38–42 mmHg. The nonpenetrating ear bars of the stereotactic frame were released. During cerebral ischemia, arterial pH was maintained at physiologic levels by intravenous infusion of bicarbonate. Vecuronium was given as a continuous infusion ( $0.1\ \text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) to maintain neuromuscular blockade. Animals were randomly assigned to one of the following treatment groups. Group 1 ( $n = 10$ ) represents the control group, with no additional treatment. Animals in group 2 ( $n = 10$ ) received  $100\ \mu\text{g}/\text{kg}$  dexmedetomidine administered intraperitoneally 30 min before the onset of ischemia. Animals in group 3 ( $n = 10$ ) were sham-operated (*i.e.*, complete instrumentation, no ischemia) with no additional treatment. After an equilibration period of 2 h, cerebral ischemia was induced by hemorrhagic hypotension and clip occlusion of the right common carotid artery. Mean arterial blood pressure was maintained within the range of 30–35 mmHg to reduce CBF in the ischemic hemisphere by 70%. After 30 min of cerebral ischemia, the clip was released and the shed blood was reperfused over 15 min. Arterial blood gases and plasma glucose concentrations were analyzed at baseline, 30 min during ischemia, 15 min after ischemia (reperfusion), and 90 min after ischemia (recovery). Blood samples for measurement of plasma catecholamine concentrations

were collected at baseline, at the end of ischemia, and 90 min after cerebral ischemia. The blood samples were centrifuged at  $4^{\circ}\text{C}$  for 10 min, and the plasma was stored at  $-70^{\circ}\text{C}$ . Brains were removed 4 h after ischemia and placed in tissue-freezing medium (Jung Tissue Freezing Medium; Leica Instruments GmbH, Nussloch, Germany), frozen in methylbutan on dry ice, and stored at  $-70^{\circ}\text{C}$ . The correct position of the microdialysis probes was verified, and histologic damage caused by the microdialysis probes was evaluated in  $7\text{-}\mu\text{m}$  brain slices, stained with hematoxylin and eosin, and animals were excluded from the study in case of major bleeding.

#### *High-performance Liquid Chromatography*

Samples for plasma norepinephrine, epinephrine, and dopamine analyses were processed using the ClinRep test kit for high-performance liquid chromatography analysis of catecholamines (Recipe Chemicals and Instruments GmbH, Munich, Germany). Plasma samples (0.25 ml) were mixed with  $50\ \mu\text{l}$  dehydroxybenzylamine (internal standard). The mixture was passed through the sample preparation column filled with aluminum oxide. The remaining particles of plasma proteins were washed out. Norepinephrine, epinephrine, and dopamine were eluted by adding  $120\ \mu\text{l}$  elution buffer. No pretreatment was necessary to analyze cerebral dialysate for extracellular norepinephrine and dopamine concentration. Samples were placed into a cooled autosampler (AS2000A; Merck Hitachi, Darmstadt, Germany). Sixty microliters of the plasma elute or  $10\ \mu\text{l}$  of the cerebral dialysate was injected into the high-performance liquid chromatography circulation system (mobile phase ClinRep; Recipe Chemicals and Instruments GmbH) for electrochemical detection (0.5 V potential). The elute and the cerebral dialysate was passed over the analytical column (ClinRep; Recipe Chemicals and Instruments GmbH) with a flow of 1.0 ml/min to the electrochemical detector (Waters 460; Waters, Milford, MA). The whole system was controlled and data were stored by the high-performance liquid chromatography systems manager software (Merck Hitachi, Darmstadt, Germany). The system was calibrated with a catecholamine standard (ClinRep; Recipe Chemicals and Instruments GmbH).

For analysis of the cerebral glutamate and aspartate concentration, the microdialysis samples were placed into an autoinjector (Gina 50 Probengeber; Dionex, Germering, Germany). Ortho-phthaldialdehyd ( $20\ \mu\text{l}$ ), diluted with boracic buffer (1:10), was mixed with  $10\ \mu\text{l}$  cerebral dialysate for derivatization. This mixture was injected into the high-performance liquid chromatography circulation system (Pumpensystem M480; Dionex) for fluorometric detection and was passed over the analytical column (Grom-Sil OAA-2,  $250 \times 4\ \text{mm}$ ; Grom, Herrenberg, Germany) with a flow of 0.8 ml/min to the fluorescence detector (Fluoreszenzdetektor RF-2000; Dionex; wavelength: extinction 280 nm–emission 475 nm).

