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Short Communication

Inhibition of medullary raphé/parapyramidal neurons prevents cutaneous vasoconstriction elicited by alerting stimuli and by cold exposure in conscious rabbits

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Abstract

In conscious rabbits, microinjection of muscimol into the medullary raphé/parapyramidal region decreased fluctuation (coefficient variation) of resting ear blood flow (from 62 ± 8 to 25 ± 4%, $P < 0.01$, $n = 8$). The muscimol injection also prevented falls in ear blood flow that normally occur in response to alerting stimuli and to cold exposure. Thus, raphé/parapyramidal neurons constitute an important brainstem center for mediating cutaneous vasoconstriction initiated by alerting stimuli and by cold exposure.

Theme: Endocrine and autonomic regulation
Topic: Cardiovascular regulation

Keywords: Ear pinna blood flow; Muscimol; Emotional stress; Thermoregulatory stress

Cutaneous vasoconstriction is part of the patterned cardiovascular response to alerting stimuli [17,18] and to cold exposure [11]. It is already established that medullary raphé/parapyramidal neurons play an important role in cutaneous vasoconstriction initiated by thermoregulatory stimuli [2,13,16]. The question arises as to whether medullary raphé/parapyramidal neurons also regulate alerting-related cutaneous vasoconstriction. In the present study, we have investigated this issue in conscious rabbits.

Male New Zealand White rabbits ($n = 8$) weighing 2.2–2.7 kg were used. One week before the experiments, an ultrasonic Doppler flow probe (Iowa Doppler Products, IA, USA) was implanted around one ear pinna artery [14]. Under halothane anesthesia, with the animal in a Kopf stereotaxic apparatus, the tip of a metal guide cannula was positioned in the fourth ventricle in the midline [10]. A rectangular shaped hole (1-mm wide laterally, 4-mm long rostrocaudally) was drilled between 5 and 9 mm posterior to lambda. The sterilized guide cannula (20–25 mm long) made from a 27 G spinal needle (Whitacre Needle, BD Medical Systems, NJ, USA) was inserted into the cerebellum until cerebral spinal fluid came out confirming that the cannula tip was set in the fourth ventricle, and the cannula was fixed in position with dental cement. The insertion point and the angle of the guide cannula were calculated on the basis of the length between bregma and lambda and of the relative angle of the bregma–lambda plane to the horizontal stereotaxic frame. If the bregma–lambda plane is horizontal and the length between bregma–lambda is 19 mm, the cannula is inserted in the midline 7.2 mm posterior to lambda at an angle of 22° from the vertical (with the tip rostral). After fixing the guide cannula in place, a stilette was inserted and advanced until the distal end touched the basi-occiput and the distance was recorded. On the day of the experiment, in conscious rabbits, intramedullary injections of Ringer or 3–5 nmol muscimol in 300–500 nl of

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Ringer were made through a silastic tube [18] inserted through the guide cannula and advanced to a point 1.5 mm less than the previously determined distance to the basi-occiput, so that the tip was positioned approximately 1 mm dorsal to the ventral surface of the medulla oblongata. Either horseradish peroxidase (Sigma-Aldrich, NSW, Australia) or β-galactosidase was included with the injection solution.

On the day of the experiment, the animal was placed in a small drape-covered cage at room temperature (25–28 °C). A series of standardized alerting stimuli were applied at intervals of approximately 2 min (a brief sound, cage tap, sudden 1 cm drop of cage, sideways movement of the cage, touching of the animals fur, removing the drape covering the cage and a pinprick (see details in [8,9,18])) were applied 30 min before intramedullary injection of Ringer or muscimol and 30 min after the injection. The Doppler flow signal, continuously recorded, was digitized and analyzed with PowerLab (ADInstruments, Castle Hill, Australia) and Chart and IgorPro (Wavemetrics, Oregon, USA) software. For characterization of basal ear pinna blood flow at rest, we calculated the mean and the coefficient of variation of the mean blood flow of a control 10-min period before the first alerting stimulus and for the period 20–30 min after muscimol injection. To assess the effect of alerting stimuli on ear pinna blood flow, before and after administration of alerting stimuli, we calculated an index based on flow values immediately pre- and post-administration of the different alerting stimuli [8]. A larger flow response index indicates a greater alerting-induced fall in flow. Previous studies in our laboratory have established the fall in ear pinna blood flow normally elicited by exposure to a cold (5–10 °C) environment in conscious rabbits [11]. In the current study, core (body) temperature was measured rectally before and after cold exposure.

