Potential Role of Medullary Raphe-Spinal Neurons in Cutaneous Vasoconstriction: An In Vivo Electrophysiological Study

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Nalivaiko, Eugene and William W. Blessing. Potential role of medullary raphe-spinal neurons in cutaneous vasoconstriction: an in vivo electrophysiological study. *J Neurophysiol* 87: 901–911, 2002; 10.1152/jn.00221.2001. In rabbits, raphe magnus/pallidus neurons form a link in the CNS pathway regulating changes in cutaneous blood flow elicited by nociceptive stimulation and activation of the central nucleus of the amygdala. To characterize relevant raphe-spinal neurons, we performed extracellular recordings from the rostral medullary raphe nuclei in anesthetized, paralyzed, mechanically ventilated rabbits. All studied neurons were antidromically activated from the dorsolateral funiculus of the spinal cord (C₆–T₂). Of 129 studied neurons, 40% were silent. The remaining neurons discharged spontaneously at 0.3–29 Hz. Nociceptive stimulation (lip squeeze with pliers) excited 63 (49%), inhibited 9 (7%), and did not affect 57 (44%) neurons. The same stimulation also elicited falls in ear pinna blood flow. In neurons activated by the stimulation, the increase in discharge preceded the fall in flow. Electrical stimulation of the spinal trigeminal tract excited 61/63 nociception-activated neurons [onset latencies range: 6–75 ms, mean: 28 ± 3 (SE) ms], inhibited 9/9 nociception-inhibited neurons (onset latencies range: 9–85 ms, mean: 32 ± 10 ms), and failed to affect 55/57 neurons insensitive to nociceptive stimulation. Neurons insensitive to nociceptive/trigeminal stimulation were also insensitive to nonnociceptive tactile stimulation and to electrical stimulation of the amygdala. They were either silent (32/45) or discharged regularly at low frequencies. They possessed long-duration action potentials (1.26 ± 0.08 ms) and slow-conducting axons (6.0 ± 0.5 m/s). These neurons may be serotonergic raphe-spinal cells. They do not appear to be involved in nociceptive-related cutaneous vascular control. Of the 63 neurons sensitive to nociceptive and trigeminal tract stimulation, 35 also responded to tactile stimulation (wide receptive field). These neurons possessed short action potentials (0.80 ± 0.03 ms) and fast-conducting axons (30.3 ± 3.1 m/s). In this subpopulation, electrical stimulation of the amygdala activated nearly all neurons tested (10/12), with a mean onset latency of 34 ± 3 ms. The remaining 28 neurons sensitive to nociceptive and trigeminal stimulation did not respond to tactile stimuli and were mainly unaffected by amygdala stimulation. It may be that fastconducting raphe-spinal neurons, with wide multimodal receptive fields and with input from the central nucleus of the amygdala, constitute the bulbospinal link in the CNS pathway regulating cutaneous blood flow in response to nociceptive and alerting stimuli.

**INTRODUCTION**

Functional roles of bulbospinal raphe neurons in the rostral medulla oblongata are still being determined (see Lovick 1993; Mason 2001 for reviews). Fields and colleagues (Behbehani and Fields 1979; Fields and Anderson 1978; Fields et al. 1977) suggested that subgroups of these neurons receive inputs from the periaqueductal gray and relay them to the spinal dorsal horn, thereby suppressing transmission of nociceptive inputs at the spinal level. Electrophysiological studies have confirmed that medullary raphe neurons change their discharge in response to nociceptive stimulation that activates the tail-flick response in rats (Anderson et al. 1977; Vanegas et al. 1984). A cardiovascular role has also been proposed for the medullary raphe region, with microstimulation studies reporting both increases and decreases in arterial pressure and sympathetic outflow (Haselton et al. 1988a; McCall 1984; Yusof and Coote 1988; Zhou and Gilbey 1995). Some electrophysiological studies of possible cardiovascular roles of bulbospinal raphe neurons have concentrated on neurons with cardiac rhythmicity (e.g., McCall and Clement 1988; Morrison and Gebber 1984, 1985; Pilovsky et al. 1995) and even in such studies, the possible cardiovascular role of the neurons has proven complex. Subclasses of medullary raphe-spinal neuron also appear to function as a key brain stem temperature control center, regulating the activity of the peripheral sympathetic nerves innervating brown fat (Morrison 1999; Morrison et al. 1999) and the tail circulation in rats (Rathner and McAllen 1999).

In rabbits, the spinal projections of neurons in the rostral medullary raphe (see Blessing and Nalivaiko 2000 for detailed discussion of anatomy) include the intermediolateral column (Haselton et al. 1988b). Low-intensity focal electrical stimulation of the raphe region in rabbits vigorously constricts the ear pinna, a major cutaneous bed, without affecting the mesenteric bed and without causing any major change in arterial pressure (Blessing et al. 1999; Nalivaiko and Blessing 1999). Similarly, selective cutaneous vasoconstriction occurs in response to nociceptive stimulation (pinching the rabbit’s lip), to electrical stimulation of the spinal trigeminal tract (containing central processes of nociceptive orofacial trigeminal neurons), and to electrical stimulation of the amygdala, a nucleus mediating cutaneous vasoconstriction in response to a salient environmental stimulus (Blessing and Nalivaiko 2000; Nalivaiko and Blessing 2001; Yu and Blessing 1998). A possible physiological role for rostral medullary raphe neurons, one that links nociceptive and cardiovascular func-
Anterior sensory evoked responses, is suggested by our observation that muscimol-mediated inhibition of neuronal function in this area entirely prevents ear pinna vasoconstriction evoked by nociceptive stimulation, by trigeminal tract stimulation, and by amygdala stimulation (Blessing and Nalivaiko 2000; Nalivaiko and Blessing 2001).

