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Gentle mechanical skin stimulation inhibits the somatocardiac sympathetic C-reflex elicited by excitation of unmyelinated C-afferent fibers.

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The effects of gentle mechanical skin stimulation on reflex discharges in cardiac sympathetic nerve evoked by somatic afferent stimulation were studied in anesthetized rats. Mass discharges were recorded from cardiac sympathetic efferent nerve while somatocardiac sympathetic A- and C-reflexes were elicited by single electrical stimuli to myelinated A- and unmyelinated C-afferent fibers of the tibial nerve. Continuous touch was applied to inner thigh skin with a force of 0.12N for 10min periods by a soft elastomer "brush" (1.1cm in diameter with 417 microcones). When touch was applied ipsilateral to the stimulated tibial nerve, the C-reflex was inhibited by up to 40% of its pre-touch amplitude, whereas the A-reflex was unaffected. Inhibition of the C-reflex started during the touch period and lasted for 15min after cessation of touching. Contralateral touch did not inhibit the C-reflex. The opioid receptor antagonist naloxone attenuated the C-reflex inhibition, but did not abolish it. The C-reflex inhibition was abolished after severing cutaneous nerves innervating inner thigh. We recorded unitary afferent activity from thigh branches of the saphenous nerve and found fibers excited by touch were low-threshold mechanoreceptive Abeta, Adelta and C fibers that have rapidly or slowly adapting properties. In all units tested, average discharge rates during touch period were less than 4Hz. The results suggest that touch-induced excitation of low threshold cutaneous mechanoreceptive fibers inhibits nociceptive transmission conveyed by C-primary-afferents, via the release of both opioid and non-opioid inhibitory mediators.

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Gentle mechanical skin stimulation inhibits the somatocardiac sympathetic C-reflex elicited by excitation of unmyelinated C-afferent fibers

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ABSTRACT

The effects of gentle mechanical skin stimulation on reflex discharges in cardiac sympathetic nerve evoked by somatic afferent stimulation were studied in anesthetized rats. Mass discharges were recorded from cardiac sympathetic efferent nerve while somatocardiac sympathetic A- and C-reflexes were elicited by single electrical stimuli to myelinated A- and unmyelinated C-afferent fibers of the tibial nerve. Continuous touch was applied to inner thigh skin with a force of 0.12 N for 10 min periods by a soft elastomer "brush" (1.1 cm in diameter with 417 microcones). When touch was applied ipsilateral to the stimulated tibial nerve, the C-reflex was inhibited by up to 40% of its pre-touch amplitude, whereas the A-reflex was unaffected. Inhibition of the C-reflex started during the touch period and lasted for 15 min after cessation of touching. Contralateral touch did not inhibit the C-reflex. The opioid receptor antagonist naloxone attenuated the C-reflex inhibition, but did not abolish it. The C-reflex inhibition was abolished after severing cutaneous nerves innervating inner thigh. We recorded unitary afferent activity from thigh branches of the saphenous nerve and found fibers excited by touch were low-threshold mechanoreceptive Aβ, Aδ and C fibers that have rapidly or slowly adapting properties. In all units tested, average discharge rates during touch period were less than 4 Hz. The results suggest that touch-induced excitation of low threshold cutaneous mechanoreceptive fibers inhibits nociceptive transmission conveyed by C-primary-afferents, via the release of both opioid and non-opioid inhibitory mediators.

1. Introduction

It is apparent from the experiences of daily life that stimulation of somatosensory receptors produces not only conscious sensations but also physiological responses. In our laboratory, we have extensively studied the mechanisms of somato-autonomic reflexes using anesthetized animals (e.g. Sato et al., 1997; Uchida et al., 1999, 2008; Hotta et al., 2005). In traditional and modern medicine, various types of somatic stimulation are used for treating visceral and other physical complaints and dysfunction, without knowing the physiological and anatomical details of how such treatments are effective (Sato et al., 2002). However, the somato-autonomic reflexes are considered to be a central component of the underlying mechanism.

In addition to evoking autonomic reflex responses such as changing heart rate, somatic afferent stimulation is known to elicit analgesic effects, for example, by leading to the release of endogenous opioids in the central nervous system (Yaksh and Elde, 1981; Han et al., 1991; Wang et al., 2005). The mechanisms by which electrical stimulation of afferent nerve fibers leads to analgesia have been examined (Woolf and Wall, 1982; Chung et al., 1984a,b), but the analgesic mechanisms associated with innocuous mechanical cutaneous stimulation have been seldom investigated.

Electrical pulse stimulation of myelinated (A) and unmyelinated (C) afferent fibers of hindlimb nerves have been found to elicit distinct sympathetic A- and C-reflex discharges, respectively, in sympathetic efferent nerves of anesthetized animals (Fedina et al., 1966; Schmidt and Weller, 1970). Since the C-reflex component can be selectively reduced by morphine administration (Ito et al., 1983; Sato et al., 1986), it has been suggested that the somato-sympathetic C-reflex may be a useful indicator to test the analgesic effects of various manipulations in anesthetized animals. The aim of the present study was to examine the effect of innocuous mechanical cutaneous stimulation on sympathetic A- and C-reflexes recorded from inferior cardiac sympathetic efferent nerve. Parts of the present results have been published in abstract form (Hotta et al., 2009a,b).

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2. Methods

The experiments were performed using male Wistar rats ($n = 35$) bred at the Tokyo Metropolitan Institute of Gerontology. Animal weights ranged from 340 to 450 g. This study was approved by the Animal Care and Use Committee of our Institution.

Animals were anesthetized with urethane. An initial dose of urethane was 1.1 g/kg i.p. A jugular vein was catheterized for i.v. administration of supplemental anesthetics and other drugs. A common carotid artery was catheterized to record arterial blood pressure and heart rate. Additional doses of urethane were administered (0.1–0.36 g/kg, i.p. or i.v.) to maintain the anesthetic level as evidenced by a stable systemic arterial blood pressure and heart rate (monitored continuously), and, when animals were not paralyzed, the absence of withdrawal reflexes. Animals were artificially ventilated via a tracheal cannula. The ventilation was monitored with a gas analyzer (Microcap, Oridion Medical, Jerusalem, Israel) and adjusted to maintain the end-tidal CO_2 level at about 3.0%. Body temperature was kept at 37–38 °C using an automatically-regulated heating pad and lamp (ATB-1100, Nihon Kohden, Tokyo).

