Furosemide inhibits the centrally-mediated pressor response to clonidine in conscious, normotensive rats

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1 The effect of furosemide on the pressor response induced by intracerebroventricular (i.c.v.) injection of clonidine was investigated in freely moving, normotensive rats with chronically implanted arterial catheters.

2 When injected i.c.v., clonidine at doses of 5 and 10 µg produced a dose-dependent pressor response and a decrease in heart rate. No depressor response was induced by clonidine.

3 Systemic (i.v.) pretreatment with furosemide (2–10 mg kg⁻¹) increased urine volume and dose-dependently inhibited the pressor response to i.c.v. clonidine (10 µg), and a long-lasting depressor response to clonidine was observed. However, furosemide treatment did not alter the bradycardia produced in response to clonidine.

4 The systemic treatment with furosemide (10 mg kg⁻¹, i.v.) had no effect on the pressor response to i.v. noradrenaline.

5 These results suggest that reduction of body fluid volume inhibits the centrally-mediated pressor response to clonidine and leads to the hypotensive effect. We also suggest that combined treatment with a diuretic increases the hypotensive efficacy of clonidine.

Keywords: Furosemide; clonidine; central pressor effect; body fluid volume; conscious rat

Introduction

We have reported that clonidine, an antihypertensive drug, increases blood pressure when injected into the cerebroventricles of conscious, normotensive rats, but not of pentobarbitone, anaesthetized rats (Kawasaki & Takasaki, 1986; Kawasaki *et al.*, 1986; Kawasaki & Takasaki, 1987a). Since central pretreatment with yohimbine, an α_2 -adrenoceptor antagonist, but not prazosin, a selective α_1 -adrenoceptor antagonist, abolished the pressor response to i.c.v. clonidine, we proposed that the response is mediated by central α_2 -adrenoceptors (Kawasaki & Takasaki, 1986). However, the precise mechanism underlying the pressor response to clonidine remains unknown.

In the clinical treatment of hypertension, combining clonidine with a diuretic has been shown to lead to a greater hypotensive effect than when either drug is given alone (Rosenman, 1975; Hui *et al.*, 1986). Thus, the hypotensive effect of clonidine is probably affected by changes in extracellular fluid volume. This gives rise to the speculation that reducing body fluid volumes may suppress the centrally-mediated hypertensive response to clonidine and subsequently the hypotensive effect may predominate. In fact, we have observed that i.c.v. injection of clonidine produced no pressor response in the conscious rat after water deprivation for 48 h, and instead caused a long-lasting depressor response (Kawasaki & Takasaki, 1987b). Therefore, in the present study, we investigated further the influence of body fluid volumes on the centrally-mediated pressor response to clonidine, using a diuretic, furosemide, in freely moving, normotensive rats.

Methods

Experimental animals

Male Wistar rats weighing 300–350 g were used in this study. The animals were given food and water *ad libitum*. They were housed in the Experimental Animal Centre of Miyazaki Medical College at a controlled ambient temperature of 22°C with 50 ± 10% relative humidity and a 12 h light/dark cycle

(light on 07 h 30 min). After surgery for chronic catheter implantation, the animals were transferred to individual cages.

Surgical procedures

Under pentobarbitone-anaesthesia (50 mg kg⁻¹, i.p.), stainless-steel guide cannulae (0.7 mm outer diameter) were implanted bilaterally into the lateral cerebroventricle as described previously (Kawasaki & Takasaki, 1986). A stainless-steel bipolar electrode was also placed above the frontal cortex to measure EEG activity. The animals were allowed to recover for 10 days before the surgery for chronic catheter implantation.

For the direct recording of blood pressure, the animals with the chronic guide cannulae and electrodes were anaesthetized with ether. Polyethylene catheters (PE 10 and PE 20) were implanted into the abdominal aorta via the left femoral artery and into the inferior vena cava via the left femoral vein as described previously (Kawasaki *et al.*, 1979; Kawasaki & Takasaki, 1986). The remainder of each catheter was passed beneath the skin to emerge on the back of the neck. Both catheters were prefilled with sterile heparinized 0.9% saline (500 iu ml⁻¹) and were flushed every 2 days. A recovery period of 1 week after surgery was allowed before starting the experiment.

Recording

On the day of the experiment, the arterial catheter was attached to polyethylene tubing, which was connected to a pressure transducer (P23ID, Statham). The arterial blood pressure (pulsatile and mean), heart rate (HR) triggered by arterial pulses, and EEG activity were measured simultaneously (while behaviour was observed) and were recorded on a polygraph (RM-6000, Nihon Kohden). Both mean blood pressure (MBP) and HR were digitized by use of a mini-computer (ATAC 450, Nihon Kohden) and printed out at 1 min intervals.

