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Figure legends

Fig.1. HR at rest and intrinsic HR (*A*) and HR sympathetic and vagal tone (*B*) obtained in sedentary (*open bars*) and exercise trained (*closed bars*) rats.

Fig.2. *A*: Transfer function from sympathetic stimulation to the HR response obtained in sedentary and exercise trained rats. Gains (*top*), phase shifts (*middle*), and coherence functions (*bottom*) are presented. *B*: Calculated step response to 1-Hz tonic sympathetic stimulation. Thick lines represent the mean, whereas thin lines indicate + SD values. The gray solid curves in the gain and step response in the right panels duplicate the means of the left panels.

Fig.3. *A*: Transfer function from vagal stimulation to the HR response obtained in sedentary and exercise trained rats. Gains (*top*), phase shifts (*middle*), and coherence functions (*bottom*) are presented. *B*: Calculated step response to 1-Hz tonic vagal stimulation. Thick lines represent the mean, whereas thin lines indicate + SD values. The gray solid curves in the gain and step response in the right panels duplicate the means of the left panels.

Fig.4. Averaged sympathetic (*A*) and vagal (*B*) gain calculated from corresponding transfer function in very low frequency (VLF), low frequency (LF), and high frequency (HF) bands.

Fig.5. HR response to stepwise sympathetic (*A*) and vagal (*B*) stimulation obtained in sedentary (*open bars*) and exercise trained (*closed bars*) rats.

Table 1. Physical characteristics

	Sedentary	Exercise Trained
Body weight (g)	642 ± 33	534 ± 33 *
Ventricular weight (g)	1.22 ± 0.03	1.17 ± 0.04 *
Ventricular weight/body weight (g kg^{-1})	1.9 ± 0.1	2.2 ± 0.1 *
Lung weight (g)	2.13 ± 0.27	1.89 ± 0.38
Lung weight/body weight (g kg^{-1})	3.3 ± 0.3	3.5 ± 0.7
Performance test (s)	1150 ± 165	1790 ± 389 *

Values are means ± SD. * $P < 0.05$ compared to Sedentary group.

Table 2. Spectral parameters of R-R interval

	Sedentary	Exercise Trained
Variance (ms^2)	87 ± 39	90 ± 32
VLF (ms^2)	73 ± 30	80 ± 30
LF (ms^2)	6.3 ± 3.4	3.1 ± 3.0
LF (%)	49 ± 11	36 ± 7 *
HF (ms^2)	8.0 ± 7.6	6.2 ± 7.1
HF (%)	51 ± 11	64 ± 7 *
LF/HF ratio	1.0 ± 0.5	0.6 ± 0.2 *

Values are means ± SD. * $P < 0.05$ compared to Sedentary group.

Table 3. HR and AP during dynamic sympathetic stimulation protocol

	Sedentary		Exercise Trained	
	Pre stimulation	During stimulation	Pre stimulation	During stimulation
AP (mmHg)	74 ± 16	68 ± 15 †	89 ± 17	84 ± 24
HR (beats·min^{-1})	377 ± 25	444 ± 23 †	381 ± 16	444 ± 26 †

Values are means ± SD. † $P < 0.05$ compared to pre-stimulation.

Table 4. Sympathetic transfer function parameters and step response

	Sedentary	Exercise Trained
Gain (beats·min^{-1}·Hz^{-1})	4.2 ± 1.5	4.5 ± 1.5
Natural frequency (Hz)	0.07 ± 0.01	0.08 ± 0.01
Damping ratio	1.96 ± 0.55	1.69 ± 0.15
Lag time (s)	0.71 ± 0.10	0.62 ± 0.11
Steady-state response (beats·min^{-1})	3.6 ± 1.6	4.2 ± 1.2
80% rise time (s)	12.9 ± 2.7	12.1 ± 3.0

Values are means ± SD. See Appendix for transfer function parameters.

Table 5. HR and AP during dynamic vagal stimulation protocol

	Sedentary		Exercise Trained	
	Pre stimulation	During stimulation	Pre stimulation	During stimulation
AP (mmHg)	72 ± 21	68 ± 15	92 ± 14	80 ± 21
HR (beats min^{-1})	373 ± 18	327 ± 38 †	372 ± 14	301 ± 32 †

Values are means ± SD. † $P < 0.05$ compared to pre-stimulation.

Table 6. Vagal transfer function parameters and step response

	Sedentary	Exercise Trained
Gain (beats·min^{-1}·Hz^{-1})	6.1 ± 3.0	9.7 ± 5.1#
Corner frequency (Hz)	0.11 ± 0.05	0.17 ± 0.09
Lag time (s)	0.10 ± 0.08	0.17 ± 0.08
Steady-state response (beats·min^{-1})	-6.7 ± 3.6	-11.2 ± 5.7#
80% fall time (s)	4.3 ± 2.2	4.3 ± 1.5

Values are means ± SD. # P = 0.06 compared to Sedentary group. See Appendix for transfer function parameters.

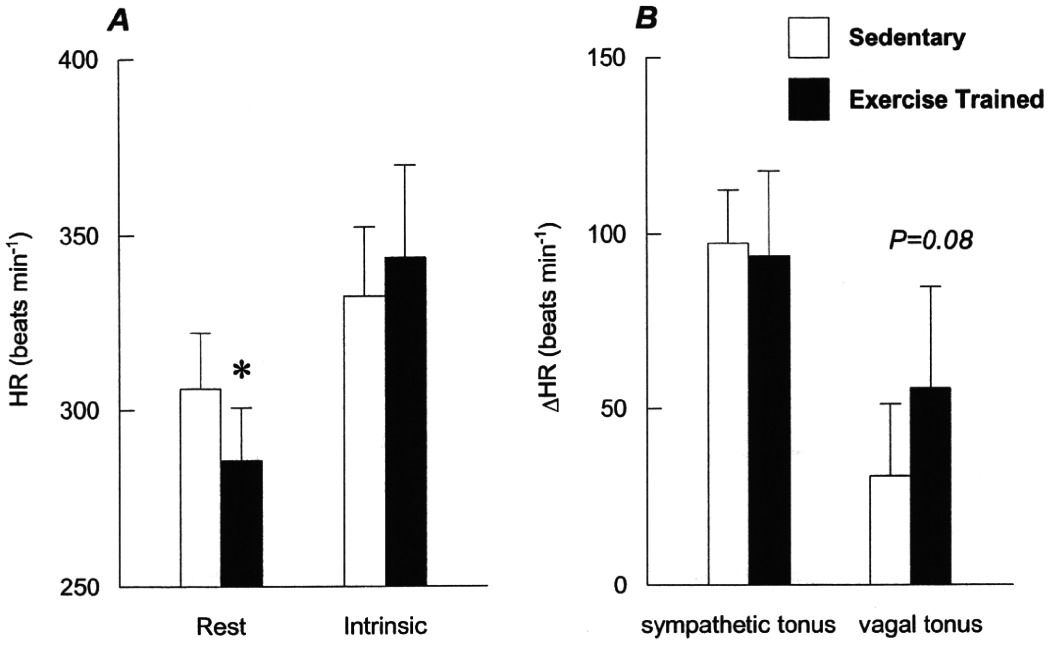
A
400
350
300
250
HR (beats min-1)
*
Rest
Intrinsic
B
150
100
50
0
ΔHR (beats min-1)
Sedentary
Exercise Trained
P=0.08
sympathetic tonus
vagal tonus