2.1. Somatocardiac sympathetic reflex discharges

With the rat in a supine position, the right second costal bone was removed. The right inferior cardiac sympathetic nerve was dissected free retropleurally, cut as close to the heart as possible, and covered with warm paraffin oil. Cardiac sympathetic efferent nerve activity was recorded from the central segment of the cardiac sympathetic nerve with platinum–iridium wire electrodes using an AC preamplifier (MEG-2100, Nihon Kohden, time constant set at 0.33 s). Vagal nerves were bilaterally cut at the cervical level to prevent vagal contamination of the sympathetic nerve activity recorded.

The right tibial nerve was dissected free from the surrounding tissues and cut close to the ankle. The central cut end segment of the nerve was placed on bipolar platinum–iridium wire electrodes for electrical stimulation. Single square pulse stimuli of 0.5 ms duration were delivered every 3 s by a digital electrical stimulator (SEN-7203, Nihon Kohden) and stimulus isolation unit (SS-202 J, Nihon Kohden). The final preparatory step was to administer gallamine triethiodide (20 mg/kg, i.v.) for immobilization.

Reflex responses of the cardiac sympathetic nerve were elicited by electrical stimulation of a tibial nerve and averaged (50 trials) by a computer (Unique Aquisition software, Unique Medical, Tokyo). Averaged responses were stored as digital signals. The size of the reflex response was measured as the area under the evoked response within 30–200 ms (A-reflex) and 200–435 ms (C-reflex) after onset of stimulation, and expressed as % of the evoked response area preceding touch.

2.2. Touch stimulation

After shaving the hair from the inner thighs and lower abdomen with a conventional clipper, touch was applied to inner thigh skin (alternatively to abdomen), either right or left, by a soft elastomer "brush" (SOMARESON type I, Toyoresin Co., Shizuoka) which was 11 mm in diameter and had 417 elastomer microcones (Fig. 1A). The microcones were arranged regularly with a pitch of 0.4 mm. The tip diameter of an individual microcone was 0.037 mm, and each had a height of 0.3 mm. The "brush" was developed recently as a device for pain relief. The potential similarities of this device's effectiveness for chronic pain relief (Dr. Y. Mukaino, personal communication) and our own experiences with physiological responses to tactile stimulation were our motivation to explore its properties. In some cases, we used a flat elastomer disc of the same size (11 mm in diameter) without microcones for comparison.

Using a robotic positioning device (miniSCARA, Dynax Co., Tokyo) and a weight attached on top of a post bonded to the brush (Fig. 1B), we applied continuous touch stimulation to the skin for a period of 10 min with a constant force of 0.12 N, or alternatively with 0.01 N or 1.1 N by changing the weight. None of these stimulus forces produced pain when applied to our forearm. We observed, by using a digital video microscope at a magnification of 300× (DS-500, Science-eye, Saitama, Japan), that the elastomer microcones bent with a force of 1.1 N, but not with forces of either 0.01 N or 0.12 N. In some cases, tapping touch was applied manually at a frequency of approximately 2 Hz.

2.3. Nerves innervating the inner thigh skin

The inner thigh area of the hindlimb is primarily innervated by the thigh branches of the saphenous nerve and posterior cutaneous nerve of thigh in rats (Swett and Woolf, 1985). The right posterior cutaneous nerves were cut at the position approx. 1 cm caudal to the third trochanter in the prone position, and the right saphenous nerves were cut at a position just caudal to the inguinal ligament in the supine position (in four animals).

2.4. Unitary afferent nerve activity

In preliminary studies, we observed that touch of the inner thigh skin evoked an afferent mass discharge from whole nerve bundles of the thigh branches of the saphenous nerve. Responses were consistently larger than those of the posterior cutaneous nerve of thigh. Thus, we recorded unitary afferent nerve activity from the thigh branches of the saphenous nerve.

After cutting the abdominal skin, the thigh branches of the saphenous nerve were separated and cut close to the inguinal ligament. These nerves were covered with warm paraffin oil and the peripheral cut segments were placed on bipolar platinum–iridium wire recording electrodes. Dissection of the nerve was performed using two forceps and a binocular microscope at 25–40× magnification until we recorded single fiber activity as described previously (Kagitani et al., 2005). Unitary action potentials were amplified (MEG-2100, Nihon Kohden), audibly monitored through connection to a speaker, visually displayed on a digital oscilloscope (TS-8500, IWATSU, Tokyo), and digitized (micro 1401, Cambridge Electronic Design, UK) for later processing (Spike 2 software, Cambridge Electronic Design, UK). The mechanical threshold within each fiber's receptive field was determined with von Frey filaments (0.03–6 mN).

2.5. Measurement of the conduction velocity of a single nerve fiber

When cutaneous touch with the microcones evoked unitary action potentials in dissected saphenous nerve fibers, a pair of needle electrodes (0.2 mm in diameter; Seirin Kasei Co., Shizuoka) were inserted into the skin near the center of the fiber's receptive field at a distance 1–2 mm apart. Isolated square wave pulses (duration 0.5 ms) were passed between these two needles at various stimulus current intensities. When electrical stimulation of the skin evoked unitary action potentials in the dissected saphenous nerve fibers, action potentials evoked either by electrical stimulation or by touch were able to be identified as being produced from a single unit by comparing the shape of the action potentials. The conduction velocity of a single nerve fiber was calculated by measuring both the length of the nerve between stimulating and recording electrodes (53–80 mm) and the latency of the action potential evoked in a dissected nerve fiber. Nerve fibers that had conduction velocities $\leqslant$2 m/s, were classified as unmyelinated C-fibers. We

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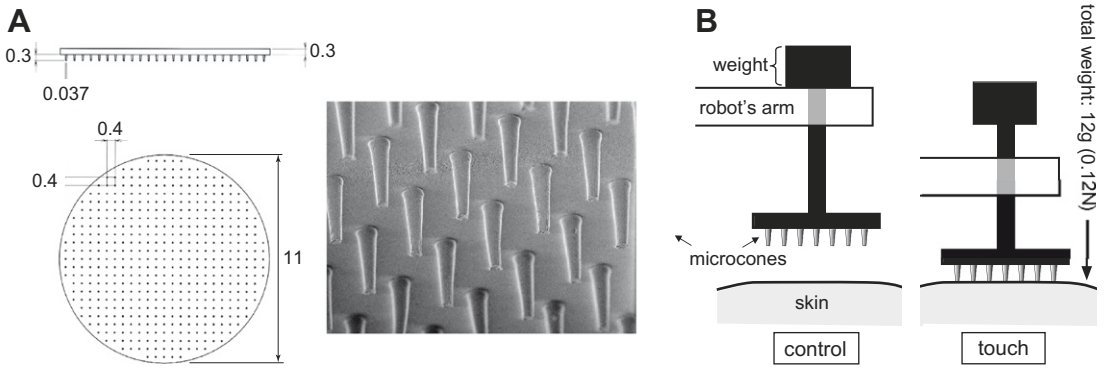

