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ORIGINAL PAPER

Age-related change in the effect of gentle mechanical cutaneous stimulation on the somato-cardiac sympathetic C-reflex

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Abstract This study examined whether aging influences the effect of gentle mechanical cutaneous stimulation (touch) on somato-cardiac sympathetic reflexes in anesthetized rats. A single electrical stimulus (15 V, 0.5 ms) applied to the tibial nerve induced somato-cardiac sympathetic A- and C-reflexes, which originated from the myelinated A- and unmyelinated C-fibers of the tibial nerve, respectively. When touch was applied to the inner thigh ipsilateral to the electrical stimulation, the C-reflex was selectively inhibited in adult rats (4–12 months), and this response was abolished by the opioid receptor antagonist, naloxone. Such an inhibitory effect of touch was impaired in aged rats (26–30 months). In contrast, depression of both A- and C-reflexes during a phenylephrine-induced rise in blood pressure was well maintained in the aged group. This study indicates that activation of the opioidergic system by gentle mechanical cutaneous stimulation attenuates with aging.

Keywords Somato-sympathetic reflex · Touch · Skin · Opioid · Aging · Baroreceptor

Introduction

In our daily life, we may experience that pain is relieved by placing hands on the skin near where we feel pain. Studies have demonstrated that tactile stimulation induces analgesia [1, 2]. However, the mechanisms by which gentle cutaneous stimulation induces analgesia have not been established. Recently, we examined the mechanism of innocuous gentle mechanical cutaneous stimulation for analgesia by recording A- and C-reflexes in the cardiac sympathetic nerve that were induced by electrical stimulation of myelinated A- and unmyelinated C-fibers, respectively, in a hindlimb somatic nerve of anesthetized adult rats [3]. In this study, gentle mechanical cutaneous stimulation was found to selectively inhibit the C-reflex, and such inhibition was the most effective when the cutaneous stimulation was applied to the inner thigh ipsilateral to the electrical stimulation. In addition, the inhibitory effect was significantly reduced by i.v. naloxone administration, indicating that endogenous opioids are involved in the effect [3]. Similar selective inhibition of the somato-cardiac sympathetic C-reflex was also observed by intrathecal (i.t.) morphine administration [4, 5]. Many of the C-primary afferents are nociceptive afferents and convey delayed pain. Therefore, gentle mechanical cutaneous stimulation is thought to inhibit nociceptive transmission conveyed by C-primary afferents in the spinal segmental level and produce pain relief.

There are various endogenous analgesic mechanisms in the body. Of those mechanisms, it is reported that the function of diffuse noxious inhibitory control induced by noxious stimulation is attenuated with aging in both rats [6] and humans (for review, see [7]). However, it is not known how aging affects the noxious inhibitory mechanism induced by innocuous stimulation at the spinal segment level. Thus, this study aimed to investigate the influence of aging on the inhibition of somato-cardiac sympathetic reflexes during gentle mechanical cutaneous stimulation. For comparison, the influence of aging on inhibition of both the A- and C-reflexes induced by excitation of arterial baroreceptors [8] was also examined.

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Methods

In this study, adult (4–12 months; $n = 8$) and aged (26–30 months; $n = 8$) Wistar female rats (160–320 g) bred at the Tokyo Metropolitan Institute of Gerontology were used. Experimental protocols were approved by the Animal Care and Use Committee of our institute. The rats were anesthetized using urethane. An initial dose of urethane (0.9–1.1 g/kg i.p.) was based on a previous study [9], and experimental methods were as described previously [3]. Arterial blood pressure and heart rate were continuously measured using a catheter placed in the carotid artery. Drugs were given through a catheter inserted in the jugular vein. Additional doses of urethane were administered as necessary (10–20% of the initial dose) in order to maintain the anesthetic level as evidenced by a stable arterial blood pressure and heart rate, and, when rats were not immobilized, the absence of body movement including withdrawal and corneal reflexes. After the completion of preparation, gallamine triethiodide (20 mg/kg i.v.) was administered to immobilize the rat.

