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Neuroprotection by the α_2-adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia

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Abstract

The present study was undertaken to explore the possible neuroprotective effect of the selective α_2-adrenoceptor agonist, dexmedetomidine in a rat model of focal cerebral ischemia. The effect of dexmedetomidine (9 μg kg^{-1}) on infarct volume was assessed and compared to that of glutamate receptor antagonists *cis*-4(phosphonomethyl)-2-piperidine carboxylic acid (CGS-19755) (20 mg kg^{-1}) or 2,3-dihydro-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) (50 mg kg^{-1}). Dexmedetomidine decreased total ischemic volume by 40% in the cortex ($P < 0.05$) compared to NaCl-treated control rats, whereas NBQX reduced the infarct by 73% in the cortex ($P < 0.001$) and by 43% in the striatum ($P < 0.01$). Dexmedetomidine infusion was associated with some minor degree of hyperglycemia and hypotension. Drug-induced kidney changes were only seen in NBQX-treated rats. These results suggest that dexmedetomidine reduced ischemic volume despite causing a minor increase in blood glucose concentrations and hypotension. Its neuroprotective efficacy was better than that produced by CGS-19775, and dexmedetomidine was safer with respect to kidney toxicity when compared to NBQX.

Keywords: α_2-Adrenoceptor agonist; Adverse side effect; Focal cerebral ischemia; Glutamate receptor antagonist; Neuroprotection; Prophylactic use

1. Introduction

Various types of glutamate receptors have been the principal targets in the development of therapeutic interventions for cerebral ischemia (Muir and Lees, 1995). In particular, antagonists acting on NMDA receptors and AMPA receptors have been shown to have neuroprotective effects against ischemic damage (Simon and Shiraishi, 1990; Gill et al., 1992; Graham et al., 1996). The clinical use of glutamate receptor antagonists available at present is limited, however, due to their potential adverse side effects (e.g., psychotomimetic effects, nephrotoxicity).

In addition to glutamate antagonists, α_2-adrenoceptor agonists have also been shown to reduce ischemic damage possibly by attenuating the excessive release of noradrenaline and glutamate during energy failure (Matsumoto et al., 1993; Talke and Bickler, 1996). There is evidence that the selective α_2-adrenergic agonist, dexmedetomidine may partially prevent neuronal damage in some models of brain ischemia. For instance, dexmedetomidine was reported to be effective in protecting against focal ischemia in rabbits (Maier et al., 1993) and in incomplete forebrain ischemia in rats (Hoffman et al., 1991) but not in severe forebrain ischemia in rats (Karlsson et al., 1995). Recently Kuhmonen et al. (1997) showed that dexmedetomidine given before occlusion followed by a continued treatment during reperfusion reduced delayed neuronal damage in global ischemia in gerbils.

In the previous studies by Hoffman et al. (1991) and Maier et al. (1993), high dexmedetomidine doses (total doses up to 100 μg kg^{-1}) were administered to rabbits and rats. Given the possible adverse systemic effects with such high doses of dexmedetomidine, the reported neuroprotection was somewhat unexpected. Until now, the relative degree of neuroprotection following dexmedetomidine has also been difficult to assess, since there are no compar-

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ative studies to glutamate receptor antagonists, for example, which have a well-established protective effect against ischemic damage.

The present study investigated the relative potency of low doses of dexmedetomidine compared to glutamate receptor antagonists in a model of rat focal ischemia. Drugs were infused continuously starting immediately after the occlusion of the middle cerebral artery with the infusion continuing throughout the onset of reperfusion. The extent of ischemic damage was determined 72 h later. In addition, possible histopathological changes in the kidneys were examined after the experiment.

2. Materials and methods

2.1. Animals

Experiments were carried out using male adult Wistar rats weighing 275–325 g. The animals had free access to food and water and were housed in individual cages in a temperature (20 ± 1°C) controlled environment with lights on from 0700 to 1900 hours. The study was approved by the Ethics Committee of the University of Kuopio and the Provincial Government of Kuopio (87Zd).