Fig. 1. Methods for gentle mechanical stimulation of the skin. Gentle mechanical stimulation was delivered using a soft elastomer "brush" which has 417 microcones regularly arranged on an 11 mm diameter sheet. The height of each microcone is 0.3 mm and its tip is flattened (0.037 mm in diameter) (A, right photograph is a scanning electron micrograph of microcones). The elastomer "brush" was applied to the inner thigh or abdomen with a force of 0.12 N for 10 min periods (B).

estimated the maximum conduction velocities of myelinated Aβ and Aδ afferent fibers, by recording the compound action potentials from whole bundles of the saphenous nerve to inner thigh in three rats, to be 55.8 m/s (mean; range: 51.4–62 m/s) and 15.6 m/s (13.3–19.4 m/s), respectively. Based on these results, we classified nerve fibers which had conduction velocities >15.6 m/s as Aβ, ⩽15.6 m/s as Aδ fibers.

2.6. Naloxone

In five animals, 2 mg/kg naloxone hydrochloride (Sigma, USA) was administered i.v. This dosage was shown in previous studies (Adachi et al., 1992; Uchida et al., 1999) to be effective for antagonizing the changes in somatocardiac sympathetic C-reflex due to high doses (20 mg/kg, i.v.) of morphine.

2.7. Data analysis

Values are expressed as means ± SEM. Statistical analysis was performed using one-way repeated-measures ANOVA followed by Dunnett's multiple comparison test or two-way repeated-measures ANOVA followed by Bonferroni correction. Statistical significance was set at the 5% level.

3. Results

3.1. Depression of the C-reflex by touch

Single shock stimulation of A- and C-afferent fibers of the tibial nerve (at 15 V with 0.5 ms pulse duration) elicited two kinds of cardiac sympathetic reflex discharges corresponding to A- and C-afferents: a short latency (about 40 ms) A-sympathetic reflex, and a long latency (about 210 ms) C-sympathetic reflex (Fig. 2A) as reported previously (Adachi et al., 1992; Uchida et al., 1999). Both reflex responses were found to be stable over the normal duration of experiments, i.e. over several hours. When A- and C-reflexes were stable for at least 10 min, we started to apply touch stimulation.

Continuous touch of the inner thigh skin on the right side (ipsilateral to the stimulated tibial nerve), by elastomer microcones with a constant force of 0.12 N for 10 min, markedly depressed the C-reflex, whereas the A-reflex was unaffected (Fig 2A). Neither heart rate nor blood pressure was affected by the touch. The time course of the effect of touch is summarized in Fig. 2B. The touch-induced depression of the C-reflex started 5 min after touch onset and the size of the C-reflex was decreased by 40 ± 12% of pre-touch

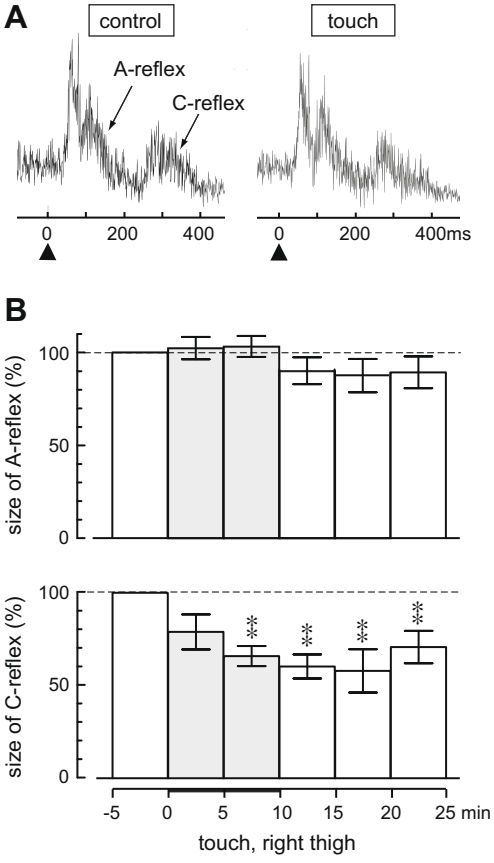

Fig. 2. The effect of touch on the somatocardiac sympathetic reflexes. (A) Specimen records of A- and C-reflexes (averages of 50 trials), elicited by single electrical stimulation of tibial nerve, before and after touch of the ipsilateral thigh. Magnitudes of the A- and C-reflexes were measured as the area under the curve of the corresponding reflex response within 30–200 ms and 200–435 ms, respectively, after onset of stimulation. (B) The graphs summarize changes in the size of the A- and C-reflexes averaged every 5 min, expressed as percentages of the pre-touch values. Shadowed columns and a horizontal line under abscissa in B indicate the period of touch. Each column and vertical bar represents mean ± SEM ($n = 5$) for the average change in amplitude (area) of evoked reflex responses. Response amplitudes of C-reflex were reduced during the touch condition compared to pre-touch levels. $^{**}p < 0.01$; significantly different from prestimulus value determined by one-way repeated-measures ANOVA followed by Dunnett's multiple comparison test.

amplitude at 5–10 min after stimulation ended (lower graph of Fig. 2B). The C-reflex, usually reaching its minimum at 0–10 min after stimulation ended, gradually returned to the prestimulus le-

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vel after 20–30 min from the end of the touch period. On the other hand, the size of the A-reflex did not change either during or after touching (upper graph of Fig. 2B). The response was reproducible in successive trials on the same animal.