Figure 1

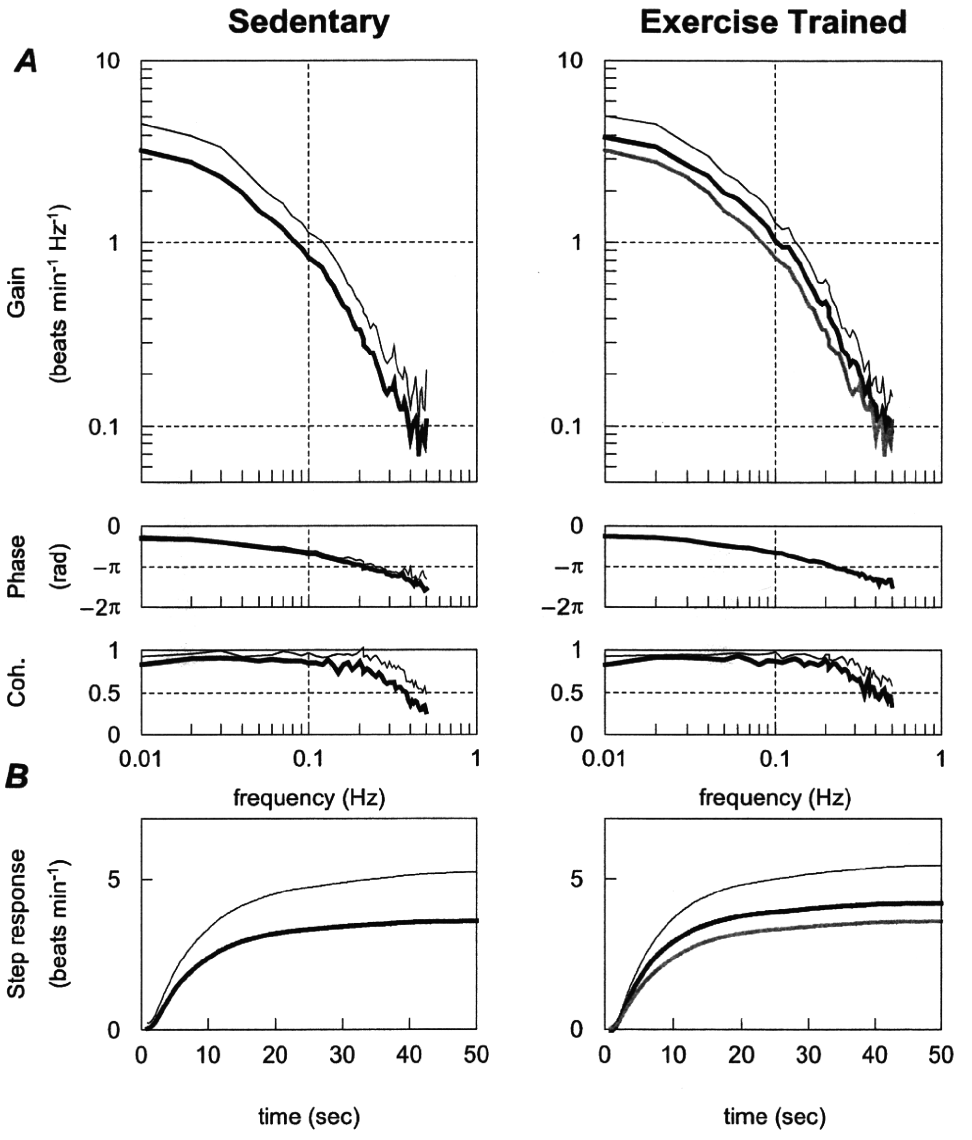
Sedentary
Exercise Trained
A
B
Gain
(beats min-1 Hz-1)
Phase
(rad)
Coh.
frequency (Hz)
Step response
(beats min-1)
time (sec)
10
1
0.1
0
−π
−2π
0.5
0.01
0.1
5
10
20
30
40
50

Figure 2

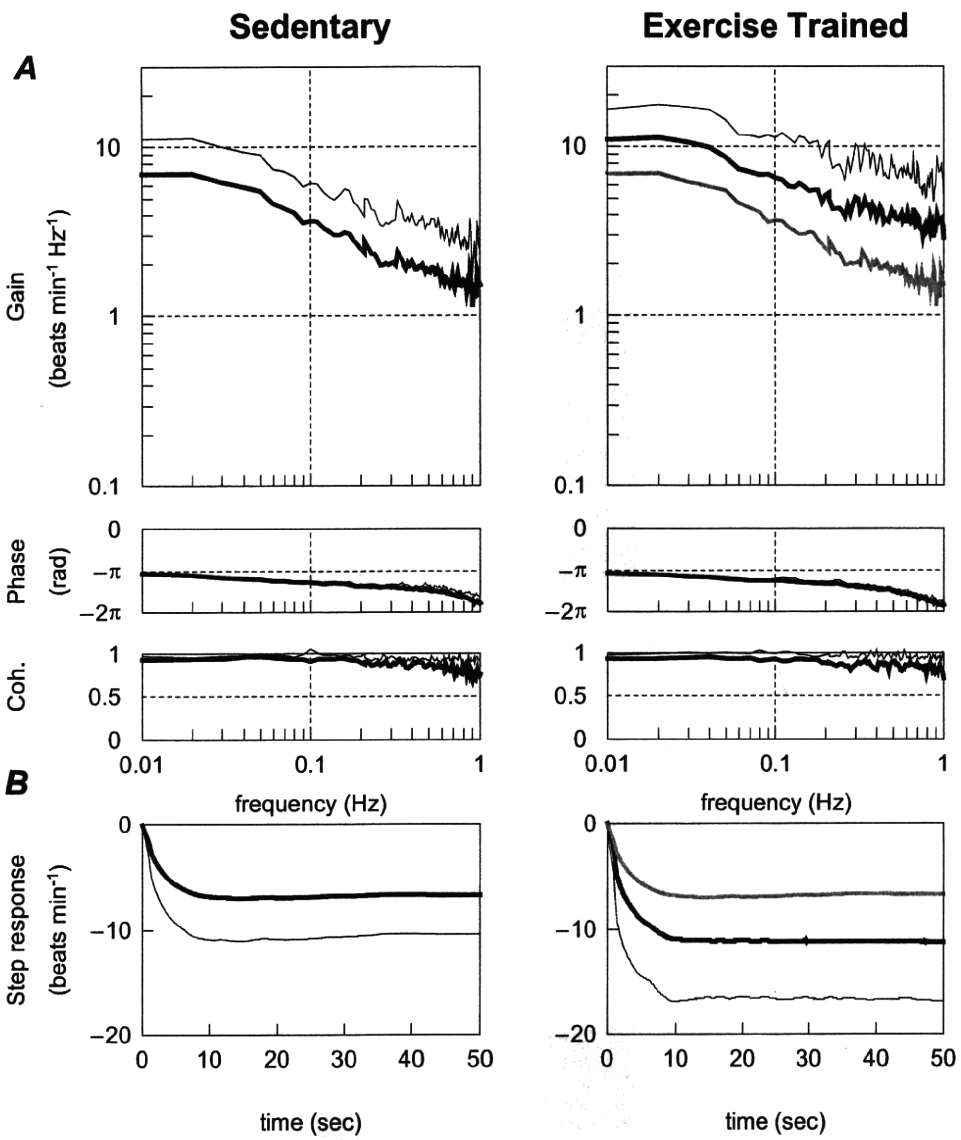
Sedentary
Exercise Trained
A
Gain
(beats min-1 Hz-1)
10
1
0.1
Phase
(rad)
0
−π
−2π
Coh.
1
0.5
0
0.01
0.1
1
frequency (Hz)
B
Step response
(beats min-1)
0
−10
−20
0
10
20
30
40
50
time (sec)