2.2. Occlusion of the middle cerebral artery

Anesthesia was initiated with 3–4% halothane (35% oxygen, 62% nitrous oxide) and maintained throughout the operation with 0.5–1% halothane delivered via a nose cone. Body temperature was maintained at 37.0 ± 0.5°C using a thermoregulatory heating unit (Harvard Homeothermic Blanket Control Unit) connected to a rectal probe. To monitor skull temperature, an additional probe was placed in the temporalis muscle (Omega, Model 680). The right femoral artery was cannulated for continuous monitoring of mean arterial blood pressure (CardioCap II, Datex) and for taking blood samples to monitor p_aO_2, p_aCO_2, pH (ABL5, Radiometer) and for blood glucose concentrations (OneTouch II, LifeScan). Heparin (40–60 IU, i.v.) was given just before the occlusion to prevent possible complications caused by blood coagulation. Subsequently, the right common carotid artery was exposed through a midline ventral cervical incision, and carefully separated from the adjacent sympathetic nerves under a stereomicroscope. The common carotid artery and the internal carotid artery were then clamped with microvascular clips. The external carotid artery was ligated distally with a nylon suture and the artery was cut for introduction of suture. A heparinized nylon suture (∅0.28 mm, rounded tip) was introduced into the stump of the external carotid artery, the internal carotid artery clip was removed, and the suture was advanced into the internal carotid artery until it passed beyond the origin of the middle cerebral artery (Longa et al., 1989). After occlusion for 90 min, the suture was removed to allow reperfusion in the middle cerebral artery territory. The stump of the external carotid artery was electrocoagulated followed by removal of the common carotid artery clip. Finally, the cervical incision was closed with silk sutures and the animals were returned to their home cages. The average time for the operation was 150 min.

2.3. Drug administration

Dexmedetomidine (Orion) and *cis*-4(phosphonomethyl)-2-piperidine carboxylic acid (CGS-19755) (Research Biochemicals) were dissolved in 0.9% NaCl. 2,3-Dihydro-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) (Tocris Cookson) was dissolved in NaOH and the pH was adjusted to 8–8.5 using concentrated HCl. Drugs were infused through a polyethylene catheter inserted into the left femoral vein at doses selected on the basis of previous studies (Simon and Shiraishi, 1990; Gill et al., 1992; Halonen et al., 1995; Graham et al., 1996; Kuhmonen et al., 1997). Dexmedetomidine was administered as an intravenous bolus (3 μg kg^{-1}) over 5 min followed by a 120 min infusion at a dose of 3 μg kg^{-1} h^{-1} (total dose 9 μg kg^{-1}) ($n = 10$). NBQX was given as an intravenous bolus (30 mg kg^{-1}) over 5 min followed by a 120 min infusion at a dose of 10 mg kg^{-1} h^{-1} (total dose 50 mg kg^{-1}) ($n = 8$) and CGS-19755 was given as an intravenous bolus (10 mg kg^{-1}) over 5 min followed by a 120 min infusion at a dose of 5 mg kg^{-1} h^{-1} (total dose 20 mg kg^{-1}) ($n = 8$). Control rats received an intravenous infusion of 0.9% NaCl ($n = 11$). The total infusion volume was adjusted to 4 ml in each experimental group.

2.4. Postoperative behavior and kidney toxicology

Each rat was subjected to a short behavioral test battery at 24, 48, and 72 h after occlusion of the middle cerebral artery. The tests included a postural reflex test, and forelimb and hindlimb placing (De Ryck et al., 1989). The weight loss of rats was also measured at same time points. After the experiment, both kidneys were preserved in neutral buffered 10% formalin for histopathological study. Kidney tissue was trimmed, processed, and embedded in paraffin wax, and sections were cut at 4 μm and stained with haematoxylin/eosin for microscopic examination in a blind manner.

