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Effects of oolong tea on renal sympathetic nerve activity and spontaneous hypertension in rats

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Abstract

In a previous study, evidence was presented that oolong tea (OT) reduced abdominal fat accumulation in diet-induced obese mice. In the study presented here, we examined the sympathetic and cardiovascular effects of intraduodenal injection of OT in urethane-anesthetized rats and found that it suppressed renal sympathetic nerve activity (RSNA) and blood pressure (BP). In addition, pretreatment with the histaminergic H3-receptor antagonist thioperamide or bilateral subdiaphragmatic vagotomy eliminated the effects of OT on RSNA and BP. Furthermore, OT drinking for 14 weeks reduced BP elevation in spontaneously hypertensive rats. These results thus suggest that OT may exert its hypotensive action through changes in autonomic neurotransmission via an afferent neural mechanism. Moreover, we found that intraduodenal injection of decaffeinated OT lowered RSNA and BP as well as OT, indicating that substances other than caffeine contained in OT may function as effective modulators of RSNA and BP.

1. Introduction

Oolong tea (OT) is widely consumed as a health drink in Japan and China and contains large amounts of various compounds [1]. Some recent studies have provided evidences that OT exerts physiological effects, and Han et al [2] confirmed that OT drinking for 10 weeks significantly prevented body fat accumulation in mice. Although it has been suggested that obesity is closely related to hypertension [3], it has not been investigated whether OT also affects blood pressure (BP) elevation. Because it is known that renal sympathetic nerves play an important role in BP regulation [4], it is possible that OT may affect renal sympathetic nerve activity (RSNA) and thus BP with a potentially hypotensive action. To determine the validity of this notion, we examined the effects of intraduodenal (ID) injection of OT on RSNA and BP in urethane-anesthetized rats as well as the effect of long-term ingestion on spontaneously hypertensive rats (SHR).

The central histaminergic system is thought to modulate cardiovascular functions via histaminergic neurotransmission [5,6]. We previously found that intracerebral ventricular (ICV) injection of low-dose histamine reduced RSNA and BP and that this effect was eliminated by pretreatment with thioperamide, a histamine H3 blocker [6]. We also found that sectioning of afferent vagal nerves eliminated sympathetic and cardiovascular responses to duodenal stimulation with *Lactobacillus johnsonii* La1 [7], which suggests the possibility of the afferent neural pathway playing a role in autonomic changes due to duodenal stimulation. We therefore hypothesized that the histaminergic system or afferent neural pathway may be involved in the effects of OT on RSNA and BP. To understand the mechanisms of the sympathetic and cardiovascular actions of OT, we investigated the effect of thioperamide or subdiaphragmatic vagotomy on the changes in RSNA and BP resulting from ID injection of OT.

2. Materials and methods

2.1. Animals

Male Wister rats weighing 300 to 330 g were used. Rats were housed in a room maintained at 24°C ± 1°C and illuminated for 12 hours (7:00 AM to 7:00 PM) everyday.

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Food and water were freely available. Rats were adapted to the environment for at least 1 week before the experiment. All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee of Osaka University.

2.2. Electrophysiological recording

On the experimental day, food was removed 3 to 4 hours before surgery. General preparation was performed as described previously [8,9]. Briefly, polyethylene catheters were inserted into the left femoral vein and the duodena cavity for intravenous and ID injections, respectively; and another catheter was inserted into the left femoral artery for BP determination under anesthesia induced by intraperitoneal injection of 1 g/kg urethane. The rats were then cannulated intratracheally, fixed in a stereotaxic apparatus, and maintained at 37.0°C to 37.5°C with a heating pad (Biomedica, Osaka, Japan). We monitored the depth of anesthesia with paw pinch tests [10]. For recording RSNA, the left renal nerve was exposed retroperitoneally through a left flank incision. The distal end of nerve was ligated and then hooked to a pair of silver wire electrodes for recording efferent nerve activity. The recording electrodes were immersed in a pool of liquid paraffin oil to prevent dehydration and for electrical insulation. The rat was allowed to stabilize for 30 to 60 minutes after placement of the recording electrodes.

Electrical change in RSNA was amplified 2000 to 5000 times with a band path of 100 to 1000 kHz monitored by an oscilloscope. Data were obtained as described previously [9]. Raw data of the nerve activity were converted to standard pulses by a window discriminator, which separated discharge from electrical background noise that remained postmortem. Both the discharge rates and the neurogram were sampled with a Power-Lab analog-to-digital converter (Colorado Springs, CO) for recording and data analysis on a computer. The catheter in the left femoral artery was connected to a BP transducer, whose output signal was amplified in a BP amplifier and averaged to produce mean arterial pressure (MAP). The electrocardiographic signal was amplified with a bioelectric amplifier to monitor the heart rate (HR). The BP and electrocardiogram were monitored with an oscilloscope, sampled with the Power-Lab, and stored on a hard disk for off-line analysis.

