

Reflex inhibition of cutaneous and muscle vasoconstrictor neurons during stimulation of cutaneous and muscle nociceptors

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Submitted 7 November 2013; accepted in final form 31 January 2014

Kirillova-Woytke I, Baron R, Jänig W. Reflex inhibition of cutaneous and muscle vasoconstrictor neurons during stimulation of cutaneous and muscle nociceptors. *J Neurophysiol* 111: 1833–1845, 2014. First published February 5, 2014; doi:10.1152/jn.00798.2013.—Cutaneous (CVC) and muscle (MVC) vasoconstrictor neurons exhibit typical reflex patterns to physiological stimulation of somatic and visceral afferent neurons. Here we tested the hypothesis that CVC neurons are inhibited by stimulation of cutaneous nociceptors but not of muscle nociceptors and that MVC neurons are inhibited by stimulation of muscle nociceptors but not of cutaneous nociceptors. Activity in the vasoconstrictor neurons was recorded from postganglionic axons isolated from the sural nerve or the lateral gastrocnemius-soleus nerve in anesthetized rats. The nociceptive afferents were excited by mechanical stimulation of the toes of the ipsilateral hindpaw (skin), by hypertonic saline injected into the ipsi- or contralateral gastrocnemius-soleus muscle, or by heat or noxious cold stimuli applied to the axons in the common peroneal nerve or tibial nerve. The results show that CVC neurons are inhibited by noxious stimulation of skin but not by noxious stimulation of skeletal muscle and that MVC neurons are inhibited by noxious stimulation of skeletal muscle but not by noxious stimulation of skin. These inhibitory reflexes are mostly lateralized and are most likely organized in the spinal cord. Stimulation of nociceptive cold-sensitive afferents does not elicit inhibitory or excitatory reflexes in CVC or MVC neurons. The reflex inhibition of activity in CVC or MVC neurons generated by stimulation of nociceptive cutaneous or muscle afferents during tissue injury leads to local increase of blood flow, resulting in an increase of transport of immunocompetent cells, proteins, and oxygen to the site of injury and enhancing the processes of healing.

cutaneous vasoconstrictor neurons; muscle vasoconstrictor neurons; postganglionic fibers; nociceptive reflex inhibition; spinal reflexes; heat-sensitive afferents; cold-sensitive afferents

SOME 15–20% of the unmyelinated fibers in the rat sural nerve and some 50% in the gastrocnemius-soleus nerve are postganglionic (Baron et al. 1988). Most of these postganglionic fibers innervate blood vessels in hairy skin or skeletal muscle, have ongoing activity, and display distinct reflexes upon physiological stimulation of cutaneous or visceral afferents (Häbler et al. 1993, 1994); a few postganglionic neurons projecting in the sural nerve innervate hairless skin and may have sudomotor function, and a few innervate deep somatic tissues (Jänig 2006). One prominent and unique reflex in cutaneous vasoconstrictor (CVC) neurons is inhibition of ongoing activity upon noxious stimulation of skin that is innervated by the postganglionic CVC neurons. Noxious stimulation of skin territories remote from the skin territory innervated by the CVC neurons

recorded from generates smaller reflex inhibition or reflex excitation or no reflexes in the CVC neurons. This inhibitory nociceptive reflex has been demonstrated in the lumbar sympathetic outflow to hindlimbs and tail of cat and rat and in the upper thoracic sympathetic outflow to head and neck of cat and rat (Bartsch et al. 2000; Boczek-Funcke et al. 1992; Grosse and Jänig 1976; Horeysek and Jänig 1974a; Jänig and Kümmel 1977; Jänig and Szulczyk 1980). It is most likely a spinal reflex (Horeysek and Jänig 1974b; Jänig and Kümmel 1981; Jänig and Spilok 1978). Muscle vasoconstrictor (MVC) neurons are excited upon stimulation of cutaneous nociceptors (Häbler et al. 1994).

The nociceptive inhibitory reflex in CVC neurons corresponds to the Lovén reflex published in 1866 (Lovén 1866). Lovén showed in rabbits that electrical stimulation of the central end of the dorsal nerve of the foot leads to a dilation of the saphenous artery but not of the ear blood vessels, with an increase of arterial blood pressure. Electrical stimulation of the central end of the posterior branch of the auricular nerve leads to a dilation of the ear blood vessels but not of the saphenous artery, again with an increase of arterial blood pressure. Lovén concluded from these results that stimulation of cutaneous afferent fibers decreases the activity in vasomotor neurons innervating the same skin territory as the afferent fibers and increases the activity in other vasomotor neurons, leading to increase of arterial blood pressure.

Here we tested in anesthetized rats the following hypotheses: 1) CVC neurons innervating the hindlimb are inhibited by stimulation of cutaneous nociceptors innervating the same hindlimb but not by stimulation of muscle nociceptors. 2) MVC neurons projecting to the hindlimb are inhibited by stimulation of muscle nociceptors of the same hindlimb but not by stimulation of cutaneous nociceptors and not or only weakly by stimulation of muscle nociceptors of the contralateral hindlimb. The cutaneous nociceptors were excited by mechanical stimulation applied to the skin or by heat or noxious cold stimuli applied to their axons (Teliban et al. 2011). The muscle nociceptors were excited by intramuscular bolus injection of hypertonic saline or by heat or noxious cold stimuli applied to their axons.

METHODS

The experiments were conducted on 10 male Wistar rats (body weight 430 ± 11.3 g). The rats were anesthetized with pentobarbital sodium (Narcoren, Merial, Hallbergmoos, Germany; 40 mg/kg ip initially, 10 mg·kg⁻¹·h⁻¹ during surgery and experiments), paralyzed (pancuronium, Organon; initial dose 1 mg/kg iv, maintenance dose 0.4 mg·kg⁻¹·h⁻¹ iv), and artificially ventilated with oxygen-enriched air.

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The mean arterial blood pressure measured via a catheter in the tail artery was always ≥ 80 mmHg. Acid-base status was regularly determined and in the range of pH = 7.4, P_{CO_2} = 35–40 mmHg, and $PO_2 \geq 100$ mmHg. Rectal temperature was kept constant at $\sim 37^\circ\text{C}$ with a servo-controlled heating blanket. At the end of the experiments, the animals were killed under deep anesthesia by an intravenous injection of a saturated potassium chloride solution. All experiments had been approved by the local animal care committee of the state administration and were conducted in accordance with the German Federal Law.

At the beginning of the experiments the sural nerve (skin nerve) or the lateral gastrocnemius-soleus nerve (or both) of the left hindlimb was (were) exposed in a hindlimb pool between ~ 5 mm proximal to the ankle and the sciatic nerve at its trifurcation ~ 10 mm distal to the sciatic notch. Additionally, the common peroneal (CP) nerve and tibial (TIB) nerve were exposed. The hindlimb pool was formed from the surrounding skin flaps and filled with warm paraffin oil. The sural nerve was isolated over a distance of ~ 6 mm ~ 20 mm rostral to the ankle and placed on a rigidly fixed black Perspex platform of 5×8 mm. The lateral gastrocnemius-soleus nerve or the sciatic nerve at its junction with the gastrocnemius-soleus nerve was also placed on a rigidly fixed black Perspex platform of similar size.

Recording from postganglionic axons. Under visual control through a stereomicroscope, thin strands with few unmyelinated fibers were isolated from the sural nerve or the lateral gastrocnemius-soleus nerve and put on the recording platinum electrode with the central end of the fibers intact (Fig. 1, *left*). The reference recording electrode was connected to the tissue nearby. Postganglionic nerve fibers were functionally identified by their ongoing activity and by their reflexes to stimulation of arterial baroreceptors and to stimulation of cutaneous or

muscle nociceptive afferents. Amplification, discrimination, and filtering of action potentials recorded extracellularly from the postganglionic fibers were the same as described by Grossmann et al. (2009a, 2009b).

Stimulation of afferent neurons. The effect of phasic stimulation of arterial baroreceptors by the diastolic-systolic changes of the arterial blood pressure on the activity in the postganglionic vasoconstrictor neurons was measured. For this purpose the activity in the postganglionic neurons was superimposed 400–600 times with respect to the R wave of the electrocardiogram at a time resolution of 20 ms. The resulting changes of the activity with respect to the R wave are called cardiac rhythmicity (CR) of the postganglionic vasoconstrictor activity (Fig. 2A and Fig. 4C, *insets*). The degree of CR was measured as the difference between the maximum activity (over 40 ms) and the minimum activity (over 40 ms) as a percentage of the maximum activity. The degree of CR was categorized as strong (CR $\geq 60\%$), middle (CR 40–60%), or weak or absent (CR $< 40\%$) (Häbler et al. 1994).

Cutaneous nociceptors of the toes were stimulated mechanically with a forceps. Unmyelinated C fibers in skeletal muscle were stimulated by hypertonic saline (5% NaCl) injected in a bolus of 0.1 ml into the ipsilateral gastrocnemius-soleus muscle or the contralateral gastrocnemius-soleus muscle (Fig. 1, *right*).