Thus bulbospinal medullary raphe neurons may constitute an important lower brain stem link in the descending pathway mediating cutaneous vasoconstriction as an integrated part of the individual’s response to nociceptive or salient stimuli.

Most data concerning electrophysiological properties and sensory fields of potentially nociceptive or cardiovascular raphe-spinal neurons have been obtained in cats (Blair and Evans 1991; Evans and Blair 1993; McCall and Clement 1989; Morrison and Gebber 1985; Yen and Blum 1984) and rats (Chiang and Pan 1985; Lumb and Morrison 1986; Martin et al. 1991; Pilowski et al. 1995; Vanegas et al. 1984; Wessendorf and Anderson 1983; Wessendorf et al. 1981). We have now used extracellular recording techniques to study the electrophysiological properties of these neurons in the anesthetized rabbit, a species in which nociceptive stimulation-induced cutaneous vascular responses are readily detectable (Blessing and Nalivaiko 2000; Yu and Blessing 1999). We antidromically identified raphe-spinal neurons from the dorsolateral funiculus and determined their response to lip squeeze and to electrical stimulation of the spinal trigeminal tract or the central nucleus of the amygdala. Because these stimuli cause cutaneous vasoconstriction by a pathway relaying in the rostral medullary raphe, we expected that similar stimuli would increase the discharge of bulbospinal neurons present in this region.

**METHODS**

Successful recording experiments were conducted in 32 male New Zealand White rabbits weighing 2.5–3.5 kg. All experimental procedures were approved by the Flinders University of South Australia Animal Ethics Committee.

**Surgical procedures**

For measurement of skin blood flow, an ultrasound Doppler flow probe (Iowa Doppler Products, IA) was implanted around the central ear artery under midazolam/hypnorm anesthesia (0.4 mg/kg im, 0.3 ml/kg im, respectively) 7–10 days prior to the experiment. On the day of the experiment, animals were anesthetized with urethane (1.5 g/kg iv over 20–30 min) administered into the marginal ear vein. When adequate anesthesia was established, an endotracheal tube was inserted via a tracheotomy. Arterial pressure was monitored via a catheter inserted in the femoral artery and connected to a pressure transducer (ADInstruments, Sydney, Australia). The Doppler flow signal from the ear artery probe was relayed to a Triton Technologies Flowmeter (San Diego, CA).

The rabbit’s head, with the neck flexed, was fixed in a modified Kopf apparatus, and the medulla oblongata was exposed by incision and retraction of the atlanto-occipital membrane. The position of the head was adjusted so that the dorsal surface of the medulla was horizontal. Animals were paralyzed with vecuronium bromide (0.5 mg/kg iv) and mechanically ventilated with humidified, oxygen-enriched air. A bilateral pneumothorax was induced. End-expiratory CO₂ was monitored and maintained at 35–40 mmHg. Spinal clamps were placed at T₂ to stabilize the vertebral column. A laminectomy was carried out to expose the spinal cord at the level of C₈–T₁. Body temperature was maintained at 38.5–39.5°C.

**Electrical stimulation of the trigeminal tract and the amygdala**

For electrical stimulation of the spinal tract of the trigeminal nerve, the tip of an insulated monopolar stainless steel electrode was positioned 0.5 mm ventral to the dorsal medullary surface, 3 mm lateral from the midline, 0.5–1.0 mm caudal to the midlevel of the area postrema. Proper positioning of the electrode was validated by the occurrence of the trigeminal depressor response (Kumada et al. 1975) associated with vigorous cutaneous vasoconstriction (Yu and Blessing 1998) in response to low-frequency electrical stimulation (5 Hz, 1 ms, 200–300 μA, 10 s train). For assessment of the sensitivity of raphe-spinal neurons to trigeminal stimulation, single pulses of similar amplitude and duration were delivered via the trigeminal electrode at intervals of 3–5 s.

For access to the amygdala, an appropriate burr hole was made using the stereotactic approach described in our previous study (Nalivaiko and Blessing 2001). An insulated monopolar stainless steel electrode was aligned perpendicular to the line connecting lambda and the point located 2 mm above bregma. Electrical stimulation of the amygdala was made 11.5–12.5 mm ventral to the dura, 4.5–5 mm lateral to the midline, 0.5 mm rostral to bregma, in the region containing the central nucleus of the amygdala and the descending outflow pathway (Schwaber et al. 1982). Proper positioning of the electrode was validated by the occurrence of cutaneous vasoconstriction in response to focal electrical stimulation (50 Hz, 1 ms, 300–500 μA, 10 s), as described previously (Nalivaiko and Blessing 2001). For the assessment of sensitivity of raphe-spinal neurons to amygdala stimulation, brief high-frequency trains of electrical pulses (200 Hz, 1 ms, 300–500 μA, 20 ms) were delivered via the amygdala electrode at intervals of 5 s. At the end of the experiment, electrical stimulation sites were marked by an anodal current (50 μA DC for 20 s).

