Regional Distribution of Vasoconstrictor Tone in Acute Spinal Rats

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Abstract Rats, chronically instrumented with an electromagnetic flow probe around the carotid, superior mesenteric, or renal artery, or the terminal aorta as well as having an arterial and venous catheter, were anesthetized with ether and submitted to high spinal cord transection. One hour later, when the rats had recovered consciousness and the arterial pressure had recovered partially, hexamethonium was intravenously injected for ganglionic blockade. Peripheral resistance (arterial pressure/regional flow) was decreased significantly by ganglionic blockade in the carotid and renal areas but not in the superior mesenteric and hindquarter (terminal aortic) areas. This suggests the presence of sizable vasoconstrictor tone to resistance vessels in the carotid and renal areas but not in the superior mesenteric and hindquarter areas in the acute spinal rat. This distribution of vasoconstrictor tone is similar to that in intact conscious rats in the resting state and suggests the possibility that the vasoconstrictor tone for resistance vessels in intact rats is also for the most part generated in the spinal cord. Spinal transection decreased blood flow in all the four regions, suggesting a decrease in cardiac output due to dilation of capacitance vessels. It is possible that, in intact rats at rest, the so-called medullary vasomotor center is sending tonic impulses for the most part to capacitance vessels.

Key words: vasomotor center, spinal vasomotor tone.

It is known, since GOLTZ (1864), that the spinal cord can be a source of tonic vasoconstrictor impulses. That this is also true in the rat is exemplified by the observation that the plateau level of arterial pressure that has recovered partially from the shock level within 1 h after high spinal transection is lowered again by ganglionic blockade (IRIUCHIJIMA and NUMAO, 1977). The spinal sympathetic tone is apparently greater than that estimated from the observed change of pressure, because of the compensatory secretion of vasopressin in pressor amounts: Injection of vasopressin antagonist lowers arterial pressure further (IRIUCHIJIMA, 1983).

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In a preceding study we estimated the regional distribution of vasoconstrictor tone in conscious rats (IRIUCHIJIMA and SAKATA, 1985). An electromagnetic flow probe was implanted around various arteries and a catheter for direct measurement of arterial pressure was inserted via a femoral artery. Percent decrease in peripheral resistance calculated as arterial pressure divided by flow was used to estimate the vasoconstrictor tone for regional resistance vessels. It was suggested that resting vasoconstrictor tone in conscious rats was not uniformly distributed but localized in the carotid and renal regions. Tone to the hindquarter and superior mesenteric areas was estimated to be immaterial in most rats.

One might doubt the validity of the above method of assessing the presence or absence of vasomotor tone using flow and pressure measurement and ganglionic blockade. However, the presence of abnormal tone in conscious spontaneously hypertensive rats (SHR; OKAMOTO and AOKI, 1963) first claimed by this method (IRIUCHIJIMA, 1985) has recently been confirmed by a different method (IRIUCHIJIMA, 1990). We consider that this has added reliability to the method with decrease in peripheral resistance on ganglionic blockade as proof for the presence of regional vasoconstrictor tone.

How is then the regional difference in vasoconstrictor tone in conscious rats with the spinal cord intact sustained? One possibility would be that supraspinal neuron pools sending out descending fibers to different spinal segments have different levels of activity. However, in the present study on spinal vasomotor activities, we obtained results which suggest the possibility that the difference is sustained for the most part as a spinal mechanism.

METHODS

Implantation of flow probes and catheters. In male Wistar rats 10–20 weeks of age weighing 250–450 g, an electromagnetic flow probe (Nihon Kohden type FC with an internal diameter of 1, 1.5, or 2 mm) was implanted around the right common carotid artery, superior mesenteric artery, left renal artery, or terminal aorta under anesthesia with thiamylal sodium (50 mg/kg, i.p.). The technical details are described elsewhere (KAWAUE and IRIUCHIJIMA, 1984). Except in rats with a probe around the terminal aorta, a polyethylene catheter for arterial pressure measurement was inserted into the right femoral artery so that the tip was placed in the terminal aorta. The arterial catheter was inserted into the left common carotid artery in rats with a probe on the terminal aorta. In all rats another catheter for injection of drugs was inserted into the right external jugular vein.