Cold- or heat-sensitive unmyelinated afferent axons in the ipsilateral CP nerve or TIB nerve (distal to the junction of the sural nerve and the nerves innervating the gastrocnemius-soleus muscle) were stimulated with a water-perfused thermode attached to the nerves (Gorodetskaya et al. 2003). Both nerves contain cutaneous afferents and muscle afferents: In the CP nerve, cutaneous afferents project in the superficial peroneal nerve innervating hairy skin and most muscle afferents through the deep peroneal nerve to the peroneal muscles. In the TIB nerve, cutaneous afferents project to the plantar skin and muscle afferents to the deep foot muscles, the posterior tibial muscle, the flexor hallucis longus muscle, and the flexor digitorum longus muscle. The temperature was measured at the site of contact with the nerve. The stimuli started from a baseline temperature of $\sim 30^\circ\text{C}$ (see Fig. 6). Heat stimuli varied in intensity between 45°C and $\sim 52^\circ\text{C}$, and cold stimuli were 5°C (Fig. 1, *right*). About 35% of cutaneous heat-sensitive afferent C fibers exhibit axonal heat sensitivity, about 40% of cutaneous nociceptive cold-sensitive C fibers exhibit axonal cold sensitivity, and all nonnociceptive cold-sensitive C fibers exhibit axonal cold sensitivity. Thus these afferent C fibers can be specifically activated by cold or heat stimuli applied to their axons (Teliban et al. 2011). Preliminary investigations of muscle afferents show that many afferent heat- and/or cold-sensitive C fibers also exhibit axonal heat or cold sensitivity (Kirillova et al. 2011; Kirillova and Jänig, unpublished observation).

Data analysis. Neural activity, temperature of the thermode for axonal stimulation of the afferent nerve fibers, arterial blood pressure, electrocardiogram, and endotracheal pressure were simultaneously fed into a computer using the Spike II system. Data analysis was performed off-line with the general-purpose capture and analysis package Spike II (Cambridge Electronic Design, Cambridge, UK). Quantitative measurements are expressed as means $\pm$ SE. For statistical analysis Student's *t*-test or the nonparametric Wilcoxon signed-rank test was used.

RESULTS

Table 1 summarizes the results described here, giving details about numbers of vasoconstrictor neurons investigated for their responses to the different noxious stimuli.

Ongoing activity. Ongoing activity was recorded from 51 fibers in 21 filaments isolated from the sural nerve and from 39 fibers in 20 filaments isolated from the lateral gastrocnemius-soleus nerve. The nerve fibers were distally cut and proximally intact, with their cell bodies in the dorsal root ganglia (afferent

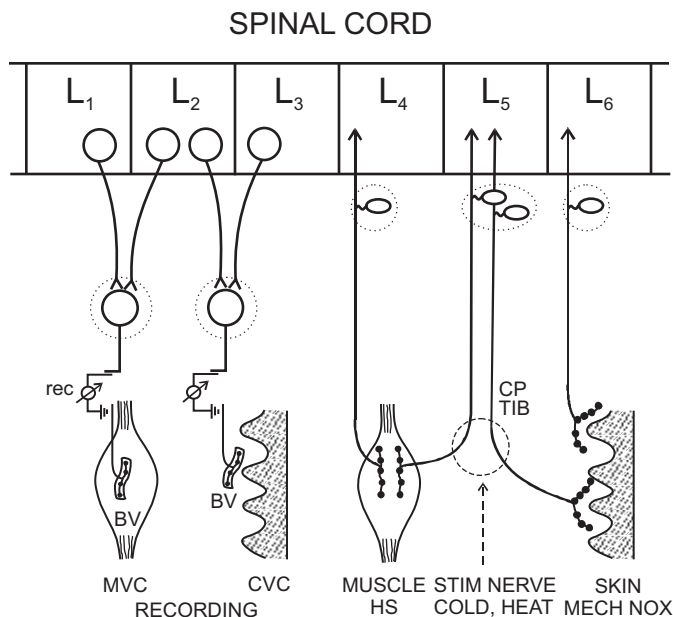


Fig. 1. Experimental approach. *Left*: activity is recorded (rec) from postganglionic cutaneous vasoconstrictor (CVC) neurons projecting in the sural nerve or from postganglionic muscle vasoconstrictor (MVC) neurons projecting in the lateral gastrocnemius-soleus nerve. The cell bodies of the postganglionic neurons are located in paravertebral ganglia L₂–L₄ (Baron et al. 1988). *Right*: stimulation of afferents. Cutaneous nociceptive afferents (and some deep nociceptive afferents) are stimulated by mechanical stimulation of the ipsilateral toes. Muscle C afferents are stimulated by hypertonic saline (HS; 0.1 ml of 5% NaCl solution) injected into the gastrocnemius-soleus muscle. Heat-sensitive afferents or cold-sensitive afferents (including nociceptive cold-sensitive afferents) are stimulated by noxious heat stimuli (43 – 52°C) or noxious cold stimuli (5°C) applied to their axons in the common peroneal (CP) nerve or tibial (TIB) nerve. The cell bodies of the afferent neurons stimulated are located in dorsal root ganglia L₄–L₆ (mostly L₅) (Baron et al. 1988). BV, blood vessel.

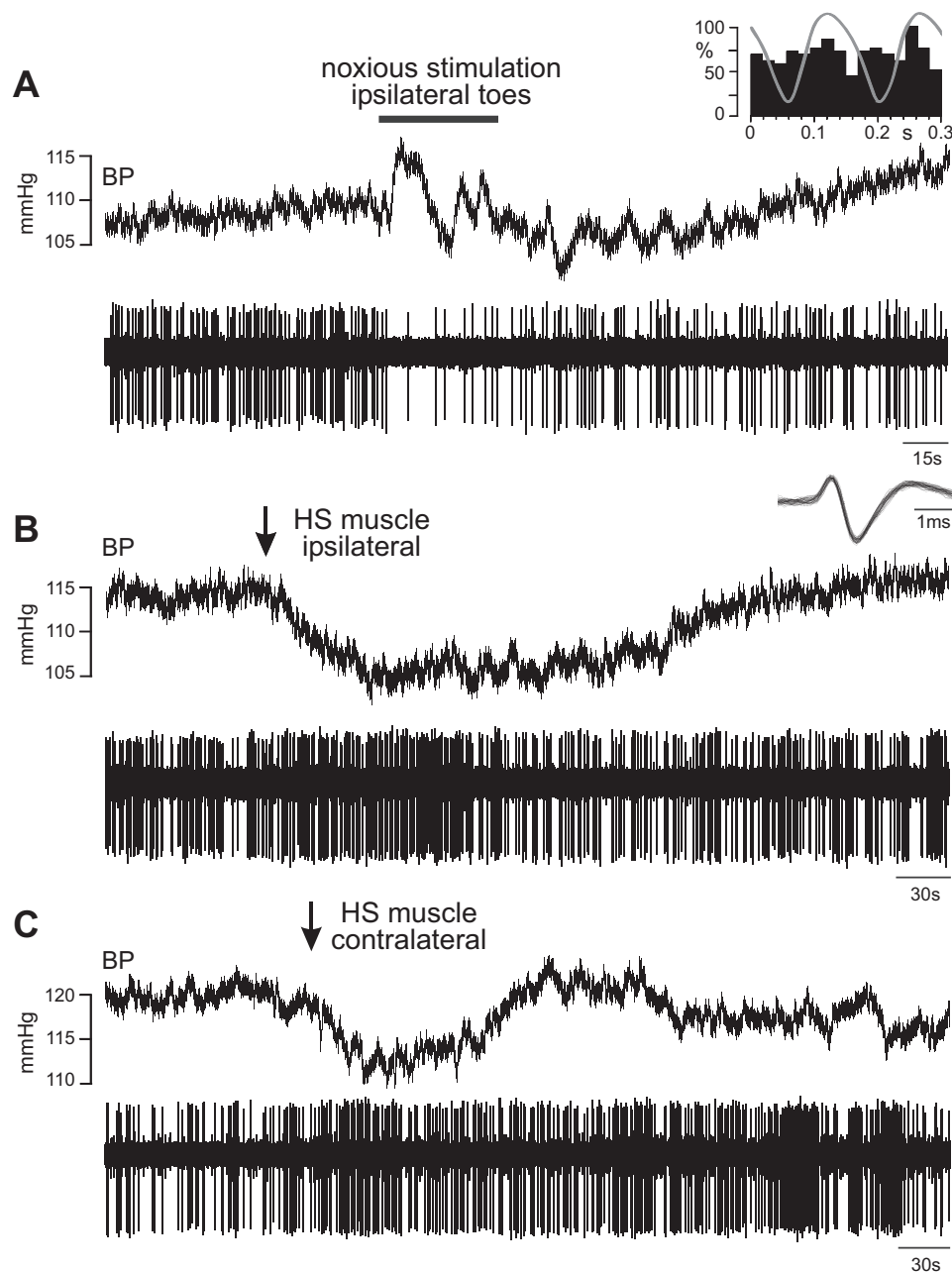


Fig. 2. CVC neuron: inhibition during mechanical noxious stimulation of the ipsilateral toes (A) and activation upon stimulation of muscle afferents by bolus injection of HS into the ipsilateral (B) or contralateral (C) gastrocnemius-soleus muscle [note the decrease of arterial blood pressure (BP) in B and C]. The filament recorded from contains 1 CVC axon (see superimposed action potentials on right in B), which exhibits no cardiac rhythmicity of its activity as shown in post-R wave histogram of CVC activity in inset on right in A. Gray curve in inset shows averaged pulsatile BP with respect to the R wave superimposed on the post-R wave histogram (total amplitude was only 4 mmHg because of our recording condition).

nerve fibers) or in the paravertebral sympathetic ganglia (postganglionic fibers) (Fig. 1, left). The ongoing activity occurred in postganglionic fibers, practically all of them being vasoconstrictor in function (Häbler et al. 1993, 1994). The ongoing activity was of central origin and synaptically transmitted from sympathetic preganglionic neurons to postganglionic neurons in the paravertebral lumbar ganglia (Jänig 2006). It was not generated in afferent neurons (e.g., not in the dorsal root ganglia; see Michaelis et al. 2000). Postganglionic fibers with ongoing activity could not be directly activated by thermal (cold or heat) stimuli applied to the sural nerve, i.e., they did not exhibit axonal cold or heat sensitivity, as is the case for thermosensitive (cold or heat sensitive) afferent C fibers (see Teliban et al. 2011; Struck et al. unpublished observation).

