

The effect of dexmedetomidine on transient focal cerebral ischemia in rats

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Abstract

To examine the possibility that the dexmedetomidine-induced cerebral vasoconstriction deteriorates ischemic brain damage, we administered dexmedetomidine during transient focal cerebral ischemia in rats. Rats received intravenous infusions of dexmedetomidine at $0.1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, and $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ during cerebral ischemia induced by the occlusion of the middle cerebral artery with a nylon suture. Further, $5\text{mg}\cdot\text{kg}^{-1}$ of yohimbine combined with dexmedetomidine were also given during the cerebral ischemia. Cerebral infarction was detected in rats receiving infusions of dexmedetomidine at $0.1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Cerebral infarct volume was significantly larger in rats receiving dexmedetomidine infusion at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ than that of rats receiving at $0.1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Treatment with yohimbine significantly decreased the dexmedetomidine-induced infarct volume by 84%. Therefore, it is suggested that the infusion of high-dose dexmedetomidine aggravates ischemic brain damage following focal cerebral ischemia in rats via the activation of alpha-2 adrenoceptor.

Key words; cerebral blood flow, cerebral infarction, cerebral ischemia

Introduction

Dexmedetomidine, an alpha-2 adrenergic agonist, has sedative, anxiolytic, analgesic, and sympatholytic actions which are thought to be beneficial for the patients. High doses of dexmedetomidine induces systemic hypertension by post-synaptic alpha-2 receptors¹⁾. However, there has been no report in testing whether high doses of dexmedetomidine which sufficiently stimulate the alpha-2B worsens ischemic brain damage following focal cerebral ischemia. Therefore, we investigated whether high doses of intravenous infusions of dexmedetomidine cause cerebral infarction following transient focal cerebral ischemia in rats.

Methods

Thirty male Sprague-Dawley rats (weighing 350~450g) were used under an approval of Institutional Animal Care and Use Committee. All rats were allowed to have free access to food and water before surgical intervention. After an intraperitoneal (i.p.) injection of pentobarbital sodium ($50\text{mg}\cdot\text{kg}^{-1}$), a tracheal tube was inserted and rats were mechanically ventilated with air using a small animal ventilator (SAR-830P, CWE, Ardmore, PA). Endtidal CO_2 was monitored (Respina 1H31, Sanei, Tokyo, Japan) and kept between 35 and 40mmHg by adjusting the ventilation. Brain surface temperature was measured with a 22-gauge stainless steel needle thermometer (PTW-301, Unique Medical, Tokyo, Japan)

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placed beneath the left temporal muscle and was maintained constant at normal throughout the experiment by the servomechanism using an overhead heating lamp. A rectal temperature probe was inserted and was maintained at 37°C during the experiment using servo-controlled heating pad. PE-50 catheters were inserted into the both femoral artery and vein to monitor mean arterial blood pressure (MAP), arterial blood gas, glucose, and to infuse drugs. Anesthesia was maintained by 5mg·kg⁻¹ pentobarbital sodium i.p. every 30min and topical infiltration of 1% lidocaine into the surgical field. Continuous infusion (0.01mg·kg⁻¹·hr⁻¹) of pancuronium was given to maintain immobilization. Rats were then placed in a stereotaxic U-frame (ST-7, Narishige, Tokyo, Japan). After an incision of the scalp, the right parietal bone was drilled thin enough to see through pial vessels with continuous flushing with saline to avoid thermal injury. The laser Doppler flow probe was then placed over the right parietal cortex, and parietal cerebral blood flow (CBF) was continuously measured using laser Doppler flowmetry (FLO-N1, Omegawave, Tokyo, Japan) as described previously². Focal cerebral ischemia was induced using a suture method originally described by Longa et al.³ Briefly, following a midline ventral cervical incision and right common carotid artery exposure, the right external carotid artery and its branches were ligated then divided. After isolating the right internal carotid artery, 4~0 monofilament nylon suture (Ethicon, Somerville, NJ) with its rounded tips were inserted from mobilized stump of external carotid artery through internal carotid artery all the way to the origin of right middle cerebral artery (MCA) until CBF was decreased by approximately 70%. After an hour of occlusion of MCA, reperfusion was performed by pulling back the nylon suture, and we confirmed that CBF returned nearly to pre-occlusion values. It has been reported that reperfusion window of this model is more than two hours³, while an hour period was chosen in the present study because an hour MCA occlusion and reperfusion was found to be enough to cause cerebral in-

