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

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Abstract

Cutaneous (CVC) and muscle vasoconstrictor (MVC) 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. The 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: CVC neurons are inhibited by noxious stimulation of skin but not by noxious stimulation of skeletal muscle. 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 cutaneous or muscle vasoconstrictor 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.

Key words: Cutaneous vasoconstrictor neurons, muscle vasoconstrictor neurons, postganglionic fibers, nociceptive reflex inhibition, spinal reflexes, heat-sensitive afferents, cold-sensitive afferents
Introduction

Some 15 to 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 neurons is inhibition of ongoing activity upon noxious stimulation of skin that is innervated by the postganglionic cutaneous vasoconstrictor (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 & Jänig 1976; Horeyseck & Jänig 1974a; Jänig & Kümmel 1977; Jänig & Szulczyk 1980). It is most likely a spinal reflex (Horeyseck & Jänig 1974b; Jänig & Kümmel 1981; Jänig & 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 has shown 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 hypothesis: (1) CVC neurons innervating the rat 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 rat 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 by pentobarbital sodium (Narcoren [Merial GmbH, Hallbergmoos, Germany], 40 mg/kg i.p. initially; 10 mg/kg per hour during surgery and the experiments), paralyzed (Pancuronium [Organon], initial dose 1 mg/kg i.v.; maintenance dose 0.4 mg/kg/h i.v.) and artificially ventilated with oxygen-enriched air. 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, Pco2=35-40 mmHg and Po2≥100 mmHg. Rectal temperature was kept constant at approximately 37°C using 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 about 5 mm proximal to the ankle and the sciatic nerve at its trifurcation about 10 mm distal to the sciatic notch. Additionally the common peroneal nerve was 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 about 6 mm about 20 mm rostral to the ankle and placed on a rigidly fixed black perspex platform of 5 x 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 the central end of the fibers being 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,b).

- Figure 1 here -

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 to 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 is called cardiac rhythmicity (CR) of the postganglionic vasoconstrictor activity (see right insets in Figs. 2A and 4C). The degree of CR was measured as the difference between the maximum activity (over 40 ms) and the minimum activity (over 40 ms) in per cent of the maximum activity. The degree of CR was categorized as strong (CR ≥ 60%), middle (CR 40% - 60%), weak or absent (CR < 40%) (Häbler et al 1994). Cutaneous nociceptors of the toes were stimulated mechanically using 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 common peroneal (CP) nerve or tibial (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 tibial 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 about 30°C (Fig. 6). Heat stimuli varied in intensity between 45 and about 51°C and cold stimuli were 5°C strong (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 axonal cold sensitivity, and all non-nociceptive cold-sensitive C-fibers 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 using the general purpose capture and analysis package Spike II (Cambridge Electronic Design Ltd, 4 Science Park, Milton Road, Cambridge, UK). Quantitative measurements are expressed as mean ± SEM. For statistical analysis the Student’s t-test or the non-parametric 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 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 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 directly be activated by thermal (cold or heat) stimuli applied to the sural nerve, i.e. they did not exhibit axonal cold or heat sensitivity, as it 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 ± SEM, 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. The cardiac rhythmicity of the activity was always strong in the postganglionic neurons innervating skeletal muscle (N=39; inset in Fig. 4C) and strong (35.3%), weak (31.4%) or absent (33.3%) in the postganglionic neurons innervating skin (N=51; inset in Fig. 2A). Numerically the cardiac rhythmicity of the ongoing activity was significantly higher in the muscle postganglionic neurons than in the cutaneous postganglionic neurons (99.6 ± 0.13% [N=39] vs 47.8 ± 4.5% [N=51], p<0.001, t-test). This quantitative difference in degree of cardiac rhythmicity 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 will call postganglionic neurons with ongoing activity projecting to skin cutaneous vasoconstrictor (CVC) neurons and postganglionic neurons with ongoing activity projecting to skeletal muscle muscle vasoconstrictor (MVC) neurons.