Spinal cord transection. After implantation, the rat was kept separately in a polyethylene cage containing wood chips. Three to 4 days later, flow and pressure were measured in the conscious, resting state in the home cage and then the rat was anesthetized with ether. In the rat, placed on the abdomen, a midline skin incision was made in the dorsal neck. The spinous process of the vertebra prominens was cut and removed. Through the space thus formed the spinal cord was transected

above the vertebra prominens with an ophthalmological scalpel under visual control.

In the rats, the vertebra prominens is not the 7th cervical as in man but the 2nd dorsal. Therefore, by the above method, the cord was transected between the 1st and 2nd dorsal vertebrae. Since this level of transection might leave some spinal segments innervating the area supplied by the common carotid below the transection, transection was made two segments higher, i.e., between the 6th and 7th cervical vertebrae in 7 more rats with the probe at the carotid artery for comparison.

A local anesthetic xylocaine jelly was applied to the wound made for transection and the skin was sutured. The ether was removed, the rat was released and returned to the home cage. Throughout these procedures and thereafter, flow and pressure were being recorded continuously.

Estimation of spinal vasoconstrictor tone. One hour after transection, with the rat in the home cage, injection of hexamethonium bromide (2.5% solution), at a rate of 0.8 mg/min for a total dose of 25 mg/kg, was made. The percent decrease in peripheral resistance calculated as arterial pressure divided by flow was used as a measure of spinal sympathetic vasoconstrictor tone for resistance vessels of the particular region. Since hexamethonium induces secretion of pressor amounts of vasopressin in spinal rats (IRIUCHIJIMA, 1983), a vasopressin antagonist, $d(CH_2)_5Tyr(Me)$ arginine vasopressin (Manning compound) was injected as a bolus (0.01 mg/kg) a few minutes after completion of the injection of hexamethonium to offset the vascular effect of this hormone.

Statistical treatment. Values are expressed as mean $\pm$ standard deviation. The paired *t*-test was used to determine the significance of successive change of a variable.

RESULTS

Figure 1 shows simultaneous recording of mean arterial pressure and carotid flow in a rat about 1 h after spinal cord transection. Intravenous infusion of hexamethonium induced a lowering of arterial pressure and a slight increase in carotid flow. The following injection of vasopressin antagonist lowered arterial pressure further with a slight decrease in carotid flow. The values of pressure and flow were read from the recording immediately before and after the infusion of hexamethonium as well as 5 min after injection of vasopressin antagonist. Calculated peripheral resistance (*r*) is entered in the figure, which decreased on ganglionic blockade and decreased further after injection of vasopressin antagonist.

Successive changes in flow in each artery and simultaneously recorded pressure, starting from the intact state through spinal transection, ganglionic blockade, and further injection of vasopressin antagonist, are tabulated in Table 1. After spinal transection all the mean flows decreased and the decreases were significant by the paired *t*-test except for the hindquarter flow.

In Fig. 2 the percent changes of regional resistance after ganglionic blockade (clear columns) and that after ganglionic blockade + injection of vasopressin

Fig. 1. Simultaneous recording of arterial pressure (AP) and carotid flow (CF) in a rat about one hour after high spinal transection (CS). Hexamethonium bromide (C6) was infused for the underlined period to a total dose of 25 mg/kg. At the arrow vasopressin antagonist (VPA) was injected at 0.01 mg/kg. *r*: calculated peripheral resistance in mmHg/(ml/min · 100 g body weight).

Table 1. Regional flows and arterial pressure before and 1 h after high spinal transection and after further hexamethonium and vasopressin antagonist injections.