The rate of ongoing activity was 1.1 ± 0.11 imp/s (mean $\pm$ SE, $n = 51$) in postganglionic neurons projecting in the sural nerve

and 1.43 ± 0.18 imp/s ($n = 39$) in postganglionic neurons projecting in the muscle nerve. CR of the activity was always strong in the postganglionic neurons innervating skeletal muscle ($n = 39$; see Fig. 4C, inset) and strong (35.3%), weak (31.4%), or absent (33.3%) in the postganglionic neurons innervating skin ($n = 51$; Fig. 2A, inset). Numerically, CR of the ongoing activity was significantly higher in the muscle postganglionic neurons than in the cutaneous postganglionic neurons [$99.6 \pm 0.13\%$ ($n = 39$) vs. $47.8 \pm 4.5\%$ ($n = 51$), $P < 0.001$, t -test]. This quantitative difference in degree of CR reflects that the muscle postganglionic neurons are involved in regulation of resistance blood vessels and therefore in regulation of arterial blood pressure and the cutaneous postganglionic neurons mainly in thermoregulation. In the remaining text we call postganglionic neurons with ongoing activity projecting to skin cutaneous vasoconstrictor (CVC) neurons and postgangli-

Table 1. Reactions of CVC and MVC neurons to stimulation of nociceptive afferent neurons innervating skin or skeletal muscle

	CVC Neurons	MVC Neurons	Change of BP
Ongoing activity, imp/s	1.1 ± 0.11 (<i>n</i> = 51)	1.42 ± 0.18 (<i>n</i> = 39)	
Cardiac rhythmicity, % of max	47.8 ± 4.5 (<i>n</i> = 51)	99.6 ± 0.13 (<i>n</i> = 39)	
Noxious toes (mechanical stimulation of ipsilateral toes 3 and 4)	∅ 6/37 ↓ 31/37	∅ 18/26 ↓ 8/26 (short)*†	↑ ↓ ‡ (5–10 mmHg)
Noxious muscle ipsilateral (injection of hypertonic saline in gastrocnemius-soleus muscle)	∅ 12/27 ↓ ↑ 3/27 (↓ short) ↑ 12/27	∅ 5/26 ↓ 21/26 (strong)	↓ 100% (16.6 mmHg)
Noxious muscle contralateral (injections of hypertonic saline in gastrocnemius-soleus muscle)	∅/↑ 6/16 ↑ 10/16	∅ 18/26 ↓ 8/26 (weak)*	↓ 100% (17 mmHg)
Heat approximately 48–52°C (stimulation of heat-sensitive axons in common peroneal n. or tibial n.)	∅ 12/37 ↓ 25/37	↓ 34/34	↓ 100% (21.5 mmHg)
Cold 5°C (stimulation of cold-sensitive axons in common peroneal n. or tibial n.)	∅ 34/34	∅ 24/24	∅ 100%

Data show reactions of cutaneous vasoconstrictor (CVC) and muscle vasoconstrictor (MVC) neurons to stimulation of nociceptors in skin or skeletal muscle (no. of neurons/total no. of neurons tested). The reactions of the CVC and MVC neurons to thermal stimulation of the common peroneal nerve or tibial nerve were put together since there was no quantitative difference between them. Values for ongoing activity and cardiac rhythmicity are means ± SE. The right column shows the changes of arterial blood pressure (BP). ∅, no change; ↑, activation or increase of BP; ↓, inhibition or decrease of BP; ∅/↑, no change or small activation; ↓ ↑, inhibition followed by activation. *Data from one and the same experiment. †Probably related to stimulation of deep somatic afferents innervating the toes. ‡Blood pressure initially increased, followed by decrease.

onic neurons with ongoing activity projecting to skeletal muscle muscle vasoconstrictor (MVC) neurons.

Blood pressure reactions to stimulation of nociceptors in skeletal muscle or skin. During mechanical noxious stimulation of the toes of the ipsilateral hindpaw the blood pressure initially increased in most cases, followed by a decrease (Fig. 2A; see also Fig. 4C). These blood pressure changes were in the range of 5–10 mmHg. Stimulation of muscle afferents by hypertonic saline injected into the ipsi- or contralateral gastrocnemius-soleus muscle was always followed by a decrease of blood pressure (range 6–50 mmHg; 16.8 ± 10.1 mmHg, mean ± SD, *n* = 32; Fig. 2, B and C, Fig. 3, B and C, Fig. 4, A and B, Fig. 5, A1 and A2). This decrease of arterial blood pressure was mostly largest to the first two injections of hypertonic saline and smaller during further injections. Therefore we used only the measurements from the first two intramuscular injections of hypertonic saline in the description of the reflexes in CVC and MVC neurons.

Stimulation of the unmyelinated axons in the CP nerve or the TIB nerve by heat (50°C) was always followed by a decrease of blood pressure (range 7.2–44.2 mmHg, 21.5 ± 10.0 mmHg, *n* = 36; Fig. 6, A1 and B1, Fig. 7). Stimulation of the unmyelinated axons in the CP or TIB nerve by noxious cold stimuli (5°C) was not accompanied by a change of blood pressure (*n* = 30; Fig. 6, A2 and B2, Fig. 7). This lack of blood pressure reaction to stimulation of nociceptive cold-sensitive afferents was not related to a conduction block of the afferent fibers (Struck et al., unpublished observation).

Reflexes in CVC neurons to noxious stimulation. Noxious stimulation of the toes of the ipsilateral hindpaw inhibited the activity in 31 of 37 CVC neurons (Fig. 2A) and did not change the activity in the remaining 6 CVC neurons. The population response of the CVC neurons in Fig. 3A illustrates that this inhibition outlasts the stimulus by >6 min. The inhibition of the CVC neurons is lateralized, i.e., it is weaker or absent when the contralateral paw is stimulated (not investigated in this study) (Häbler et al. 1994; Jänig 1985, 2006).

Noxious stimulation of muscle afferents of the ipsilateral hindlimb by hypertonic saline injected into the gastrocnemius-soleus muscle activated most CVC neurons; this acti-

vation either occurred immediately or, more often, was delayed (Fig. 2B). Three CVC neurons showed a weak decrease of activity in the first minute. In the population response, the activity in the CVC neurons slowly increased by ~25% after injection of hypertonic saline and remained increased for up to 6 min after hypertonic saline injection (Fig. 3B). Noxious stimulation of muscle afferents of the contralateral hindlimb by hypertonic saline injected into the gastrocnemius-soleus muscle increased the activity in the CVC neurons by ~50%. This increase occurred immediately after hypertonic saline injection and decreased to the control level in ~6 min (Fig. 2C, Fig. 3C).

Excitation of afferent axons by heat stimuli applied to the ipsilateral CP or TIB nerve inhibited the activity in the CVC neurons (Fig. 6A1, Fig. 7A). This inhibition was graded (Fig. 8A). The threshold generating this inhibition was in the range of 48–52°C (Fig. 8C). Stimulation of afferent axons in the CP or TIB nerve by noxious cold stimuli (5°C) had no significant effect on the activity in the CVC neurons (Fig. 6A2, Fig. 7A).

Reflexes in MVC neurons to noxious stimulation. Stimulation of nociceptive muscle afferents by a bolus injection of hypertonic saline into the gastrocnemius-soleus muscle of the ipsilateral hindlimb inhibited the activity in 21 of 26 MVC neurons investigated (Fig. 4A, Fig. 5A1). This inhibition started immediately and lasted for ~3–4 min after intramuscular injection of hypertonic saline. Stimulation of nociceptive muscle afferents of the contralateral hindlimb by hypertonic saline either had no effect (Fig. 4B, Fig. 5A2) or inhibited the activity in the MVC neurons (Fig. 5A2). This inhibition was seen in only one of six experiments.

Noxious stimulation of the toes of the ipsilateral paw mostly had no effect or weakly excited 18 of 26 MVC neurons (Fig. 4C, Fig. 5B). Eight MVC neurons were inhibited (Fig. 5B). This inhibition was observed in only one of six experiments. It was much shorter than the inhibition of the activity of CVC neurons generated by noxious stimulation of the toes (compare Fig. 3A with Fig. 5B). Interestingly, the MVC neurons showing inhibition to stimulation of nociceptors in contralateral skeletal muscle and to noxious stimulation of ipsilateral toes were from the same experiment.