2.3. Experimental protocol

Baseline measurements of the RSNA and MAP were made for 5 minutes before ID injection of OT (OT beverage bottled by SUNTORY, Osaka, Japan), decaffeinated OT (DOT; manufactured by SUNTORY), or water (2 mL). To confirm the dose response to OT, concentrate solution of OT or OT diluted 10 times in water, respectively, was injected. The indicated parameters were recorded for 60 minutes after the injection. To investigate the effect of thioperamide, a histaminergic H3-receptor antagonist, a brain cannula made of polyethylene tubing was inserted into the left lateral cerebral ventricle under pentobarbital anesthesia 1 week before the experiment [11]. Thioperamide maleate (2 μg/10 μL artificial cerebrospinal fluid [aCSF], Sigma-Aldrich, St Louis, MO) was administered 15 minutes before the OT injection using the brain cannula. At the end of the experiment, hexamethonium chloride (10 mg/kg, intravenously) was administered to block action potentials of postganglionic neural activity to determine the noise level of the recording. Afterward, animals were killed with an overdose of urethane. In some rats (n = 4), vagotomy of afferent nerves was performed before OT injection. For cutting the subdiaphragmatic vagus nerve, the stomach was retracted through a midline abdominal incision; and the nerve bundles of anterior and posterior vagi were dissected from the esophagus and sectioned using an ophthalmic clip. Control rats (n = 4) received a sham operation without the application of cutting. After vagotomy treatment, the experiment was carried out.

2.4. BP measurement in SHR

To examine the effects of OT on BP in SHR, 3-week–old male SHR (n = 10) and normotensive Wister-Kyoto rats (WKY) (n = 9) received OT for 14 weeks. Each group was further divided into 2 subgroups, which were, respectively, made to drink water or OT. Rats were allowed ad libitum access to these solutions and food, which were measured weekly during the experimental term. Systolic blood pressure (SBP) measurement of the animals was performed weekly on conscious animals by the tail-cuff method (BP-98A; Softorn, Tokyo, Japan). On the experimental day, food and solutions were removed 3 to 4 hours before BP measurement; and then the measurement was performed from 2:00 PM to 3:00 PM. Just after experiment, blood was collected from decapitated rats for analysis of blood glucose level. After death, abdominal (epididymal, perirenal, and mesenteric) adipose tissues were dissected; and total abdominal fat was weighed.

2.5. Data analysis

The RSNA and MAP were measured during each 5-minute period after ID injection and analyzed by digital signal processing analyses. All data were expressed as means ± SEM. Mann-Whitney *U* test was used to compare basal levels in each group. Percentage changes from the baseline values were calculated for the RSNA. Absolute value changes from the baseline were calculated for MAP and HR. Two-way analysis of variance (ANOVA) was applied to compare group responses of the RSNA, MAP, HR, and SBP.

3. Results

Typical recordings of RSNA and BP in the 60 minutes after an intestinal injection of OT are shown in Fig. 1. Water