farcion with high dose of dexmedetomidine during the course of preliminary experiments. After 30min observation from reperfusion, the brains of rats were rapidly removed and cooled in ice-cold saline for 10min. Using a dissecting matrix (RBM4000C, ASI instruments, Warren, MI), six 2mm-thick coronal slices were cut out and immersed in 2% 2,3,5-triphenyl-tetrazolium-hydrochloride (TTC) (Wako, Osaka, Japan) at 37°C for 30min and stored in 10% neutral buffered 10% formalin as described previously⁴. Infarct volume was calculated by measuring the unstained area using digital camera (Cybershot, Sony, Tokyo, Japan) connected to a computer and an image analysis software (Adobe photoshop 6.0, Adobe systems, San Jose, CA), then multiplying the area by slice thickness (2mm) and summing the volume of each slice altogether. Thereafter, the slices were embedded in paraffin and cut into 6μm sections and stained with hematoxylin and eosine (HE) to further confirm the infarction histologically. Since current study was focused in the vasoconstrictive effect of high dose of dexmedetomidine on the focal cerebral ischemia in early phase, we measured the infarct volume immediately after the experiment and did not survive animals any longer.

Experimental protocols

Infusion of each drug was started 30min before MCA occlusion and continued throughout the experiments unless noted. Control (pre-occlusion) values were taken just before the MCA occlusion. Rats were assigned into one of four following groups. In the first three groups, experiments were performed to examine the dose dependent effect of dexmedetomidine on focal ischemia. Group 1 rats (n=8) received intravenous infusion of vehicle (saline) alone. Rats in group 2 (n=8), group 3 (n=8) received intravenous infusion of dexmedetomidine (Abott, North Chicago, IL) at 0.1μg·kg⁻¹·min⁻¹, and 1μg·kg⁻¹·min⁻¹ respectively. Rats in group 4 (n=6) received i.p. 5mg·kg⁻¹ of yohimbine (Sigma, St. Louis, MO), an alpha-2 adrenoceptor antagonist, 30min prior to cerebral ischemia in addition to infusion of dexmede-

tomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Results were expressed mean \pm SD. For statistics, analysis of variance (ANOVA) with Student-Newman-Keuls test or Chi analysis was used. A $p<0.05$ was considered significant.

Results

Control (pre-occlusion) values of MAP, heart rate, blood glucose are presented in **Table 1**. Compared with vehicle (saline)-treated rats, the control value of MAP was significantly higher in rats receiving dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. The control value of heart rate was significantly lower in rats receiving dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and those receiving yohimbine combined with an infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ when compared with vehicle-treated rats. The control value of blood glucose was significantly higher in rats receiving an

infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

CBF normalized by taking the control (pre-occlusion) value as 100% in rats from groups 1 to 4 (study for dose-dependency) are shown in **Fig. 1**. During focal cerebral ischemia, CBF decreased in a similar degree (approximately 40% of control value) in these four groups of rats. At the reperfusion period, rats receiving dexmedetomidine at $0.1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (group 2) showed slightly but significantly higher CBF compared to vehicle-treated rats. As shown in **Fig. 2**, in both the ischemic and the reperfusion periods, MAP remained significantly higher in rats receiving dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (group 3) compared to rats receiving lower doses of dexmedetomidine or rats receiving vehicle alone. In rats receiving yohimbine combined with dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, MAP was not different from that of vehicle-treated rats.