The mobile phase A consisted of 23 mm sodium acetate adjusted with HCl to a pH of 6.0. The mobile phase B consisted of 600 ml methanol and 50 ml acetonitril. The gradient was changed as follows: after beginning, 100% phase A-0% phase B; after 29.8 min, 79% phase A-21% phase B; after 32.5 min, 47% phase A-53% phase B; after 34 min, 0% phase A-100% phase B. The analysis refers to a four-point standard curve of a custom-made standard.

#### Statistical Analysis

Data (mean  $\pm$  SD) of four consecutive time points were evaluated: before hemorrhagic hypotension (baseline), at 30 min of cerebral ischemia (ischemia), 15 min after cerebral ischemia upon reinfusion of the withdrawn blood (reperfusion), and 90 min after cerebral ischemia (recovery).

Data of variables that are available from two hemispheres and at each time point (CBF and cerebral neurotransmitter concentrations) were subjected to a two-way repeated-measurements analysis of variance with the two within-group factors (time<sup>2</sup> and hemisphere), the between-groups factor (group), and all possible interaction terms (time<sup>2</sup>  $\times$  hemisphere; time<sup>2</sup>  $\times$  group; hemisphere  $\times$  group; time<sup>2</sup>  $\times$  hemisphere  $\times$  group). To evaluate the following three hypotheses, respective *post hoc* analyses were performed in a stepwise manner, if the respective interaction terms of this global test were significant ( $P < 0.05$ ).

Differences between groups during ischemia: Once "time<sup>2</sup>  $\times$  group" or "time<sup>2</sup>  $\times$  hemisphere  $\times$  group" proved to be significant in the global test ( $P < 0.05$ ), another two-way repeated-measurements analysis of variance was performed with the within-groups factor (time<sup>2</sup>), the between-groups factor (group), and their interaction term separately for each hemisphere. Once "time<sup>2</sup>  $\times$  group" proved to be significant ( $P < 0.05$ ), values during ischemia of the respective hemisphere were analyzed with a factorial analysis of variance, with group as independent factor and, if significant ( $P < 0.05$ ), followed by three unpaired *t* tests ( $P < 0.05/3 = 0.016$  for multiple comparison correction).

Differences between baseline and ischemia within each group and each hemisphere: Once "time<sup>2</sup>  $\times$  group" or "time<sup>2</sup>  $\times$  hemisphere  $\times$  group" proved to be significant in the global test ( $P < 0.05$ ), another two-way repeated-measurements analysis of variance was performed with the within-groups factor (time<sup>2</sup>), the between-groups factor (group), and their interaction term separately for each hemisphere. Once "time<sup>2</sup>  $\times$  group" proved to be significant ( $P < 0.05$ ), values at baseline and during ischemia were compared using paired *t* tests in each group and each hemisphere ( $P < 0.05/6 = 0.008$  for multiple comparison correction).

Differences between hemispheres during ischemia within each group: Once "time<sup>2</sup>  $\times$  hemisphere" or "time<sup>2</sup>  $\times$  hemisphere  $\times$  group" proved to be significant

in the global test ( $P < 0.05$ ), another two-way repeated-measurement analysis of variance was performed with the within-groups factor (hemisphere), the between-groups factor (group), and their interaction term. Once "hemisphere  $\times$  group" proved to be significant ( $P < 0.05$ ), values during ischemia of the ipsilateral and the contralateral side were compared using paired *t* tests separately in each group ( $P < 0.05/3 = 0.016$  for multiple comparison correction).

Data of variables that are available at each time point (physiologic variables and plasma catecholamine concentrations) were subjected to a two-way repeated-measurements analysis of variance with the within-groups factors (time<sup>2</sup>), the between-groups factor (group), and their interaction term (time<sup>2</sup>  $\times$  group). To evaluate the following two hypotheses, respective *post hoc* analyses were performed in a stepwise manner if "time<sup>2</sup>  $\times$  group" was significant ( $P < 0.05$ ).