In order to maintain physiological conditions during the experiment, the rat was artificially ventilated via a catheter inserted into the trachea. The ventilation was monitored (Microcap, Oridion Medical, Jerusalem, Israel) and adjusted to maintain the end-tidal CO_2 level at approximately 3%. In addition, rectal temperature was measured, and core body temperature was controlled at approximately 37.5°C using a heat pad and lamp (ATB-1100, Nihon Kohden, Tokyo).

Mass discharges were recorded in the right inferior cardiac sympathetic nerve, and the somato-cardiac sympathetic reflex was evoked by applying a single electrical shock (15 V, 0.5 ms) to the tibial nerve. The tibial nerve was electrically stimulated every 3 s, and evoked reflex discharges were averaged 50 times (Unique Acquisition software, Unique Medical, Tokyo) and stored on a computer. The averaged reflex discharges were quantified by measuring the area under the curve off-line using the computer software.

Following fur-trimming, the inner thigh skin was continuously touched for 10 min using a soft elastomer brush (SOMARESON I, gifted by Toyoresin, Shizuoka) ipsilateral to the electrically stimulated tibial nerve. This brush had approximately 400 microcones on the surface of the flat disc (11 mm in diameter), and the tip of the microcone was flattened. A robot was used for stable weight application of touch (6, 12 or 24 g). For each rat, one or multiple kinds of weight were tested. It was confirmed using a digital video microscope (DS-500, Science-eye, Saitama, Japan) that these weights did not bend the microcones.

To examine whether endogenous opioids contribute to the effect of touch, the opioid receptor antagonist, naloxone hydrochloride (Sigma, USA), was administered (2 mg/kg i.v.). This dose of naloxone has been reported to be sufficient to reverse the influence of a high dose of morphine on somato-cardiac sympathetic reflexes [5, 10].

At the end of the experiment, one of two doses of the α1 receptor agonist, phenylephrine, was administered intravenously to both age groups, and the pressor response was induced for examining the effect of baroreceptor stimulation on somato-cardiac sympathetic reflexes. For three of four rats in each group, phenylephrine was given as an initial bolus injection (0.4 ml at 50 μg/ml) and then as a continuous infusion (1.0 ml at 10 μg/ml, approximately 0.1 ml/10 s). For the remaining rats, 0.4 and 1.0 ml (250 μg/ml) of phenylephrine were given as a bolus injection and a continuous infusion, respectively. Arterial blood pressure and reflex discharges were averaged for 2.5 min immediately before and immediately after phenylephrine administration and compared.

Statistical analyses were performed using a commercialized statistics package (GraphPad Prism4, USA). The effect of mechanical cutaneous stimulation on somato-cardiac sympathetic reflexes was tested using the one-way repeated measures ANOVA followed by the Dunnett's multiple comparison test. The influence of baroreceptor stimulation on blood pressure and reflex discharges was tested using the paired t test. Data were expressed as mean ± SEM unless otherwise stated. The statistical significance level was set at $p < 0.05$.

Results

In 8 adult rats, the effect of touch with 12 g of weight, which was the same weight used in our previous study [3], was examined after stable recording of A- and C-reflexes were confirmed (latency 32.2 ± 1.2 and 186.9 ± 11.9 ms, respectively). The C-reflex was selectively inhibited by touch with 12 g, but the A-reflex was unchanged (Fig. 1A). Additionally, the effects of half of the weight, 6 g, and twice the weight, 24 g, were also examined. The effect of touch with 6 g on both reflexes was similar to 12 g; however, there was no effect of touch using 24 g. Thus, the effects of 6 or 12 g weights on A- (Fig. 1Ba) and C- (Fig. 1Bb) reflexes were treated as mean of mean values. An inhibition of C-reflex started to occur during 5–10 min of the touch period and continued until 5–10 min after touch was terminated. Such an effect became maximal at 0–5 min after touch termination and reached 88.2 ± 3.3% of the pre-touch level (Fig. 1Bb). The A-reflex tended to be slightly inhibited, but not statistically significantly.