- Figure 2 and Figure 3 here -
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 (Figs. 2A, 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 mm Hg; 16.8 ± 10.1 mm Hg, mean ± SD, N = 32; Figs. 2B,C, 3B,C, 4A,B, 5A,B). 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 to the first two intramuscular injection of hypertonic saline in the description of the reflexes in the CVC and MVC neurons.

Stimulation of the unmyelinated axons in the common peroneal nerve or the tibial nerve by heat (50 °C) was always followed by a decrease of blood pressure (range 7.2 - 44.2 mm Hg; 21.5 ± 10.0 mm Hg, N = 36; Figs. 6A1, 6B1, 7). Stimulation of the unmyelinated axons in the common peroneal or tibial nerve by noxious cold stimuli (5°C) was not accompanied by a change of blood pressure (N = 30; Figs. 6A2, 6B2, 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 (see Struck et al unpublished).

Reflexes in CVC neurons to noxious stimulation

Noxious stimulation of the toes of the ipsilateral hindpaw inhibited the activity in 31/37 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 more than 6 mins. 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 activation occurred either immediately or more often was delayed (Fig. 2B). Three CVC neurons showed a weak decrease of activity in the first min. In the population response the activity in the CVC neurons slowly increased by about 25% after injection of hypertonic saline and remained increased for up to 6 minutes 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 about 50%. This increase occurred immediately after
hypertonic saline injection and decreased to the control level in about 6 mins (Fig. 2C,
Fig. 3C).

Excitation of afferent axons by heat stimuli applied to the ipsilateral common
peroneal (CP) or tibial (TIB) nerve inhibited the activity in the CVC neurons (Fig. 6A1,
7A closed circles). This inhibition was graded (Fig. 8A). The threshold generating this
inhibition was in the range of 48 to 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 (Figs. 6A2, 7A triangles).

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
the 21/26 MVC neurons investigated (Figs. 4A, 5A1). This inhibition started
immediately and lasted for about 3-4 mins after intramuscular injection of hypertonic
saline. Stimulation of nociceptive muscle afferents of the contralateral hindlimb by
hypertonic saline had either no effect (Figs. 4B, 5A2 closed circles) or inhibited the
activity in the MVC neurons (Figs. 5A2 triangles). This inhibition was seen in only one
out of 6 experiments.

Noxious stimulation of the toes of the ipsilateral paw mostly had no effect or weakly
excited 18/26 MVC neurons (Fig. 4C, Fig. 5B closed circles). Eight MVC neurons were
inhibited (Fig. 5B triangles). This inhibition was observed in only one out of 6
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).

Interesting 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.

Stimulation of the afferent axons in the ipsilateral CP or TIB nerve by heat
inhibited the activity in all MVC neurons investigated (Figs. 6B1, 7B closed circles).
This inhibition was stronger in the MVC neurons than in the CVC neurons and was graded (Figs. 7, 8). The threshold of the inhibition in the MVC neurons was in the range of 43 to 48°C and significantly lower than in the CVC neurons (p<0.001, $\chi^2$-test; Fig. 8C). 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 (Figs. 6B2, 7B triangles).

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): (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 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 common peroneal or the tibial nerve which contain muscle and cutaneous afferents leads to reflex inhibition of both CVC and MVC neurons. This inhibition is stronger in MVC than in CVC neurons. (4) Stimulation of the axons of nociceptive cold-sensitive afferent neurons projecting in the common peroneal or tibial nerve has no effect on the activity in the 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 cardiac rhythmicity 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 (Fig. 5B, Table 1). Muscle nociceptors were stimulated by injecting 0.1 ml hypertonic saline into the ipsilateral or contralateral gastrocnemius-soleus muscle. This stimulus probably also excites non-nociceptive unmyelinated or thinly myelinated muscle afferents (Mense 1993, 2009). The mechanism by which hypertonic saline excites afferent fibers is unclear (Kress & 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 common peroneal or tibial nerve. Both nerves contain cutaneous afferents and muscle afferents. Heat stimulation of a skin nerve specifically excites about a 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 which are cold- and/or mechanosensitive only. Noxious cold stimulation of a skin nerve excites all non-nociceptive cold-sensitive afferent axons and about 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 4 times stronger than the cold sensitivity (Kirillova et al 2011). The strong heat sensitivity of unmyelinated 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 very little the activity in MVC neurons (Fig. 5B); heat stimulation of the common peroneal or tibial nerve inhibited both CVC and MVC neurons, the inhibition of activity in MVC neurons being stronger than in CVC neurons (Figs. 7, 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 common peroneal or tibial 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, left side). 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 & 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 & Jänig 1976; Horeyseck & Jänig 1974a; Jänig & Kümmel 1977).