Differences between groups during ischemia: Values during ischemia were analyzed with a factorial analysis of variance, with "group" as independent factor and, if significant ( $P < 0.05$ ), followed by three unpaired *t* tests ( $P < 0.05/3 = 0.016$  for multiple comparison correction).

Differences between baseline and ischemia within each group: Values at baseline and during ischemia were compared using paired *t* tests in each group ( $P < 0.05/3 = 0.016$  for multiple comparison correction).

All variables are presented as mean  $\pm$  SD. Statistical analyses were performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL).

## Results

Table 1 shows the physiologic variables. According to the study protocol, mean arterial blood pressure was decreased in control animals (group 1) and in dexmedetomidine-treated animals (group 2) during cerebral ischemia compared with sham-operated animals (group 3). There were no differences for arterial oxygen and carbon dioxide tensions within (baseline *vs.* ischemia) and between groups. In control animals and animals treated with dexmedetomidine, plasma glucose concentration decreased during ischemia compared with baseline. During ischemia, plasma glucose concentration was higher with sham-operated animals compared with control animals and dexmedetomidine-treated animals.

Table 2 shows the results of the statistical analysis testing the differences between both hemispheres. As expected, the decrease of cortical CBF (laser Doppler flowmetry) was more severe in the ischemic hemisphere compared with the nonischemic hemisphere in control animals (group 1) and animals treated with dexmedetomidine (group 2). Cerebral norepinephrine and glutamate concentration was significantly higher in the ischemic hemisphere compared with the nonisch-

**Table 1. Mean Arterial Blood Pressure (MAP), Arterial Blood Gas Tensions, and Plasma Glucose Concentration during Baseline, Ischemia, Reperfusion, and Recovery (90 min after Cerebral Ischemia)**

	Group	Time				Significance	
		Baseline	Ischemia	Reperfusion	Recovery	Time × Group	Baseline vs. Ischemia
MAP (mmHg)	Control	118 ± 8	33 ± 2†	127 ± 20	98 ± 16	<i>P</i> < 0.001	<i>P</i> < 0.001
	Dexmedetomidine	133 ± 8	31 ± 1†	128 ± 21	70 ± 6		<i>P</i> < 0.001
	Sham operated	120 ± 9	122 ± 11*	123 ± 10	125 ± 10		ns
Pao <sub>2</sub> (mmHg)	Control	135 ± 25	140 ± 16	145 ± 35	137 ± 32	ns	na
	Dexmedetomidine	116 ± 15	122 ± 6	110 ± 21	100 ± 4		na
	Sham operated	108 ± 16	95 ± 22	103 ± 10	109 ± 9		na
Paco <sub>2</sub> (mmHg)	Control	40 ± 2	37 ± 4	40 ± 3	39 ± 4	ns	na
	Dexmedetomidine	42 ± 1	41 ± 5	43 ± 3	41 ± 4		na
	Sham operated	40 ± 4	41 ± 3	40 ± 4	42 ± 3		na
Glucose (mg/dl)	Control	55 ± 9	44 ± 5†	50 ± 7	71 ± 9	<i>P</i> < 0.001	<i>P</i> < 0.05
	Dexmedetomidine	77 ± 6	50 ± 6†	56 ± 7	69 ± 10		<i>P</i> < 0.001
	Sham operated	57 ± 6	60 ± 5*	60 ± 11	61 ± 11		ns

Mean ± SD; \* *P* < 0.05 compared with control group during ischemia; † *P* < 0.05 compared with sham operated animals during ischemia. ns = not significant; na = not applicable.

emic hemisphere in control animals (group 1) and animals receiving dexmedetomidine (group 2).

Figure 1 shows the cortical CBF in the ischemic (fig. 1A) and the nonischemic (fig. 1B) hemisphere before, during, and after cerebral ischemia. CBF was measured in arbitrary perfusion units. According to the study protocol, during cerebral ischemia CBF was decreased in control animals (group 1) and in animals treated with the dexmedetomidine (group 2) but not sham-operated animals (group 3). This decrease in CBF was also observed in the nonischemic hemisphere.