**Electrophysiological recordings**

Single-unit extracellular recordings in raphe magnus/pallidus region were made using tungsten glass-coated microelectrodes (tip diameter: 2–4 μM, impedance: 2–4 MΩ) and a NL102 DC preamplifier (NeuroLog), filtered at 100 Hz to 3 kHz and digitized (sampling rate of 10 kHz) with MacLab (ADInstruments). Recordings sites were limited to the raphe region involved in the control of cutaneous vascular tone in rabbits (Blessing and Nalivaiko 2000; Blessing et al. 1999; Nalivaiko and Blessing 1999, 2001). The recording microelectrode, held in the micromanipulator at an angle of 10° (tip rostral), was lowered into the brain stem through the floor of the fourth ventricle, 0.5–0.8 mm rostral to the obex (defined as the rostral edge of the area postrema), not more than 0.3 mm lateral to the midline. In some experiments, following electrophysiological recordings, the tungsten electrode was replaced by a stainless steel electrode, and recording sites were labeled by passing anodal current (1 μA DC for 15 s).

Raphé-spinal neurons were identified by antidromic activation from the dorsolateral funiculus of the spinal cord. A monopolar glass-covered tungsten stimulating electrode was inserted 0.8 mm lateral to the midline at the level of C₆–T₁. Electrode depth was adjusted so that electrical stimulation (50 Hz, 1 ms, 30–100 μA) evoked a rise in arterial pressure accompanied by ear pinna vasoconstriction. During the search procedure, the dorsolateral funiculus was constantly stimulated at 1 Hz with pulses of 2-ms duration. In the last six rabbits studied, the stimulating current was increased to 3–4 mA for more efficient excitation of unmyelinated fibers. Standard criteria for antidromic activation included constant latency of activation from the spinal cord, ability to follow high-frequency stimulation and, when possible, a collision test. For on-line calculation of neuronal discharge rate, and construction of peristimulus time histograms, signals were fed into a window discriminator (NL201, NeuroLog) and analyzed with a Macintosh G3 computer using Chart or Scope software (ADInstruments).
Protocol

Once the antidromically activated raphe-spinal neuron was identified, its response to nociceptive tactile stimuli was assessed by touching fur on the hind paw, front paw, back, neck, and face. Next, sensitivity to nociceptive stimulation was assessed by firmly squeezing the rabbit’s lip once with pliers for 2 s. The neuron was also assessed for its response to electrical stimulation of the spinal tract of trigeminal nerve (single pulse: 300 μA, 1 ms) and of the central amygdala (20-ms train, 200 Hz, 300 μA, 1 ms). Peristimulus time histogram for trigeminal- and amygdala-evoked responses were computed on-line by averaging ≥30 sweeps.

Data storage and analysis

Analog signals for skin blood flow, end-expiratory CO₂, and arterial pressure were digitized (40 Hz) with MacLab 16 s (ADInstruments) and displayed and stored on a G3 Apple Macintosh computer. These signals, together with amplified signal from extracellular microelectrode, were also stored on magnetic tape for subsequent off-line analysis.

An additional off-line procedure was implied to validate the identity of antidromically evoked action potentials. Neuronal firing recorded on the magnetic tape was sampled at 10 kHz by Chart software (ADInstruments), and all spikes differing from the background noise were detected. Subsequently, 10 antidromically activated spikes were averaged; the averaged amplitude and duration (at 50% height) was noted. Units were included if averaged antidromically evoked action potentials coincided with the group (or with 1 of the groups in the case where several units were recorded simultaneously) of segregated spikes detected by the software.

Neuronal responsiveness to tactile and nociceptive stimulation was evaluated on-line by audio monitoring and by rate-meter histograms (NL201 Window Discriminator and NL600 Pulse Integrator, NeuroLog). Off-line, Chart software was used to construct rate meter histograms following mechanical stimulation and for computing of peristimulus time histograms following electrical stimulation of the spinal tract of the trigeminal nerve or the amygdala. Effects of mechanical (tactile and nociceptive) and electrical stimulation were statistically assessed by the cumulative sum method (Davey et al. 1986) using IgorPro software (WaveMetrics). The potential relation between neuronal discharge and cardiac or respiratory rhythmicity was assessed by the peristimulus time histogram technique (30 sweeps averaged) using arterial pressure or CO₂ signals, respectively, as a trigger pulse (Gieroba et al. 1995). Analysis of variance with repeated measures and Fisher’s protected t-test were used to determine the significance of differences in measured variables. A χ² test was used to assess differential sensitivity to amygdala stimulation in tactile-sensitive versus insensitive cells. Values presented in the text are means ± SE.

Histology

At the end of the experiment, the animals were given an overdose of pentobarbitone sodium and were perfused transcardially with aldehyde fixative. The brains were removed, blocked and sectioned on a freezing microtome (50 μm). Sections were stained with the Perl’s Prussian blue reaction for the detection of stimulation and recording sites.

RESULTS

General electrophysiological properties of rabbit raphe-spinal neurons

Extracellular recordings were performed from 129 antidromically identified raphe-spinal neurons in 32 rabbits. Rosstrocaudal location of recording sites was limited to the previously described raphe area, centered just caudal to the caudal pole of the facial nucleus, extending for ~0.5 mm both rostrally and caudally (Blessing and Nalivaiko 2000; Blessing et al. 1999; Nalivaiko and Blessing 1999, 2001) where pharmacological inhibition of neurons resulted in suppression of cutaneous vasoconstriction elicited by nociceptive stimuli or by electrical stimulation of the forebrain areas (see DISCUSSION).