In chronic spinal cats mechanical or heat stimulation of cutaneous nociceptors of the hindpaw inhibits cutaneous vasoconstrictor 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 (Horeyseck & Jänig 1974b; Jänig & Kümmel 1981; Jänig & Spilok 1978). Thus this reflex is most likely organized at the level of the spinal cord.

Absence of the inhibitory reflex in CVC neurons to stimulation of cutaneous nociceptive cold afferents

Noxious cooling of a skin nerve excites almost all non-nociceptive cold-sensitive afferent axons and about 40% of the nociceptive cold-sensitive afferent axons (Teliban et al 2011). Surprisingly, this noxious afferent stimulus applied to the common peroneal or tibial 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 which 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 has for the hindlimb 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 non-nociceptive cool-sensitive and nociceptive cold-sensitive afferent nerve fibers (by cold stimulation of the common peroneal or tibial 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 only 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). This lack of activation of CVC neurons during stimulation of cold-sensitive afferent axons may have two reasons: (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 non-nociceptive input in cold-sensitive afferent axons generated by cold stimuli applied to the common peroneal or tibial nerve was probably too small, although these cold stimuli practically activate all non-nociceptive 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 are CVC neurons already strongly activated. Thus an additional cold afferent input from a restricted source (here hindlimb paw) may be not sufficient to further activate the CVC neurons by supraspinal integration (Owens et al 2002).
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). Alternatively is the inhibition of the MVC pathway during stimulation of nociceptors in skeletal muscle 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 one. The increase of blood flow in the ipsilateral hindlimb is abolished after injection of the neurotoxic substance kainic acid or 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 Korim et al (2011) showing that electrical sciatic nerve stimulation which is suprathreshold for C-fibers generates decrease of activity in the ipsilateral lumbar sympathetic trunk and increase of activity in the contralateral one, 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 due to 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 rostral ventrolateral medulla (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 (Figs. 4B and 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 & Sved 2011).

The Lovén reflex

The inhibitory reflex has first been described by Christian Lovén in rabbits for the skin while working in Carl Ludwig’s laboratory in Leipzig (Lovén 1866). Lovén found 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 a 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 which 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 his results as being generated by decrease of activity in vasoconstrictor neurons and 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 other organs 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 of cutaneous nociceptive afferents.

Studies on humans

In the literature it 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 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 to 60 min leads to progressive decrease of the muscle sympathetic nerve activity, arterial blood pressure and heart rate in about 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 & Wallin (1987) have shown that painful intraneural electrical microstimulation in the superficial peroneal nerve at the ankle, at a strength that excites thinly myelinated (Aδ) 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-
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 cutaneous vasoconstrictor 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 & 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 tetraplegic patients.

Pathophysiological implications

How do these putative 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 vasodilatation which 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, based on 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 (Jänig & Baron 2003; Baron & Jänig 2013).

3. The inhibitory spinal systems may change plastically during chronic tissue injury (inflammation, nerve injury) leading to decrease of functioning of these spinal inhibitory systems and to the development of positive feedback systems. These changes may also occur in patients with complex regional pain syndrome (CRPS) (Baron & Jänig 2013, Jänig 2009, 2013, Jänig & 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).
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 immunocompetent 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?
References


Acknowledgements

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**Figure 1**

*Experimental approach. Left side:* 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 the paravertebral ganglia L2 to L4 (Baron et al 1988). *Right side:* 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 ones) are stimulated by noxious heat stimuli (43 - 52°C) or noxious cold stimuli (4 - 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 the dorsal root ganglia L4 – L6 (mostly L5) (Baron et al 1988). BV, blood vessel.