Figure 2A shows the plasma norepinephrine concentration before, during, and after cerebral ischemia. In

**Table 2. Statistical Analysis of Differences between Both Hemispheres for Local Cerebral Blood Flow and Neurotransmitter Concentrations in the Dialysate**

Group	Significance	Significance	
		Hemisphere × Group	Hemisphere
Local Cerebral Blood flow	Control		<i>P</i> < 0.05
	Dexmedetomidine	<i>P</i> < 0.05	<i>P</i> < 0.05
	Sham-operated		ns
Cerebral Norepinephrine Concentration	Control		<i>P</i> < 0.001
	Dexmedetomidine	<i>P</i> < 0.05	<i>P</i> < 0.001
	Sham-operated		ns
Cerebral Dopamine Concentration	Control		na
	Dexmedetomidine	ns	na
	Sham-operated		na
Cerebral Glutamate Concentration	Control		<i>P</i> < 0.05
	Dexmedetomidine	<i>P</i> < 0.05	<i>P</i> < 0.05
	Sham-operated		ns
Cerebral Aspartate Concentration	Control		na
	Dexmedetomidine	ns	na
	Sham-operated		na

time<sup>2</sup> × hemisphere or time<sup>2</sup> × hemisphere × group was significant in the global test (*P* < 0.05) for all tested variables.

ns = not significant; na = not applicable.

control animals, plasma norepinephrine concentration was increased during cerebral ischemia compared with baseline. In contrast, treatment with dexmedetomidine decreased plasma norepinephrine concentration compared with control animals during cerebral ischemia. Plasma epinephrine concentration was also decreased with dexmedetomidine compared with control animals (fig. 2B) during cerebral ischemia, whereas plasma dopamine concentrations did not change in any group (data not shown).

Figure 3 shows norepinephrine (fig. 3A) and glutamate concentrations (fig. 3B) in the cerebral cortex and hippocampus before, during, and after cerebral ischemia in the ischemic and nonischemic hemisphere. Compared with baseline, the cerebral norepinephrine and glutamate concentrations were increased during ischemia in the ipsilateral but not in the contralateral hemisphere in control animals. Dexmedetomidine did not affect the ischemia-induced elevation of cerebral norepinephrine and glutamate concentrations. Likewise, brain aspartate was elevated in the control and dexmedetomidine-treated animals (data not shown). The cerebral dopamine concentrations did not change over time in any group (data not shown).

## Discussion

Consistent with previous investigations using this animal model, the current results show that cerebral ischemia (induced by hemorrhagic hypotension and clipping of the right common carotid artery) causes an increase in plasma norepinephrine and epinephrine but not in dopamine concentrations. This elevation in peripheral sympathetic tone did not occur in the presence of the  $\alpha_2$ -agonist dexmedetomidine. Likewise, cerebral norepinephrine, glutamate, and aspartate, but not dopamine concentrations were increased during ischemia. How-