Experimental protocols

For i.c.v. injection of the drugs, a stainless-steel injection cannula (0.35 mm outer diameter) was lowered into the lateral cerebroventricle through the guide cannula. The injection cannula was attached to an extension polyethylene tube (PE 20), which was connected to a Hamilton microsyringe (25 µl).

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The venous catheter was connected to a piece of polyethylene tubing approximately 60 cm long (PE 20, containing 0.2 ml).

When the animal was completely relaxed and a drowsy EEG pattern (i.e., high voltage and slow waves) was recorded for over 5 min, the drugs were injected. Clonidine or the equivalent volume of 0.9% saline was injected i.c.v. with a micro-injector in a volume of 5 μ l through the right guide cannula. There was no significant change in MBP and HR after i.c.v. injection of saline.

Furosemide (2, 5 and 10 mg kg⁻¹) or 0.9% saline (1 ml kg⁻¹) was injected i.v. through the extension tubing 60 min before i.c.v. injection of clonidine. Noradrenaline was injected i.v. with stepwise increases in the dose every 10–20 min. After i.v. injection, the tubing was flushed for 60 s with 0.3 ml of sterile saline.

In some rats, urine volume was measured for 60 min after i.v. furosemide (2, 5 and 10 mg kg⁻¹) or 0.9% saline (1 ml kg⁻¹).

Each animal received only one dose of clonidine and one pretreatment at 1 week intervals on each occasion. At the end of each experiment the catheters were sealed with stoppers and the animals were returned to their home cages.

After completion of the experiment the animals were anaesthetized with large doses of pentobarbitone. To determine the site of injection cannula placement, 5 μ l of dye was injected and the brain was removed. Correct guide cannula placement was indicated by the diffusion of dye in the ventricle.

Statistical analysis

All values are expressed as mean $\pm$ s.e.mean. Statistical analysis was performed using the unpaired *t* test and one way ANOVA. A *P* value less than 0.05 was considered statistically significant.

Drugs

The following drugs were used: clonidine HCl (Nippon C.H. Boehringer Sohn, Kawanishi, Japan), furosemide (Lasix inj.,

Hoechst Japan, Tokyo, Japan), (–)-noradrenaline HCl (Sigma Chemical Co., St. Louis, MO, U.S.A.). All drugs were dissolved in sterile 0.9% saline.

Results

Pressor response to i.c.v. clonidine

When injected i.c.v., clonidine at doses of 5 and 10 μ g produced a dose-dependent and long-lasting rise in blood pressure concomitant with a decrease in heart rate as shown in Figures 1, 2 and 3. No depressor response to clonidine was observed (Figures 1 and 2). Clonidine injection (5 and 10 μ g) caused a marked EEG drowsy pattern (Figure 1) and the animal was sedated for more than 60 min.

Effect of furosemide treatment

As shown in Figures 1, 2 and 3, pretreatment with 10 mg kg⁻¹ i.v. furosemide abolished the pressor response to i.c.v. clonidine (5 and 10 μ g), and there was a marked depressor response after clonidine injection. Furosemide treatment did not affect the clonidine-induced decrease in heart rate and EEG drowsy pattern.

Furosemide at doses of 2–10 mg kg⁻¹ increased urine volume in a dose-dependent manner, as shown in Figure 4a. After i.v. furosemide, the basal MBP and HR before i.c.v. injection of clonidine were 95.3 $\pm$ 2.1 mmHg and 324.8 $\pm$ 16.4 beats min⁻¹ (1 ml kg⁻¹ saline control), 98.8 $\pm$ 3.4 mmHg and 317.8 $\pm$ 8.5 beats min⁻¹ (2 mg kg⁻¹ furosemide), 96.3 $\pm$ 1.4 mmHg and 305.2 $\pm$ 6.0 beats min⁻¹ (5 mg kg⁻¹ furosemide) and 104.3 $\pm$ 1.5 mmHg and 359.0 $\pm$ 17.5 beats min⁻¹ (10 mg kg⁻¹ furosemide), respectively. Pretreatment with furosemide dose-dependently inhibited the clonidine (10 μ g)-induced pressor response, and a depressor response to clonidine appeared. However, this treatment did not affect the clonidine-induced decrease in HR.

Figure 1 Typical records of the cardiovascular response to i.c.v. clonidine (10 μ g) and the effect of systemic (i.v.) treatment with furosemide (10 mg kg⁻¹) in the conscious, normotensive rat. (a) Saline treatment (1 ml kg⁻¹, i.v.); (b) furosemide treatment, which was carried out 1 week after saline treatment in the same animal. ECO, electrocorticogram. HR, heart rate. BP, blood pressure.

Figure 2 Changes in mean blood pressure (MBP) and heart rate (HR) following i.c.v. clonidine ($10\mu\text{g}$) in vehicle (saline 1 ml kg^{-1} , i.v., $\bigcirc$)-and furosemide (10 mg kg^{-1} , i.v., $\bullet$)-treated rats. $n = 6$ for both groups. The rats were treated 60 min before clonidine injection. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the vehicle (saline) control.