The effects of different force or mode of touch on the C-reflex were tested in seven rats. Touch with a constant force of one tenth (0.01 N, $n = 4$) depressed the C-reflex (maximally by 27 ± 9% at 5–10 min after touch onset) in a manner similar to touch with a force of 0.12 N. Touch with a constant force of 10 times (1.1 N, $n = 4$) slightly depressed the C-reflex, but the effect was not statistically significant. A similar effect was observed during touch with a force of 0.12 N applied by a flat elastomer disc without microcones ($n = 3$). Furthermore, the C-reflex was not affected by tapping touch at 2 Hz (0.01 N, $n = 1$; 0.12 N, $n = 3$).

3.2. Effects of touch of various skin areas on the C-reflex

The effects on the C-reflex of touch delivered to three different skin areas were studied in eight rats. The stimulated areas were indicated by either filled circles or crosses in Fig. 3B: right abdomen, right thigh and left thigh. Graphs of the size of the C-reflex in Fig. 3A demonstrate both depressive (a) and ineffective (b) effects on the C-reflex. These graphs were obtained by touch stimulation at skin areas referred to the areas in B indicated by arrows. The C-reflex was depressed by stimulation of two areas located in the thigh and abdomen on the right side (ipsilateral to the stimulated tibial nerve; indicated by filled circles in Fig. 3B). The other area tested located on the thigh on the left side (contralateral to the stimulated tibial nerve; indicated by a cross in Fig. 3B) was ineffective for C-reflex suppression. When touch was applied to the right abdomen, the C-reflex was depressed by up to 19 ± 5% of its pre-touch amplitude (Fig. 3Aa). The magnitude of depressive effect was less than half of that induced by touch to the right thigh.

3.3. Naloxone administration

The effect of touch on the C-reflex after intravenous administration of the opioid receptor antagonist naloxone (2 mg/kg) was examined in five rats in order to define the role of endogenous opioids in mediating the touch-induced depression of the C-reflex. At 10 min after injection of naloxone, the baseline level of the C-reflex before touch had not changed. C-reflex depression by touch stimulation remained (open circles in Fig. 4), but the extent of C-reflex depression was approximately half that produced by touch stimulation in the absence of naloxone (filled circles in Fig. 4). The C-reflex was depressed by only 23 ± 5% of its pre-touch amplitude at 5–10 min after touch onset, and significant depression lasted for 5 min after the end of touch stimulation. Naloxone significantly reduced the depressive effect of touch stimulation ($p < 0.01$, by two-way repeated ANOVA), and post hoc Bonferroni correction revealed that such a reduction was significant after the stimulation period ended.

3.4. Afferent nerve transection

Transection of the afferent nerves innervating the inner thigh skin abolished the C-reflex depression induced by touch stimulation delivered to the thigh. We tested this in four rats by transecting the saphenous nerve and posterior cutaneous nerve ipsilateral (right side) to the touch stimulated thigh skin. The baseline level of the C-reflex before touch was not affected, whereas C-reflex depression by touch stimulation was completely prevented in every case (Fig. 5). C-reflex depression following stimulation of the ipsilateral abdomen was preserved (tested in two rats).

3.5. Unitary activity of the saphenous nerve

Unitary afferent nerve activity of the saphenous nerve branches innervating the inner thigh skin was recorded from the peripheral

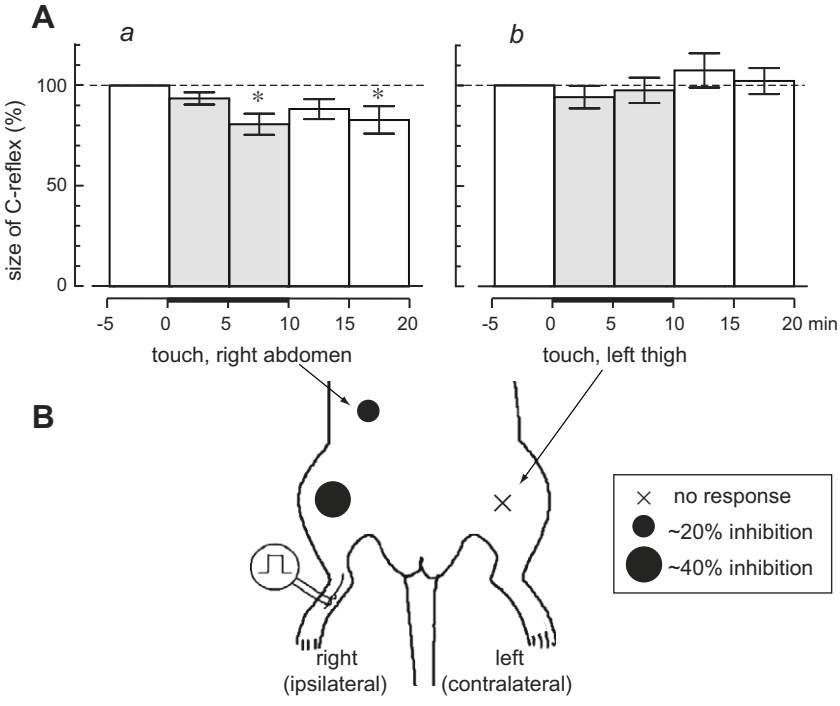

Fig. 3. The effects of touch applied onto the ipsilateral abdomen (Aa) and the contralateral thigh (Ab) on the somatocardiac sympathetic C-reflex. (A) The graphs summarize ($n = 5$) changes in the size of the C-reflex averaged every 5 min, expressed as percentages of the pre-touch values. See Fig. 2 for further details. * $p < 0.05$. (B) The stimulated areas indicated by filled circles or crosses. The result of the ipsilateral thigh stimulation is also included for a comparison purpose. Note that the size of circles indicates the degree of C-reflex inhibition but not the size of stimulated area.

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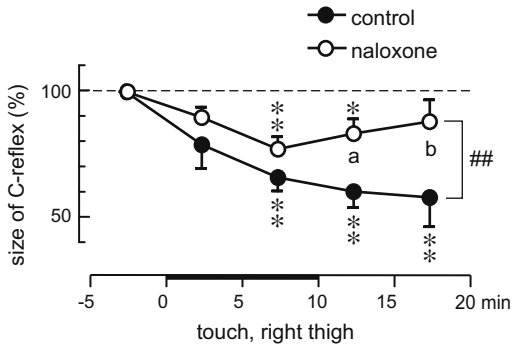

Fig. 4. The effects of naloxone (2 mg/kg, i.v.) on the inhibition of the C-reflex by touch applied onto the ipsilateral thigh. The effect of touch on the somatocardiac sympathetic C-reflex before (filled circles) and after (open circles) naloxone. Each point and vertical bar represents mean ± SEM ($n = 5$). * $p < 0.05$, ** $p < 0.01$; significantly different from prestimulus values. ## $p < 0.01$; significant difference between control and after naloxone by two-way repeated ANOVA followed by Bonferroni correction (a. $p < 0.05$, b. $p < 0.01$).