Table 1 Control (pre-occlusion) values of mean arterial blood pressure, heart rate, and blood glucose

	Group1 n=8 (vehicle)	Group2 n=8 ($0.1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Group3 n=8 ($1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Group4 n=6 (+YOH)
MAP (mmHg)	107 \pm 17	101 \pm 20	148 \pm 17*	108 \pm 17
HR (beats \cdot min $^{-1}$)	403 \pm 38	360 \pm 45	315 \pm 28**	279 \pm 45**
BG (mg \cdot dl $^{-1}$)	105 \pm 17	131 \pm 16*	161 \pm 15**	135 \pm 16*

Data are expressed as mean \pm SD.

* $p<0.05$ vs. vehicle-treated rats (Group1). ** $p<0.01$ vs. vehicle-treated rats (Group1).

MAP=mean arterial blood pressure; HR=heart rate; BG=blood glucose; YOH=yohimbine.

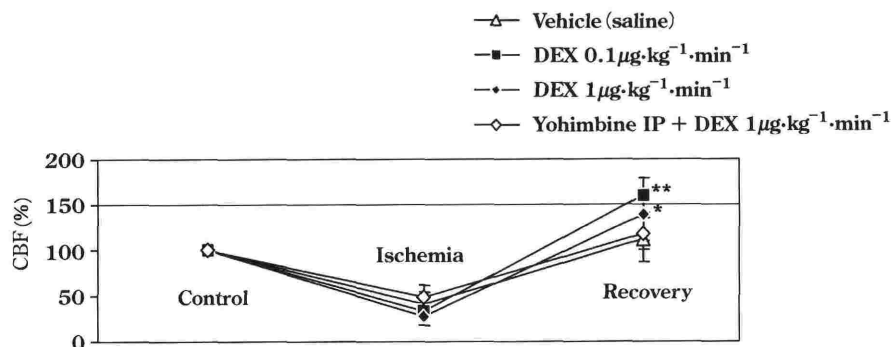


Figure 1 Cerebral blood flow measured by laser Doppler flowmetry at control, during ischemia, and reperfusion.

During ischemia, cerebral blood flow was decreased in a similar degree in rats receiving an infusion of vehicle, dexmedetomidine at 0.1, and $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, and yohimbine plus an infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

* $p<0.05$ vs. vehicle-treated rats; ** $p<0.01$ vs. vehicle-treated rats.

CBF=cerebral blood flow; DEX=dexmedetomidine; IP=intraperitoneal injection.

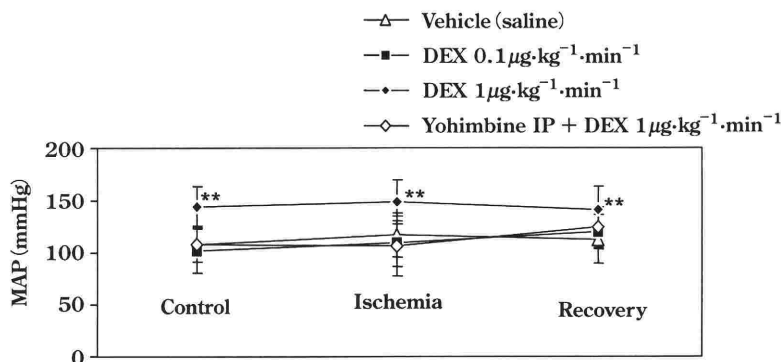


Figure 2 Mean arterial blood pressure at control, during ischemia, and reperfusion.

Mean arterial blood pressure was significantly higher in rats receiving an infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ compared to vehicle-treated rats throughout the experimental period.

** $p < 0.01$ vs. vehicle-treated rats.

MAP=mean arterial blood pressure; DEX=dexmedetomidine; IP=intraperitoneal injection.