Variables	<i>n</i>	Intact	CS	C6	VPA
Carotid flow	5	1.47 ± 0.34	0.89 ± 0.28*	0.97 ± 0.26	0.88 ± 0.23
Sup. mes. flow	7	4.54 ± 0.73	2.84 ± 1.00**	2.44 ± 0.70	2.30 ± 0.66
Renal flow	7	1.90 ± 0.31	1.52 ± 0.33*	1.52 ± 0.33	1.43 ± 0.44
Hindq. flow	6	4.91 ± 1.22	3.96 ± 1.67	3.23 ± 1.05	2.96 ± 1.30
Arterial p.	25	113 ± 9	97 ± 13	82 ± 10	68 ± 10

Flow in ml/(ml · 100 g body weight) and pressure in mmHg. *n*, number of rats; CS, 1 h after spinal transection (cord section) at Th 1; C6, after injection of hexamethonium bromide at 25 mg/kg; VPA, 5 min after further injection of vasopressin antagonist at 0.01 mg/kg. Mean ± S.D. * Indicates significant decrease of flow from the intact value by the paired *t*-test at *p* < 0.01, ** at *p* < 0.005.

antagonist (shaded columns), both in reference to the resistance before blockade, are graphically presented. Peripheral resistance decreased significantly after ganglionic blockade in the carotid and renal areas. These changes were more marked after further injection of vasopressin antagonist. However, in the superior mesenteric and hindquarter areas, peripheral resistance did not change significantly after ganglionic blockade. Even further injection of vasopressin antagonist did not induce appreciable change in peripheral resistance in these two areas.

Changes in carotid flow and arterial pressure after spinal transection between the 6th and 7th cervical vertebrae and following ganglionic blockade and vasopressin antagonist injection are presented in Table 2. By and large these variables were similar to those after transection at Th 1 (Table 1). The percent decrease in carotid resistance after ganglionic blockade was $-14.5 \pm 15.2\%$, significant at *p* < 0.1 and

Fig. 2. Percent decrease in peripheral resistances after ganglionic blockade with hexamethonium bromide (25 mg/kg, clear columns) and after further injection of vasopressin antagonist (0.01 mg/kg, shaded columns), both in reference to the value before hexamethonium. n in parenthesis. Mean $\pm$ S.D. Decimals: significant levels by paired t -test.

Table 2. Carotid flow and arterial pressure before and after spinal transection between the 6th and 7th cervical vertebrae, two segments higher than before.

Variables	Intact	CS	C6	VPA
Carotid flow	1.44 ± 0.43	$1.08 \pm 0.33^{**}$	1.03 ± 0.30	1.04 ± 0.27
Arterial p.	109 ± 6	98 ± 15	80 ± 15	68 ± 11

$n=7$. See Table 1 for abbreviations, units, and symbols.

not at the usual significant level of $p < 0.05$, but that after ganglionic blockade + vasopressin antagonist injection was $-27.5 \pm 10.8\%$, significant at $p < 0.001$. This indicated the presence of vasoconstrictor tone in the carotid area after spinal transection at C6 as at Th 1.

DISCUSSION

On the assumption that decreases in peripheral resistance on ganglionic blockade indicate the presence of vasoconstrictor tone, in acute spinal rats, sizable tone was present in the carotid and renal areas but not in the superior mesenteric and hindquarter areas. This uneven distribution of vasoconstrictor tone was similar to that observed in intact rats (IRIUCHIJI and SAKATA, 1985).

It is possible that the vasoconstrictor tone is sustained spinally even in intact

rats. Otherwise, the distribution of tonic activity over the supraspinal neuron pools controlling the respective spinal vasomotor neuron pools for regional vascular beds should coincide with that over the spinal ones when separated from the supraspinal influence. It seems to us that the latter possibility is less likely. In both carotid and renal areas, the decrease in peripheral resistance in spinal rats on ganglionic blockade + vasopressin antagonist injection was similar to that in intact rats on ganglionic blockade (IRIUCHIJIMA and SAKATA, 1985). This suggests that the magnitude of the tonic discharge was comparable.