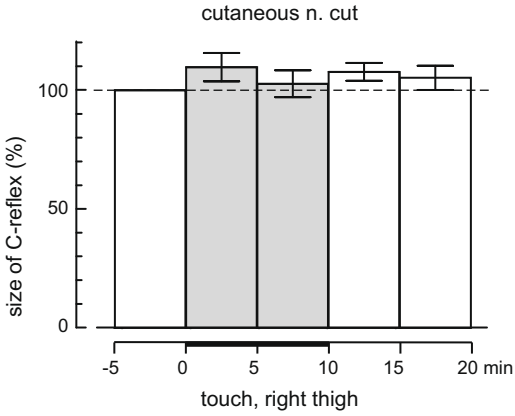

Fig. 5. Transection of nerves innervating the ipsilateral thigh skin abolished the inhibitory action of thigh-touch on the C-reflex ($n = 4$). See Fig. 2 for further details.

cut end segment of the nerve branches in 10 rats. Continuous touch of the inner thigh skin, with a force of 0.12 N for 10 min, activated Aβ, Aδ and C single unitary afferent fibers in the saphenous nerve.

A total of 32 units that responded to touch were recorded after dissection. The conduction velocities of these 32 units ranged between 0.7 and 42.3 m/s, thus belonging to Aβ, Aδ and C fibers. The mean conduction velocity recorded from afferents classified as belonging to Aβ, Aδ and C fibers were 28.3 ± 1.8 m/s ($n = 18$), 11.0 ± 0.7 m/s ($n = 8$), and 0.9 ± 0.04 m/s ($n = 6$), respectively. Mechanical stimulation (von Frey filament) thresholds were determined in 26 units and ranged from 0.03 mN to 4 mN, i.e., within the non-noxious range. In each unit, the increase in average discharge rate during touch stimulation varied, but was always less than 4 Hz.

Fig. 6 illustrates examples of unitary afferent recordings from Aβ (A and B) and C (C) fibers in response to touch. Panels Aa, Ba, and Ca illustrate action potentials recorded from different individual fibers during touch stimulation (left) and those evoked by electrical stimulation of the receptive field (right). The shapes of the action potentials elicited by both touch and electrical stimulation were identical. Thus, both action potentials were considered to originate from the same unit. Fig. 6 panels Ab, Bb, and Cb show action potentials and their histograms in the units during touch stimulation for 10 min.

The dynamic responses of units varied. Touch excited the unit shown in Fig. 6Ab throughout touch period, with a high dynamic response at the onset (slowly adapting SAI unit). The initial period of high frequency firing ceased within 30 s after the onset of touch, but the unit continued to discharge with a gradually increasing firing rate until the end of touch period. Twelve units (eight units were identified as Aβ fibers and four identified as Aδ fibers) responded to touch stimulation in this manner and the mean discharge rate during the 10-min touch period was at 1.59 ± 0.37 Hz ($n = 12$; Fig. 7A). The unit shown in Fig. 6Bb was excited by touch, however there was no dynamic response at the onset. Action potential rates gradually increased during the touch period (slowly adapting SAII unit). Five units (Aβ: $n = 3$; Aδ: $n = 2$) exhibited this type of response to touch. The mean discharge rate of the action potential during the touch period in the five units was 0.22 ± 0.16 Hz (Fig. 7B).

In the unit shown in Fig. 6Cb, touch elicited a response that was similar to the SAI unit mentioned above, except that there was a high dynamic response at the offset of touch as well as the onset of touch. The slower conduction velocities were used to identify these units as C-fibers. We recorded six such units and the mean discharge rate during touch stimulation was 0.33 ± 0.10 Hz ($n = 6$; Fig. 7C).

Some of these slowly adapting units had low levels of spontaneous activity before touch (e.g., Fig. 6Cb). The cessation of touch was often associated with a decrease in the discharge rate of the units. However, increased firing rates relative to pre-touch level remained in some units for 10 min or longer after the touch stimulation ended (e.g., Fig. 6Ab).

There was one other type of unitary afferent response. Units of this group did not discharge spontaneously and were transiently activated at the onset and offset of touch (rapidly adapting RA unit) (Aβ: $n = 7$; Aδ: $n = 2$). The majority of these units had high instantaneous discharge rates of 100–200 Hz.

4. Discussion

4.1. Selective inhibition of the C-reflex

In the present study, we demonstrated for the first time that touch of the skin depresses the somatocardiac sympathetic C-reflex, but not the A-reflex. In anesthetized rats, a similar selective suppression of somatocardiac sympathetic C-reflexes has been obtained by intrathecal injection of opioids, such as morphine (Adachi et al., 1992), or μ- or δ-opioid receptor agonists (Sato et al., 1995) at the lumbar spinal cord.

From the hindlimb, the basic somatocardiac sympathetic reflex arc is comprised of afferents running in the tibial nerve, the reflex center is in the brain stem (Sato et al., 1997) and the efferent limb is the set of cardiac sympathetic nerves that emerge from the spinal cord at the first to sixth thoracic (T1–6) segments in rats (Strack et al., 1988). The C-reflex is mediated by primary-afferent C-fibers and we speculate that its depression by touch stimulation is a segmental inhibition on the C-afferent input, because (1) heart rate was not affected by touch in these vagotomized rats, (2) the A-reflex was also not affected, (3) touch applied to abdomen (closer to efferent output) was less effective than touch applied to thigh (closer to afferent input), and (4) contralateral touch was ineffective.