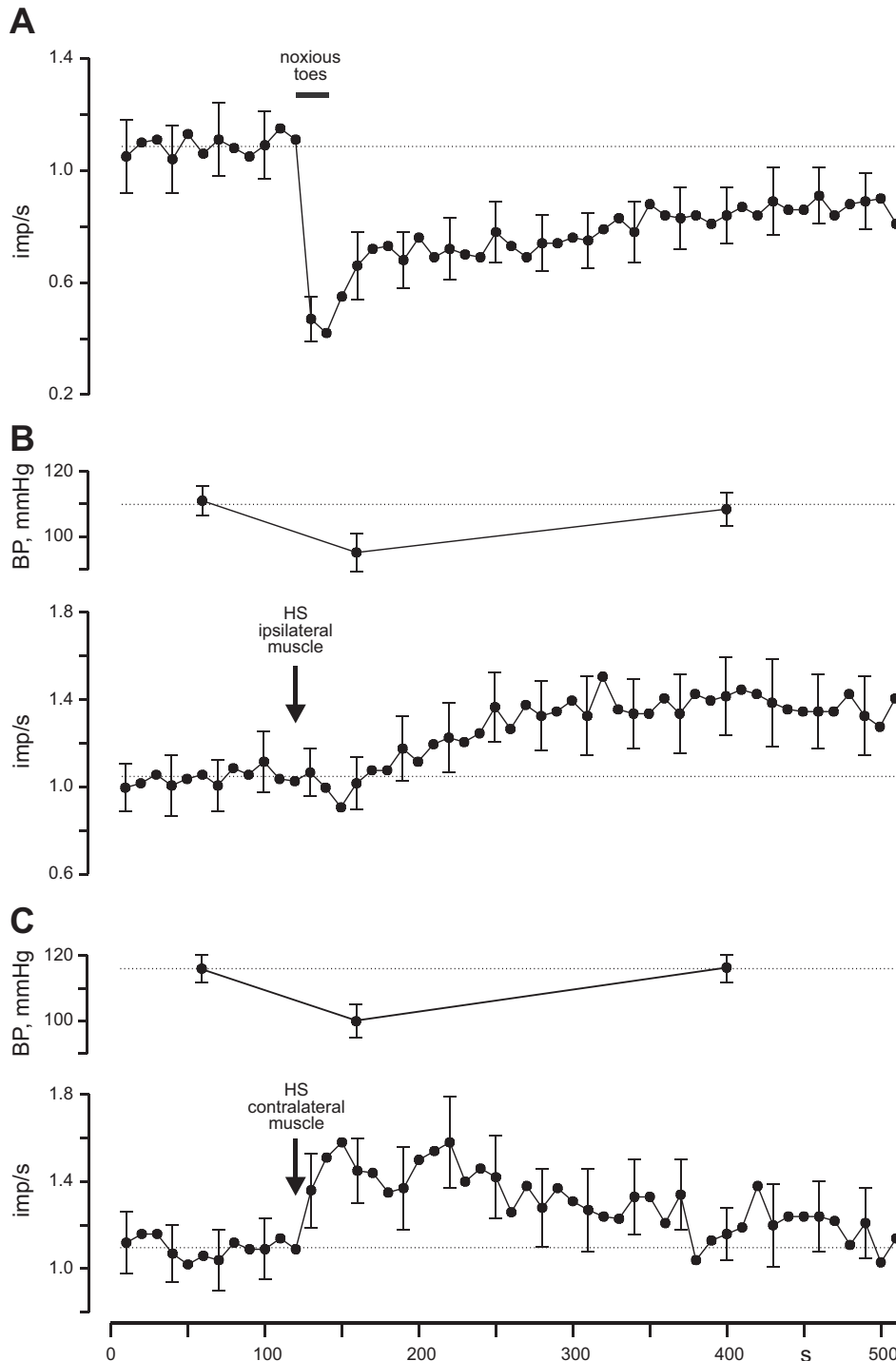


Fig. 3. CVC neurons. *A*: mechanical noxious stimulation of ipsilateral toes; $n = 37$ neurons recorded in 15 filaments. *B*: injection of HS into ipsilateral gastrocnemius-soleus muscle; $n = 27$ neurons recorded in 10 filaments. *C*: injection of HS into contralateral gastrocnemius-soleus muscle; $n = 16$ neurons recorded in 6 filaments. Note the decrease of BP during stimulation of muscle afferents. Means $\pm$ SE.

Stimulation of the afferent axons in the ipsilateral CP or TIB nerve by heat inhibited the activity in all MVC neurons investigated (Fig. 6*B1*, Fig. 7*B*). This inhibition was stronger in the MVC neurons than in the CVC neurons and was graded (Fig. 7, Fig. 8). The threshold of the inhibition in the MVC neurons was in the range of 43–48°C and significantly lower than in the CVC neurons ($P < 0.001$, χ^2 -test; Fig. 8*C*). Excitation of afferent axons in the CP or TIB nerve by noxious cold stimuli of 5°C had no effect on the activity in the MVC neurons (Fig. 6*B2*, Fig. 7*B*).

The inhibitory reflexes in MVC neurons appear to be sensitive to the depth of anesthesia. They were relatively weak

when the rats were in a relatively light anesthetic state and were enhanced after an intravenous injection of pentobarbital (4 measurements).

DISCUSSION

The main results of the experiments reported here are (Table 1) as follows. 1) CVC neurons innervating the rat hindlimb are inhibited by stimulation of cutaneous nociceptors of the ipsilateral hindlimb. Stimulation of muscle nociceptors of the ipsi- or contralateral hindlimb excited the CVC neurons. The reflex

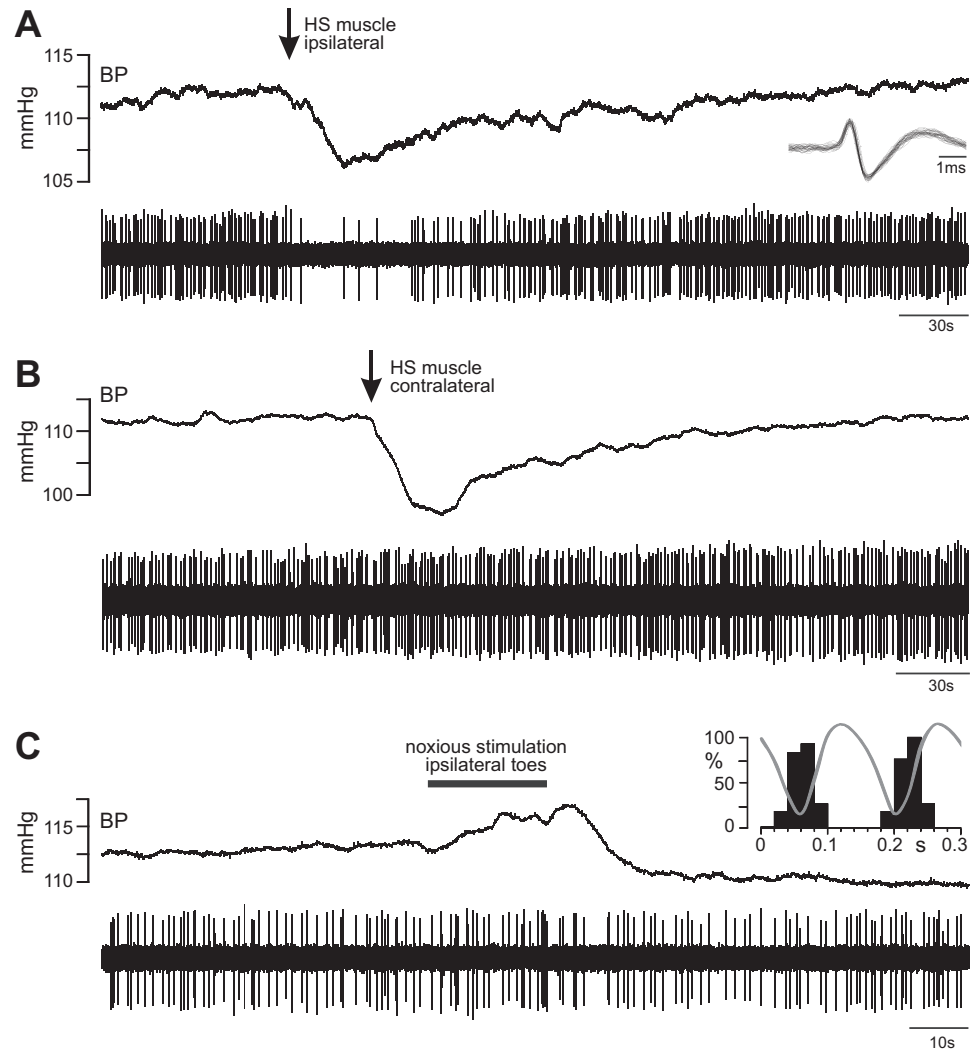


Fig. 4. MVC neuron: inhibition after stimulation of muscle afferents by bolus injection of HS into the ipsilateral gastrocnemius-soleus muscle (A), no effect after stimulation of muscle afferents by HS injected into the contralateral gastrocnemius-soleus muscle (B), and no effect or weak activation during noxious mechanical stimulation of the ipsilateral toes (C). Note the decrease of BP following stimulation of muscle afferents. The filament recorded from contained 1 MVC axon (see superimposed action potentials on right in A), which exhibited strong cardiac rhythmicity of its activity as shown in post-R wave histogram on right in C. Gray curve in inset shows the averaged pulsatile BP with respect to the R wave of the electrocardiogram (total amplitude was only 2 mmHg because of our recording condition). The beginning of the activation of the MVC neuron coincides with the decrease of BP, taking a conduction time of action potentials from the spinal cord to the recording site of 100 ms into account (Häbler et al. 1994).

inhibition and excitation were long-lasting. 2) MVC neurons innervating the hindlimb are strongly inhibited by stimulation of muscle nociceptors of the ipsilateral hindlimb but only weakly inhibited or not inhibited by stimulation of muscle nociceptors of the contralateral hindlimb. Stimulation of cutaneous nociceptors of the ipsilateral hindlimb had mostly no effect or weak excitatory or inhibitory effects on the MVC neurons. 3) Stimulation of the axons of nociceptive heat-sensitive afferent neurons projecting in the CP or TIB nerve, which contain muscle and cutaneous afferents, leads to reflex inhibition of both CVC and MVC neurons. This inhibition is stronger in MVC neurons than in CVC neurons. 4) Stimulation of the axons of nociceptive cold-sensitive afferent neurons projecting in the CP or TIB nerve has no effect on the activity in CVC and MVC neurons.