An example of a recording site is presented in Fig. 1A. Nearly all raphe-spinal units were located between 2.5 and 5.5 mm from the dorsal medullary surface, with maximal neuronal density at ~4.5 mm. Many (52/129, 40%) of the antidromically activated neurons were silent. The remainder had spontaneous activity ranging from 0.3 to 29 Hz (Fig. 2A), with the discharge pattern distributed as follows: very low (<1 Hz) frequency (6/129, 5%); low frequency (1–10 Hz; 31/129, 24%), high frequency (10–25 Hz) regular (25/129, 19%); high-frequency oscillating (15/129, 12%). Conduction velocities of antidromically activated axons ranged from <1 to 70 m/s (Fig. 2B) with a mean of 21 m/s. There was an apparent tendency of fast-conducting neurons to be located more ventrally. Of 31 neurons recorded at depth ≤3.8 mm, only 5 had conducting velocities faster than 20 m/s, while for 98 more ventral neurons, 44 conducted at this or higher speeds (Fig. 2C). None of the recorded raphe-spinal neurons possessed cardiac- or respiratory-related rhythmicity.

Sensitivity of raphe-spinal neurons to lip squeeze and light touch of the body and to electrical stimulation of the spinal trigeminal tract and the amygdala

All neurons were assessed for their sensitivity to nociceptive stimuli (lip squeeze), tactile nonnociceptive stimuli applied to
different areas of the body, and electrical stimulation of the spinal tract of trigeminal nerve. Nociceptive stimulation increased the discharge rate in 63 neurons (49%), did not affect 57 neurons (44%), and inhibited the remaining 9 neurons (7% of total population or 12% of spontaneously active neurons). Our classification of recorded neurons is given in Fig. 3. As previously described (Blessing and Nalivaiko 2000), nociceptive stimulation also elicited sudden falls in ear pinna blood flow. Excitatory responses of neurons activated by nociceptive stimulation always preceded these episodes of cutaneous vasoconstriction by 1.5–2 s (Fig. 4). In contrast, when nociceptive stimulation inhibited the discharge rate, response onset did not correlate with the onset of cutaneous vasoconstriction, and in several instances, the decrease in discharge occurred after ear pinna flow commenced to fall. Electrical stimulation of the spinal tract of the trigeminal nerve excited 61/63 (97%) neurons activated by nociceptive stimulation, affected only 2/57 (5%) of neurons insensitive to such stimulation, and inhibited 9/9 (100%) neurons inhibited by nociceptive stimulation.

Multiple tactile stimuli (nonnociceptive as described in METHODS) delivered to various bodily locations activated 35/63 neurons also activated by nociceptive stimulation (27% of the total raphe-spinal neuronal population). These neurons will be referred to as multimodal. The remaining 28/63 neurons activated by nociceptive stimuli (21%) were insensitive to tactile stimulation.
stimulation and thus will be referred to as nociceptive neurons. Nonnociceptive tactile stimuli did not affect any of the 57 neurons insensitive to nociceptive stimulation and caused inhibition in 2/9 neurons inhibited by such stimulation.

Forty of 129 raphe-spinal neurons were additionally assessed for sensitivity to electrical stimulation of the central amygdala. Amygdala stimulation excited 14/40 (33%) of the neurons studied, distributed as follows: 3/13 nociceptive, 10/12 multimodal, and 1/15 insensitive to nociceptive stimulation. Amygdala stimulation did not affect any neurons inhibited by nociceptive stimulation nor did the stimulation inhibit any neuron examined. Nearly all of the neurons sensitive to amygdala stimulation were found at 4.5–5.5 mm from the dorsal medullary surface. Amygdala stimulation sites are shown in Fig. 1B.

**Neurons activated by nociceptive stimulation**

Raphe-spinal neurons activated by the nociceptive stimulation varied with respect to the pattern and rate of their spontaneous discharge, their reactivity to the stimulation, and their conduction velocity. Of 63 cells activated by the nociceptive stimulation, 14 (22%) were silent, 6 (10%) discharged at a very low rate (<1 Hz), 14 (22%) discharged irregularly at frequencies <10 Hz, 15 (25%) exhibited a regular high-frequency (10–25 Hz) discharge pattern, and 13 (21%) possessed a very characteristic cyclical pattern, with their discharge rate showing regular smooth transitions from 0–5 to 12–25 Hz, with a cycle period of 20–50 s.

Nociceptive stimulation resulted in clearly detectable excitatory responses. In silent neurons, such stimuli evoked short-lasting (1–5 s) bursts of activity. In spontaneously active neurons, lip squeeze usually evoked a more vigorous response, with discharge rate increasing three- to sixfold compared to resting value. The duration of such responses varied, ranging from 3–5 s transients to 40–50 s periods of increased activity. No correlation was found between amplitude and duration of pain-induced responses.

A subpopulation of neurons sensitive to nociceptive stimulation was composed of cells sensitive to nonnociceptive tactile stimuli (touching fur on back and front paws, neck, back, and face). Such stimulation resulted in excitatory responses smaller in amplitude and duration compared with those elicited by lip squeeze. Most of these neurons responded to stimulation of all areas and thus probably possessed a receptive field of the whole body. An example of a recording from such a multimodal neuron is shown in Fig. 5A.

The conduction velocity of neurons sensitive to nociceptive stimulation ranged from 7 to 71 m/s. Histogram analysis revealed a bimodal distribution of this parameter, with peaks at 12–18 and 28–34 m/s. A bias toward lower conduction velocity was found in nociceptive (20 ± 2 m/s) compared with multimodal neurons (30 ± 3 m/s, P < 0.05). Another difference between two neuronal subpopulations was in the mean duration of action potentials. In multimodal neurons, it was shorter (0.80 ± 0.03 ms) than in nociceptive neurons (0.98 ± 0.06 ms, P < 0.01). A scatter plot (axon conduction velocity vs. action potential duration) of neurons exited by lip squeeze is shown in Fig. 6A.