4.2. Activation of low threshold A- and C-afferent fibers

The present study showed all three populations of cutaneous afferents (classified on the basis of conduction velocity as Aβ, Aδ, or C fibers) were activated during the touch period, suggesting possible contributions of various groups of afferents to the C-reflex inhibition. There have been several studies that addressed afferent

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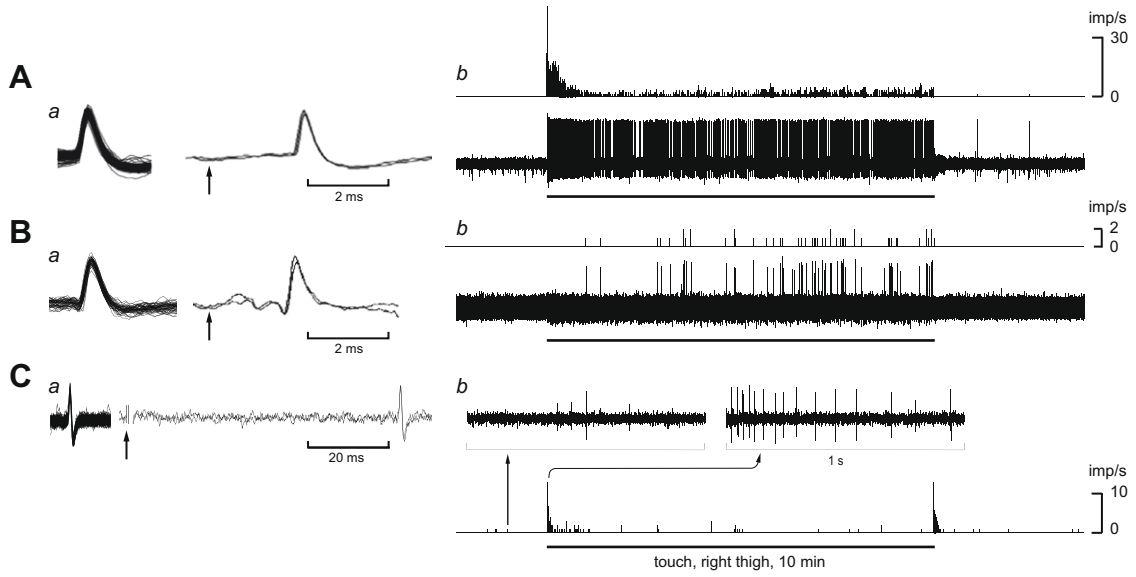

Fig. 6. Examples of unitary afferent fiber recordings in the saphenous nerve. (A and B) Aβ slowly adapting fibers with (SAI; A) and without (SAII; B) dynamic sensitivity. (C) C fiber. (Aa, Ba and Ca) Action potentials superimposing during the touch stimulation (left) and those evoked by electrical stimuli of the receptive field (right). The latency of the electrically evoked action potential, the distance between the recording and stimulating electrodes and the calculated conduction velocity were 2.7 ms, 75 mm and 27.8 m/s (Aβ fiber) for A; 2.2 ms, 65 mm and 29.5 m/s for B (Aβ fiber); 67.7 ms, 60 mm and 0.9 m/s for C (C fiber). (Ab, Bb and Cb) Responses of each unitary afferent to touch (graph shows a spike histogram). A horizontal line indicates the period of touch stimulation.

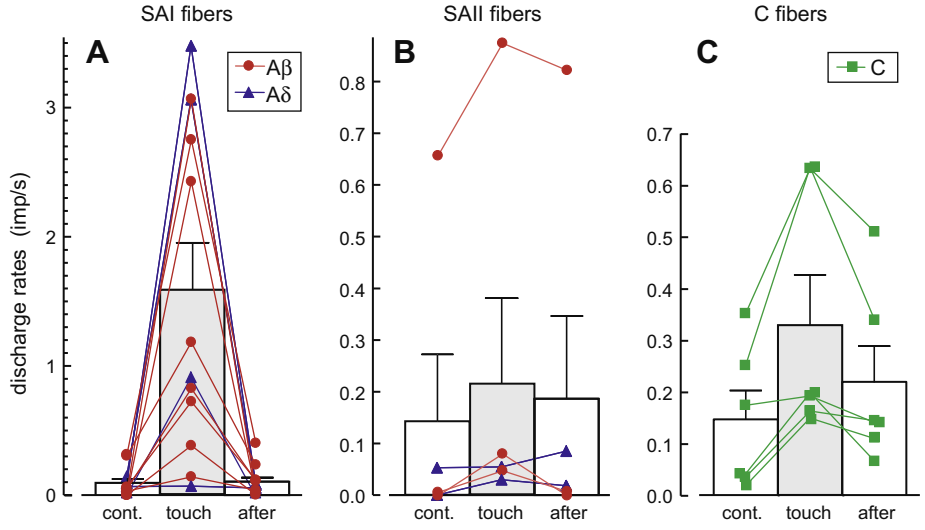

Fig. 7. Summary of slowly adapting single fiber activity before (for 5 min), during (for 10 min) and after (for 10 min) touch stimulation. (A) A fibers with dynamic sensitivity (SAI). (B) A fibers without dynamic sensitivity (SAII) (●: Aβ fiber, ▲: Aδ fiber). (C) C fibers (■). Each column and vertical bar represents mean ± SEM.

fiber contributions to analgesia based on data produced by selective electrical stimulation of different groups of fibers. Activation of Aβ afferents for analgesia has been demonstrated with electrical stimulation techniques (Pomeranz and Paley, 1979; Woolf and Wall, 1982), and contributions from Aδ and C afferents for peripheral stimulation-induced analgesia have also been suggested (Chung et al., 1984b; Ikeda et al., 1999). However, in contrast to these previous studies using electrical stimulation in which not only low-threshold mechanoreceptive fibers but also high threshold nociceptive fibers (>40 mN) were activated, the Aβ, Aδ and C fibers activated by touch in this study were only low threshold (<4 mN) mechanoreceptive fibers. Low-threshold mechanoreceptive C-fibers are reportedly rich in hairy skin where we applied touch, but not in glabrous skin, in rats (Lynn and Carpenter, 1982; Fang et al., 2005) and in humans (Vallbo et al., 1999; Wessberg et al., 2003). The touch-induced excitations of low-threshold mechanoreceptive fibers of all three populations (Aβ, Aδ and C) of afferents appear to contribute to C-reflex inhibition.

4.3. Sensory receptor types

The afferents innervating hairy skin are generally divided into rapidly and slowly adapting varieties on the basis of their response to stepwise indentation and flexing of the hairs (Sato et al., 1997). Both rapidly adapting (RA) units and slowly adapting (SA) units were excited by touch. Both subtypes of SA units, those with (SAI) and those without (SAII) high dynamic sensitivity, were excited by touch. Among these responses, slowly developing tonic excitation in SAI and SAII units may be important for eliciting the C-reflex inhibition, because inhibition of the C-reflex also devel-

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oped slowly. It is interesting that cutaneous touch stimulation with soft microcones depressed the C-reflex whereas stimulation with a flat surface of the same material applied with the same force did not produce the depressive effect. This may be because even slight movements of the animal (with arterial pulsation or breathing) are able to vibrate these microcones across the skin. Such a slight movement may lead to longer lasting discharge in the afferent fibers which may be important in the C-reflex inhibition.