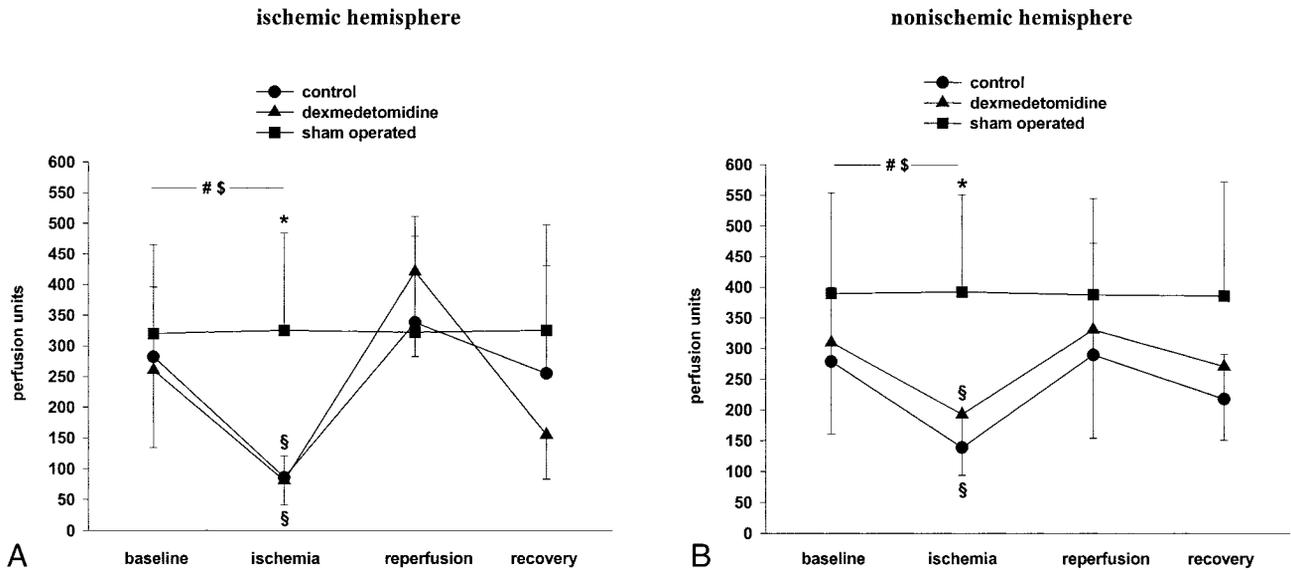


Fig. 1. Cortical cerebral blood flow (CBF) in the ischemic (A) and nonischemic (B) hemisphere before, during, and after cerebral ischemia. \* $P < 0.05$  compared with control group during ischemia; § $P < 0.05$  compared with sham-operated animals during ischemia; # $P < 0.05$ , baseline versus ischemia within control group; \$ $P < 0.05$ , baseline versus ischemia within dexmedetomidine-anesthetized animals.

ever, the ischemia-induced increase of cerebral norepinephrine and glutamate could not be suppressed by administration of dexmedetomidine. This suggests that neuroprotection observed with dexmedetomidine<sup>1,5</sup> is not related to presynaptic inhibition of catecholamine and glutamate release in the brain.

Cerebral ischemia is associated with an increase in circulating and extracellular brain catecholamine concentrations.<sup>1,2,6,7</sup> Interventions to reduce sympathetic tone (e.g., administration of ganglionic blocking agents, anesthetics, or  $\alpha_2$  agonists) improved neurologic outcome. Neuroprotection observed with reduced sympathetic activity may be related to several mechanisms. (1) Catecholamines stimulate cerebral metabolic rate for ox-

xygen,<sup>8,9</sup> an effect that further imbalances the ratio between cerebral oxygen demand and oxygen supply. (2) High catecholamine concentrations also increase the sensitivity of pyramidal neurons to excitatory neurotransmitters such as glutamate,<sup>10</sup> which results in elevated intracellular  $Ca^{2+}$  concentrations with consecutive activation of intracellular catabolic enzymes (excitotoxicity). (3) Catecholamines may exert a direct neurotoxic effect when exposed to neuronal tissue in excessive concentrations.<sup>11</sup> (4) It is also possible that increased sympathetic activity decreases perfusion in the ischemic penumbra because ischemic hypotension would produce greater decrease in CBF in sympathetically intact rats (i.e., sympathetic vasoconstriction) as compared