2.5. Estimation of ischemic damage

The animals were decapitated 72 h after occlusion of the middle cerebral artery and their brains were prepared for 2,3,5-triphenyltetrazolium chloride (TTC) staining. Brains were rapidly removed from the skull and cooled in ice-cold saline for 10 min. Using a dissecting matrix, eight serial 2-mm coronal slices were cut and immediately im-

mersed in 2% TTC in 0.1 M phosphate buffer at 37°C for 10 min and stored thereafter in neutral buffered 10% formalin (Lundy et al., 1986). Estimation of the infarcted area in the striatum and cortex was performed within 2–3 days of staining using an image analysis system (Imaging Research) and a DAGE MTI CCD-72 series camera (DAGE.MTI). The area of infarction was determined according to the indirect method of Swanson et al. (1990). Areas of surviving gray matter were outlined with a mouse-controlled cursor separately for each hemisphere and automatically recognized. The difference between the size of an intact area in the contralateral hemisphere and the respective residual area in the ipsilateral hemisphere was taken as the infarcted area. Total infarct volume was calculated by multiplying the infarct area by the thickness of the slice and by summing together the infarct volumes of each slice.

2.6. Statistical analysis

Statistical differences in the various physiological parameters during operation (at baseline, during occlusion and during reperfusion) between different experimental groups and NaCl controls were analyzed by using analysis of variance (ANOVA) for repeated measures. If the treatment × time interaction in ANOVA was significant, pairwise tests were made using the Bonferroni correction. Differences in the volume of ischemic damage between different experimental groups were analyzed by using one-way analysis of variance. Duncan's test was used as a post-hoc test. Behavioral scores were statistically analyzed using the Kruskall–Wallis test.

3. Results

3.1. Physiological variables during occlusion of the middle cerebral artery

The physiological variables monitored at baseline and during occlusion and during reperfusion are shown for each experimental group in Table 1. There were no differences in blood gases or blood pH between any of experimental groups and the saline-treated control animals. Skull temperature, as measured from the temporalis muscle, decreased slightly during occlusion in rats given dexmedetomidine (treatment × time interaction, $F(2,34) = 3.85$, $P < 0.05$). Dexmedetomidine also increased blood glucose concentrations (treatment × time interaction, $F(2,34) = 6.99$, $P < 0.01$). Dexmedetomidine decreased mean arterial blood pressure (treatment × time interaction, $P < 0.05$) whereas NBQX seemed to increase mean arterial blood pressure (treatment × time interaction, $F(2,30) = 2.83$, $P = 0.075$).

3.2. Postoperative observations

The average body weight decreased 14–19% during the 3-day follow-up period, but there were no significant differences in weight loss between any of the drug treatment groups or NaCl-treated control rats. Behavioral scores (the sum of scores from the behavioral tests) were not different between any of the experimental groups at 24, 48 or 72 h after occlusion of the middle cerebral artery, though rats receiving NBQX performed better in behavioral tests, but due to high variation among the behavioral scores the difference compared to saline controls did not

Table 1
The effect of drug treatment on physiological variables during focal cerebral ischemia

Experimental group		Temperature (C°)		p_aO_2 (mm Hg)	p_aCO_2 (mm Hg)	pH	Mean arterial pressure (mm Hg)	B-glucose (mmol l^{-1})
		Skull	Rectal					
NaCl ($n = 11$)	Baseline	36.5 ± 0.2	37.0 ± 0.2	106 ± 9	46 ± 10	7.36 ± 0.03	91 ± 4	6.7 ± 0.8
	Occlusion	36.6 ± 0.4	37.0 ± 0.4	93 ± 10	46 ± 8	7.35 ± 0.02	103 ± 13	5.2 ± 0.5
	Reperfusion	36.6 ± 0.6	36.9 ± 0.5	88 ± 21	42 ± 12	7.31 ± 0.03	84 ± 10	5.4 ± 0.7
Dexmedetomidine ($n = 10$)	Baseline	36.2 ± 0.6	36.8 ± 0.3	112 ± 10	51 ± 10	7.34 ± 0.04	92 ± 5	7.3 ± 0.7
	Occlusion	36.0 ± 0.5[a]	36.8 ± 0.4	90 ± 18	42 ± 9	7.36 ± 0.04	97 ± 8	7.8 ± 1.6[b]
	Reperfusion	36.4 ± 0.4	36.9 ± 0.3	84 ± 15	47 ± 9	7.31 ± 0.03	78 ± 11	7.7 ± 1.8[b]
NBQX ($n = 8$)	Baseline	36.4 ± 0.5	37.0 ± 0.3	105 ± 12	49 ± 6	7.37 ± 0.03	91 ± 4	7.0 ± 1.1
	Occlusion	36.3 ± 0.5	37.0 ± 0.2	91 ± 11	47 ± 6	7.35 ± 0.04	114 ± 8	5.5 ± 0.6
	Reperfusion	36.3 ± 0.4	36.6 ± 0.4	89 ± 3	49 ± 6	7.33 ± 0.04	100 ± 7	5.9 ± 0.7
CGS-19755 ($n = 8$)	Baseline	36.3 ± 0.3	36.8 ± 0.1	102 ± 11	49 ± 6	7.35 ± 0.03	94 ± 4	6.8 ± 0.5
	Occlusion	36.4 ± 0.3	37.0 ± 0.1	93 ± 12	45 ± 12	7.33 ± 0.02	99 ± 8	5.6 ± 1.0
	Reperfusion	36.5 ± 0.3	36.9 ± 0.2	83 ± 6	50 ± 12	7.28 ± 0.03	85 ± 8	5.5 ± 1.2