One might think that we should obtain the same result without regional differences in vasoconstrictor tone if the ganglionic blocker hexamethonium bromide specifically dilated resistance vessels in the carotid and renal areas. However, after regional sympathectomy, the regional resistance in these areas did not decrease significantly on ganglionic blockade (IRIUCHIJIMA and SAKATA, 1985). This also excludes renal autoregulation as the cause of the decrease in renal resistance to ganglionic blockade. If it were due to autoregulation, it should survive the denervation. We are skeptical about the presence of such autoregulation of renal blood flow as observed by REIN (1931) in anesthetized dogs with his Thermoströmuhr in conscious rats. In anesthetized dogs, too, LANGSTON *et al.* (1960) have observed that a change in perfusion pressure always results in a corresponding change in renal blood flow when the kidney was not subjected to surgical trauma.

Injection of vasopressin antagonist does not change arterial pressure significantly in intact rats before and after ganglionic blockade or in spinal rats before ganglionic blockade but decreases it significantly in spinal rats after ganglionic blockade (IRIUCHIJIMA, 1983). This finding suggests that ganglionic blockade increases the secretion of vasopressin in spinal rats, which may offset the effect of ganglionic blockade to decrease regional resistance by blocking sympathetic vasoconstrictor impulses. When the resistance after further administration of the vasopressin antagonist was compared to the resistance before ganglionic blockade, the mean percent decrease was greater in the carotid and renal areas than the respective decrease by ganglionic blockade alone. However, the changes were still insignificant in the superior mesenteric and hindquarter areas (Fig. 2). The observed regional difference in sensitivity of peripheral resistance to vasopressin antagonist may have reflected the fact that peripheral vessels are most sensitive to vasopressin in the kidney and skin (PAGE, 1987). The proportion of cutaneous flow to the total flow should be far greater in the carotid than in the terminal aorta.

TAYLOR and SCHRAMM (1987) and OSBORN *et al.* (1987) have observed that, in anesthetized rats, spinal transection reduces lumbar sympathetic activity and increases renal activity. Their observation may be interpreted to indicate that there is sizable tone in the hindquarters before transection and that renal tone is greater after transection than before. This apparent discrepancy with our results seems to be explained by assuming that anesthesia excites lumbar sympathetic activity and inhibits renal activity (IRIUCHIJIMA and SAKATA, 1985).

The level of spinal transection between the 1st and 2nd dorsal vertebrae might

have been inappropriate for the study of the spinal vasomotor tone to the vascular area supplied by the carotid artery: Some segments supplying this area might have been left above the transection. We repeated the experiment with the carotid flow in 7 more rats by transecting the spinal cord 2 segments higher, between the 6th and 7th cervical vertebrae. Qualitatively, we obtained the same result for the presence of vasoconstrictor tone in the carotid area. Although the decrease in carotid resistance by ganglionic blockade alone was not significant ($0.05 < p < 0.1$), that after further administration of the vasopressin antagonist was highly significant ($p < 0.001$). It seems that the majority of spinal preganglionic neurons supplying the carotid area are present below Th 1.

After spinal transection the mean value of the regional flow decreased in all the four arteries examined (Table 1). This suggests that cardiac output decreased after spinal transection and further that capacitance vessels were receiving sizable tone from the supraspinal level. A marked decrease in cardiac output on spinal transection has been reported by YARDLEY *et al.* (1989) in anesthetized cats.

Admitting that the tone to the carotid and renal areas are sustained at the spinal level even before spinal transection, we may say that, in intact conscious rats at rest, the so-called medullary vasomotor tone is principally directed to capacitance vessels, not to resistance vessels.

In summary, ganglionic blockade with hexamethonium bromide in rats 1 h after high spinal transection significantly decreased peripheral resistance in the carotid and renal areas but not in the superior mesenteric and hindquarter areas. This suggests, as in intact rats, vasoconstrictor tone is abundant in the former areas but not in the latter areas in acute spinal rats.

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