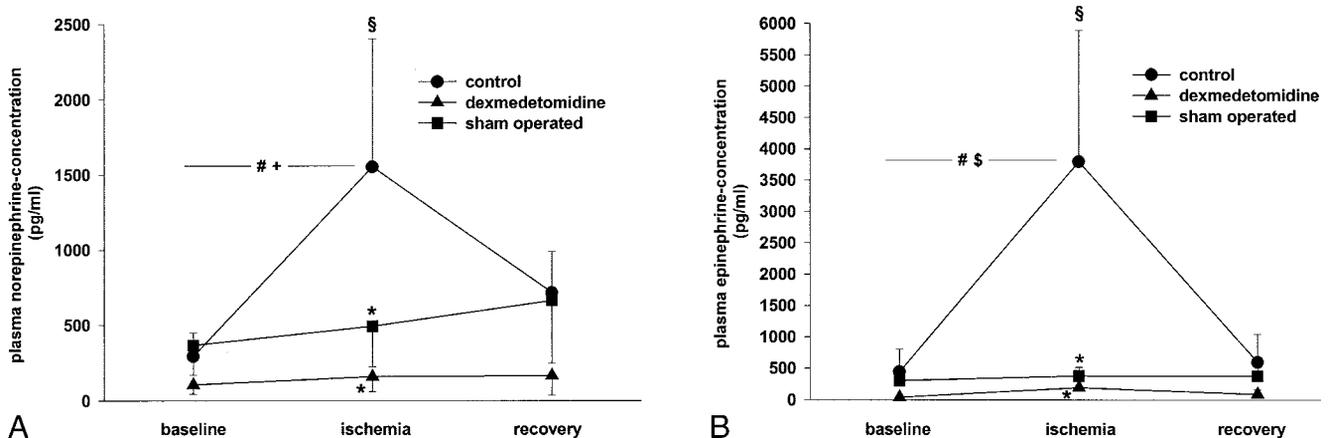


Fig. 2. Plasma norepinephrine (A) and epinephrine (B) concentration before, during, and after cerebral ischemia. Dexmedetomidine suppressed the plasma norepinephrine and epinephrine concentration during cerebral ischemia. \* $P < 0.05$  compared with control group during ischemia; § $P < 0.05$  compared with sham-operated animals during ischemia; # $P < 0.05$ , baseline versus ischemia within control group; \$ $P < 0.05$ , baseline versus ischemia within dexmedetomidine-treated animals; + $P < 0.05$ , baseline versus ischemia within sham-operated animals.