The present finding that low frequency discharges were observed during the touch period in cutaneous low-threshold mechanoreceptive fibers indicates that relatively low frequency excitation of cutaneous low-threshold mechanoreceptive fibers is sufficient to inhibit the C-reflex. The efficacy of low frequency activity is in accord with a previous report that low-frequency stimulation (0.5–5 Hz) of somatic afferent fibers induces: (1) long-term depression at primary afferent synapses with substantia gelatinosa neurons in rats (Sandkühler et al., 1997; Ikeda et al., 1999), (2) long-lasting inhibition of the reflex activation of cardiovascular system evoked by application of bradykinin to the gallbladder in anesthetized cats (Li et al., 1998) and (3) long-term decreases in perceived pain in humans (Klein et al., 2004), and also that low-frequency (about 0.2 Hz) cutaneous stroke with a cotton bud can reduce size of allodynia area for a long time in patients with neuropathic pain (Love-Jones et al., 2009). This suggestion is supported by our result that tapping touch, which presumably produces high frequency activation of RA and SAI units, was ineffective for the C-reflex inhibition.

4.4. Opioid and non-opioid receptor contributions

C-reflex depression induced by touch stimulation was reduced, but not abolished, by the administration of naloxone. This result suggests that endogenous opioids may play a significant role, while a naloxone-resistant, i.e., non-opioid receptor-mediated inhibitory mechanism, such as GABA and glutamate (Tjen-A-Looi et al., 2007; Sandkühler et al., 1997; Ikeda et al., 1999), may also be involved. Endogenous opioids are released in the CNS during electrical stimulation of somatic afferent nerves (Yaksh and Elde, 1981; Han et al., 1991; Wang et al., 2005). Our findings suggest that touch can promote release of endogenous opioids in the CNS. In anesthetized animals, opioids inhibit the C-reflex, by acting at the spinal sites in both rats (Adachi et al., 1992; Sato et al., 1995; Uchida et al., 1999) and cats (Sato et al., 1985, 1986). On the other hand, by acting at brain stem sites, they facilitate the C-reflex in rats (Adachi et al., 1992; Sato et al., 1995; Li et al., 1996), and suppress both the A- and C-reflexes in cats (Kato et al., 1992). Such findings are consistent with our speculation that touch-induced inhibition of the C-reflex occurs at spinal levels. However, we cannot exclude the possibility of other sites of opioid action, such as brain or peripheral nerve because naloxone was administered intravenously. Further study, e.g., intrathecal application of naloxone, is needed to clarify site of opioid action contributing to the touch effect.

4.5. Cutaneous afferents for touch sensation

C-reflex depression by touch stimulation of the inner thigh was totally abolished after severing the ipsilateral saphenous nerve and posterior cutaneous nerve which innervate the skin where touch was applied. The use of local pharmacological blockade of nerve activity is an alternative to nerve transection. Complete transection of a nerve can make it difficult or impossible to determine if changes in evoked responses may actually be due to nerve fibers themselves becoming unresponsive. Depression of the C-reflex was blocked by nerve transection over the entire post-transection period, while C-reflex depression following stimulation of the abdomen with its intact afferent nerves was preserved, indicating that non-specific failure was not likely to be a significant confounding phenomenon. Therefore, we conclude that cutaneous afferent nerves are essential for the inhibitory effects demonstrated in this study.

We studied the characteristics of each single afferent fiber responding to touch, using a nerve fiber dissecting technique and found that cutaneous afferent fibers were activated during touch stimulation and usually ceased or decreased firing within a few seconds after the end of stimulation. Remarkably, the inhibition of the C-reflex induced by touch persisted for 10 min after the end of stimulation. Opioid receptors contribute to the long-lasting depression because naloxone was able to decrease the duration of C-reflex depression, but since C-reflex depression outlasts the change in afferent fiber activity by minutes, a central component to maintain depression in the absence of afferent input is necessary.

5. Conclusion

The present results demonstrate in rats that gentle mechanical cutaneous stimulation can inhibit spinal synaptic transmission of C-afferent volleys into the somatocardiac sympathetic reflex pathways. Inhibition of the C-reflex is mediated by opioid and non-opioid systems, most likely at the spinal segmental neural pathway, triggered by low-frequency input arising from low threshold cutaneous mechanoreceptors with myelinated and unmyelinated fibers. Many of the C-primary-afferents are nociceptive afferents conveying delayed pain, so that the present results may be related to the mechanisms that gentle mechanical skin stimulation can produce pain relief. The mechanism presented here may explain a part of the clinical outcomes of physical therapy, and may also have a role for social conditioning, i.e., to learn how skin contact with others is beneficial. Furthermore, our findings may support the use of the device with microcones in the therapy of pain, although studies on normal human subjects are important as the next step.

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References

Adachi T, Sato A, Sato Y, Schmidt RF. Depending on the mode of application morphine enhances or depresses somatocardiac sympathetic A- and C-reflexes in anesthetized rats. Neurosci Res 1992;15:281–8.

Chung JM, Fang ZR, Hori Y, Lee KH, Willis WD. Prolonged inhibition of primate spinothalamic tract cells by peripheral nerve stimulation. Pain 1984a;19:259–75.

Chung JM, Lee KH, Hori Y, Endo K, Willis WD. Factors influencing peripheral nerve stimulation produced inhibition of primate spinothalamic tract cells. Pain 1984b;19:277–93.

Fang X, McMullan S, Lawson SN, Djouhri L. Electrophysiological differences between nociceptive and non-nociceptive dorsal root ganglion neurones in the rat in vivo. J Physiol 2005;565:927–43.

Fedina L, Katunskii AY, Khayutin VM, Mitsányi A. Responses of renal sympathetic nerves to stimulation of afferent A and C fibres of tibial and mesenterial nerves. Acta Physiol Acad Sci Hung 1966;29:157–75.

