Cutaneous vascular bed is not involved in arterial pressure changes elicited by increasing or decreasing the activity of inhibitory vasomotor neurons in caudal ventrolateral medulla in rabbits

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Received 6 June 2000; accepted 6 July 2000

Abstract

We determined whether caudal ventrolateral medulla (CVLM) vasodepressor neurons tonically inhibit vasomotor tone in the ear in anesthetized rabbits. Injection of 1-glutamate (10 nmol in 100 nl) into the CVLM decreased arterial pressure and increased superior mesenteric conductance. Ear conductance decreased (0.43 ± 0.06 to 0.33 ± 0.05 cm s⁻¹ per mmHg, n = 15 injections, 12 rabbits, P < 0.01). Conversely, bilateral injection of γ-aminobutyric acid (100 nmol in 100 nl) increased arterial pressure and decreased superior mesenteric conductance. At the same time ear conductance increased (0.39 ± 0.09 to 0.48 ± 0.27 cm s⁻¹ per mmHg, n = 8 injections, eight rabbits, P < 0.05). Results suggest that ear vessels are not tonically inhibited by the CVLM vasodepressor neurons. Presympathetic motoneurons regulating cutaneous flow may be excited, rather than inhibited, by the CVLM neurons. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Caudal ventrolateral medulla; Vasomotor neurons; Arterial pressure

The caudal ventrolateral medulla (CVLM) contains inhibitory vasomotor neurons; activation of these neurons causes a fall in arterial pressure and, conversely, inhibition of the neurons causes a rise in arterial pressure, changes mediated by appropriate alteration in peripheral sympathetic vasomotor tone [2]. The question arises as to which peripheral vascular beds are involved in these changes in sympathetic vasomotor activity. Electrophysiological recordings from post-ganglionic sympathetic nerves indicate that changes in renal vasomotor tone contribute to the arterial pressure changes, and similar recordings from the lumbar sympathetic chain confirm that hindlimb vessels (presumably principally skeletal muscle vessels) are also involved [1,5,8,9]. Measurement of regional blood flow confirms that appropriate changes in hindlimb and renal vascular tone contribute to the changes in arterial pressure evoked from the CVLM [9]. In addition, flow studies in rat show that the superior mesenteric bed is involved in arterial pressure changes evoked from both the CVLM and the rostral ventrolateral medulla (RVLM), [9] and our recent study in the rabbit confirms the role of the RVLM in regulation of the mesenteric bed [3].

Our rabbit studies [3,4] also show that blood flow to the rabbit ear pinna, a predominantly cutaneous bed, is principally controlled by presympathetic motoneurons in the raphe/parapyramidal region. In our present experiments in rabbits we have determined whether focal excitation or inhibition of the CVLM vasomotor neurons alters ear pinna blood flow in the manner expected on the hypothesis that decrease in vasomotor tone in the cutaneous bed contributes to decreases in arterial pressure elicited from the CVLM and conversely. We injected excitatory and inhibitory amino acids into the CVLM and compared blood flow changes in the ear with simultaneously measured changes in the superior mesenteric artery.

New Zealand White rabbits (n = 17, 2.2–3 kg) were anesthetized with fentanyl citrate (0.1 mg/kg (i.m.)) and fluanisone (3 mg/kg (i.m.)) and midazolam (2 mg/kg (i.m.)) and prepared with Doppler ultrasonic flow probes (Iowa Doppler Products, IA) chronically implanted around the superior mesenteric artery, with wires left subcutaneously in the cervical region [3]. Animals were allowed to recover and remained in the animal house for at least 1 week. Animals were then anesthetized with urethane (1.25–
1.5 g/kg (i.v.) infused over 30 min), a tracheostomy was performed and animals were paralyzed with vecuronium bromide (0.75 mg/kg (i.v.)) and mechanically ventilated with oxygen so that peak CO₂ was between 30 and 35 mmHg. Rectal temperature was kept at 38.5–39.5°C. A second Doppler probe was positioned around the central ear artery to record ear pina blood flow. The urethane was sometimes supplemented with halothane to increase baseline ear pina flow [3]. A catheter was inserted into one femoral artery for recording arterial pressure and

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>AP (mmHg)</th>
<th>Ear conductance (cm⁻¹ mmHg⁻¹)</th>
<th>Mesenteric conductance (cm⁻¹ mmHg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>l-glu</td>
<td>GABA</td>
<td>l-glu</td>
</tr>
<tr>
<td>Pre-inject</td>
<td>80 ± 5</td>
<td>85 ± 9</td>
<td>0.43 ± 0.06</td>
</tr>
<tr>
<td>Post-inject</td>
<td>94 ± 4*</td>
<td>97 ± 9**</td>
<td>0.33 ± 0.05*</td>
</tr>
<tr>
<td>n</td>
<td>15 (12)</td>
<td>15 (12)</td>
<td>6</td>
</tr>
</tbody>
</table>

* Numbers of injections and numbers of rabbits (brackets) for each experimental condition are given. Significantly different from pre-injection value, *P < 0.05; **P < 0.01.

Fig. 1. Ear and superior mesenteric Doppler signals and arterial pressure changes evoked by (A) unilateral injection of l-glutamate (10 nmol in 100 nl) or by (B) bilateral injection of GABA (100 nmol in 100 nl) into the caudal ventrolateral medulla (CVLM). The conductance traces were obtained by dividing the mean flow signal by the mean arterial pressure signal and normalizing the pre-injection value to 100%.
heart rate (HR). The flow probes were connected to a Triton Technologies (San Diego, CA) flowmeter and the analogue output was digitized (40 Hz sampling rate) and displayed using MacLab (ADInstruments, Sydney, Australia) and an Apple Macintosh G3 computer. Other data obtained from some of the present rabbits have been used in another paper [3].