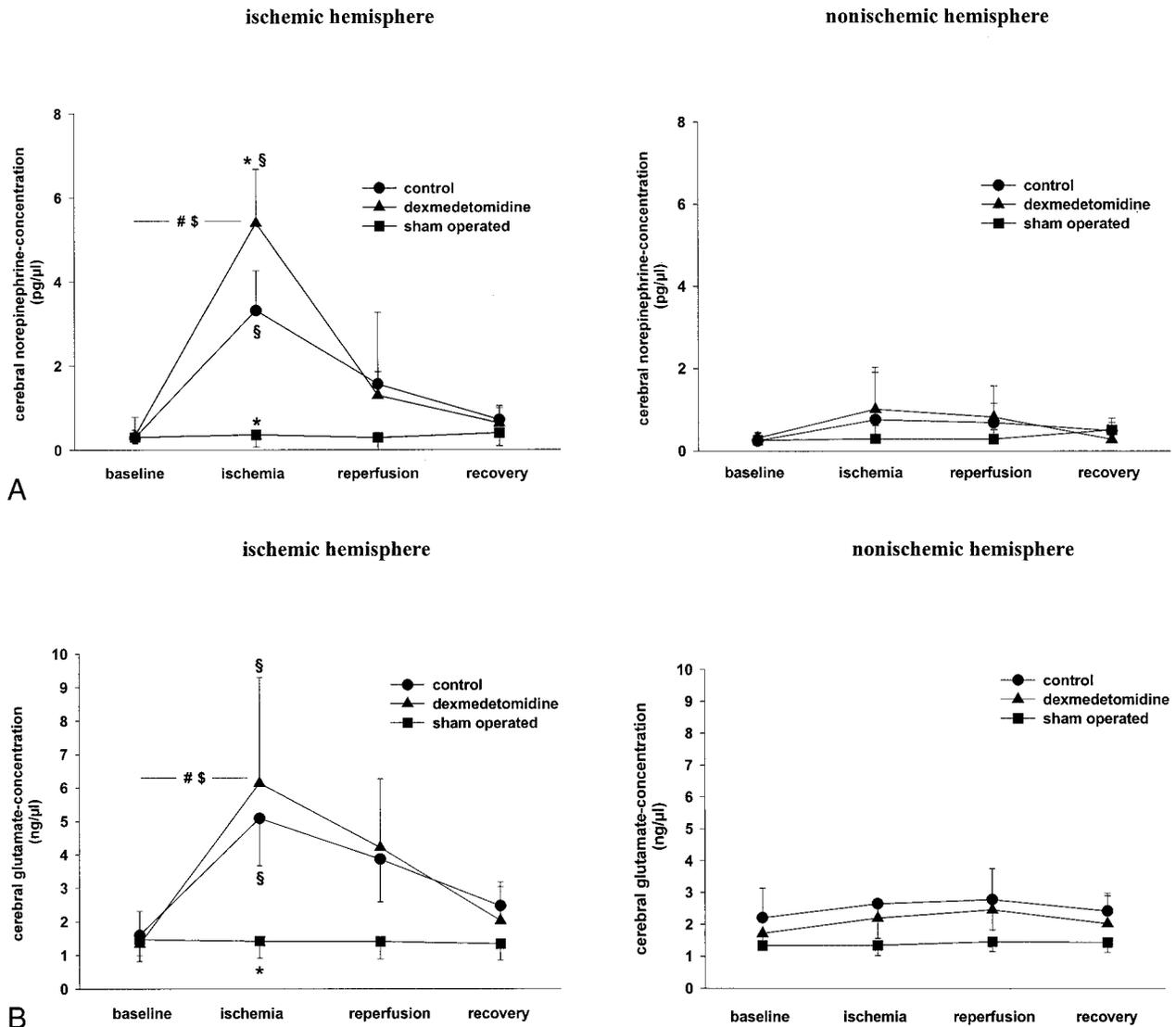


Fig. 3. Cerebral norepinephrine concentration (A) and glutamate concentration (B) in the ischemic and nonischemic hemisphere before, during, and after cerebral ischemia. Both cerebral norepinephrine and glutamate concentrations increased in the ischemic hemisphere during ischemia irrespective of treatment. \* $P < 0.05$  compared with control group during ischemia; § $P < 0.05$  compared with sham-operated animals during ischemia; # $P < 0.05$ , baseline versus ischemia within control group; § $P < 0.05$ , baseline versus ischemia within dexmedetomidine-anesthetized animals.

with animals subjected to ganglionic blockade.<sup>12</sup> Therefore, suppression of catecholamine concentrations may be neuroprotective by economizing the ratio between cerebral oxygen demand and oxygen supply, reducing excitotoxicity, reducing toxic effects, or improving perfusion in the ischemic penumbra.

We expected a suppression of the ischemia-induced increase in cerebral catecholamine concentration in the presence of dexmedetomidine. This assumption was based on the current understanding of the pharmacologic mechanisms of  $\alpha_2$ -adrenergic agonists and a study in rabbits subjected to global ischemia in which norepinephrine concentration in the striatum was less with dexmedetomidine compared with control animals.<sup>13</sup> However, dexmedetomidine did not attenuate the stress response to incomplete hemispheric ischemia (group 2)

in the current study. It is possible that the different results between the two studies are related to differences in regions of interest (*i.e.*, the striatum represents dopaminergic territory) or the use of different ischemia models. The data also suggest that presynaptic stimulation of  $\alpha_2$ -adrenergic receptors with consecutive decrease in extracellular catecholamine concentrations occurs to a lesser extent than previously believed.

The excitatory amino acid neurotransmitter glutamate triggers neuronal death when released in excessive concentrations during cerebral ischemia.<sup>14</sup> In hippocampal rat brain slices, dexmedetomidine suppressed the excessive glutamate release during potassium chloride-evoked depolarization or hypoxic stress.<sup>15</sup> It was therefore expected that dexmedetomidine would decrease the ischemia-induced glutamate release in the current

study. However, during cerebral ischemia, the elevation of extracellular brain glutamate concentrations was similar between rats treated with dexmedetomidine compared with control animals. Consistently, dexmedetomidine did not decrease the hippocampal glutamate concentration in a model of global cerebral ischemia in rabbits.<sup>16</sup> These data suggest that the neuroprotective potential of dexmedetomidine is not related to changes in extracellular glutamate concentrations. An *in vitro* study in cultured astrocytes has shown that dexmedetomidine activates the oxidative metabolism of glutamine, a precursor of the neurotoxic glutamate, and thereby reduces the glutamate availability.<sup>17</sup> However, our results did not demonstrate a reduction in cerebral glutamate concentration, an effect that would have been expected from the mechanism of glutamine disposal suggested by Huang *et al.*,<sup>17</sup> and, therefore, this mechanism is not supposed to be a major factor of brain protection with dexmedetomidine.