Electrical stimulation of the spinal tract of trigeminal nerve caused activation in 26/28 (93%) nociceptive neurons and in all multimodal neurons (Fig. 5B). As revealed by peristimulus time histograms, the latency of these responses was very variable from cell to cell (mean: 28 ± 3 ms, range: 6–75 ms) as shown in Fig. 5C.

Electrical stimulation of the central amygdala activated 10/12 tested multimodal neurons, with mean latency of 34 ± 3 ms (range: 20–65 ms). The same stimulation activated 3/11 tested nociceptive neurons, with latencies of 30, 40, and 80 ms (Fig. 7). This differential sensitivity to the amygdala stimulation in multimodal versus nociceptive cells was statistically significant (P < 0.025). We were unable to detect any difference with respect to firing pattern or response to nociceptive stimulation between these neuronal subpopulations.

**Neurons inhibited by nociceptive stimulation**

Inhibitory responses to nociceptive stimulation could be observed only in spontaneously active raphe-spinal neurons. Of 77 such spontaneously active neurons (representing 60% of total population of antidromically activated neurons), 9 (12%) were inhibited by lip squeeze (Fig. 3). Three had a regular firing pattern in the range of 20–26 Hz. Two had a cyclical discharge, oscillating between 7–9 and 10–18 Hz. The remaining neurons fired irregularly at frequencies >10 Hz. Nociceptive stimulation clearly decreased the discharge rate in this neuronal population (Fig. 8A). The spontaneous firing rate was reduced to ≤50% in three neurons, whereas three others became silent. The duration of the inhibitory response varied...
from 2 to 13 s. In two of nine neurons inhibited by nociceptive stimulation, inhibitory responses were also elicited by tactile stimuli from wide receptive fields. This inhibition was smaller in amplitude and shorter in duration compared with the response induced by nociceptive stimulation.

The mean conduction velocity of neurons inhibited by nociceptive stimulation was 25 ± 3 m/s (range: 11–45 m/s). Mean action potential duration was 0.92 ± 0.07 ms (range: 0.5–1 ms). Electrical stimulation of the spinal tract of the trigeminal nerve caused inhibitory responses in all neurons of this population (Fig. 8B). The mean latency of these responses was 32 ± 10 ms (Fig. 8C). No response was observed in one pain-inhibited neuron assessed for its sensitivity to electrical stimulation of the central amygdala.

**Neurons insensitive to nociceptive stimulation**

Of 57 pain-insensitive neurons, none was affected by non-nociceptive tactile stimuli, and only 2 cells were slightly activated by electrical stimulation of the trigeminal tract. Of 15 neurons tested for sensitivity to electrical stimulation of the central amygdala, only 1 was weakly excited. An example of a recording obtained from a neuron insensitive to nociceptive stimulation is shown in Fig. 9.

Similarly to both neuronal groups described in the preceding text, a subpopulation of raphe-spinal neurons insensitive to nociceptive stimulation was also composed of cells possessing nonuniform electrophysiological properties. Greater variability was observed in both conduction velocities (range: 0.6–54 m/s) and action potential duration (range: 0.6–1.8 ms). Raster plots of conduction velocity versus AP duration revealed a tendency for slow-conducting neurons to possess action poten-

**FIG. 6.** A: raster plot (axon conduction velocity vs. action potential duration) of neurons exited by lip squeeze. ○, nociceptive neurons (sensitive only to lip squeeze); ●, multimodal neurons (sensitive to lip squeeze and to multiple tactile stimuli). B: raster plot of neurons insensitive to lip squeeze. Slow-conducting/long AP neurons separated from fast-conducting/short AP neurons by straight line passing at a slope of 20.8 cm*s⁻¹m*s⁻¹.

**FIG. 7.** Excitatory responses to the electrical stimulation of the central amygdala of raphe-spinal neurons activated by nociceptive stimulation. A: peristimulus time histogram obtained following amygdala stimulation in the same multimodal neuron as shown in Fig. 5. - - -, onset of the 20-ms train of electrical pulses delivered at 200 Hz. Inset: fragment of raw data showing excitatory response to the amygdala stimulation (sweep duration: 800 ms; ⋄, stimulus artifact). B: histogram of the onset latencies of excitatory responses elicited by stimulation of the central amygdala (30 sweeps).

**FIG. 8.** Properties of raphe-spinal neuron inhibited by nociceptive stimulation. A: instantaneous frequency plot showing neuronal activity at rest and following touching fur on the back leg, front leg, and neck, and following lip squeeze (>). Left inset: collision test (sweep duration 15 ms). Right inset: fragment of raw record obtained immediately after presentation of the nociceptive stimulus (sweep duration: 3.5 s). Note another neuron, of smaller amplitude, unaffected by the stimulus. B: peristimulus time histogram obtained in the same neuron following electrical stimulation of the spinal trigeminal tract (30 sweeps). - - -, the single electric pulse. Inset: fragment of raw data showing inhibitory response to trigeminal stimulation (sweep duration: 800 ms; ⋄, stimulus artifact). C: histogram of the onset latencies of inhibitory responses elicited by stimulation of the spinal trigeminal tract (combined data from all raphe-spinal neurons inhibited by nociceptive stimulation).
tials longer than those of fast-conducting ones (Fig. 6B). We thus subdivided nonresponding neurons into two subgroups on the basis of these two properties. “Fast-conducting/short action potential” neurons were separated from “slow-conducting/long action potential” cells by a straight line passing at the slope of 20.8 m/s−1 ms−1. The mean conduction velocity for these two groups of neurons was 25 ± 4 and 6 ± 0.5 m/s, respectively. Mean action potential duration was 0.85 ± 0.04 and 1.26 ± 0.08 ms, respectively. The validity of this division was confirmed by finding that these two subgroups differed in their firing patterns. Fast neurons either discharged regularly at frequencies of 12–30 Hz (5/12) or were silent (7/12). Most (32/45, 71%) slow neurons were silent and 13 others had a low-frequency (<8 Hz) discharge rate. Thus the electrophysiological profile of these slow neurons possibly indicates that they may be serotonergic (see DISCUSSION).