Figure 3

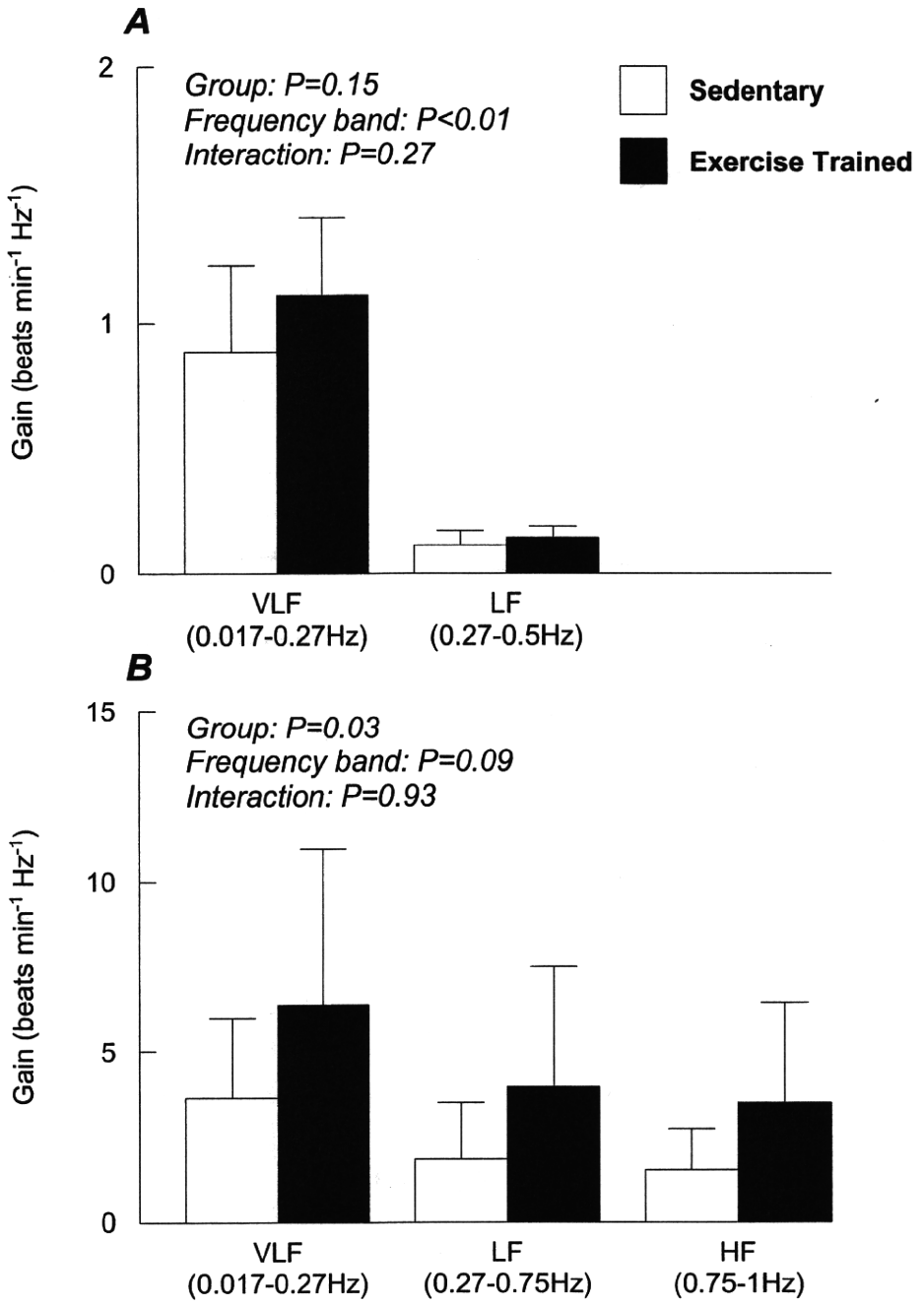
A
Group: P=0.15
Frequency band: P<0.01
Interaction: P=0.27
Sedentary
Exercise Trained
Gain (beats min-1 Hz-1)
2
1
0
VLF
(0.017-0.27Hz)
LF
(0.27-0.5Hz)
B
Group: P=0.03
Frequency band: P=0.09
Interaction: P=0.93
Gain (beats min-1 Hz-1)
15
10
5
0
VLF
(0.017-0.27Hz)
LF
(0.27-0.75Hz)
HF
(0.75-1Hz)