Experiments using isolated canine stellate ganglia have shown that dexmedetomidine inhibited the synaptic conduction by decreasing the postsynaptic response to a late, slow excitatory presynaptic stimulation through an  $\alpha_2$ -receptor-mediated process.<sup>18</sup> In addition, studies in isolated primary cortical neurons (*i.e.*, tissue deprived from glial supporting layers and presynaptic receptors) demonstrate neuroprotection with dexmedetomidine, an effect that was reversible by the  $\alpha_2$ -receptor antagonist yohimbine.<sup>19</sup> It is therefore possible that dexmedetomidine is neuroprotective by postsynaptic action with consecutive reduction in  $Ca^{2+}$  currents.

In the current model of incomplete cerebral ischemia in which decreased sympathetic tone was always associated with improved outcome,<sup>1-3</sup> plasma but not extracellular brain catecholamine concentrations were suppressed. Similarly, intraischemic plasma catecholamine concentrations were lower with the neuroprotective anesthetics isoflurane or ketamine compared with control rats anesthetized with fentanyl-nitrous oxide that were subjected to incomplete or near-complete forebrain ischemia,<sup>20</sup> whereas brain catecholamine concentrations were elevated with any background anesthetic technique. Consistently, in rats in which brain norepinephrine stores were depleted 7 days before forebrain ischemia, no differences in plasma catecholamine concentrations and histopathologic damage were evident when compared with control animals, despite significant differences in cerebral norepinephrine concentrations.<sup>21</sup> These results support the view that circulating catecholamines rather than cerebral catecholamine concentrations mediate neuroprotection after cerebral ischemia.

The purpose of the current study was to investigate whether neuroprotection observed with dexmedetomidine in previous investigations<sup>1</sup> using the same ischemia model involves changes of cerebral neurotransmitter concentrations. To enable comparison between the cur-

rent and previous investigations, we repeated the study protocol with respect to the background anesthetic technique (fentanyl-nitrous oxide) and the dosage of dexmedetomidine. The use of this background anesthetic technique has the advantage of preserved cerebrovascular autoregulation in rats,<sup>22</sup> but concern was expressed as to the adequacy of analgesia and sedation. However, there is strong evidence that fentanyl-nitrous oxide provides adequate anesthesia in nonstressed animals, as rats given fentanyl-nitrous oxide demonstrate an identical electrophysiologic pattern (electroencephalogram decreased to  $\delta$  frequency) compared with animals receiving 1.0 minimum alveolar concentration isoflurane or desflurane.<sup>3</sup> At the end of preparation before termination of isoflurane, all surgical incisions were infiltrated with 0.5% bupivacaine. Thereafter, rats were not exposed to any painful stimuli. Therefore, fentanyl-nitrous oxide seems to provide sufficient analgesia and sedation in rats not exposed to surgical stress. The dose of dexmedetomidine used during the current study results from previous experiments using this ischemia model, where 10  $\mu\text{g}/\text{kg}$  dexmedetomidine produced moderate and 100  $\mu\text{g}/\text{kg}$  dexmedetomidine produced profound improvement of neurologic outcome. Likewise, studies in rats have shown that a hypnotic anesthetic effect, as characterized by loss of righting reflex, occurs at doses greater than 100  $\mu\text{g}/\text{kg}$  dexmedetomidine intraperitoneal.<sup>23</sup> We therefore decided to assess cerebral and peripheral neurotransmitter concentrations using a dose of 100  $\mu\text{g}/\text{kg}$  dexmedetomidine based on our previous experiences and the dose-dependent hypnotic anesthetic action of dexmedetomidine *via* activation of central  $\alpha_2$ -adrenoceptors.<sup>23</sup>

In conclusion, dexmedetomidine did not inhibit the intraischemic increase of cerebral extracellular catecholamine or glutamate concentrations, whereas peripheral catecholamine concentrations were suppressed. These data indicate that modulation of cerebral catecholamine and glutamate release is not related to the neuroprotective effect of dexmedetomidine previously demonstrated in this model.

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