Following naloxone administration, basal levels of A- and C-reflexes did not change. However, the inhibitory

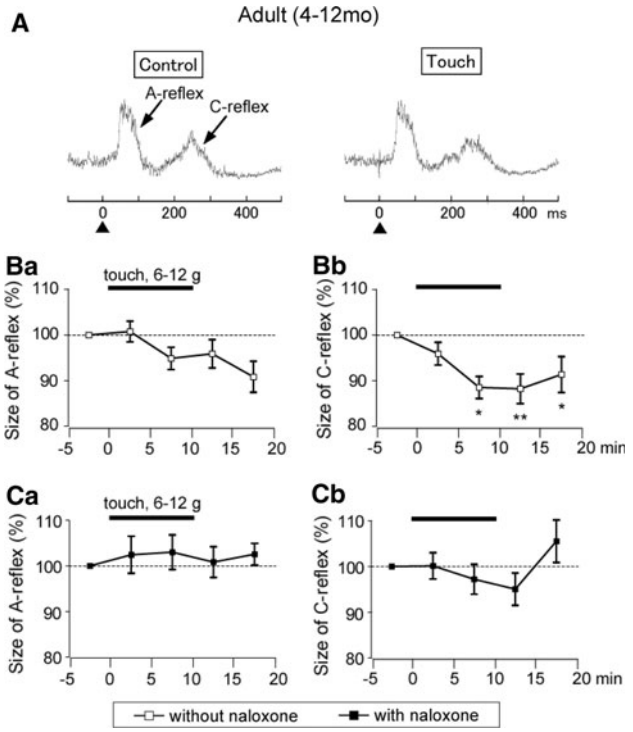

Fig. 1 The effect of touch on the somato-cardiac sympathetic reflex in adult rats. Representative records of A- and C-reflexes (averaged 50 times) before and during touch (12 g) are shown (**A**). At the point of the triangle (*filled triangle*) located under the time axis, a single electrical shock was applied to the tibial nerve. The graphs show the effect of touch (6–12 g) on A- (**a**) and C- (**b**) reflexes without (**B**) and with (**C**) i.v. naloxone administration (8 adult rats). The *horizontal line* in the *graph* indicates a period of 10-min touch. Data are expressed as changes with respect to pre-touch level (100%). *Each symbol* and *vertical bar* expresses mean ± SEM. $*p < 0.05$, $**p < 0.01$; significantly different from pre-touch value as determined by the one-way repeated measures ANOVA followed by Dunnett's multiple comparison test

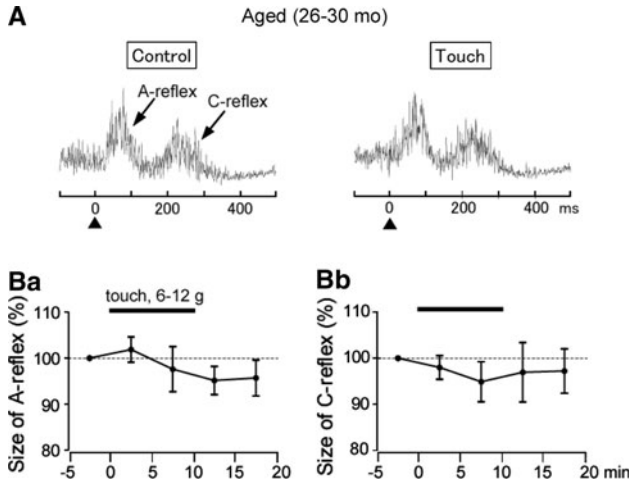

Fig. 2 The effect of touch on the somato-cardiac sympathetic reflex in aged rats. Representative records of A- and C-reflexes (averaged 50 times) before and during touch (6 g) are shown (**A**). At the point of the triangle (*filled triangle*) located under the time axis, a single electrical shock was applied to the tibial nerve. The graphs (**B**) show the effect of touch (6–12 g) on A- (**a**) and C- (**b**) reflex discharges (8 aged rats). See Fig. 1 for further details

effect of touch on C-reflex discharge disappeared (Fig. 1Cb).