Figure 4

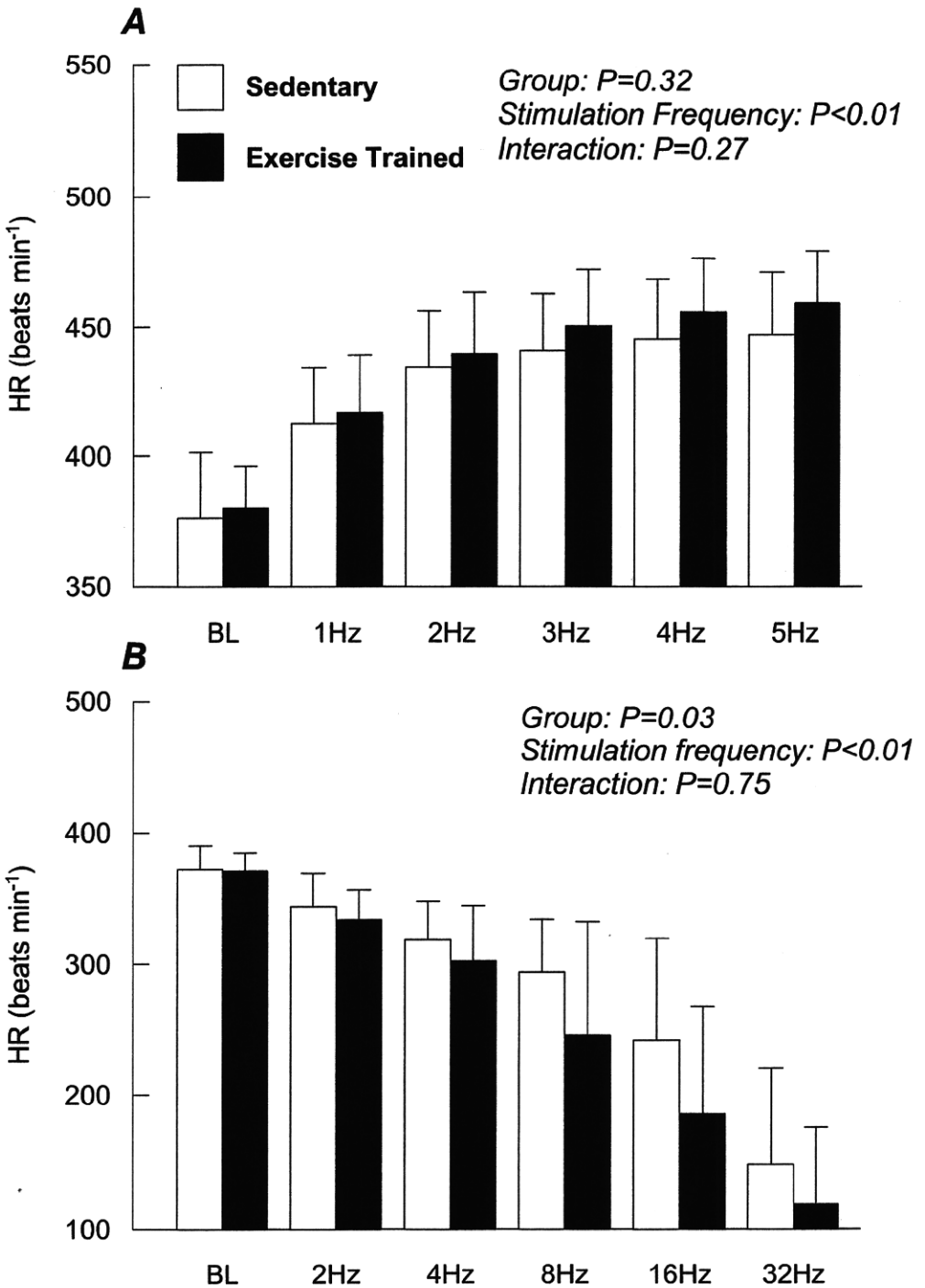
A
550
500
450
400
350
HR (beats min-1)
Sedentary
Exercise Trained
Group: P=0.32
Stimulation Frequency: P<0.01
Interaction: P=0.27
BL
1Hz
2Hz
3Hz
4Hz
5Hz
B
500
400
300
200
100
HR (beats min-1)
Group: P=0.03
Stimulation frequency: P<0.01
Interaction: P=0.75
BL
2Hz
4Hz
8Hz
16Hz
32Hz

Figure 5

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Involvement of the mechanoreceptors in the sensory mechanisms of manual and electrical acupuncture

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ABSTRACT

The modalities of acupuncture can be broadly classified into manual acupuncture (MA) and electroacupuncture (EA). Although MA has been reported to cause winding of tissue around the needle and subsequent activation of the sensory mechanoreceptors and nociceptors, the sensory mechanisms of acupuncture stimulation are not fully understood. To test the hypothesis that the involvement of the mechanoreceptors in the sensory mechanism is different in MA and EA, we examined the effects of a stretch-activated channel blocker gadolinium on the hemodynamic responses to hind limb MA and EA in anesthetized rats (n=9). Gadolinium significantly attenuated the MA-induced bradycardic response (−22±5 vs. −10±3 bpm, P<0.05) and tended to attenuate the MA-induced depressor response (−30±5 vs. −18±4 mm Hg, P=0.06). On the other hand, gadolinium significantly attenuated both the EA-induced bradycardic (−22±5 vs. −9±4 bpm, P<0.01) and depressor responses (−32±6 vs. −15±5 mm Hg, P<0.01). These results indicate that the mechanoreceptors are involved in the sensory mechanisms for both MA and EA.

1. Introduction

Acupuncture has been used to modulate autonomic nervous activity and cardiovascular function (Kimura and Sato, 1997; Lin et al., 2001). The modalities of acupuncture can be broadly classified into two categories: manual acupuncture (MA) and electroacupuncture (EA). MA and EA induce similar changes in the functional magnetic resonance imaging signal in the human brain (Napadow et al., 2005). Neural mechanisms involved in acupuncture have been the focus of investigations. The effects of EA are considered to be related to stimulation of finely myelinated (group III) and unmyelinated (group IV) fibers, which activate opioid receptors in the rostral ventrolateral medulla to inhibit sympathetic outflow (Chao et al., 1999). Depletion of group IV fibers by neonatal capsaicin treatment reduces the influence of EA on the pressor responses to mechanical stimulation of visceral organs (Tjen-A-Looi et al., 2005). The extensive network of tangential cutaneous axons, coupled with their communications with the large numbers of Merkel cells, might be considered a new division of the autonomic nervous system: the cutaneous intrinsic visceral afferent nervous system (Silberstein, 2009).

Although cardiovascular responses induced by acupuncture-like stimulation are known to be reflexes mediated via somatic afferent nerves, visceral afferent nerves and autonomic efferent nerves (Sato et al., 1994, 2002; Tjen-A-Looi et al., 2005; Uchida et al., 2007; Yamamoto et al., 2008; Silberstein, 2009), the sensory mechanisms of MA and EA that initiate afferent nerve discharge are not fully understood. Langevin et al. (2001) proposed that MA causes winding of tissues around the needle and subsequent activation of sensory mechanoreceptors and nociceptors, and also suggested that changes in extracellular milieu induced by MA are important factors for neuromodulation. Burnstock (2009) proposed that mechanical deformation of the skin leads to the release of ATP from keratinocytes, fibroblasts and other cells; then the sensory nerves are activated through purinergic receptors. Although EA may induce MA-like stimuli via electrical twitching of surrounding tissues, EA may also directly depolarize sensory axons and nerve terminals adjacent to the needle and induce reflex responses. If the direct depolarization is the major sensory mechanism of EA, inhibition of mechanoreceptors would not zsignificantly attenuate the effects of EA. On the other hand, if the mechanical stimulation plays a dominant role in the sensory mechanism of EA, inhibition of mechanoreceptors would significantly attenuate the effects of EA.

Among mechanoreceptors, mechanosensitive ion channels detect mechanical stimuli and transduce these stimuli into electrical signals in sensory neurons. Gadolinium chloride is widely used experimentally as an inhibitor of stretch-activated ion channels and physiological responses of tissues to mechanical stimulation (Adding et al., 2001). To test the hypothesis that the contribution of mechanoreceptors in the sensory mechanism differs in MA and EA, we examined the effects of gadolinium on the hemodynamic responses to MA and EA in anesthetized rats.

