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Vascular Areas Where Clonidine Induces Vasodilation in Hypertensive and Normotensive Rats

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Iriuchijima, J. *Vascular Areas Where Clonidine Induces Vasodilation in Hypertensive and Normotensive Rats.* Tohoku J. Exp. Med., 1999, **189** (1), 29-36 —— The aim of this study was to find vascular areas where clonidine decreases the regional vascular resistance when this drug lowers arterial pressure in conscious spontaneously hypertensive rats and normotensive control rats. Arterial pressure was observed with an indwelling catheter at a carotid. Blood flow was measured with an electromagnetic flow probe implanted around the renal artery or the superior mesenteric artery. Regional vascular resistance was calculated as arterial pressure divided by blood flow. Intravenous bolus injection of clonidine at a dose to decrease arterial pressure decreased renal resistance and superior mesenteric resistance. Quantitatively, the combined effect of the decrease in these two resistances was enough to account for the decrease in arterial pressure. Although clonidine is thought to inhibit sympathetic nerve activity centrally, the above vasodilator effect is not ascribable to this inhibitory mechanism: Sympathetic activity to be inhibited does not seem to be present in the superior mesenteric area and clonidine similarly decreased renal vascular resistance even after renal denervation. ———— clonidine; renal artery; superior mesenteric artery; SHR; renal nerve

Since a substantial part of the hypertension in spontaneously hypertensive rats (SHR) (Okamoto and Aoki 1963) is sustained by abnormal sympathetic vasoconstrictor tone in the hindquarters (Iriuchijima 1985, 1988) and since clonidine is thought to inhibit sympathetic nerve firing rate (Schmitt and Schmitt 1969; Kobinger 1978; Hieble and Kolpak 1993), clonidine is expected to decrease the hindquarter vascular resistance (HQR). In reality, however, clonidine at a dose enough to decrease arterial pressure does not decrease HQR in SHR (Iriuchijima 1997). In normotensive control rats (NCR) clonidine increases HQR, which is ascribable to compensatory sympathetic excitation (Teranishi and Iriuchijima 1997).

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Our interest was stimulated by the question what vascular areas contribute to the hypotension on clonidine in SHR and NCR by decreasing the regional resistance. Renal flow was first studied and the research was extended to superior mesenteric flow.

Methods

Rats

SHR used in this study were either those obtained from Japan Charles River Co. (Yokohama), or their descendants bred in our laboratory. Arterial pressure was higher in the latter group, because mating was done among rats with higher pressure. NCR were Wistar rats obtained from the same distributor. In total 23 SHR (268 ± 31 g, 14.0 ± 3.7 week-old, mean $\pm$ s.d.) and 16 NCR (395 ± 43 g, 13.6 ± 2.1 week-old) were used in this study.

Implantation

All the operations were performed under anesthesia with thiamylal sodium (50 mg/kg, i.p.). For observation of arterial pressure an indwelling catheter was placed in the right common carotid. Another catheter was inserted into the right external jugular vein for intravenous drug injection. An electromagnetic flow probe (type FC, Nihon Kohden, Tokyo) with an internal diameter of 1 mm was implanted around the left renal artery and that of 1.5 mm around the superior mesenteric artery. In each rat only one probe was implanted. In some rats all nerve tissues surrounding the renal artery and vein were removed near the hilus before implantation of a flow probe around the renal artery. The wire with a plug from the probe and the catheter ends, filled with heparinized saline (10 U of heparin/ml) and plugged with a short piece of stainless steel wire, were tunneled subcutaneously to exit between the scapulae.

Recording

After implantation, each rat was kept separately in a white $35 \times 37 \times 17$ cm polyethylene cage containing wood chips. Water and food pellets were given ad libitum. Two to 4 days after implantation, after the rat had resumed eating and drinking, the polyethylene tube from a pressure transducer was connected to the arterial catheter and the cable from the flowmeter circuit (MFV-3100, Nihon Kohden) to the plug of the flow probe. Pressure and flow signals were smoothed with an RC (resistance-capacitance) low pass filter of a time constant of 1 seccond and recorded with a rectangular pen-writer.