Group data were analyzed by repeated measures analysis of variance, with comparison of post-injection or -cooling values with pre-injection or -cooling values. Fisher's protected t-test was use to determine significant differences, with the significant threshold set at the 0.05 level. All data were showed mean ± S.E.M.

The effect of cold exposure was then assessed by transferring the animal (in its cage) from room temperature to a 5–10 °C environment [12]. The animal remained in the cold environment for 30 min and was then returned to the room temperature environment for an additional 30 min. Body temperature was measured by temporary insertion of a thermometer into the rectum before and after the cold exposure. The animal was then deeply anaesthetized with pentobarbitone (80 mg/kg i.v.), and the brain was fixed by transcardiac perfusion of a mixture of formaldehyde (final concentration, 4%) and glutaraldehyde (final concentration, 0.5%) solution. The brain was removed for histological demonstration of injection sites by visualization of β-galactosidase or HRP reaction products.

Injection of Ringer did not affect either resting ear pinna flow or alerting-related fall in ear pinna blood flow (Table 1). After intramedullary injection of muscimol (n = 8), rabbits appeared grossly normal, with normal breathing. Resting ear blood flow increased within 5 min of the muscimol injection and stabilized at a higher level (Fig. 1A and Table 1). Fluctuations in ear blood flow in the resting condition were dramatically reduced, so that the coefficient of variation for mean blood flow was reduced from 62 ± 8% before muscimol to 25 ± 4% after muscimol (Table 1). The sudden falls in ear pinna blood flow normally elicited by alerting stimuli were substantially reduced after muscimol (Fig. 2 and Table 1).

After the alerting stimuli experiment, rabbits were transferred from the room temperature environment to the cold environment. Muscimol injection entirely prevented the fall in ear pinna blood flow normally elicited by cold exposure, as demonstrated in Fig. 1A. Mean ear pinna blood flow for the period 10–20 min after transfer to the cold cage was 53 ± 3 cm/s, which is significantly greater than 7 ± 3 cm/s ($P < 0.01$, $n = 5$) of the corresponding value in Ringer-treated animals, and not significantly different from the pre-transfer value of 52 ± 3 cm/s ($P > 0.05$, n = 8) (Table 2). During the 30-min cold exposure after injection of muscimol, core body temperature decreased from 39.0 ± 0.1 to 37.8 ± 0.2 °C ($P < 0.05$, $n = 4$), which was lower than 39.2 ± 0.1 °C, the corresponding value of Ringer-treated animals ($P < 0.01$, n = 4) (Table 2).

An example of muscimol injection sites marked in this case by HRP reaction product is shown in Fig. 3. The injection sites were within 1 mm lateral from the ventral midline medulla between the levels of the rostral half of the inferior olive and of the caudal half of the facial nuclei and the injectate spread between the levels. The injectate tended to spread ventrolaterally around the pyramidal tracts and dorsally along the electrode track.

Table 1

	Muscimol		Ringer	
	Pre-injection	Post-injection	Pre-injection	Post-injection
(a) Resting ear pinna flow signal (cm/s)				
	26 ± 4 (8)	54 ± 7 (8)*	60 ± 18 (5)	60 ± 16 (5)[a]
(b) Coefficient of variation for ear pinna blood flow (%)				
	62 ± 8 (8)	25 ± 4 (8)*	36 ± 10 (5)	34 ± 13 (5)[a]
(c) Fall in blood flow by alerting stimuli (flow response index, %)				
Sound	50 ± 6 (8)	6 ± 2 (8)*	63 ± 3 (5)	57 ± 5 (5)[a]
Tap cage	60 ± 4 (8)	10 ± 3 (8)*	71 ± 1 (5)	68 ± 3 (5)[a]
Drop cage	54 ± 5 (8)	7 ± 2 (8)*	70 ± 6 (5)	67 ± 3 (5)[a]
Move cage	60 ± 6 (8)	5 ± 1 (8)*	69 ± 5 (5)	66 ± 5 (5)[a]
Touch fur	67 ± 3 (8)	8 ± 4 (8)*	72 ± 4 (5)	67 ± 3 (5)[a]
Remove drape	70 ± 3 (7)	9 ± 4 (7)*	73 ± 5 (5)	65 ± 2 (5)[a]
Pinprick	67 ± 4 (8)	22 ± 7 (8)*	70 ± 5 (5)	68 ± 4 (5)[a]

Value in brackets is the number of rabbits.

[a] $P > 0.05$ not significantly different from values for pre-injection.

* $P < 0.01$ significantly different from values for pre-injection.