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2. Methods

2.1. Surgical preparation

Animal care was provided in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Physiological Society of Japan. All protocols were reviewed and approved by the Animal Subject Committee at the National Cerebral and Cardiovascular Center. Male Wister Kyoto rats weighing from 310 to 460 g were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and ventilated mechanically via a tracheal tube with oxygen-enriched room air. The depth of anesthesia was maintained by continuous intravenous infusion of pentobarbital sodium (20–25 mg kg^{-1} h^{-1}) through a double lumen catheter inserted into the right external carotid vein. Ringer solution (6 mg kg^{-1} h^{-1}) was administered to maintain fluid balance. Arterial blood pressure (AP) was measured using a catheter inserted into the right common carotid artery. Heart rate (HR) was determined from AP using a cardiotachometer. Body temperature was maintained at approximately 38 °C using a heating pad.

2.2. MA and EA stimulations ($n=9$)

With the animal in the supine position, both hind limbs were lifted to obtain a better view of the lateral sides of the lower legs. An acupuncture needle with a diameter of 0.2 mm (CE0123, Seirin-Kasei, Japan) was inserted into a point below the knee joint just lateral to the tibia in the left or right leg. For MA stimulation, the acupuncture needle was inserted to a depth of 5–10 mm and manually twisted clockwise and counter-clockwise, and moved up and down at a frequency of 1–2 Hz for a duration of 120 s. Two to three MA trials were conducted with an intervening interval of more than 5 min within which AP and HR returned to the respective pre-stimulation values. For EA stimulation, another acupuncture needle was inserted into a point approximately 1 cm from the above-mentioned needle toward the ankle joint and used as the ground. EA was applied for 120 s using an isolator connected to an electrical stimulator (SEN 7203, Nihon Kohden, Japan). The pulse width and the stimulus current were set at 500 μs and 5 mA, respectively. The stimulation frequency was set at 10 Hz in six and at 20 Hz in three of the nine rats. The pulse duration was based on previous studies (Tjen-A-Looi et al., 2005; Yamamoto et al., 2008; Uchida et al., 2008). The amplitude and frequency were selected so that the magnitudes of reflex hemodynamic responses became comparable to those induced by MA before gadolinium administration. In each animal, two to three EA trials were conducted with an intervening interval of more than 5 min within which AP and HR returned to the respective pre-stimulation values.

Gadolinium chloride hexahydrate was dissolved in saline at a concentration of 20 mM (Nakamoto and Matsukawa, 2007). After performing MA and EA under control conditions, we administered the gadolinium solution intravenously (2 ml/kg). After 10 min, we repeated MA and EA. The acupuncture needle positions were kept unchanged between MA and EA trials as well as before and after the gadolinium administration.

In a supplemental protocol ($n=7$ additional rats), to examine the possibility that simple insertion of needles caused significant hemodynamic influences, an acupuncture needle (CE0123, Seirin-Kasei, Japan) was only inserted into a point below the knee joint just lateral to the tibia in the left or right leg and placed for a duration of 120 s. Needle was inserted to a depth of 5–10 mm.

2.3. Aortic depressor nerve stimulation ($n=6$)

Using a pair of platinum electrodes, we identified the aortic depressor nerve (ADN) running along the common carotid artery, based on the AP pulse-synchronous nerve activity monitored through a loud speaker. After a depressor response to brief electrical stimulation of the nerve was confirmed, the electrodes and the nerve were fixed and insulated by silicone glue (Kwik-Sil, World Precision Instruments, FL, USA). The nerve fibers caudal to the electrodes were then crushed by a tight ligature so that only the afferent fibers directed to the central nervous system were stimulated. In four of the six rats, the right ADN was stimulated. In the remaining two rats, the left ADN was stimulated because of failure to stimulate the right ADN properly. The ADN was stimulated for 120 s at a frequency of 50 Hz (pulse width: 2 ms, voltage: 2 V). ADN stimulation was repeated with an interval of 5 min until the AP and HR responses appeared to be reproducible under control conditions. We then administered the gadolinium solution intravenously (20 mM, 2 ml/kg). After 10 min, we repeated the ADN stimulation.

2.4. Data analysis

Data were digitized using a 16-bit analog-to-digital converter (Contec, Japan) and stored at 200 Hz on a laboratory computer system. First, AP and HR data were averaged every 10 s. Averaged time courses of AP and HR responses were then obtained from two to three trials of MA, EA or ADN stimulation in each animal. Next, the effects of MA, EA or ADN were examined using repeated-measures one-way analysis of variance (ANOVA) followed by Dunnett's test (Glantz, 2002). The baseline data point immediately before stimulation was treated as a single control point for the Dunnett's test. Finally, the maximum effect of MA, EA or ADN stimulation was quantified by the differences between the minimum and baseline values for AP and HR (ΔAP and ΔHR). The effects of gadolinium on ΔAP and ΔHR were examined by a paired-t test (Glantz, 2002). The differences were considered significant at $P<0.05$. Data are presented in mean ± SE values.

3. Results

Fig. 1 depicts the averaged time courses of AP and HR responses to MA ($n=9$ rats). MA gradually decreased AP and HR under control conditions. The minimum AP and HR were reached near the end of the MA stimulation period. After the cessation of MA, AP and HR gradually returned toward the respective baseline values. Intravenous gadolinium administration significantly decreased baseline AP from 138 ± 5 to 120 ± 5 mm Hg ($P<0.01$) but had no significant effect on baseline HR (379 ± 10 vs. 383 ± 7 bpm). Following gadolinium administration, although MA also decreased AP and HR significantly, ΔAP tended to be attenuated (−30 ± 5 vs. −18 ± 4 mm Hg; 68 ± 16% of the pre-gadolinium; $P=0.06$) and ΔHR was significantly attenuated (−22 ± 5 vs. −10 ± 3 bpm; 57 ± 23% of the pre-gadolinium; $P<0.05$) compared to control conditions.

Fig. 2 depicts the averaged time courses of AP and HR responses to EA ($n=9$ rats). Under control conditions, EA decreased AP and HR. Both responses reached almost a steady state at approximately 1 min of EA stimulation. AP and HR remained decreased during the rest of the EA stimulation period, and gradually returned toward the respective baseline values after the cessation of EA. Intravenous gadolinium administration significantly decreased baseline AP from 140 ± 5 to 123 ± 7 mm Hg ($P<0.01$) but did not affect baseline HR (385 ± 9 vs. 384 ± 7 bpm). Following gadolinium administration, although EA significantly decreased AP, the decrease in HR was only significant at 55 s of EA stimulation. ΔAP (−32 ± 6 vs. −15 ± 5 mm Hg; 38 ± 11% of the pre-gadolinium; $P<0.01$) and ΔHR (−22 ± 5 vs. −9 ± 4 bpm; 37 ± 14% of the pre-gadolinium; $P<0.01$) were attenuated significantly compared to control conditions.