DISCUSSION

This is the first description of electrophysiological and physiological properties of antidromically identified rostral medullary raphe-spinal neurons in the rabbit since the brief account by Haselton and colleagues (1988b). The stimulating electrode was placed in the dorsolateral funiculus of the lower cervical cord so that it is likely that we antidromically activated raphe neurons projecting to the intermediolateral column as well as neurons that might project to the spinal dorsal horn (Basbaum et al. 1978). We classified all antidromically activated neurons according to whether their discharge was increased, decreased, or unchanged by a strong nociceptive stimulus (squeezing of the lip). As expected, neurons activated by lip squeeze were also activated by electrical stimulation of the spinal trigeminal tract, a CNS pathway containing the centrally projecting axons of unmyelinated and thinly myelinated nociceptive afferents from the orofacial region. Similarly, neurons inhibited by lip squeeze were also inhibited by trigeminal tract stimulation, and neurons unaffected by lip squeeze were also unaffected by trigeminal tract stimulation.

There was a relationship between the conduction velocity of the antidromically activated neurons and their response to lip squeeze/trigeminal stimulation. Nearly all neurons activated or inhibited by such stimulation had axonal conduction velocities >10 m/s. Conversely, the majority of neurons unaffected by nociceptive stimulation had relatively slow conduction velocities, <10 m/s. These slowly conducting neurons could also be distinguished by virtue of their longer action potential duration and their tendency to be silent or to discharge at low frequencies. Because extracellular recordings do not allow detection of inhibitory responses in silent neurons, it is possible that some or all of the silent fast-conducting nonresponding neurons might belong to the raphe-spinal neuronal group inhibited by nociceptive stimulation. More than half of neurons excited by nociceptive stimuli were also excited by nonnociceptive tactile stimulation of the fur of various bodily regions, indicative of a wide receptive field. Neurons insensitive to nociceptive stimulation were also insensitive to nonnociceptive tactile stimulation of the fur. Because somatosensory responses in raphe-spinal neurons are anesthetic dependent (Blair and Evans 1993), our data should be interpreted with caution.

Although there are many in vivo electrophysiological studies of neurons in the raphe magnus/pallidus region, with emphasis on their nociceptive/antinociceptive functions (see Mason 2001 for review), only a few studies have confined themselves to the examination of neurons with identified spinal projections, addressing the question of their conduction velocity. In early studies performed in cats, Anderson et al. (1977) reported fast-conducting neurons excited by both nociceptive and nonnociceptive tactile stimulation with wide receptive fields. Nociceptive-activated neurons with wide receptive field for nonnociceptive stimuli were described in the rat by Wessendorf and Andersen (1983) and by Fields and colleagues (1995); conduction velocities of their axons ranged 0.9–33 m/s. In two other studies (Chiang and Pan 1985; Lumb and Morrison 1986), conduction velocity of rat raphe-spinal cells was found to be in the range of 1–28 m/s. Vanegas et al. (1984) found in rats that fast-conducting raphe-spinal neurons were more likely to be activated by nociceptive stimulation. Similar findings were reported in conscious rat studies by Martin et al. (1991). Fast-conducting (10–70 m/s) raphe-spinal neurons with wide receptive fields sensitive to the nociceptive stimulation have also been described in primates (Willcockson et al. 1983). Thus the consensus from studies in which the question has been specifically addressed is that majority of raphe-spinal neurons sensitive to nociceptive stimulation tend to have fast-conducting axons (usually >6–8 m/s) which suggests their nonserotonergic nature (see following text). This agrees with our findings.

Identification of putative raphe-spinal presympathetic neurons constricting the cutaneous vascular bed

Our previous microinjection studies in rabbits (Blessing et al. 1999) show that excitation of neurons in the raphe region causes intense vasoconstriction in the cutaneous bed without greatly affecting arterial pressure and without changing flow in the mesenteric bed. Inhibition of neuronal function in the same medullary region entirely prevents cutaneous vasoconstriction initiated by nociceptive stimulation (Blessing and Nalivaiko 2000). Thus the raphe region contains neurons whose activation leads to cutaneous vasoconstriction. In our present electrophysiological study, the recording sites were concentrated in this same region, just caudal to the caudal pole of the facial nucleus, extending for ~0.5 mm both rostrally and caudally, so that it is likely that our sample of 129 raphe-spinal neurons
included at least some cells whose activity constricts the cutaneous bed. Neurons insensitive to noxious stimulation could not be the cells mediating cutaneous vasoconstriction elicited by this form of stimulation. Similarly, because pharmacological inhibition of neurons in the raphe region under study results in dilatation of cutaneous vessels (Blessing and Nalivaiko 2000), neurons inhibited by noxious stimulation are unlikely to mediate cutaneous vasoconstriction. These considerations suggest that putative presympathetic neurons mediating cutaneous vasoconstriction elicited by noxious stimulation belong to the subpopulation of nociception-activated raphe-spinal neurons.