In 8 aged rats, somato-cardiac sympathetic A- and C-reflexes were stably recorded (Fig. 2A). The latencies of A- and C-reflexes were 29.3 ± 2.4 and 170.0 ± 4.7 ms, respectively, and there were no differences from those in adult rats. The applications of 6 g ($n = 5$), 12 g ($n = 6$) and 24 g ($n = 5$) of touch were attempted, but those weights had little influence on A- or C-reflexes (Fig. 2A) except in one rat in which the C-reflex was obviously inhibited [at 0–5 min after touch; decreased to 42% (6 g), 81% (12 g) and 80% (24 g) of the pre-touch level]. By summarizing the responses of touch to 6 or 12 g of weight as in the adult rat group, it was found that there was no significant change in A- or C-reflexes in the aged group (Fig. 2Ba, b).

After stable recordings of blood pressure and both reflexes were confirmed, phenylephrine was administered intravenously (4 rats in each group). There was no difference in basal blood pressure between the adult and aged rat groups (73.8 ± 8.3 and 70.8 ± 3.7 mmHg, respectively). In response to phenylephrine administration, blood pressure sharply increased, and a similar degree of pressor response between both the adult and aged groups was observed (adult Δ58 ± 13.3 mmHg, aged Δ56 ± 16.6 mmHg) (Fig. 3a). By phenylephrine administration, somato-cardiac sympathetic A- and C-reflexes were both inhibited (adult decreased to 60.6 ± 13.4 and 66.8 ± 7.9% of the pre-touch level, respectively; aged 53.5 ± 15.2 and 56.3 ± 13.4%, respectively), and there was no difference in response between the groups (Fig. 3b).

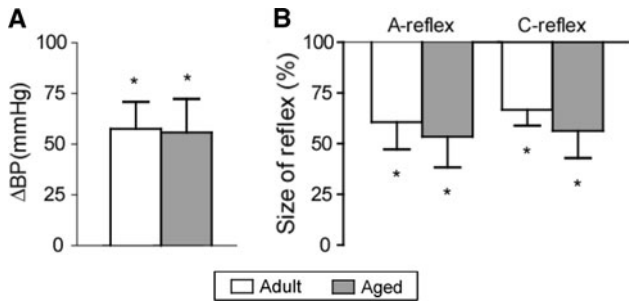

Fig. 3 The effect of i.v. phenylephrine administration on blood pressure (**a**) and reflex discharges (**b**). The graphs show changes in blood pressure (ΔBP) (mmHg) and reflex discharges (%) recorded for 2.5 min after i.v. phenylephrine administration with respect to pre-administration value. *Open* and *shaded columns* indicate the results of adult and aged rats, respectively (4 rats in each group). *Each column* and *vertical bar* expresses mean ± SEM. $*p < 0.05$; significantly different from pre-administration value as determined by the paired *t* test

Discussion

This study demonstrated that touch applied to the inner thigh ipsilateral to the electrically stimulated tibial nerve selectively inhibited the somato-cardiac sympathetic C-reflex in adult female rats, and endogenous opioids are involved in such an inhibition. These results were consistent with our previous study in adult male rats [3], whereas there are partial contradictions that (1) the extent of the effect of touch on the C-reflex was smaller and (2) naloxone administration completely blocked the effect of touch in the present study. A possible reason for the first point is that the release of endogenous opioids in female rats is less than in male [11]. Thus, it seems that the extent of C-reflex inhibition by touch in female adult rats (the present study) was smaller than in male adult rats [3]. With respect to the second point, as naloxone administration significantly but not completely blocked the effect of touch in our previous study [3], there may also be a gender difference in the release of naloxone-resistant inhibitory mediators.

In addition, the present study employed aged rats to investigate the influence of aging on the effect of touch observed in adult rats. So far, there have been no reports on reflex discharges evoked by somatosensory stimulation recorded in the cardiac sympathetic nerves in aged animals. The present study is the first to demonstrate that cardiac sympathetic A- and C-reflexes are evoked by electrical stimulation applied to somatic afferents in aged rats as in adult ones. This result is consistent with a report by Suzuki et al. [9] that showed the response of cardiac sympathetic nerve activity to noxious pinch stimulus in 24–27- and 32–36-month-old rats was not different from in 4–7-month-old rats. Further, we examined the effect of touch on the evoked somato-cardiac sympathetic reflexes in aged rats and found that reflex discharges were not inhibited by touch in most of the rats. Hence, it was clarified that the C-reflex inhibitory effect of touch was attenuated with age.