Values at baseline (baseline), at 45 min after occlusion of the middle cerebral artery (occlusion) and at 20 min after reperfusion (reperfusion) are expressed as mean ± S.D. Statistical significance (analysis of variance for repeated measures followed by pairwise tests using the Bonferroni correction); [a]$P < 0.01$, [b]$P < 0.001$ (compared to NaCl group).

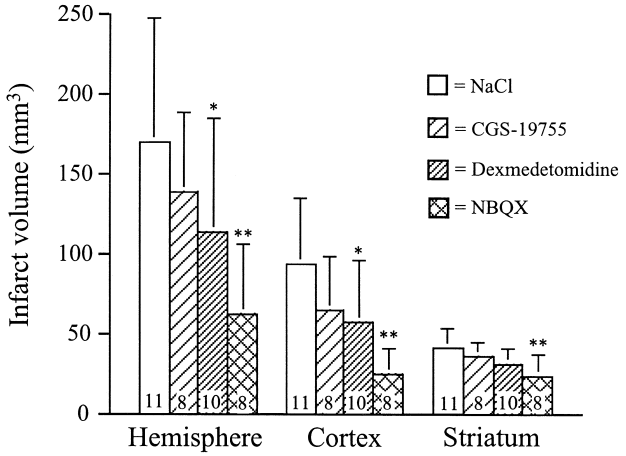

Fig. 1. The effect of drug treatment on infarct volume (mm^3) determined from 2,3,5-triphenyltetrazolium (TTC)-stained sections at 72 h after occlusion of the middle cerebral artery. The bars represent the mean ($\pm$S.D.). The number of animals is indicated inside each bar. Statistical significance (one-way analysis of variance followed by Duncan's test); $^{*}P < 0.05$, $^{**}P < 0.01$ (compared to NaCl group).

reach statistical significance. Histopathological analyses of hematoxylin/eosin-stained kidney sections revealed drug-related changes only in the NBQX group. In cortex, there were many dilated tubules lined by flattened epithelial cells. Also mineralisation was observed in a few tubules. In medulla, there were many dilated collecting ducts with and without hyperplastic changes in the epithelium. Occasionally slight interstitial infiltrations of subchronic inflammatory cells including fibroblasts were seen in medulla. The yellow drug substance was seen in some dilated tubules in cortex and medulla.

3.3. Effect of drug treatment on infarct volume

The total infarct volumes following drug treatment are shown in Fig. 1. There was an overall significant difference between the groups when comparing the whole cerebral hemisphere ($F(4,40) = 3.87$, $P < 0.01$), the cortex ($F(4,40) = 5.11$, $P < 0.01$), and the striatum ($F(4,40) = 3.13$, $P < 0.05$). Intergroup analyses revealed that dexmedetomidine decreased ischemic damage by 33.1% in the whole hemisphere ($P < 0.05$) and by 39.6% in the cortex ($P < 0.05$). NBQX reduced infarct volume by 63.0% in the whole hemisphere ($P < 0.01$), by 73% in the cortex ($P < 0.01$) and by 43% in the striatum ($P < 0.01$).