We did not find raphe-spinal neurons whose discharge clearly corresponded to the apparently spontaneous changes in ear pinna vascular resistance sometimes observed in anesthetized rabbits. In investigations of central regulation of autonomic function, it is never easy to correlate the discharge of an individual neuron with an integrated motor output. The delay from electrical activation of the raphe to the onset of cutaneous vasoconstriction depends on the intensity of the stimulus, presumably at least partially because change in blood flow is a complex integrated response, reflecting the discharge of many different raphe neurons.

Stimulation of the spinal trigeminal tract (Yu and Blessing 1998) and of raphe magnus/pallidus (Nalivaiko and Blessing 1999) both produce similar cutaneous vascular responses, with latencies of ~2 s. The trigemino-raphe latency in the present study was 5–35 ms and the raphe-spinal latency (determined antidromically) was from 1.5 to 20 ms, so that most of the 2-s delay is in the periphery. Approximately one-half of raphe-spinal neurons examined increased their discharge in response to lip squeeze and trigeminal tract stimulation (Fig. 3). This increase in discharge preceded the commencement of ear pinna vasoconstriction. Some neurons displayed prolonged increases in discharge to a single shock to the trigeminal tract (e.g., the cell in Fig. 5 that responded for ≥800 ms). Some cells increased their discharge for as long as 1.5 s after a single trigeminal tract shock. Lip squeeze increased the discharge of some raphe-spinal cells for periods as long as 50 s. However, with our present evidence, it is difficult to more precisely relate the increase in discharge of raphe-spinal units to the vasomotor change. The full vasoconstrictor response to trigeminal tract stimulation requires a train of impulses (Yu and Blessing 1998), not just a single impulse. Thus any conclusion concerning the functional role of the various classes of raphe-spinal neurons examined in our study must be based on indirect evidence.

Previous electrophysiological investigations of raphe-spinal neurons have considered the possibility of a cardiovascular role for these cells. The main criterion for such a role has been the response of the neurons to baroreceptor-derived inputs (generally fairly minimal in agreement with the findings in the present study), and the studies have focused on neurons with slowly conducting axons, usually <5 m/s (Barman and Gebber 1985; McCall and Clement 1989; Morrison and Gebber 1985; Pilowsky et al. 1995). None of the neurons examined in the current study had a clearly established cardiac rhythmicity indicating their baro-sensitivity. It is thus possible to suggest that cutaneous vascular control is not directly associated with changes in systemic arterial pressure. In good accord with this hypothesis is the finding that activation or inhibition of the cutaneous vasomotor center in the raphe region does not affect systemic arterial pressure in rabbits (Blessing and Nalivaiko 2000; Blessing et al. 1999; Nalivaiko and Blessing 1999).

An alternative approach to the establishment of a cardiovascular role for raphe-spinal cells has been to stimulate the medullary raphe and to record the evoked discharge either in spinal sympathetic neurons (Morrison 1993) or in peripheral sympathetic axons (Huangfu et al. 1994; Yusof and Coote 1988; Zhou and Gilbey 1995). These studies were conducted before it was realized that presympathetic neurons in the raphe magnus/pallidus selectively regulate sympathetic discharge to cutaneous vessels and to brown fat rather than to skeletal muscle/mesenteric/renal beds usually investigated with recordings from lumbar and splanchnic sympathetic nerves (Huangfu et al. 1994; Morrison 1993; Zhou and Gilbey 1995). Activation of raphe neurons evokes relatively little discharge in the splanchnic nerves (Morrison 1999). As far as we are aware, no studies have excluded a contribution from fast-conducting raphe-spinal neurons to sympathetic discharge in cutaneous beds. We consider that the vigorous manner in which the majority of the fast-conducting neurons were excited by noxious stimulation (and by stimulation of the amygdala, see following text) makes it likely that they mediate the vigorous cutaneous vasoconstriction elicited by this stimulation. This evidence suggests that a subpopulation of the fast-conducting raphe-spinal neurons described in the present study functions as cutaneous presympathetic vasomotor neurons.

The present study is the first to describe excitatory actions of amygdala stimulation on medullary raphe-spinal neurons. In our previous study in anesthetized rabbits, we found that electrical stimulation of the amygdala elicited vigorous constriction in the cutaneous bed without affecting arterial pressure or flow to the mesenteric bed (Nalivaiko and Blessing 2001). In this study, we demonstrated that neurons in the raphe area constitute an essential brain stem relay for cutaneous vasoconstriction elicited by electrical stimulation of the central nucleus of the amygdala (Nalivaiko and Blessing 2001). This result is important because work from our laboratory in conscious rabbits (Yu and Blessing 1999, 2001) has demonstrated that neural circuitry in the amygdala is essential for the cutaneous (but not mesenteric) vasoconstriction that occurs when the rabbit detects a salient environmental stimulus, one that signals potential danger to the animal’s well being. Clearly, it is possible that the underlying amygdalo-spinal pathway mediating this vasoconstriction also relays in the raphe region. In the present study, we found that electrical stimulation of the amygdala excited the subclass of raphe-spinal neurons that was activated by both noxious stimulation and by nonnociceptive tactile stimulation. This subpopulation of neurons may well mediate amygdala-induced cutaneous vasoconstriction.