In the supplemental protocol ($n=7$ rats), the insertion of an acupuncture needle alone did not significantly change AP (138 ± 9 vs. 138 ± 9 mm Hg) or HR (399 ± 20 vs. 400 ± 20 bpm).

Fig. 3 shows the averaged time courses of AP and HR responses to ADN stimulation ($n=6$ rats). ADN stimulation decreased AP and HR under control conditions. The minimum AP and HR were reached at 15 s of ADN stimulation. Both parameters remained decreased during the rest of the ADN stimulation period, and returned toward the respective

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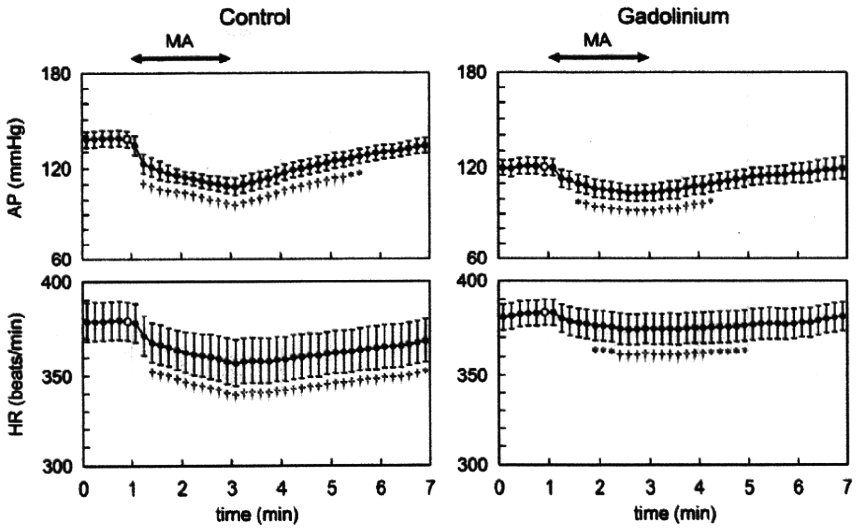

Fig. 1. Time courses of arterial pressure (AP) and heart rate (HR) responses induced by manual acupuncture (MA) averaged from 9 rats. MA gradually decreased AP and HR under control conditions (left) and after gadolinium administration (right). Gadolinium treatment tended to attenuate the AP response and significantly attenuated the HR response induced by MA, compared to control conditions. Data are mean ± SE values. $^{*}P<0.05$ and $^{\dagger}P<0.01$ versus the control data point (open circle) immediately before the application of MA.

baseline values after the cessation of ADN stimulation. AP and HR appeared to recover more rapidly compared to those observed after MA and EA. Intravenous gadolinium administration significantly decreased baseline AP from 126 ± 4 to 118 ± 2 mm Hg ($P<0.01$) but had no significant effect on baseline HR (373 ± 13 vs. 369 ± 11 bpm). Following gadolinium administration, ADN stimulation significantly decreased AP and HR. Neither ΔAP (−43 ± 7 vs. −49 ± 3 mm Hg) nor ΔHR (−27 ± 8 vs. −34 ± 5 bpm) was attenuated compared to control conditions.

4. Discussion

We have shown that ion channels blocked by gadolinium are implicated in the hypotensive and bradycardic effects of acupuncture at the hind limb in rats, irrespective of technique.

4.1. Effects of gadolinium on AP and HR responses to MA and EA

Insertion of acupuncture needle alone did not change AP and HR significantly, indicating that continuous stimulation either by MA or EA was necessary to induce sustained AP and HR responses. Mechanoreceptors are thought to play an important role in the sensory mechanism of MA. Because gadolinium blocks mechanosensitive ion channels in sensory neurons (Cho et al., 2002), we hypothesized that intravenous administration of gadolinium would attenuate the AP and HR responses to MA. As expected, ΔAP tended to be attenuated after gadolinium administration (Fig. 1, top). However, since gadolinium also decreased baseline AP, it is uncertain whether the attenuation of ΔAP was mainly attributable to the inhibition of reflex response to MA or to the decreased baseline AP. On the other hand, gadolinium did not significantly affect baseline HR and

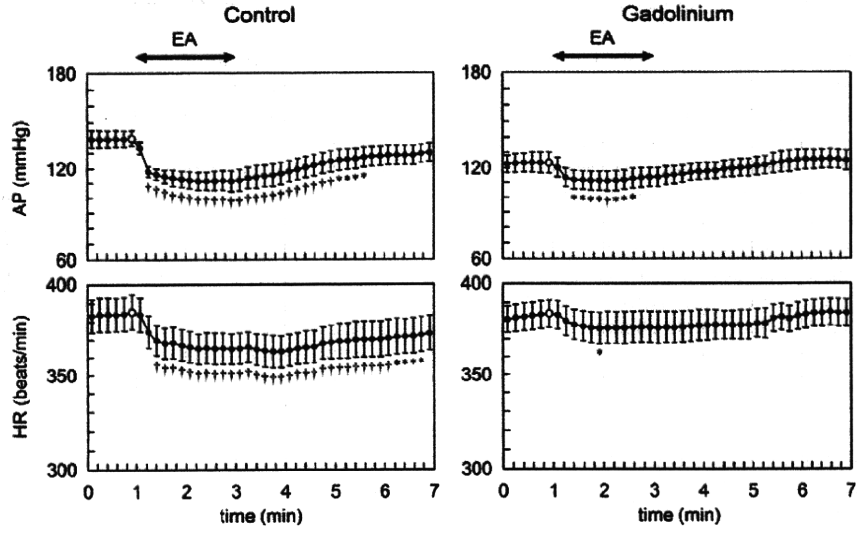

Fig. 2. Time courses of AP and HR responses induced by electroacupuncture (EA) averaged from 9 rats. EA gradually decreased AP and HR under control conditions (left) and after gadolinium administration (right). Gadolinium significantly attenuated both AP and HR responses induced by EA, compared to control conditions. Data are mean ± SE values. $^{*}P<0.05$ and $^{\dagger}P<0.01$ versus the control data point (open circle) immediately before the application of EA.