**Figure 2**

*Cutaneous vasoconstrictor (CVC) neuron. Inhibition during mechanical noxious stimulation of the ipsilateral toes (A) and activation upon stimulation of muscle afferents by bolus injection of hypertonic saline into the ipsilateral (B) or contralateral gastrocnemius-soleus muscle (C) (note the decrease of arterial blood pressure [BP]) in B and C). The filament recorded from contains one CVC axon (see superimposed action potentials on the right in B) which exhibits no cardiac rhythmicity of its activity as shown in the post-R-wave histogram of the CVC activity in the inset on the right in A. The grey curve in the inset shows the averaged pulsatile arterial blood pressure with respect to the R-wave superimposed on the post-R-wave histogram (the total amplitude was only 4 mm Hg due to our recording condition).*

**Figure 3**

*Cutaneous vasoconstrictor neurons. A. Mechanical noxious stimulation of ipsilateral toes. N = 37 neurons. B. Injection of hypertonic saline (HS) into the ipsilateral gastrocnemius-soleus muscle. N = 27 neurons recorded in 10 filaments. C. Injection of hypertonic saline (HS) into the ipsilateral gastrocnemius-soleus muscle. N = 16 neurons*
recorded in 6 filaments. Note the decrease of arterial blood pressure (BP) during stimulation of muscle afferents. Means ± SEM.

Figure 4

**Muscle vasoconstrictor (MVC) neuron.** Inhibition after stimulation of muscle afferents by bolus injection of hypertonic saline (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 arterial blood pressure (BP) following stimulation of muscle afferents. The filament recorded from contained one MVC axon (see superimposed action potentials on the right in A) which exhibited strong cardiac rhythmicity of its activity as shown in the post-R-wave histogram on the right in C. The grey curve in the inset shows the averaged pulsatile arterial blood pressure with respect to the R-wave of the electrocardiogram (the total amplitude was only 2 mm Hg due to our recording condition). The beginning of the activation of the MVC neuron coincides with the decrease of blood pressure taking a conduction time of action potentials from the spinal cord to the recording site of 100 ms into account (Häbler et al 1994).

Figure 5

**Muscle vasoconstrictor neurons.** A1. Injection of hypertonic saline (HS) into the ipsilateral gastrocnemius-soleus muscle (n = 26 neurons recorded in 10 filaments). A2. Injection of hypertonic saline (HS) into the contralateral gastrocnemius-soleus muscle (n = 26 neurons recorded in 10 filaments). The neurons show either no change of their activity (N = 18, closed circles) or are inhibited (N = 8, triangles). The neurons showing inhibition were from one experiment. B. Noxious mechanical stimulation of ipsilateral toes (n = 26). Neurons show either weak activation or no change of activity (N = 18, closed circles) or inhibition of activity (N = 8, triangles). The neurons showing inhibition were from the same experiment as the MVC neurons showing inhibition to stimulation of the contralateral muscle (triangles in A2). Note the decrease of arterial blood pressure (BP) during stimulation of muscle afferents. Mean ± SEM.

Figure 6

**Noxious heat stimulation of axons elicit inhibitory reflexes in vasoconstrictor neurons but not noxious cold stimulation.** A1, 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 common peroneal nerve (CP; 50°C in A1, 48°C in B1). Note the decrease of arterial blood pressure. A2, B2. No effect during excitation of cold-sensitive afferents by noxious cold stimulation of the common peroneal nerve (5°C). BP, blood pressure.

Figure 7
Activity in CVC and MVC neurons during stimulation of afferent fibers by heat or noxious cold stimuli applied to the common peroneal nerve or tibial 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 the decrease of arterial blood pressure (BP) during heat stimulation and no change of BP during noxious cold stimulation. Mean + SEM.

Figure 8
Graded responses of CVC neurons (n=11, A) and MVC neurons (n=17, B) to heat stimulation of the common peroneal (CP) or tibial (TIB) nerve. (C) Thresholds of inhibition of CVC or MVC neurons to heat stimulation of the CP or TIB nerve.