The rabbit's head was held in a Kopf device with the neck flexed, and the dorsal surface of the medulla was exposed by incision and retraction of the atlanto-occipital membrane. L-glutamate (10 nmol in 100 nl, unilateral injections lasting 3–4 s) or γ-aminobutyric acid (GABA) (100 nmol in 100 nl, sequential bilateral injections using a single micropipette, each injection 3–4 s, and total injection period approximately 1 min) was injected into the CVLM using glass micropipettes and a hand held air pressure injection system. The volume of injectate was monitored by using the operating microscope to observe the movement of the fluid meniscus. Coordinates for injection were 0.75 mm caudal to the rostral border of the area postrema in the midline, 3.0 mm lateral from the midline and 3.0 mm below the dorsal medullary surface at the point of entry of the pipette. Either horseradish peroxidase or Beta-galactosidase was included with the injectate to mark either the L-glutamate or the GABA injection site. The enzymes were detected by the DAB procedure and by the XGAL reaction, respectively. All chemicals were from Sigma Chemical Co., USA.

The cardiovascular effect of the L-glutamate injections was assessed as the maximal change occurring approximately 15 s after the unilaterlal injection. The cardiovascular effect of the GABA injections was assessed as the maximal change occurring approximately 90 s after the second of the bilateral injections.

CVLM injection of L-glutamate decreased arterial pressure (Table 1). As documented in Fig. 1A and Table 1, superior mesenteric conductance increased as arterial pressure fell, and decreased as arterial pressure increased, in accordance with the idea that conductance in this bed contributes to the changes in arterial pressure. These changes contrasted with those observed for ear conductance. As arterial pressure fell in response to injection of L-glutamate, ear flow also fell (Fig. 1A), to a degree that indicated an overall decrease in ear conductance, rather than the expected increase (Table 1).

CVLM injection of GABA increased arterial pressure (Table 1). As shown in Fig. 1B and Table 1, as arterial pressure rose superior mesenteric conductance decreased, indicating that vasoconstriction in this bed contributes to the rise in arterial pressure. A contrasting change in ear flow was again observed. As arterial pressure increased ear flow also increased, to the extent that ear vascular conductance was increased by the GABA injection, not decreased (Fig. 1B and Table 1).

An example of a CVLM injection site, defined by the spread of horseradish peroxidase reaction product, is shown in Fig. 2.

Our present results in rabbits, taken together with previous findings in both rats and rabbits, indicate that changes in superior mesenteric vascular tone contribute to changes in arterial pressure evoked by alteration of neuronal function in either the CVLM or the RVLM. The vasomotor effects elicited from the CVLM are thought to be mediated by an inhibitory GABAergic projection to the RVLM [11]. Our present superior mesenteric results thus fit well with the hypothesis that CVLM vasomotor neurons tonically inhibit superior mesenteric presympathetic motoneurons in the RVLM.

The superior mesenteric results contrast with those obtained for the ear vascular bed. Vasomotor neurons in the CVLM appear to regulate ear flow quite differently to the manner in which they regulate mesenteric flow. L-glutamate and GABA injections into the CVLM altered ear pinna vascular conductance in a manner opposite to that expected on the hypothesis that changes in the caliber of the vessels in this bed contribute to the accompanying changes in arterial pressure. It thus seems most unlikely that the CVLM vasoconstrictor neurons have a strong tonic inhibitory influence on the relevant presympathetic neurons regulating the ear pinna bed. Indeed it appears that the CVLM neurons, presumably via indirect connections, have a small tonically excitatory influence on the ear vascular presympathetic

![Fig. 2. Diagram of a transverse section through the CVLM injection site region, with the injection site (arrow) marked by the peroxidase-DAB reaction. Abbreviations: AP, area postrema; cc, central canal; dmnoX, dorsal motor nucleus of the vagus; io, inferior olive; LRN, lateral reticular nucleus; nTS, nucleus tractus solitarius; p, pyramidal tract; TS, tractus solitarius; Vap, spinal nucleus of the trigeminal nerve; Vsp, spinal tract of the trigeminal nerve; XII, hypoglossal nucleus.](image-url)
neurons. The present results do not bear directly on the
neuroanatomical location of these cells, but our findings
are consistent with the idea that they might be located
outside the RVLM. As noted in the introduction, other
evidence from our laboratory suggests that the ear vascula-
ture is controlled principally from the raphe/parapyramidal
region rather than from the RVLM.

Experiments in rats also indicate that the CVLM vasode-
pressor neurons do not appear to tonically inhibit the RVLM
presympathetic motoneurons responsible for secretion of
epinephrine from the adrenal medulla [7]. Combined
_Phaseolus vulgaris_ and transneuronal viral tracing studies
from our laboratory do suggest that there is a direct projec-
tion from the CVLM region to RVLM presympathetic adre-
nal motoneurons in this species [6], so that the appro-
priate functional experiments in the rabbit should prove most
interesting.

Our present data add to a growing body of evidence that
the brainstem regulation of cutaneous blood vascular tone is
anatomically and physiologically separate from the cor-
responding regulation of renal, mesenteric and skeletal muscle
vascular tone. The CVLM vasodepressor neurons have a
major tonically active inhibitory effect on the vascular
tone of these three latter beds, but this tonic inhibition
does not extend to the cutaneous bed.

Our research was supported by the National Health and
Medical Research Council, by the National Heart Founda-
tion of Australia and by the Neurosurgical Research Foun-
dation of South Australia. Mr Joseph Garcia, Ms Robyn
Flook and Ms Sarah Kennedy provided technical assistance.

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