Statistics

The paired t-test was used for the significance of a successive change in a variable. The U-test was used for the difference in a variable between two groups when the difference in the s.d.'s was over 100%. p-Values of <0.05 were consid-

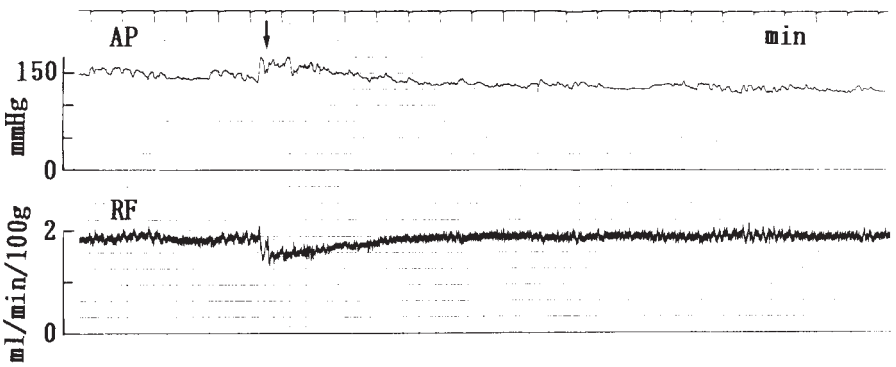

Fig. 1. Effects of clonidine (5 μg/kg, i.v., at the arrow) on arterial pressure (AP) and renal flow (RF). SHR, 300 g, 18 week-old. Note that, after a brief increase in AP and a decrease in RF, AP decreased to a level lower than the original level, while RF recovered almost to the original level.

ered to indicate statistical significance.

Results and Discussion

Effects of clonidine on renal flow

Fig. 1 shows one example of a recording of arterial pressure and renal flow in an SHR when clonidine was injected in a bolus at 5 μg/kg. This dose was selected as appropriate for studying the acute depressor effect of this drug because the effect was greater than that at 2 μg/kg and comparable with that at 10 μg/kg (Iriuchijima 1997). On injection, after an initial brief rise of arterial pressure and a concomitant decrease in renal flow, the pressure gradually decreased and reached a lower plateau level in 20 minutes, by which time renal flow had almost recovered the original level. Renal flow resistance (RR) calculated as arterial pressure divided by renal flow decreased.

Fig. 2 summarizes the results from 9 SHR and 6 NCR. Twenty minutes after clonidine injection RR was lower than the original level significantly at $p<0.01$ by the paired t-test in SHR and at $p<0.01$ in NCR. This time point, 20 minutes after injection, was selected as that when the variables were to be compared with the controls, because arterial pressure had reached a lower stationary level by this time and was maintained for at least half an hour thereafter (Iriuchijima 1997).

In a previous study we proposed an equation relating a change in peripheral conductance to its contribution to the corresponding change in arterial pressure (Iriuchijima 1997):

$$\partial P/\partial g = -P^2/Q, \tag{1}$$

or, approximately,

$$\triangle P = (-P^2/Q)\triangle g, \tag{2}$$

where P = mean arterial pressure, g = peripheral conductance, and Q = minute volume.