Han JS, Chen XH, Sun SL, Xu XJ, Yuan Y, Yan SC, et al. Effect of low- and high-frequency TENS on Met-enkephalin-Arg-Phe and dynorphin A immunoreactivity in human lumbar CSF. Pain 1991;47:295–8.

Hotta H, Sato A, Schmidt RF, Suzuki A. Cerebral regional cortical blood flow response during joint stimulation in cats. Neuroreport 2005;16:1693–5.

Hotta H, Schmidt RF, Uchida S, Watanabe N. Gentle mechanical skin stimulation inhibits the somatocardiac sympathetic C-reflex elicited by excitation of unmyelinated C-afferent fibers. J Physiol Sci 2009a;59(Supp. 1):473.

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Hotta H, Schmidt RF, Uchida S, Watanabe N. The cutaneous afferents contributing to inhibitions of the sympathetic reflex elicited by excitation of unmyelinated C-afferent fibers in anesthetized rats. Neurosci Res 2009b;65(Suppl. 1):58.

Ikeda H, Asai T, Randić M, Murase K. Robust suppression of afferent-induced excitation in the rat spinal dorsal horn after conditioning low-frequency stimulation. J Neurophysiol 1999;82:1957–64.

Ito K, Nakamura H, Sato A, Sato Y. Depressive effect of morphine on the sympathetic reflex elicited by stimulation of unmyelinated hindlimb afferent nerve fibers in anesthetized cats. Neurosci Lett 1983;39:169–73.

Kagitani F, Uchida S, Hotta H, Aikawa Y. Manual acupuncture needle stimulation of the rat hindlimb activates groups I, II, III and IV single afferent nerve fibers in the dorsal spinal roots. Jpn J Physiol 2005;55:149–55.

Kato J, Meguro K, Sato A, Sato Y. The effects of morphine administered into the vertebral artery on the somatosympathetic A- and C-reflexes in anesthetized cats. Neurosci Lett 1992;138:207–10.

Klein T, Magerl W, Hopf HC, Sandkühler J, Treede RD. Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. J Neurosci 2004;24:964–71.

Li P, Pitsillides KF, Rendig SV, Pan HL, Longhurst JC. Reversal of reflex-induced myocardial ischemia by median nerve stimulation: a feline model of electroacupuncture. Circulation 1998;97:1186–94.

Li WM, Sato A, Sato Y, Schmidt RF. Morphine microinjected into the nucleus tractus solitarius and rostral ventrolateral medullary nucleus enhances somatosympathetic A- and C- reflexes in anesthetized rats. Neurosci Lett 1996;221:53–6.

Love-Jones SJ, Besson M, Steeds CE, Brook P, Chizh BA, Pickering AE. Homotopic stimulation can reduce the area of allodynia in patients with neuropathic pain. Eur J Pain 2009;13:942–8.

Lynn B, Carpenter SE. Primary afferent units from the hairy skin of the rat hind limb. Brain Res 1982;238:29–43.

Pomeranz B, Paley D. Electroacupuncture hypalgesia is mediated by afferent nerve impulses: an electrophysiological study in mice. Exp Neurol 1979;66:398–402.

Sandkühler J, Chen JG, Cheng G, Randić M. Low-frequency stimulation of afferent Adelta-fibers induces long-term depression at primary afferent synapses with substantia gelatinosa neurons in the rat. J Neurosci 1997;17:6483–91.

Sato A, Li P, Campbell JL. Acupuncture: is there a physiological basis? Int Congr Ser 2002;1238:1–182.

Sato A, Sato Y, Schmidt RF. Modulation of somatocardiac sympathetic reflexes mediated by opioid receptors at the spinal and brainstem level. Exp Brain Res 1995;105:1–6.

Sato A, Sato Y, Schmidt RF. The impact of somatosensory input on autonomic functions. Rev Physiol Biochem Pharmacol 1997;130:1–328.

Sato A, Sato Y, Suzuki A, Swenson RS. The effects of morphine administered intrathecally on the somatosympathetic reflex discharges in anesthetized cats. Neurosci Lett 1986;71:345–50.

Sato A, Sato Y, Swenson RS. Effects of morphine on somatocardiac sympathetic reflexes in spinalized cats. J Auton Nerv Syst 1985;12:175–84.

Schmidt RF, Weller E. Reflex activity in the cervical and lumbar sympathetic trunk induced by unmyelinated somatic afferents. Brain Res 1970;24:207–18.

Strack AM, Sawyer WB, Marubio LM, Loewy AD. Spinal origin of sympathetic preganglionic neurons in the rat. Brain Res 1988;455:187–91.

Swett JE, Woolf CJ. The somatotopic organization of primary afferent terminals in the superficial laminae of the dorsal horn of the rat spinal cord. J Comp Neurol 1985;231:66–77.

Tjen-A-Looi SC, Li P, Longhurst JC. Role of medullary GABA, opioids, and nociceptin in prolonged inhibition of cardiovascular sympathoexcitatory reflexes during electroacupuncture in cats. Am J Physiol Heart Circ Physiol 2007;293:H3627–35.

Uchida S, Kagitani F, Hotta H. Mechanism of the reflex inhibition of heart rate elicited by acupuncture-like stimulation in anesthetized rats. Auton Neurosci 2008;143:12–9.

Uchida S, Suzuki A, Hotta H, Sato A. The effects of morphine on supraspinal and propriospinal somatocardiac reflexes in anesthetized rats. Neurosci Lett 1999;269:161–4.

Vallbo ÅB, Olausson H, Wessberg J. Unmyelinated afferents constitute a second system coding tactile stimuli of the human hairy skin. J Neurophysiol 1999;81:2753–63.

Wang Y, Zhang Y, Wang W, Cao Y, Han JS. Effects of synchronous or asynchronous electroacupuncture stimulation with low versus high frequency on spinal opioid release and tail flick nociception. Exp Neurol 2005;192:156–62.

Wessberg J, Olausson H, Fernström KW, Vallbo ÅB. Receptive field properties of unmyelinated tactile afferents in the human skin. J Neurophysiol 2003;89:1567–75.

Woolf CJ, Wall PD. Chronic peripheral nerve section diminishes the primary afferent A-fibre mediated inhibition of rat dorsal horn neurones. Brain Res 1982;242:77–85.

Yaksh TL, Elde RP. Factors governing release of methionine enkephalin-like immunoreactivity from mesencephalon and spinal cord of the cat in vivo. J Neurophysiol 1981;46:1056–75.

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