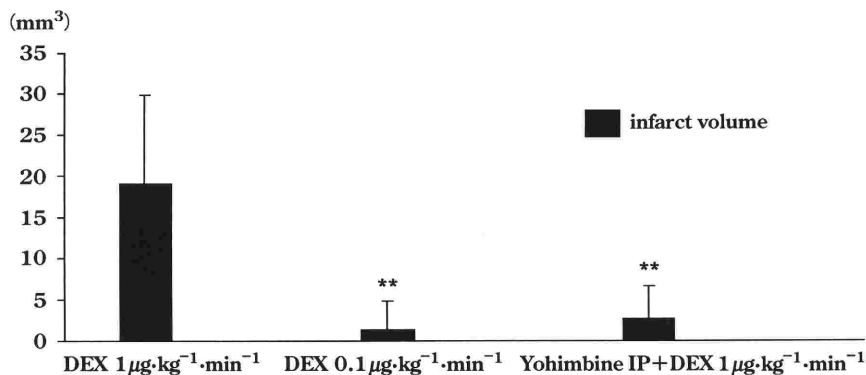


Figure 3 Infarct volumes in rats receiving an infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $0.1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, and $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ combined with yohimbine.

Infarct volume was significantly reduced in rats receiving dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ combined with yohimbine.

** $p < 0.01$ compared with rats receiving an infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

DEX=dexmedetomidine; IP=intraperitoneal injection.

Cerebral infarction was detected in three groups of rats. Those were rats receiving an infusion of dexmedetomidine at $0.1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (group 2), rats treated with yohimbine plus an infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (group 4), and rats receiving infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (group 3). No cerebral infarction was detected in rats receiving vehicle. As presented in **Fig. 3**, cerebral infarct volume was significantly higher in rats receiving an infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ compared to that from rats receiving at $0.1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of dexmedetomidine. Also, as shown in the **Fig. 3**, treatment of yohimbine com-

bined with dexmedetomidine significantly reduced cerebral infarct volume by 84% compared to that of rats receiving an infusion of dexmedetomidine at $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Subsequent histological examination of each brain slice with HE staining revealed the occurrence of the ischemic changes in neurons.

Discussion

Alpha-2 adrenoceptors have an important role in regulating the cardiovascular system⁵. An intravenous bolus administration of dexmedetomidine, a highly selective alpha-2 agonist, has been shown to produce initial hypertension via the activation of pe-

peripheral α -2B adrenoceptors including those in cerebral vessels^{1,6)}. Based on previous studies both in animals and humans^{7,8)}, this dexmedetomidine-induced vasoconstrictive action is thought to become dominant when administering the drug in a rapid manner or in a high dose. Consistent with these previous reports, during infusion of dexmedetomidine at a rate of $1\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, mean arterial blood pressure was significantly elevated in the present study, indicating the vasoconstriction of peripheral resistance arteries. In these conditions, cerebral infarction was observed in our rats. Therefore, it is suggested that the activation of α -2B receptors in cerebral vasculature plays an important role in augmenting cerebral infarction. Our results that yohimbine reduced the infarct volume further supports the involvement of α -2 adrenoceptors to the dexmedetomidine-induced cerebral infarction.

The neuroprotective effects of dexmedetomidine following incomplete ischemia⁹⁾, convulsion¹⁰⁾ and focal cerebral ischemia¹¹⁾ have been reported. Suppression of the sympathetic nervous system which reduces norepinephrine release in ischemic brain is thought to be one of the protective mechanisms of dexmedetomidine in these studies. Our results are different from these previous studies. Focal ischemia (present study) vs. incomplete ischemia (unilateral carotid ligation plus hemorrhagic hypotension), or route of administration (intravenous infusion vs. intraperitoneal injection) could explain the difference.

Since the present study was conducted to examine the acute cerebral vasoconstrictive effect of dexmedetomidine on focal ischemic brain, we did not survive animals for days. However, a recent study indicates that dexmedetomidine exerts neuroprotective effect by modulating apoptosis¹²⁾. Clearly, future studies focused on the effects of dexmedetomidine on delayed neuronal death or apoptosis will be needed.