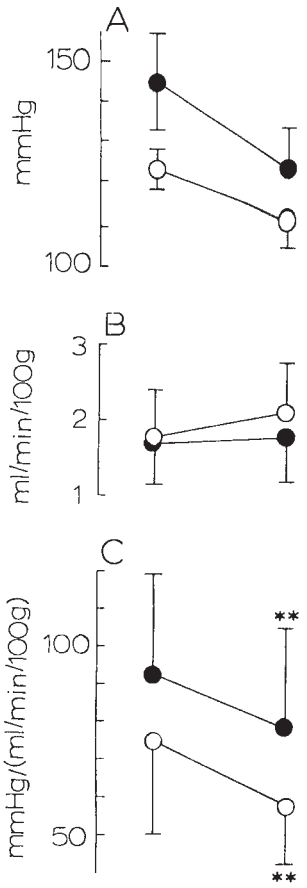

Fig. 2. Mean±S.D. of arterial pressure (A), renal flow (B), and renal resistance (C) from 9 SHR (280±31 g, 15±3 week-old) (filled circles) and 6 NCR (398±50 g, 14±2 week-old) (open circles), before and 20 minutes after clonidine (5 μg/kg, i.v.). In both SHR and NCR, clonidine decreased renal resistance significantly. $^{**}p<0.01$.

As read from Fig. 2, mean renal resistance decreased from about 92 mmHg/(ml/min/100 g) to 78 in SHR on clonidine. Taking the inverse, renal conductance increased from about 0.011 (ml/min/100 g)/mmHg to 0.013, an increase of about 0.002. Substituting this value in equation (2) as well as P=145 mmHg (Fig. 2) and Q=22 (ml/min/100 g) (Iriuchijima 1983), we obtain, approximately,

$$\triangle P_{calc}=2 \text{ mmHg}.$$

This figure, even doubled to consider the contralateral kidney also, is far smaller than the actual decrease in mean arterial pressure, i.e.,

$$145-123=22 \text{ mm Hg}=\triangle P_{meas}.$$

The corresponding value for NCR was

$$\triangle P_{calc}=2 \text{ mmHg}$$

against

$$\triangle P_{meas}=123-111=10 \text{ mmHg}.$$

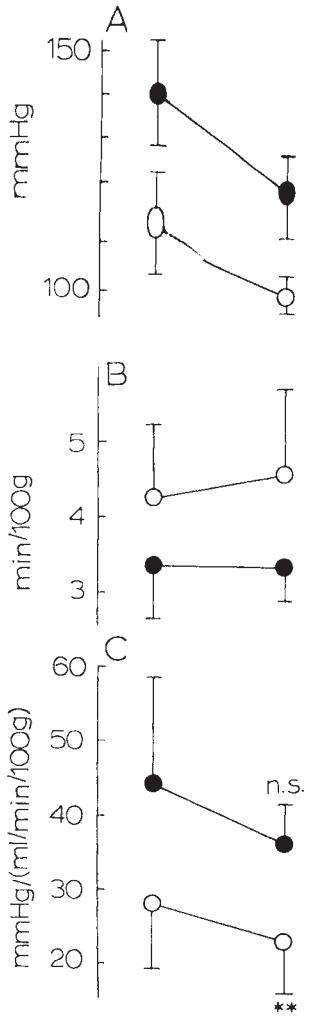

Fig. 3. Mean±S.D. of arterial pressure (A), superior mesenteric flow (B), and superior mesenteric resistance (C) from 7 SHR (261±23 g, 14±3 week-old) (filled circles) and 5 NCR (408±23 g, 13±2 week-old) (open circles), before and 20 minutes after clonidine (5 μg/kg, i.v.). In NCR, clonidine decreased superior mesenteric resistance significantly. In SHR, although the decrease was not significant, the mean superior mesenteric resistance was decreased considerably by clonidine. n.s., not significant. $^{**}p<0.01$.

These calculations suggested to us that we seek another vascular area which contributes additionally to the pressure decrease on clonidine. Since HQR is known not to decrease on clonidine in either SHR or NCR (Iriuchijima 1997), we selected superior mesenteric resistance (SMR) to be examined next.

Effects of clonidine on superior mesenteric flow

Previously, we observed that SMR was not decreased by ganglionic blockade with hexamethonium bromide (Iriuchijima and Sakata 1985; Iriuchijima 1986), which suggests there is no sizable sympathetic tone in the resistanace vessels of the superior mesenteric area. Therefore, at first, we did not expect SMR to decrease on clonidine.