Effects of pressor response to i.v. noradrenaline

As shown in Figure 5, systemic treatment with 10 mg kg^{-1} i.v. furosemide did not affect the dose-response curve for pressor responses induced by i.v. injected noradrenaline (0.1 – $1.0\mu\text{g kg}^{-1}$).

Discussion

The present study shows that i.c.v. clonidine produces a dose-dependent and long-lasting pressor response in conscious rats, and does not lower blood pressure. This finding is in accord with previous reports that i.c.v. clonidine produces a hypertensive response only in conscious rats (Kawasaki & Takasaki, 1986; Kawasaki *et al.*, 1986; Kawasaki & Takasaki, 1987a).

Figure 3 Effect of systemic treatment with furosemide (10 mg kg^{-1} , i.v., hatched columns) on the pressor and depressor responses induced by i.c.v. injected clonidine (5 and $10\mu\text{g}$) in conscious, normotensive rats. $n = 6$ in treated and control groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the vehicle (saline, open columns)

Figure 4 Effect of systemic treatment with furosemide (2 – 10 mg kg^{-1} , i.v.) on the urine volume (a) and the pressor response (b, circles), depressor response (b, squares) and decrease in heart rate (c) induced by i.c.v. clonidine (Clon, $10\mu\text{g}$) in conscious normotensive rats. The abscissa scale indicates dose of furosemide; S being saline (1 ml kg^{-1} , i.v.) treatment. The rats were treated 60 min before clonidine injection. ** $P < 0.01$; *** $P < 0.001$, compared with the saline control.

The pressor response to clonidine is abolished by pentobarbitone anaesthesia (Kawasaki & Takasaki, 1986; 1987a), which has no effect on α_2 -adrenoceptors (Armstrong *et al.*, 1982), and by central (i.c.v.) pretreatment with the α_2 -adrenoceptor antagonist, yohimbine (Kawasaki & Takasaki, 1986). Therefore, we propose that the pressor response to i.c.v. injected clonidine is of central origin and is mediated by central α_2 -adrenoceptors. However, it has recently been proposed that the hypotensive effect of clonidine is mediated not only by

Figure 5 Effect of systemic treatment with furosemide (10 mg kg^{-1} , i.v., $\bullet$) and vehicle (saline 1 ml kg^{-1} , i.v., $\bigcirc$) on the pressor response induced by i.v. noradrenaline (0.1 – $1.0\mu\text{g kg}^{-1}$) in conscious normotensive rats. $n = 6$ for both groups. The rats were treated 60 min before noradrenaline injection.

α_2 -adrenoceptors but also by imidazoline-preferring receptors (Bousquet *et al.*, 1986). Since yohimbine binds selectively to α_2 -adrenoceptors but not to imidazoline-preferring receptors (Lehmann *et al.*, 1989), central α_2 -adrenoceptors are mainly responsible for the pressor response to i.c.v. clonidine.

In conscious rats treated by furosemide, i.c.v. injected clonidine produced little pressor response and instead induced a marked depressor response. The inhibitory effect of furosemide on the pressor response was dose-dependent and was correlated with increased urine volume, indicating that the inhibition is associated with loss of body fluid volumes. These results are consistent with our previous report that the clonidine-induced pressor response was abolished by water deprivation for 48 h (Kawasaki & Takasaki, 1987b). The present finding that clonidine produces a marked depressor response in rats treated with furosemide is also consistent with the previous observation of a depressor response to clonidine in water-deprived rats. Collectively, the results suggest that clonidine has both hypertensive and hypotensive effects, which are mediated by central α_2 -adrenoceptors. It seems likely that the pressor component of clonidine masks the hypotensive effect and that reduced body fluid volumes affects only the pressor component.

Changes in sodium ion concentration *in vitro* (Woodcock & Murley, 1982) and sodium balance *in vivo* (Diop *et al.*, 1984) have been shown to alter the binding properties of central and peripheral α_2 -adrenoceptor agonists. Therefore, it is possible that the inhibition of the clonidine-induced pressor response after furosemide treatment might be due to alterations in the binding activities of α_2 -adrenoceptors. These changes in binding activity would result from electrolyte alterations, which are well known to be caused by furosemide. However, this is unlikely because furosemide did not inhibit either the hypotensive effect or the drowsy EEG pattern induced by clonidine, which are mediated by central α_2 -adrenoceptors (Florio *et al.*, 1975; Kobinger, 1978; Kostowski *et al.*, 1984). Furthermore, the present results show that furosemide treatment in conscious rats does not affect the pressor response to i.v. noradrenaline, a mixed α_1 - and α_2 -adrenoceptor agonist. Thus, it seems likely that electrolyte alterations by furosemide at doses used in this study have little effect on the binding activities of central and peripheral α_2 -adrenoceptors.

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