The postganglionic fibers recorded from were by functional criteria postganglionic vasoconstrictor fibers. The rates of ongoing activity in these neurons were in the range of those in postganglionic CVC and MVC neurons as reported in the literature for rats (Häbler et al. 1994, 1999). The degree of CR of the activity in these neurons, which is a measure of arterial baroreceptor control, was high in the MVC neurons and medium to low in the CVC neurons.

Stimulation of nociceptive afferents. In this study we used various stimuli exciting nociceptive afferents from skeletal muscle or skin to work out the inhibitory reflex circuits associated with the final CVC or MVC pathway. Cutaneous nociceptors were excited by mechanical stimulation of the toes of the ipsilateral hindpaw. This stimulus also excites a few deep somatic nociceptive afferents, which may be responsible for a weak inhibitory effect on a few MVC neurons and for the small decrease of arterial blood pressure following the increase (Table 1). Muscle nociceptors were stimulated by injecting 0.1 ml of hypertonic saline into the ipsilateral or contralateral gastrocnemius-soleus muscle. This stimulus probably also excites nonnociceptive unmyelinated or thinly myelinated muscle afferents (Mense 1993, 2009). The mechanism by which hypertonic saline excites afferent fibers is unclear (Kress and Reeh 1996; Mense 2009). The afferent terminals may be unspecifically excited by high extracellular sodium concentrations or by glutamate released locally (Tegeder et al. 2002).

Heat- or cold-sensitive C afferents were stimulated by heat or cold stimuli applied to the axons in the CP or TIB nerve. Both nerves contain cutaneous afferents and muscle afferents. Heat stimulation of a skin nerve specifically excites about a

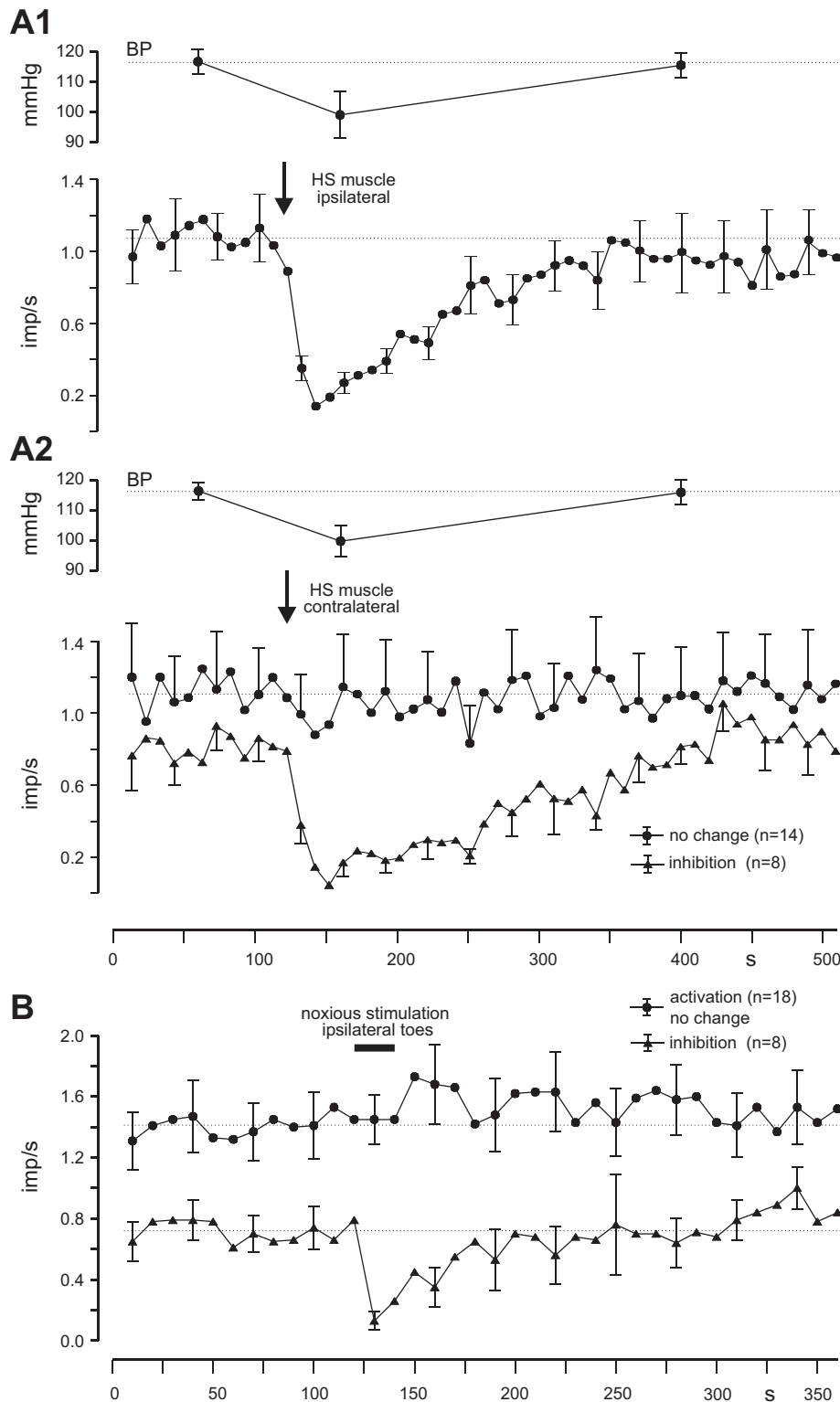


Fig. 5. MVC neurons. *A1*: injection of HS into the ipsilateral gastrocnemius-soleus muscle ($n = 26$ neurons recorded in 10 filaments). *A2*: injection of HS into the contralateral gastrocnemius-soleus muscle ($n = 26$ neurons recorded in 10 filaments). The neurons either show no change of their activity ($n = 18$, ●) or are inhibited ($n = 8$, ▲). The neurons showing inhibition were from 1 experiment. *B*: noxious mechanical stimulation of ipsilateral toes ($n = 26$). Neurons show either weak activation or no change of activity ($n = 18$, ●) or inhibition of activity ($n = 8$, ▲). The neurons showing inhibition were from the same experiment as the MVC neurons showing inhibition to stimulation of the contralateral muscle (*A2*, ▲). Note the decrease of BP during stimulation of muscle afferents. Means $\pm$ SE.

third of the unmyelinated axons of the heat-sensitive afferent neurons as identified from their cutaneous receptive field; however, it does not excite axons of afferent neurons that are cold sensitive and/or mechanosensitive only. Noxious cold stimulation of a skin nerve excites all nonnociceptive cold-sensitive afferent axons and $\sim 40\%$ of the nociceptive cold-sensitive afferent axons (Teliban et al. 2011). We have not yet

investigated the axonal cold or heat sensitivity of muscle afferents systematically as we have done for cutaneous afferent neurons (Struck et al., unpublished observations). However, preliminary results show that many muscle C afferents have axonal cold or heat sensitivity, the heat sensitivity probably being about four times stronger than the cold sensitivity (Kirilova et al. 2011). The strong heat sensitivity of unmyelinated