Contrary to the expectation, clonidine did decrease SMR as it did RR (Fig. 3). The decrease was significant at $p<0.01$ in NCR. In SHR it was not significant but the mean SMR decreased considerably. Thus

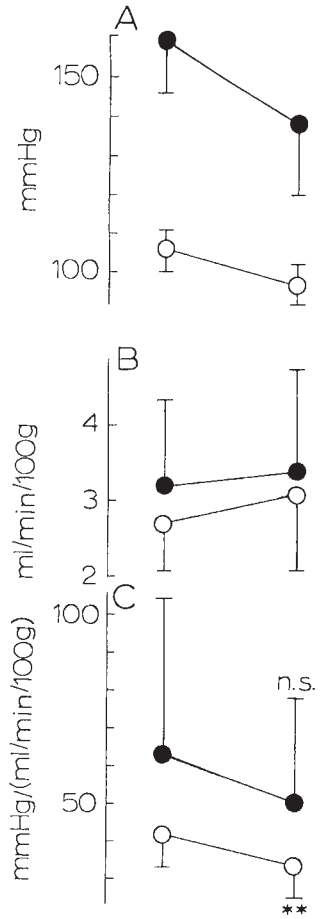

Fig. 4. After renal denervation. Mean±S.D. of arterial pressure (A), renal flow (B), and renal resistance (C) from 7 SHR (254±28 g, 15±2 week-old) (filled circles) and 5 NCR (377±41 g, 14±2 week-old) (open circles), before and 20 minutes after clonidine (5 μg/kg, i.v.). In NCR, clonidine decreased renal resistance significantly. In SHR, the mean renal resistance was decreased by clonidine. n.s., not significant. $^{**}p<0.01$.

$$\triangle P_{calc} = 4 \text{ mmHg for SHR}$$

and

$$\triangle P_{calc} = 5 \text{ mmHg for NCR.}$$

In other words, if we considered vasodilation on clonidine in the bilateral kidneys and the superior mesenteric area (mostly intestine) together, it was enough to account for the hypotension induced by this drug.

Clonidine after renal denervation

In both SHR and NCR alike, we surmize that sympathetic vasoconstrictor tone is present in the resistance vessels of the renal area because ganglionic blockade decreases RR (Iriuchijima and Sakata 1985; Iriuchijima 1986). The decrease in RR is not ascribable to renal autoregulation because the decrease is absent after renal denervation (Iriuchijima and Sakata 1985) and because auto-

regulation is quite imperfect in the rat (Hope and Clausen 1982; Iriuchijima 1998).

The above finding that clonidine decreased SMR, on which vasoconstrictor tone does not seem present, also suggested the possibility that the decrease in RR on clonidine was not due to inhibition of the sympathetic tone—all the more so because clonidine does not inhibit the abnormal hindquarter tone in SHR (Iriuchijima 1997).

In 7 SHR and 5 NCR the effects of clonidine on arterial pressure and renal flow were observed after renal denervation. The results are summarized in Fig. 4. RR was significantly decreased in NCR ($p<0.01$). The decrease was not significant in SHR, although the mean value decreased considerably.

The percent change in RR on clonidine in NCR was $-21.4\pm7.7\%$ in the rats with renal nerves intact and $-20.0\pm9.9\%$ in those after denervation. Thus, we conclude that the dilator effect of this drug on renal resistance vessels is similar, with or without renal nerves.

In NCR with renal nerves intact the mean$\pm$S.D. for RR before clonidine was 75 ± 24.9 mmHg/(ml/min/100 g). In those after denervation it was 41.4 ± 8.5. They were significantly different by the U-test at $p<0.05$. The difference is ascribable to the vasodilation after denervation.

In conclusion, we do not have any evidence to indicate that clonidine inhibits sympathetic tone in the CNS of SHR and NCR, at least when this drug is used acutely as done in this study.

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