Although we have shown that the high dose of dexmedetomidine causes cerebral infarction, several factors may interfere with our results and interpretation. First, it is possible that high-dose of dexmedetomidine-induced hyperglycemia exacerbates brain

injury after cerebral ischemia. However, the level of blood glucose in our rats is lower than that of affecting cerebral infarct volume¹³⁾. In addition, in a preliminary study, we infused glucose to elevate blood glucose levels near the highest range of which obtained by dexmedetomidine infusion and no cerebral infarction was observed (data not shown), suggesting the relative small impact of dexmedetomidine-induced hyperglycemia on infarct volume in the present study. Second, the effects of basal anesthetics should be considered. We used pentobarbital and infiltration of local anesthetics for the maintenance of anesthesia because pentobarbital has been reported to have no effect on cerebral vessels¹⁴⁾. We avoided using urethane and xylazine both of which are α -2 adrenoceptor agonists considering the possible confounding effect with dexmedetomidine. Clinically, it is suggested that a high-dose dexmedetomidine should be avoided in patients with decreased cerebral blood flow such as cerebral vasospasm after subarachnoid hemorrhage.

In summary, high doses of intravenous infusion of dexmedetomidine augments cerebral infarction following focal cerebral ischemia in rats by stimulation of α -2 adrenoceptors.

References

- 1) MacMillan LB, Hein L, Smith MS, et al: Central hypotensive effects of the α 2a-adrenergic receptor subtype. *Science* 1996; 273: 801-3.
- 2) Okamoto H, Ito O, Roman RJ, et al: Role of inducible nitric oxide synthase and cyclooxygenase-2 in endotoxin-induced cerebral hyperemia. *Stroke* 1998; 29: 1209-18.
- 3) Longa EZ, Weinstein PR, Carlson S, et al: Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989; 20: 84-91.
- 4) Hatfield RH, Mendelow AD, Perry RH, et al: Triphenyltetrazolium chloride (TTC) as a marker for ischaemic changes in rat brain following permanent middle cerebral artery occlusion. *Neuropathol Appl Neurobiol* 1991; 17: 61-7.
- 5) Ruffolo RR Jr, Nichols AJ, Stadel JM, et al: Pharmacologic and therapeutic applications of α 2-adrenoceptor subtypes. *Annu Rev Pharmacol Toxicol* 1993; 33: 243-79.
- 6) Ganjoo P, Farber NE, Hudetz AG, et al: In vivo effects of

- dexmedetomidine on laser-Doppler flow and pial arteriolar diameter. *Anesthesiology* 1998; 88: 429-39.
- 7) Kamibayashi T, Maze M: Clinical uses of α_2 -adrenergic agonists. *Anesthesiology* 2000; 93: 1345-9.
 - 8) Ebert TJ, Hall JE, Barney JA, et al: The effects of increasing plasma concentrations of dexmedetomidine in humans. *Anesthesiology* 2000; 93: 382-94.
 - 9) Hoffman WE, Kochs E, Werner C, et al: Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat. Reversal by the α_2 -adrenergic antagonist atipamezole. *Anesthesiology* 1991; 75: 328-32.
 - 10) Halonen T, Kotti T, Tuunanen J, et al: α_2 -adrenoceptor agonist, dexmedetomidine, protects against kainic acid-induced convulsions and neuronal damage. *Brain Res* 1995; 693: 217-24.
 - 11) Jolkkonen J, Puurunen K, Koistinaho J, et al: Neuroprotection by the α_2 -adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia. *Eur J Pharmacol* 1999; 372: 31-6.
 - 12) Engelhard K, Werner C, Eberspacher E, et al: The effect of the α_2 -agonist dexmedetomidine and the N-methyl-D-aspartate antagonist S(+)-ketamine on the expression of apoptosis-regulating proteins after incomplete cerebral ischemia and reperfusion in rats. *Anesth Analg* 2003; 96: 524-31.
 - 13) Kawai N, Keep RF, Betz AL: Hyperglycemia and the vascular effects of cerebral ischemia. *Stroke* 1997; 28: 149-54.
 - 14) Hatano Y, Nakamura K, Moriyama S, et al: The contractile responses of isolated dog cerebral and extracerebral arteries to oxybarbiturates and thiobarbiturates. *Anesthesiology* 1989; 71: 80-6.