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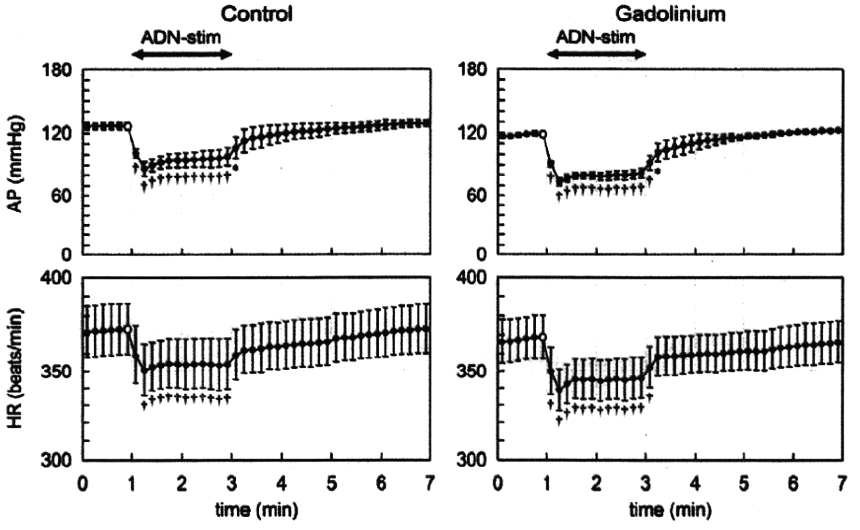

Fig. 3. Time courses of AP and HR responses induced by electrical stimulation of the aortic depressor nerve (ADN-stim) averaged from 6 rats. ADN-stim decreased AP and HR under control conditions (left) and after gadolinium administration (right). Gadolinium did not attenuate the AP and HR responses induced by ADN-stim, compared to control conditions. Data are mean ± SE values. $^{*}P<0.05$ and $^{\dagger}P<0.01$ versus the control data point (open circle) immediately before the application of ADN-stim.

significantly attenuated ΔHR induced by MA (Fig. 1, bottom). Judging from the HR response, it is conceivable that gadolinium inhibits the reflex hemodynamic responses to MA.

We assumed that direct depolarization of sensory axons and nerve terminals adjacent to the needle could be the major sensory mechanism of EA. In fact, direct electrical stimulation of muscle afferent fibers evokes a variety of cardiovascular responses similar to those induced by EA (Sato et al., 1981). If direct depolarization is the major sensory mechanism for EA, inhibition of mechanoreceptors would have no significant effect on EA, because the results of the ADN stimulation protocol indicates that the axonal conduction would not be blocked even after gadolinium administration once the afferent nerve is discharged (Fig. 3). Contrary to this assumption, gadolinium significantly attenuated ΔAP and ΔHR induced by EA (Fig. 2), suggesting that the mechanoreceptors play an important role in the sensory mechanism of EA, as in the case of MA. EA probably causes electrical twitching of surrounding tissues and exerts MA-like stimulation through the mechanoreceptors.

Despite the significant contribution of mechanoreceptors to the sensory mechanisms of both MA and EA, the fact that the hemodynamic responses to MA and EA were not entirely abrogated after gadolinium administration indicates the presence of sensory mechanisms other than the mechanosensitive ion channels. Not all capsaicin-sensitive neurons are mechanosensitive, and gadolinium has no effect on capsaicin-induced calcium transient in sensory neurons (Gschossmann et al., 2000). Depletion of group IV fibers by neonatal capsaicin treatment reduces the influence of EA on the pressor responses to mechanical stimulation of visceral organs (Tjen-A-Looi et al., 2005), suggesting an importance of capsaicin-sensitive neurons in the mechanisms of acupuncture. Nociceptive neurons are therefore a likely candidate for the residual sensory mechanism after gadolinium administration. The group IV C-fiber tactile afferents is known to be widely distributed in the skin of mammals (Wessberg et al., 2003). These fibers could be regarded as a cutaneous intrinsic visceral afferent nervous system (Silberstein, 2009). In addition, the present results do not rule out the possibility that direct depolarization of sensory axons or nerve terminals occurs during EA. Albeit this assumption, EA seemed to have received even greater influence from gadolinium than MA (Figs. 1 and 2). Because MA with needle movements can cause greater deformations in the adjacent extracellular milieu compared to EA, MA may have induced signal transductions other than mechanosensitive ion channels, such as integrin-linked signal transduction pathways (Aplin et al., 1998), resulting in the greater residual hemodynamic responses after gadolinium administration. Further studies are required in the future to solve this question.

4.2. Effects of gadolinium on the AP and HR responses to ADN stimulation

Gadolinium decreased baseline AP, suggesting actions other than the inhibition of mechanosensitive ion channels. For instance, gadolinium has been shown to block voltage-gated calcium, sodium and potassium channels (Adding et al., 2001). To exclude the possibility that gadolinium attenuates the reflex hemodynamic responses to MA and EA via nonspecific mechanisms such as the inhibition of central autonomic neurotransmission, we performed the ADN stimulation experiment. Gadolinium did not attenuate ΔAP and ΔHR induced by ADN stimulation (Fig. 3). It is unlikely, therefore, that gadolinium inhibits the central autonomic neurotransmission from afferent to efferent nerve activities or significantly blunted the AP and HR responses to changes in autonomic nerve activities.

4.3. Implication of MA and EA

Although the present results indicate that MA and EA may share a common sensory mechanism, EA may be more flexible than MA in terms of its application for biomedical engineering because the effects of EA can be controlled quantitatively by adjusting the stimulation current and stimulation frequency. As an example, a previous study from our laboratories has demonstrated that servo-controlled hind limb electrical stimulation can reduce AP at a prescribed target level in anesthetized cats (Kawada et al., 2009). EA can be applied continuously using a stimulating device without the attendance of an acupuncturist once the needle is properly positioned. Continuous electrical stimulation of auricular acupuncture points for 48 h/week has been shown to be more effective than auricular acupuncture without electrical stimulation for the treatment of chronic cervical pain in an outpatient population (Sator-Katzenschlager et al., 2003). Although further studies are required, EA delivered via a dedicated stimulating device may be an additional modality to the treatment of cardiovascular diseases.

4.4. Limitations

First, the present study was conducted under pentobarbital anesthesia. Because anesthesia affects the autonomic tone, AP and HR