Figure 9
Organization of the inhibitory nociceptive reflexes of the cutaneous vasoconstrictor (CVC) system (right) and of the muscle vasoconstrictor (MVC) system (left): A hypothesis. Stimulation of muscle nociceptive afferent neurons leads to inhibition 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 peanglionic neurons. The nociceptive inhibitory reflex systems to the CVC and the MVC pathway are separated. They are largely lateralized and under differential supraspinal control. BV, blood vessel.

Table 1
Reactions of cutaneous vasoconstrictor (CVC) neurons and muscle vasoconstrictor (MVC) neurons to stimulation of nociceptive afferent neurons innervating skin or skeletal muscle.
Kirillova et al

Fig. 2

A

noxious stimulation
ipsilateral toes

115
110
105
mmHg

BP

B

BP

ipsilateral

115
110
105
mmHg

C

HS muscle
contralateral

120
115
110
mmHg

Kirillova et al
Fig. 2
Kirillova et al
Fig. 3
A

HS muscle ipsilateral

B

HS muscle contralateral

C

noxious stimulation ipsilateral toes

Kirillova et al
Fig. 4
Kirillova et al. 
Fig. 7

A

CVC

B

MVC

BP

imp/s

mmHg

heating

cooling
supraspinal control

L1,2

MVC CVC

L4-6

nociceptive afferents BV

BV

SKELETAL MUSCLE

PRINCIPAL

SKIN

Kirillova et al
Fig. 9
Reactions of cutaneous vasoconstrictor (CVC) neurons and muscle vasoconstrictor (MVC) neurons to stimulation of nociceptors in skin or skeletal muscle (number of neurons/total number 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. Ongoing activity and cardiac rhythmicity: mean ± SEM. The right column shows the changes of arterial blood pressure (BP). Ø, no change; ↑, activation or increase of BP; ↓, inhibition or decrease of BP; Ø/↑, no change or small activation; ↓↑, inhibition followed by activation.

### Table 1

<table>
<thead>
<tr>
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<th>CVC neurons</th>
<th>MVC neurons</th>
<th>Change of BP</th>
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<tbody>
<tr>
<td><strong>Ongoing activity (imp/s)</strong></td>
<td>1.1 ± 0.11 (n=51)</td>
<td>1.42 ± 0.18 (n=39)</td>
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<tr>
<td><strong>Cardiac rhythmicity (% of max)</strong></td>
<td>47.8 ± 4.5 (n=51)</td>
<td>99.6 ± 0.13 (n=39)</td>
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<td><strong>Noxious toes</strong></td>
<td>Ø 6/37</td>
<td>Ø/↑ 18/26</td>
<td>↑ ³ (5-10 mmHg)</td>
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<td>(mechanical stimulation of</td>
<td>↓ 31/37</td>
<td>↓ 8/26 (short)²</td>
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<td>ipsilateral toes 3 &amp; 4)</td>
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<tr>
<td><strong>Noxious muscle ipsilateral</strong></td>
<td>Ø 12/27</td>
<td>Ø 5/26</td>
<td>↓ 100% (16.6 mmHg)</td>
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<tr>
<td>(injection of hypertonic saline</td>
<td>↓↑ 3/27 (↓ short)</td>
<td>↓ 21/26 (strong)</td>
<td></td>
</tr>
<tr>
<td>in gastrocnemius-soleus muscle)</td>
<td>↑ 12/27</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Noxious muscle contralateral</strong></td>
<td>Ø/↑ 6/16</td>
<td>Ø 18/26</td>
<td>↓ 100% (17 mmHg)</td>
</tr>
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<td>(injections of hypertonic saline</td>
<td>↑ 10/16</td>
<td>↓ 8/26 (weak)¹</td>
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<td>in gastrocnemius-soleus muscle)</td>
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<tr>
<td><strong>Heat ~48-52°C</strong></td>
<td>Ø 12/37</td>
<td>Ø 34/34</td>
<td>↓ 100% (21.5 mmHg)</td>
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<tr>
<td>tibial n.)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Cold 5°C</strong></td>
<td>Ø 34/34</td>
<td>Ø 24/24</td>
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¹ Data from one and the same experiment.
² Probably related to stimulation of deep somatic afferents innervating the toes.
³ The blood pressure initially increased followed by decrease.