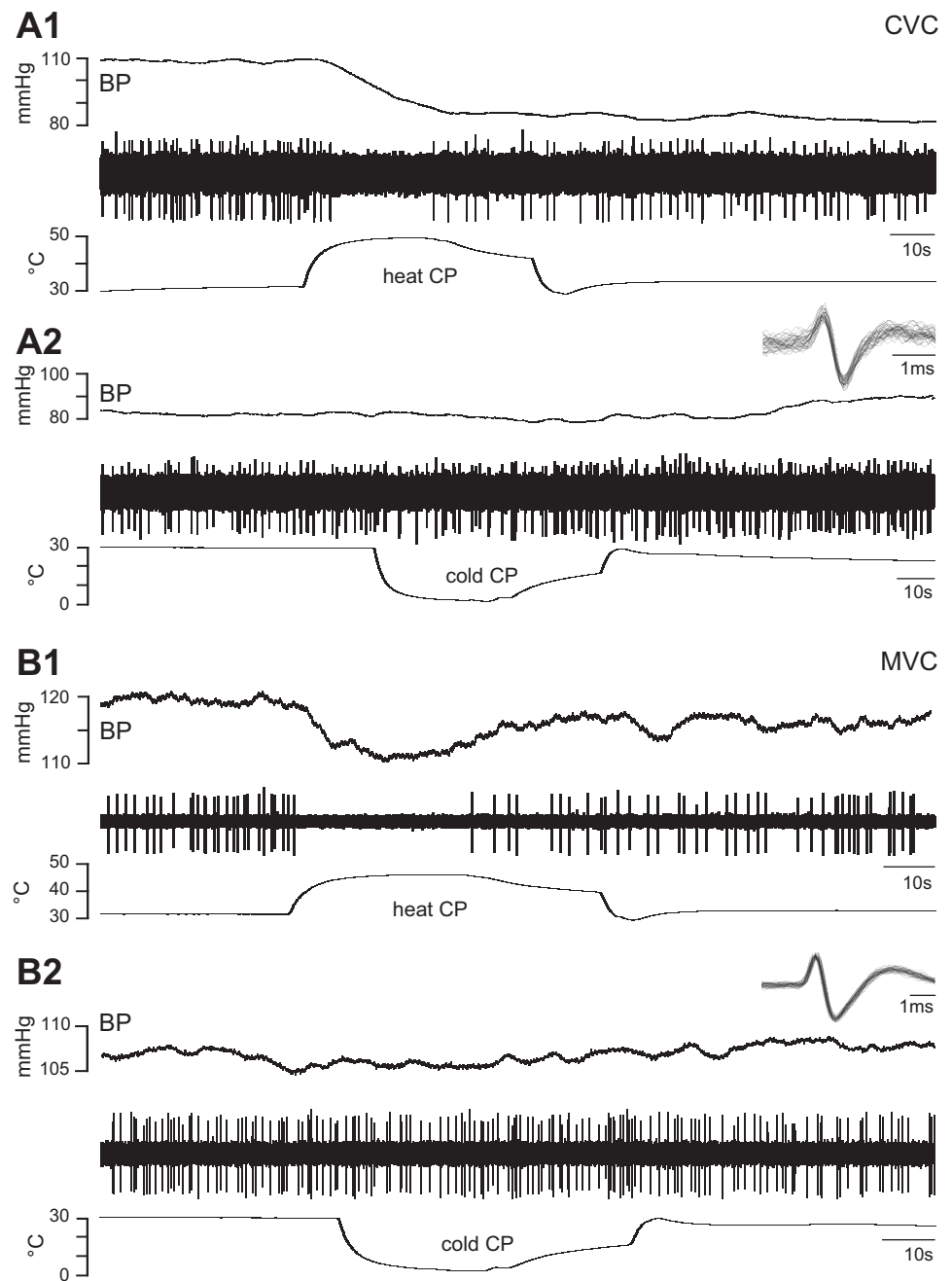


Fig. 6. Noxious heat stimulation, but not noxious cold stimulation, of axons elicits inhibitory reflexes in vasoconstrictor neurons. *A1* and *B1*: inhibition of activity in a CVC neuron (*A1*) and in a MVC neuron (*B1*) during excitation of heat-sensitive afferents by heat stimulation of the axons in the CP nerve (50°C in *A1*, 48°C in *B1*). Note the decrease of BP. *A2* and *B2*: no effect during excitation of cold-sensitive afferents by noxious cold stimulation of CP nerve (5°C).

muscle afferents is also indirectly supported by the results reported here: noxious stimulation of skin (toes of the hindpaw) inhibited the activity in CVC neurons but not or only very little the activity in MVC neurons (Fig. 5*B*); heat stimulation of the CP or TIB nerve inhibited both CVC and MVC neurons, the inhibition of activity in MVC neurons being stronger than in CVC neurons (Fig. 7, Fig. 8). These results can only be explained on the basis of a strong axonal heat sensitivity of unmyelinated muscle afferents.

Inhibitory reflexes in CVC neurons to stimulation of cutaneous nociceptors. The depression of CVC neurons during noxious mechanical stimulation of skin or noxious heating of cutaneous nociceptor axons in a nerve (here CP or TIB nerve) is most likely mediated by an inhibitory reflex pathway between cutaneous nociceptive primary afferent neurons and

sympathetic preganglionic CVC neurons synaptically connected to postganglionic CVC neurons (Fig. 9, *right*). This inhibition to noxious cutaneous stimulation has also been shown to exist in sympathetic preganglionic lumbar or thoracic neurons (Bartsch et al. 2000; Boczek-Funcke et al. 1992; Jänig and Szulczyk 1980). It has a spatial organization, i.e., it is particularly prominent in postganglionic neurons innervating the same skin territory as the stimulated afferent nociceptive neurons (Grosse and Jänig 1976; Horeysek and Jänig 1974a; Jänig and Kümmel 1977).

In chronic spinal cats mechanical or heat stimulation of cutaneous nociceptors of the hindpaw inhibits CVC neurons innervating the hindpaw. This inhibitory reflex is accompanied by an increase of blood flow through skin in the cat hindpaw, outlasts the noxious stimulus, and is lateralized (Horeysek and

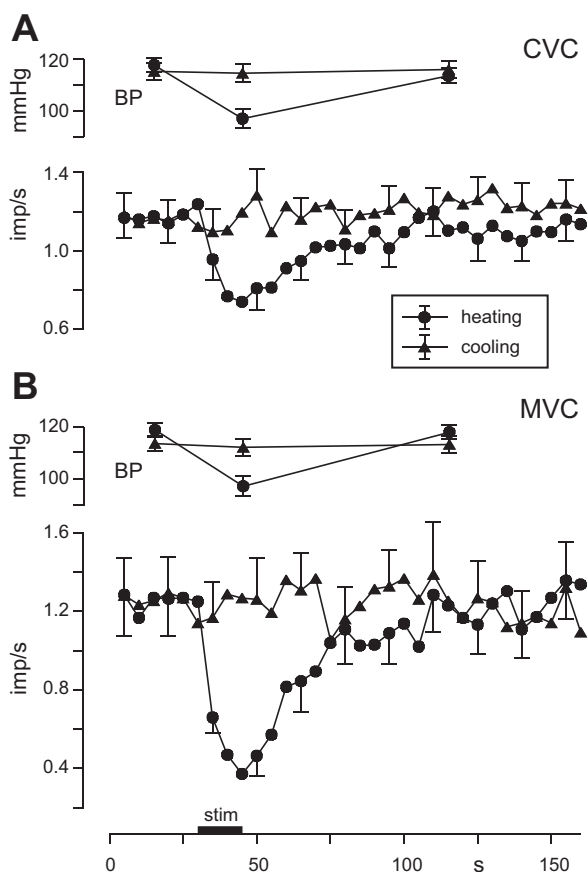


Fig. 7. Activity in CVC and MVC neurons during stimulation of afferent fibers by heat or noxious cold stimuli applied to CP nerve or TIB nerve. *A*: responses of CVC neurons to stimulation of heat-sensitive axons (52°C , $n = 37$ neurons recorded in 20 filaments) or of cold-sensitive axons (5°C , $n = 34$ neurons recorded in 17 filaments). *B*: responses of MVC neurons to stimulation of heat-sensitive axons (48°C , $n = 34$ neurons recorded in 17 filaments) or of cold-sensitive axons (5°C , $n = 24$ neurons recorded in 13 filaments). Note decrease of BP during heat stimulation and no change of BP during noxious cold stimulation. Means $\pm$ SE.

Jänig 1974b; Jänig and Kümmel 1981; Jänig and Spilok 1978). Thus this reflex is most likely organized at the level of the spinal cord.

Absence of inhibitory reflex in CVC neurons to stimulation of cutaneous nociceptive cold afferents. Noxious cooling of a skin nerve excites almost all nonnociceptive cold-sensitive afferent axons and $\sim 40\%$ of the nociceptive cold-sensitive afferent axons (Teliban et al. 2011). Surprisingly, this noxious afferent stimulus applied to the CP or TIB nerve generated no reflex inhibition of the activity in the postganglionic CVC neurons (and no change of arterial blood pressure; Table 1). The total number of impulses in the population of cutaneous cold-sensitive nociceptive C fibers generated by a stimulus of 5°C applied to their axons is about as high as the total number of impulses in the population of heat-sensitive C fibers generated by a stimulus of 50°C applied to their axons. This estimate is based on the numbers of cold-nociceptive or heat-nociceptive unmyelinated axons in the sural nerve, on the percentages of these afferent fibers excited by noxious cold (5°C) or heat stimuli (50°C) applied to a skin nerve, and on the discharge rates during these noxious stimuli (Struck et al., unpublished observations; Teliban et al. 2011). The complete absence of

reflex inhibition of CVC neurons projecting in the sural nerve during noxious cold stimulation argues that the inhibitory spinal reflex pathway connected to the CVC pathway (Fig. 9) is not activated by nociceptive cold-sensitive afferent neurons even if they are additionally heat-sensitive. We conclude that nociceptive heat-sensitive but cold-insensitive cutaneous afferent neurons and nociceptive cold-sensitive afferent neurons activate different groups of spinal second-order neurons that have different functions.