4. Discussion

The present data provide further evidence that the selective α_2-adrenergic agonist, dexmedetomidine, has neuroprotective effect in transient focal cerebral ischemia. A relatively low dose of dexmedetomidine (a total dose of 9 $\mu g\ kg^{-1}$) which had minute systemic effects was shown to reduce infarct volume in the cerebral cortex. The efficacy of dexmedetomidine was better compared to that of the competitive NMDA receptor antagonist, CGS-19755. It is unlikely that this neuroprotection is simply due to a delay in infarct maturation since the ischemic damage was determined 72 h after the occlusion.

Variations in the physiological parameters that were observed following drug administrations were mild. The increase in blood glucose concentration following dexmedetomidine has been previously reported (Hoffman et al., 1991). Although hyperglycemia preceding ischemia exacerbates the outcome from ischemic damage (Wass and Lanier, 1996), it was recently shown that the same is true for ischemic hyperglycemia (Kawai et al., 1997). Thus, the hyperglycemia induced by dexmedetomidine should aggravate ischemic damage rather than provide protection. This was clearly not the case, though blood glucose concentration was elevated in the dexmedetomidine-treated rats. Mean arterial blood pressure seemed to be decreased during the operation after dexmedetomidine. It is unlikely, however, that such a transient and small decrease in blood pressure could significantly affect infarct volume (Zhu and Auer, 1995). Drug-induced alterations in the brain or body temperature may also have effects on ischemic volume (Morikawa et al., 1992). However, skull and rectal temperature in the dexmedetomidine-treated rats did not differ from controls during operation except for a transient decrease after the bolus injection of dexmedetomidine. There was no evidence of long-term hypothermia after the operation at the dexmedetomidine dose applied.

In previous studies, the anti-ischemic effect of dexmedetomidine has been shown at much higher doses (up to 100 $\mu g\ kg^{-1}$) (Hoffman et al., 1991, 1993; Maier et al., 1993; Karlsson et al., 1995), which is surprising in light of evidence indicating that dexmedetomidine has a U-shaped dose response curve in experimental models associated with excitotoxic cell death (Halonen et al., 1995; Kuhmonen et al., 1997). Although direct comparisons between these reports and our study are difficult to make due to the use of different species and routes of administration, the dexmedetomidine dose required for its neuroprotective properties in the present study was much lower (9 $\mu g\ kg^{-1}$) and did not produce any serious systemic adverse side effects. In the preliminary experiments high dexmedetomidine doses (15 and 27 $\mu g\ kg^{-1}$) were used, but the neuroprotective effects were not significant possibly because of the detrimental hyperglycemia induced by these doses (data not shown). Another possible explanation is the nonspecific activation of α_1-adrenoceptors as is the case of higher doses of clonidine in reversing the beneficial effects of lower doses in pentylenetetrazole-induced epileptic seizures (Papanicolaou et al., 1992). However, it is unlikely that α_1-adrenoceptor agonism could contribute to the present results since dexmedetomidine is a more selective α_2-adrenoceptor agonist than clonidine (Savola and Virtanen, 1991).

The mechanism(s) underlying the neuroprotective ef-

fects of α_2-adrenoceptor agonists is not known. The effect may be mediated in part by catecholaminergic neurotransmission. Dexmedetomidine may attenuate the excessive release of noradrenaline induced by ischemia by the activation of presynaptic α_2-adrenoceptors (Globus et al., 1989; Matsumoto et al., 1993). This could alleviate potential detrimental effects of metabolizing excessive noradrenaline which can lead to the formation of free radicals. Although the pathophysiological significance of free radicals in ischemic brain damage is still controversial, inhibition of oxidative deamination of catecholamines decreases H_2O_2 production during reperfusion (Simonson et al., 1993; Suzuki et al., 1995). The involvement of free radicals in ischemic brain damage is further supported by partial neuroprotection following antioxidants such as superoxide dismutase (Chan, 1998). In addition, the neuroprotective action of α_2-adrenoceptor agonists may be due to a postsynaptic reduction in neuronal excitability and/or a possible presynaptic decrease in glutamate release (Bickler and Hansen, 1996; Talke and Bickler, 1996). However, although dexmedetomidine seemed to decrease evoked glutamate release from hippocampal slices, it did not affect NMDA receptor mediated changes in intracellular Ca^{2+} (Talke and Bickler, 1996).