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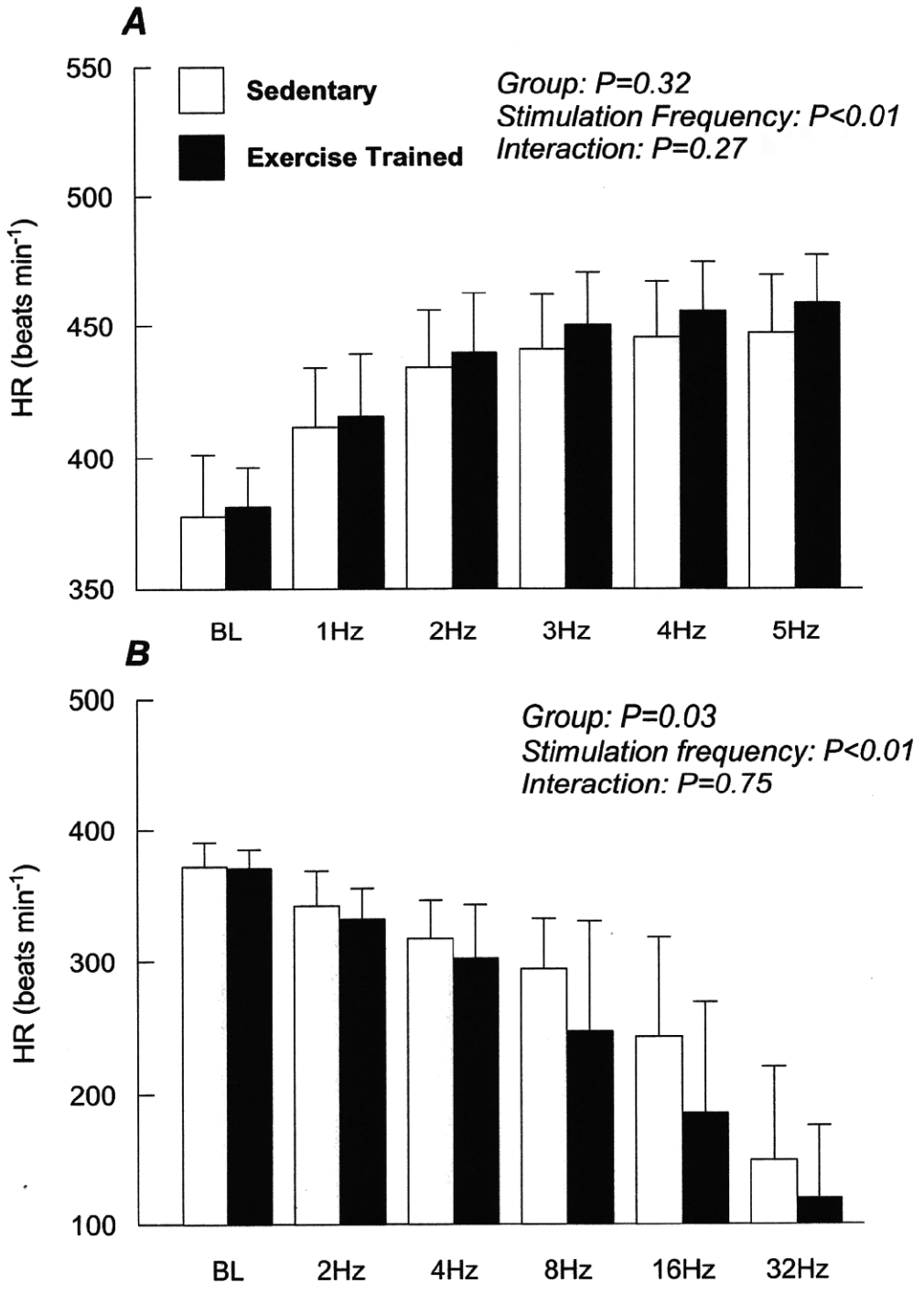
A
Sedentary
Exercise Trained
Group: P=0.32
Stimulation Frequency: P<0.01
Interaction: P=0.27
HR (beats min-1)
550
500
450
400
350
BL
1Hz
2Hz
3Hz
4Hz
5Hz
B
Group: P=0.03
Stimulation frequency: P<0.01
Interaction: P=0.75
HR (beats min-1)
500
400
300
200
100
BL
2Hz
4Hz
8Hz
16Hz
32Hz

Figure 5

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Involvement of the mechanoreceptors in the sensory mechanisms of manual and electrical acupuncture

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ABSTRACT

The modalities of acupuncture can be broadly classified into manual acupuncture (MA) and electroacupuncture (EA). Although MA has been reported to cause winding of tissue around the needle and subsequent activation of the sensory mechanoreceptors and nociceptors, the sensory mechanisms of acupuncture stimulation are not fully understood. To test the hypothesis that the involvement of the mechanoreceptors in the sensory mechanism is different in MA and EA, we examined the effects of a stretch-activated channel blocker gadolinium on the hemodynamic responses to hind limb MA and EA in anesthetized rats (n=9). Gadolinium significantly attenuated the MA-induced bradycardic response (−22±5 vs. −10±3 bpm, P<0.05) and tended to attenuate the MA-induced depressor response (−30±5 vs. −18±4 mm Hg, P=0.06). On the other hand, gadolinium significantly attenuated both the EA-induced bradycardic (−22±5 vs. −9±4 bpm, P<0.01) and depressor responses (−32±6 vs. −15±5 mm Hg, P<0.01). These results indicate that the mechanoreceptors are involved in the sensory mechanisms for both MA and EA.

1. Introduction

Acupuncture has been used to modulate autonomic nervous activity and cardiovascular function (Kimura and Sato, 1997; Lin et al., 2001). The modalities of acupuncture can be broadly classified into two categories: manual acupuncture (MA) and electroacupuncture (EA). MA and EA induce similar changes in the functional magnetic resonance imaging signal in the human brain (Napadow et al., 2005). Neural mechanisms involved in acupuncture have been the focus of investigations. The effects of EA are considered to be related to stimulation of finely myelinated (group III) and unmyelinated (group IV) fibers, which activate opioid receptors in the rostral ventrolateral medulla to inhibit sympathetic outflow (Chao et al., 1999). Depletion of group IV fibers by neonatal capsaicin treatment reduces the influence of EA on the pressor responses to mechanical stimulation of visceral organs (Tjen-A-Looi et al., 2005). The extensive network of tangential cutaneous axons, coupled with their communications with the large numbers of Merkel cells, might be considered a new division of the autonomic nervous system: the cutaneous intrinsic visceral afferent nervous system (Silberstein, 2009).

Although cardiovascular responses induced by acupuncture-like stimulation are known to be reflexes mediated via somatic afferent nerves, visceral afferent nerves and autonomic efferent nerves (Sato et al., 1994, 2002; Tjen-A-Looi et al., 2005; Uchida et al., 2007; Yamamoto et al., 2008; Silberstein, 2009), the sensory mechanisms of MA and EA that initiate afferent nerve discharge are not fully understood. Langevin et al. (2001) proposed that MA causes winding of tissues around the needle and subsequent activation of sensory mechanoreceptors and nociceptors, and also suggested that changes in extracellular milieu induced by MA are important factors for neuromodulation. Burnstock (2009) proposed that mechanical deformation of the skin leads to the release of ATP from keratinocytes, fibroblasts and other cells; then the sensory nerves are activated through purinergic receptors. Although EA may induce MA-like stimuli via electrical twitching of surrounding tissues, EA may also directly depolarize sensory axons and nerve terminals adjacent to the needle and induce reflex responses. If the direct depolarization is the major sensory mechanism of EA, inhibition of mechanoreceptors would not zsignificantly attenuate the effects of EA. On the other hand, if the mechanical stimulation plays a dominant role in the sensory mechanism of EA, inhibition of mechanoreceptors would significantly attenuate the effects of EA.

Among mechanoreceptors, mechanosensitive ion channels detect mechanical stimuli and transduce these stimuli into electrical signals in sensory neurons. Gadolinium chloride is widely used experimentally as an inhibitor of stretch-activated ion channels and physiological responses of tissues to mechanical stimulation (Adding et al., 2001). To test the hypothesis that the contribution of mechanoreceptors in the sensory mechanism differs in MA and EA, we examined the effects of gadolinium on the hemodynamic responses to MA and EA in anesthetized rats.

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