The latency of the raphe excitatory response elicited by stimulation of the amygdala was ~30 ms compared with a range from 5 to 60 ms from the spinal trigeminal tract. The duration of the excitatory response for trigeminal tract stimulation was variable. It was not unusual for the raphe-spinal neuron to increase its discharge for as long as 1.5 s even after a single electrical stimulus. In contrast, amygdala stimulation usually increased the discharge of the bulbospinal neuron for a brief period only, usually <150 ms. These differences suggest the possibility that the excitatory pathway from the amygdala to the raphe is mainly direct, whereas the excitatory pathway
from the trigeminal tract to the raphe appears to be complex, probably involving both monosynaptic and polysynaptic pathways traversing different levels of the brain stem and possibly even the forebrain. There is little established neuroanatomical data regarding projections from the spinal trigeminal nucleus to the raphe pallidus area. On the other hand, Hermann et al. (1997) reported retrogradely labeled neurons in the central nucleus of the amygdala after tracer injection in raphe magnus/pallidus in rats, and the projection has also been observed in rabbits (Haselton et al. 1988b), so the excitatory influence of the amygdala observed in our study could be mediated by a direct projection.

The amygdalo-raphe projection could also relay in the hypothalamus (Pittman et al. 1981; Wallace et al. 1992) or in the periaqueductal gray (Behbehani and Fields 1979; Carrive et al. 2000; Lovick 1993). Kishi et al. (2000) described the effects of hypothalamic stimulation on baro-insensitive neurons located at the medial border of the rostral ventrolateral medulla (RVLMT) in rabbits at the same rostrocaudal level as neurons recorded in our study. The sensitivity of their cells to nociceptive or tactile sensory stimulation was not tested. However, their orthodromic activation from the hypothalamus preceded cutaneous vasoconstriction. The mean conduction velocity of the subpopulation of these neurons antidromically activated from the spinal cord was threefold faster than that of RVLMT-baro-sensitive neurons. These baro-insensitive neurons thus resemble multisensory cells reported in the present paper, and it may be that both studies examined similar neuronal populations. We focused on the midline raphe area, where pharmacological blockade of cutaneous vasomotor function is most efficient, whereas Kishi et al. (2000) may have recorded from parapyramidal cutaneous presympathetic neurons located more laterally.

Potential role of slow conducting raphe-spinal neurons

We found that slowly conducting raphe-spinal neurons were generally insensitive to nociceptive stimulation. These are the neurons usually considered in studies of the potential cardiovascular role of raphe magnus/pallidus. Bulbospinal serotonin-synthesizing neurons, a subpopulation of these neurons, have a strong projection to sympatetic preganglionic neurons in the intermediolateral column (Blessing et al. 2001; Jensen et al. 1995; Loewy 1981; Smith et al. 1998). At present there is no consensus concerning functional cardiovascular roles for these neurons. In combined electrophysiological/immunohistochemical studies of the total medullary raphe neuronal population, serotonergic neurons possessed slow and regular discharge rate (Gao and Mason 2000; Mason 1997). In our study, only antidromically activated neurons were analyzed, and most of the slow-conducting neurons were silent, making it impossible to identify serotonin (5-HT) as the neurotransmitter on the basis of the discharge pattern. So far no studies have correlated axonal conduction velocity with immunohistochemical identification of individual serotonin-containing neurons. Their conduction velocity is considered to be slow, consistent with their unmyelinated axons, a conclusion supported by indirect evidence (Wessendorf et al. 1981). In our study, slow-conducting raphe-spinal neurons with an electrophysiological profile characteristic of a serotonergic phenotype were unresponsive to nociceptive stimulation, in agreement with the findings of Mason (1997, 1999) in rats.

Although no definite cardiovascular role has been established for raphe magnus/pallidus 5-HT cells, there is a large body of evidence relating CNS serotonergic function with temperature regulation (Gudelsky et al. 1986; Muller et al. 1988; Nakamura and Sakaguchi 1990), and thus it may be that the medullary 5-HT cells are involved in the control of cutaneous vasculature related to thermoregulatory function (Nakamura and Sakaguchi 1990) via an independent, slow-conducting raphe-spinal pathway.

Are raphe-induced vasoconstriction and antinociception mediated by the same raphe-spinal neurons?

Functional studies of the rostral medullary raphe usually focus either on nociception/antinociception or on the cardiovascular system, rarely on both subjects. However, available neuroanatomical evidence clearly indicates that the raphe projects both to the spinal dorsal horns and to the intermediolateral column, as well as to the ventral horn (Antal et al. 1996; Basbaum et al. 1978; Skagerberg and Bjorklund 1985). In a combined functional and anatomical study, Light (1985) identified individual raphe-spinal axons that send terminals to both the dorsal horn and the intermediolateral column.

Alerting-related cutaneous vasoconstriction presumably functions, at least in part, to shunt blood away from the surface of the body, the region most likely to be damaged by agents in the external environment. In this context, the vasoconstriction can be viewed as an integral part of the general protective response, which also includes antinociception. Certainly in rabbits, focal electrical stimulation of this area elicits both antinociception (Sotgiu 1987) and, as we have demonstrated, cutaneous vasoconstriction (Blessing et al. 1999; Nalivaiko and Blessing 1999). Stimulation of the amygdala, a brain region intimately involved in processing of potentially dangerous environmental stimuli (LeDoux 1994) causes both cutaneous vasoconstriction (Nalivaiko and Blessing 2001) preceded by excitation of multimodal neurons (the present study) and antinociception (Helmstetter et al. 1998; Kalivas et al. 1982). Thus it could well be that an individual raphe neuron, via complex axonal branching, innervates multiple regions of the spinal cord, including both the dorsal horn and the intermediolateral column, thereby functioning in an integrative capacity to coordinate the different components of the nociceptive/antinociceptive response.

In conclusion, present electrophysiological findings are consistent with our hypothesis that raphe magnus/pallidus neurons with fast-conducting direct projections to the intermediolateral column are responsible for constricting the cutaneous vascular bed during alerting responses.

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