An alternative explanation for the complete absence of reflex inhibition of postganglionic CVC neurons upon stimulation of nociceptive cold-sensitive afferent axons could be this: The inhibitory reflex pathway to the preganglionic CVC neurons, which for the hindlimb has its origin in the spinal lumbar dorsal horn and which is normally activated by cutaneous nociceptive afferent neurons, is inhibited by the concomitant massive activation of nonnociceptive cool-sensitive and nociceptive

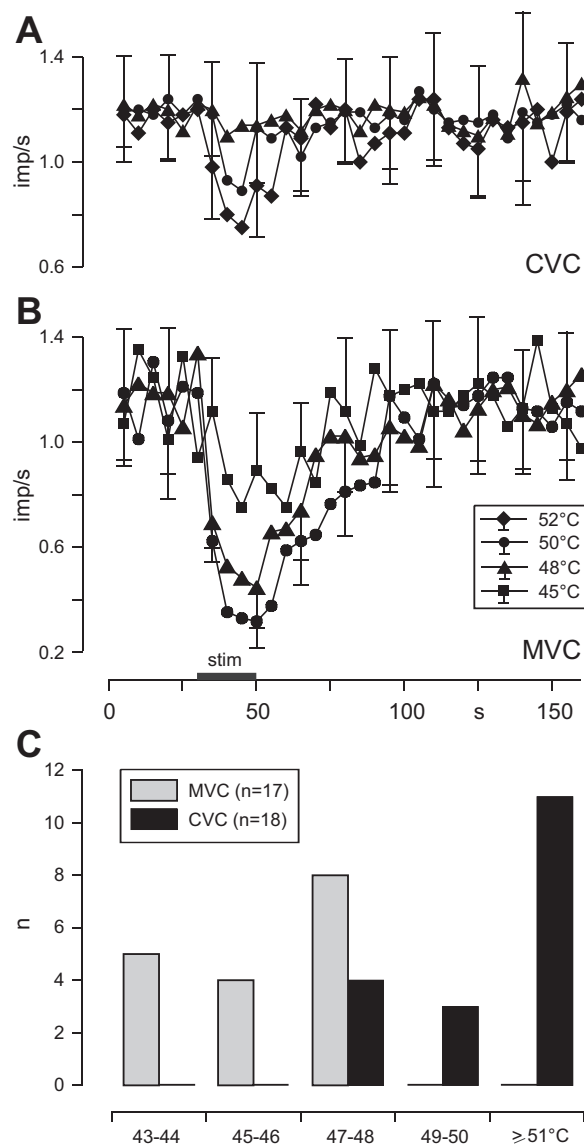


Fig. 8. *A* and *B*: graded responses of CVC neurons ($n = 11$, *A*) and MVC neurons ($n = 17$, *B*) to heat stimulation of CP or TIB nerve. *C*: thresholds of inhibition of CVC or MVC neurons to heat stimulation of CP or TIB nerve.

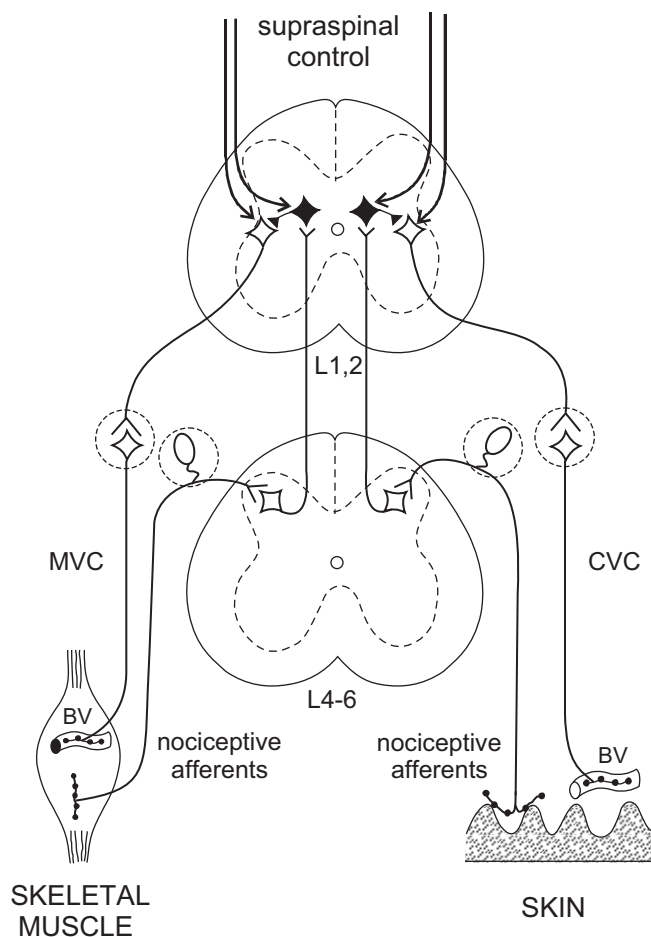


Fig. 9. Organization of the inhibitory nociceptive reflexes of the CVC system (right) and of the MVC system (left): a hypothesis. Stimulation of muscle nociceptive afferent neurons leads to inhibition of MVC neurons but not of CVC neurons. Stimulation of cutaneous nociceptive afferent neurons inhibits ipsilaterally projecting CVC neurons but not MVC neurons. The inhibitory reflexes are organized at the level of the spinal cord, the inhibitory interneurons being possibly located in the same segments as the preganglionic neurons. The nociceptive inhibitory reflex systems to the CVC and the MVC pathway are separated. They are largely lateralized and under differential supraspinal control.

cold-sensitive afferent nerve fibers (by cold stimulation of the CP or TIB nerve). At present we test this hypothesis.

No postganglionic CVC neuron showed at least a short-lasting reflex excitation to noxious cold stimuli or somewhat longer excitatory responses to mild cold stimuli of 12–20°C applied to the CP or TIB nerve activating almost solely axons of type 1 cold-sensitive afferent neurons. All cold stimuli up to 5°C strongly activate type 1 cold-sensitive C fibers (Teliban et al. 2011). There may be two reasons for this lack of activation of CVC neurons during stimulation of cold-sensitive afferent axons: 1) Activation of CVC neurons by cool stimuli applied to skin may require a widespread activation of thermal cool afferent input, e.g., from the whole trunk; thus our nonnociceptive input in cold-sensitive afferent axons generated by cold stimuli applied to the CP or TIB nerve was probably too small, although these cold stimuli activate practically all nonnociceptive cool-sensitive afferents in the respective nerve (Teliban et al. 2011). 2) We kept the core temperature of our rats close to 37°C (measured in the rectum). At this temperature CVC

neurons are already strongly activated. Thus an additional cold afferent input from a restricted source (here the hindlimb paw) may be not sufficient to further activate the CVC neurons by supraspinal integration (Owens et al. 2002).

Inhibitory reflexes in MVC neurons. Stimulation of skeletal muscle nociceptors was invariably followed by inhibition of activity in MVC neurons. This inhibition of MVC activity was most powerful when muscle nociceptors ipsilateral to the MVC pathway were stimulated and was largely lateralized (Fig. 5A). The inhibition of MVC activity was regularly accompanied by a decrease of arterial blood pressure, showing that all MVC neurons supplying the ipsilateral hindlimb (and some supplying the contralateral hindlimb) were inhibited, leading to vasodilation and decrease of peripheral resistance of the arterial system.

We hypothesize that the decrease of MVC activity during stimulation of muscle nociceptors is mediated by an inhibitory spinal reflex circuit that is largely lateralized and entirely separated from the inhibitory reflex circuit associated with the final CVC pathway (Fig. 9, left). An alternative is that the inhibition of the MVC pathway during stimulation of nociceptors in skeletal muscle is mediated by a supraspinal pathway. Cravo et al. (2003) have shown in rats that repetitive electrical stimulation of the sciatic nerve suprathreshold for afferent C fibers increases the blood flow in the ipsilateral hindlimb and decreases the blood flow in the contralateral hindlimb. The changes of blood flow can be explained by decrease of activity in MVC neurons innervating the ipsilateral hindlimb and increase of activity in MVC neurons innervating the contralateral hindlimb. The increase of blood flow in the ipsilateral hindlimb is abolished after injection of the neurotoxic substance kainic acid or injection of bicuculline, a blocker of GABAergic transmission, into the contralateral rostral ventrolateral medulla (RVLM). The authors conclude that the increase of blood flow in the hindlimb during sciatic nerve stimulation is mediated by excitatory sympathetic premotor neurons in the contralateral RVLM that are inhibited during electrical sciatic nerve stimulation and project to the ipsilateral final MVC pathway. The results of Cravo et al. (2003) are indirectly supported by the study of Korim et al. (2011) showing that electrical sciatic nerve stimulation that is suprathreshold for C fibers generates decrease of activity in the ipsilateral lumbar sympathetic trunk and increase of activity in the contralateral trunk, the decrease being mediated by the contralateral RVLM. Experiments on rats with chronically interrupted spinal cord will be necessary to show that the inhibitory reflex in MVC neurons generated by stimulation of muscle nociceptors is organized at the level of the spinal cord.