Age-related changes in the number and functions of cutaneous afferents as well as opioid release and its receptors may be involved in the attenuation of C-reflex inhibition by touch. Reinke and Dinse [12] reported that the mechanical threshold of cutaneous sensory receptor units (rapidly and slowly adapting; RA and SA) in the plantar nerve using von Frey hair in 24–27-month-old rats was similar to that in 3- and 6-month-old rats; however, the ratio of the myelinated nerve fibers of the SA unit to RA unit decreased in the 24–27-month-old animals. In addition to such myelinated fibers, low-threshold mechanoreceptive C fibers in hairy skin of rats [13] were also activated during the touch period [3]. The proportion of mechanoreceptive C fibers relative to mechanoinsensitive C fibers was decreased in older humans (41–67 vs. 21–36 years old) [14]. Since it was thought that the low frequency activity of SA and low threshold C fiber mechanoreceptor units, which continued during the touch period, contributed to an inhibitory effect on reflex discharge [3], a reduction in the ratio of these units may be related to the attenuation of the touch effect.

In terms of the influence of aging on the opioid system, Crisp et al. [15] reported that greater doses of μ- and δ-opioid receptor agonists (i.t.) were necessary in aged rats (25–26 months old) for comparable delay in the latency of the tail-flicking response to heat stimulation as compared to young (5–6-month-old) and mature (15–16-month-old) rats. Additionally, using autoradiography, Hoskins et al. [16] showed that the B_{max} value of μ-opioid receptor agonist (i.t.) was not different among different rat age groups (young 5–6 months old, mature 15–16 months old, aged 25–26 months old), whereas the K_d value was greater in aged rats (25–26 months old) than in younger rats (5–6 and 15–16 months old). The results suggest that there is a decrease in the affinity of the agonist for the spinal μ-opioid receptor. As a subtype of opioid receptor, κ-opioid receptor is also known to contribute to analgesia. The κ-opioid receptor agonists have been reported to be more effective for inhibiting the tail-withdrawal response to hot water immersion in aged rats (21 months old) than in young rats (3 months old) [17]. Therefore, the present study results seem to show an association with age-related changes in μ- and δ-opioid receptors, but not the κ-opioid receptor.

Accompanying the pressor response to i.v. phenylephrine administration, somato-sympathetic A- and C-reflexes were both inhibited as reported previously [8, 18]. This inhibitory effect was abolished by transection of the aortic and carotid sinus nerves, indicating that this response was induced by excitation of arterial baroreceptors [8]. We showed that the same magnitude of pressor response was induced by the same doses of i.v. phenylephrine administration, and the A- and C-reflexes were depressed to the same extent in both adult and aged groups. The age-related decrease in adrenergic responsiveness of cardiovascular function is known. However, there seems to be a gender difference in such a change, as it has been reported that the phenylephrine-induced pressor response was attenuated in aged male rats [19] but not in female ones [20]. Kurosawa et al. [19] examined the effect of phenylephrine-induced (i.v.) changes in blood pressure and adrenal nerve activity in 4- and 26-month-old rats, and showed that there was no difference in the extent of inhibition of adrenal nerve activity between these groups when the same degree of blood pressure increase was induced. Taken together, it can be summarized that inhibitory effects of baroreceptor excitation on sympathetic reflexes (the present study) and tonic activity [19] are not influenced by aging.

The present study found that only the mechanism for selective C-reflex inhibition by touch was attenuated by aging, but not the overall inhibitory mechanisms for the reflex. Hence, our results suggest that an endogenous analgesic mechanism caused by gentle cutaneous stimulation in daily life attenuates during aging, which may be one of the causative factors of persistent pain in elderly people.

Conflict of interest The authors declare that they have no conflict of interest.

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