The present study is to our knowledge the first one that compares the safety and anti-ischemic effect of an α_2-adrenoceptor agonist with those of glutamate receptor antagonists. These pharmacologically different type of drugs all provided partial protection. The rank order of potency for their neuroprotective effects was NBQX $\gg$ dexmedetomidine $>$ CGS-19755. Blockade of AMPA receptors with NBQX was superior in preventing ischemic damage. NBQX decreased total ischemic volume by 43–73%, which is more extensive than that previously reported by Gill et al. (1992) and Graham et al. (1996) following permanent occlusion the middle cerebral artery. The present drug dosing and, in particular, infusion lasting over the onset of reperfusion may contribute to the ischemic outcome. The degree of protection using dexmedetomidine was 33–40%, which was better than that obtained with CGS-19755. CGS-19755 seems to be less effective after transient as compared to permanent occlusion of the middle cerebral artery in which it decreased total infarct volume up to 72% (Simon and Shiraishi, 1990). In the rabbit model of focal ischemia, dexmedetomidine decreased ischemic volume in the cortex by 18% at 6 h after occlusion (Maier et al., 1993), but this protection may have been merely due to a delay in infarct development.

Although glutamate receptor antagonists are at an advanced stage of development in the therapy of acute stroke, the severe side effects produced by these compounds limits their use in clinical use (Muir and Lees, 1995). It should to be emphasized that the profile of adverse effects of dexmedetomidine, NBQX and CGS-19755 is quite different. The major adverse effect of NBQX is kidney toxicity which has restricted its clinical development. Precipitation of NBQX in the kidney nephron was also seen in our study. However, foreign body giant cell formation, which typically accompanies crystal deposition, was absent in the present histopathological evaluation suggesting that other mechanisms may also be responsible for the observed kidney lesion (Greaves, 1990). At present, more water soluble AMPA analogs are available, but their safety and anti-ischemic effect remains to be determined (Cordon et al., 1998). CGS-19755, on the other hand, produces severe psychotomimetic side-effects including hallucinations and paranoid reactions (Grotta et al., 1995) to such an extent that clinical trials with CGS-19755 have been suspended (Davis et al., 1997). Compared with the glutamate receptor antagonists, dexmedetomidine has been found to be safe perioperatively and it is now under clinical development in phase III studies.

Previous examples warrant that caution should be taken when extending results from animal studies of stroke into the clinical setting (Grotta, 1994). However, given the known beneficial effects of dexmedetomidine and other α_2-adrenoceptor agonists in surgical patients (e.g., sedation and attenuation of sympathetic and cardiovascular responses) (Maze and Tranquilli, 1991) along with their probable anti-ischemic effect, their use as an anesthetic adjuvant may greatly benefit patients at high risk of cerebral ischemia. Invasive procedures such as cardiac surgery, endarterectomy, and endovascular therapy are all associated with an identifiable risk for cerebrovascular events and central nervous system impairment (Harrison et al., 1989; Swain, 1993; Ergin et al., 1994; Pugsley et al., 1994). For example, following coronary artery bypass surgery, 36% of patients still showed some neuropsychological deficits 8 weeks after surgery (Harrison et al., 1989). Administration of dexmedetomidine prior or during such operations might attenuate not only the hemodynamic responses to anesthesia and surgery, but also harmful consequences of possible ischemic injury (Fisher et al., 1994). The initial clinical studies in patients undergoing vascular and cardiac surgery have shown that while dexmedetomidine decreased intraoperative sympathetic tone, patients may require supplemental pharmacological intervention to support blood pressure and heart rate (Talke et al., 1995; Jalonen et al., 1997). Large scale studies will reveal whether hypotension and bradycardia restrict the prophylactic use of dexmedetomidine in preventing cerebral ischemic events associated with certain surgical operations.

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