During stimulation of muscle nociceptors the arterial blood pressure decreased, largely because of inhibition of the activity in MVC neurons projecting to the ipsilateral hindlimb. This decrease of arterial blood pressure should induce unloading of arterial baroreceptors, which subsequently generates a disinhibition of sympathetic premotor neurons in the RVLM (by decrease of the activity in the inhibitory interneurons of the baroreceptor reflex pathway in the caudal ventrolateral medulla) and an activation of MVC neurons. However, the MVC neurons projecting to the hindlimb contralateral to activated muscle nociceptors were not activated during decrease of arterial blood pressure (Fig. 4B and Fig. 5A2, Table 1). This result implies 1) that the arterial baroreceptor reflex circuit to

the MVC pathway is inhibited bilaterally during stimulation of muscle nociceptors, preventing in this way an activation of MVC neurons (during unloading of arterial baroreceptors), and 2) that the mechanism underlying the generation of ongoing activity in the MVC neurons is not inhibited (Guyenet 1990; Jänig 2006; Schreihofer and Sved 2011).

The Lovén reflex. The inhibitory reflex was first described by Christian Lovén for the skin in rabbits while working in Carl Ludwig's laboratory in Leipzig (Lovén 1866). Lovén found out that electrical stimulation of the central stump of the dorsal nerve of the hindpaw, a branch of the superficial peroneal nerve (the nervus dorsalis pedis, which is a skin nerve), leads to dilation of the saphenous artery, no vasodilation but sometimes vasoconstriction in the ear, and increase of blood pressure. Stimulation of the central stump of the posterior branch of the auricular nerve generates vasodilation in the ear, no vasodilation but sometimes vasoconstriction of the saphenous artery, and increase of blood pressure. He concluded that vasomotor fibers innervating the saphenous artery or ear blood vessels must be different from vasomotor fibers responsible for increase in blood pressure and that vasodilation of skin vessels is generated reflexly by stimulation of afferents that innervate the same skin territory that is innervated by the cutaneous vasomotor fibers or a territory close by (Lovén 1866). Lovén did not use the terms "vasoconstrictor fibers" and "vasodilator fibers." Therefore he did not comment on his results as being generated by decrease of activity in vasoconstrictor neurons or activation of vasoconstrictor neurons. Bayliss (1908) reproduced the Lovén reflex in dogs by measuring plethysmographically the volume change of the hindlimb, which is largely dependent on blood flow through skeletal muscle. Electrical stimulation of the distally cut lumbar dorsal root L₆ elicited a vasodilation and electrical stimulation of the median nerve a vasoconstriction in the hindlimb. Bayliss and various authors of textbook chapters describing the Lovén reflex (Bard 1968; Bell et al. 1950; Detweiler 1979; Hamilton 1950) believed that the reflex vasodilation resulting from stimulation of (probably nociceptive) afferents supplying the same tissues as the efferent sympathetic fibers is likely to be a general phenomenon everywhere in the body. Thus the results of Lovén were generalized to apply also to organs other than the skin, although Lovén never investigated the reflex inhibition in other organs. This would imply that there exist several specific inhibitory sympathetic reflex pathways to the vasculature of various tissues or organs, each being defined by the (nociceptive) afferent input from the tissue and by the sympathetic outflow to the tissue. However, this idea has never been systematically tested. Here we have shown for the first time that MVC neurons are specifically inhibited upon stimulation of nociceptive muscle afferents innervating the same extremity but not stimulation of cutaneous nociceptive afferents.

Studies on humans. In the literature the idea is propagated that cutaneous pain is accompanied by increase of arterial blood pressure and heart rate whereas deep somatic pain is often associated with decrease of arterial blood pressure and heart rate (Feinstein et al. 1954; Lewis 1942). This belief has recently been challenged by investigations showing that both muscle and cutaneous pain generated experimentally by bolus injections of hypertonic saline either into the tibialis anterior muscle or into the overlying skin were accompanied by increase in muscle sympathetic nerve activity, arterial blood

pressure, and heart rate (Burton et al. 2009). Further investigations of the same group showed that infusion of hypertonic saline into the tibialis anterior muscle over 40–60 min leads to progressive decrease of muscle sympathetic nerve activity, arterial blood pressure, and heart rate in ~50% of the subjects tested and to progressive increase in the remaining subjects tested (Fazalbhoy et al. 2012). Skin sympathetic nerve activity initially increased, followed by a sustained decrease with the corresponding changes of skin blood flow (initial decrease followed by sustained increase; Hall et al. 2012). These results obtained in humans appear to be at variance with our results. We hypothesize that the putatively spinal inhibitory reflexes mediating the inhibition of CVC or MVC neurons upon noxious stimulation of skin or skeletal muscle are not readily seen in awake human beings. This does not mean that these inhibitory reflexes are absent in humans.

Blumberg and Wallin (1987) have shown that painful intra-neural electrical microstimulation in the superficial peroneal nerve at the ankle, at a strength that excites thinly myelinated (A δ) nociceptive afferents, elicits reflex dilation (increased blood flow) in skin areas lying adjacent to, as well as in, the territory of the stimulated nerve. In addition to causing vasodilation ipsilaterally, this stimulus also evokes a lesser vasodilator response in the contralateral limb. The vasodilation in the ipsi- and contralateral skin is abolished by local anesthesia of the nerve proximal to the stimulation site. Finally, the dilation is enhanced by body cooling (i.e., when the activity in CVC neurons is high), arguing that the reflex vasodilation is produced by decrease of activity in CVC neurons and not by activation of cutaneous vasodilator neurons. Thus this reflex in human beings described by Blumberg and Wallin (1987) appears to be very similar to the inhibitory reflex in CVC neurons elicited by cutaneous noxious stimuli in anesthetized cats and rats. Whether this inhibitory reflex in humans is spinal has to be shown, e.g., in experimental studies on chronic paraplegic or quadriplegic patients.

Pathophysiological implications. How do these putatively spinal nociceptive inhibitory vasoconstrictor reflexes behave under pathophysiological conditions, i.e., under chronic activation of the nociceptive system (e.g., during chronic inflammation or chronic nerve injury)? The potential clinical implications of our findings are considerable. These implications are expressed in five hypotheses.

1) The inhibitory spinal systems are switched on during injury of peripheral tissues, furthering healing of the affected tissues. Thus inflammation of peripheral tissues sensitizes nociceptors; the inhibitory reflex in the vasoconstrictor pathway to the inflamed tissue is activated, and the blood flow through the tissue increases.

2) The vasodilation that is observed in the innervation territory of a partially injured nerve, and in the innervation territory of neighboring nerves, is usually thought to be due to lesion of sympathetic vasoconstrictor axons. However, in light of our present findings a second mechanism (spinal inhibition of activity in preganglionic CVC or MVC neurons) might also be involved. This mechanism may operate in patients with complex regional pain syndrome (CRPS) in the acute phase (Baron and Jänig 2013; Jänig and Baron 2003).

3) The inhibitory spinal systems may change plastically during chronic tissue injury (inflammation, nerve injury), leading to decrease of functioning of these spinal inhibitory sys-

tems and to the development of positive feedback systems. These changes may also occur in patients with CRPS (Baron and Jänig 2013; Jänig 2009; Jänig and Baron 2003).

4) The inhibitory spinal systems are involved in generation of autonomic changes (e.g., increase of blood flow) in referred zones of patients following deep somatic tissue injury (Vecchiet et al. 1993).

5) The inhibitory spinal system(s) connected to deep somatic tissues is (are) activated during manual interventions at the paraspinal deep tissues in patients with functional diseases involving deep somatic tissues or viscera. The manual interventions activate deep somatic small-diameter myelinated and unmyelinated high- and low-threshold afferents. This afferent activation leads to increase of blood flow mediated by the inhibitory reflex(es) through the deep somatic tissues, to relief of pain, and to cure of the functional diseases (King et al. 2011).

In conclusion, stimulation of muscle nociceptors leads to inhibition of MVC activity but not of CVC activity and stimulation of cutaneous nociceptors to inhibition of CVC activity but not of MVC activity. These inhibitory reflexes are spatially organized. Thus our results clearly show that the inhibitory reflex mechanisms are function specific as far as skeletal muscle and skin are concerned. We hypothesize that tissue injury activating and sensitizing nociceptors generates via reflex inhibition of vasoconstrictor activity local increase of blood flow resulting in an increase of transport of immuno-competent cells, proteins, and oxygen to the site of injury and enhancing the process of healing. Thus this inhibitory reflex is a tissue-protecting reflex that enhances the blood flow through injured and inflamed tissues, e.g., by neuropeptides and other compounds released by nociceptive nerve fibers, mast cells, and macrophages. Is the blood flow through other tissues, such as joint, bone, or visceral organs (e.g., heart, kidney), also controlled by "private" inhibitory sympathetic, possibly spinal circuits that mediate increase of blood flow during injury of the tissues?

ACKNOWLEDGMENTS

The authors are grateful to Hans-Georg Schaible for critical reading of this manuscript.

GRANTS

This research was supported by the German Research Foundation, Grünenthal, and Pfizer.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: I.K.-W., R.B., and W.J. conception and design of research; I.K.-W. performed experiments; I.K.-W. analyzed data; I.K.-W., R.B., and W.J. interpreted results of experiments; I.K.-W. and W.J. prepared figures; I.K.-W. and W.J. drafted manuscript.

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