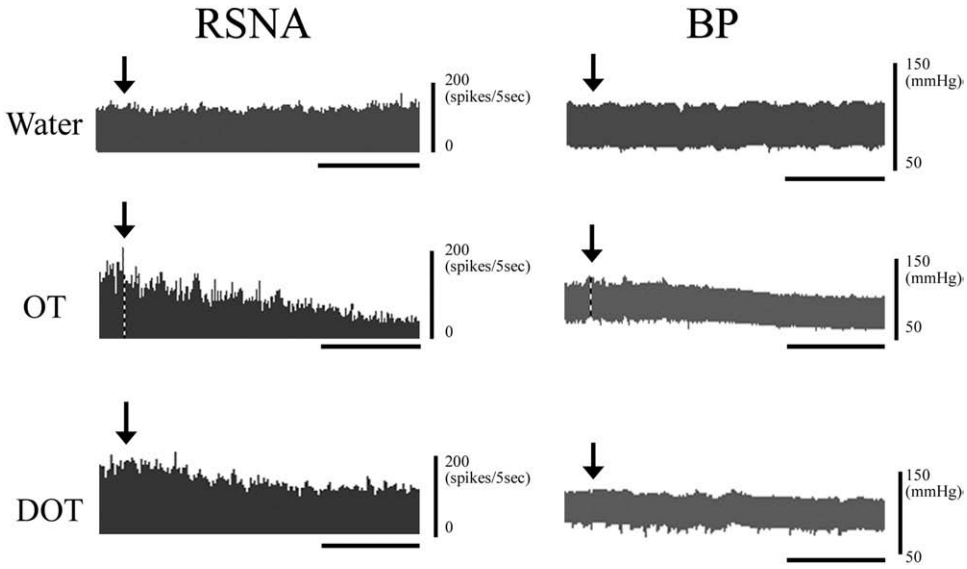

Fig. 1. Effects of ID injection of OT on RSNA and BP. Typical recordings of RSNA and BP of rats injected intraduodenally with water, OT, or DOT. The arrows indicate the time of injection. The horizontal bars represent 20 minutes.

injection did not affect RSNA or BP, but injection of either OT or DOT significantly suppressed RSNA and BP in urethane-anesthetized rats. The RSNA and MAP were gradually suppressed after OT injection (Fig. 2A, B), with maximum suppression of both values occurring at 50 to 60 minutes (60.6% ± 20.4% for RSNA and −15.4 mm Hg ± 10.4 mm Hg for MAP). Heart rate was not influenced by injection of OT or DOT (Fig. 2E, F). Moreover, water injection did not significantly alter levels of RSNA, MAP, and HR at least up to 60 minutes after the injection. Fig. 2B, D shows that diluted solution (1/10) of OT affected RSNA and MAP to a lesser extent than concentrate solution (1). Moreover, RSNA and MAP were markedly suppressed after DOT injection, with maximum suppressions occurring at 60 minutes (63.0% ± 9.6% for RSNA and 13.2 mm Hg ± 6.8 mm Hg for MAP). These data indicate that some substances except for caffeine in OT might cause renal sympathetic and cardiovascular responses. No significant differences were detected among the 4 groups in the respective basal values at 0 minute (Table 1).

Fig. 3 shows the effects of intragastric injection of water or OT on RSNA, MAP, and HR. The RSNA (Fig. 3A), MAP (Fig. 3B), and HR (Fig. 3C) were gradually suppressed after OT injection, with maximum suppression of values occurring at 55 to 60 minutes (66.4% ± 12.9% for RSNA, −13.6 mm Hg ± 4.6 mm Hg for MAP, and −52.0 beats per minute ± 14.2 beats per minute for HR). No significant differences were detected among the 2 groups in the respective basal values at 0 minute (Table 1).

We examined the effects of ICV injection of thioperamide on the changes in RSNA, MAP, and HR caused by OT injection (Fig. 4). Oolong tea significantly suppressed RSNA and MAP (Fig. 4A, B) in rats compared with water-injected rats preinjected with aCSF. However, the RSNA and MAP changes induced by OT were eliminated by pretreatment with thioperamide. Basal (0 minute) RSNA, MAP, and HR values in rats pretreated with aCSF or thioperamide are shown in Table 1; and the differences in respective basal values were not statistically significant.

We examined the effect of vagotomy on the autonomic and cardiovascular changes elicited by ID injection of OT (Fig. 5). As expected, RSNA and MAP were markedly suppressed by OT in sham-operated rats (Fig. 5A, B). In contrast, ID injection of OT did not affect RSNA and MAP in vagotomized rats. Heart rate was not affected by OT injection in both groups (Fig. 5C). Basal (0 minute) values of RSNA, MAP, and HR in sham-operated and vagotomized rats are shown in Table 1; and the respective basal values were not significantly different.

Finally, we examined SBP changes in SHR and WKY provided with the water or the OT (Fig. 6). At the beginning of the experiment (4 weeks old), no statistically significant differences were observed in the SBP levels of rats in 2 experimental groups among both the WKY and SHR. As shown in Fig. 6A, the SBP was progressively elevated in SHR during the experimental period (14 weeks); but the development of hypertension was significantly suppressed by OT drinking. At 18 weeks old, SBP of the water and OT groups in SHR was 195.5 ± 3.8 and 176.0 ± 5.1 mm Hg, respectively. Moreover, the weights of abdominal (epididymal, perirenal, and mesenteric) fat and body and the blood glucose level of OT-treated groups were lower than those of the water group (Table 2). On the other hand, in WKY, mild elevation of SBP was observed during experimental period; but the elevation in OT-treated group was lower than that in the water group (Fig. 6B). At 18 weeks old, the SBP of the water and OT groups in WKY was 129.8 ± 3.5 and 123.8 ± 3.4 mm Hg, respectively. In addition, the abdominal fat

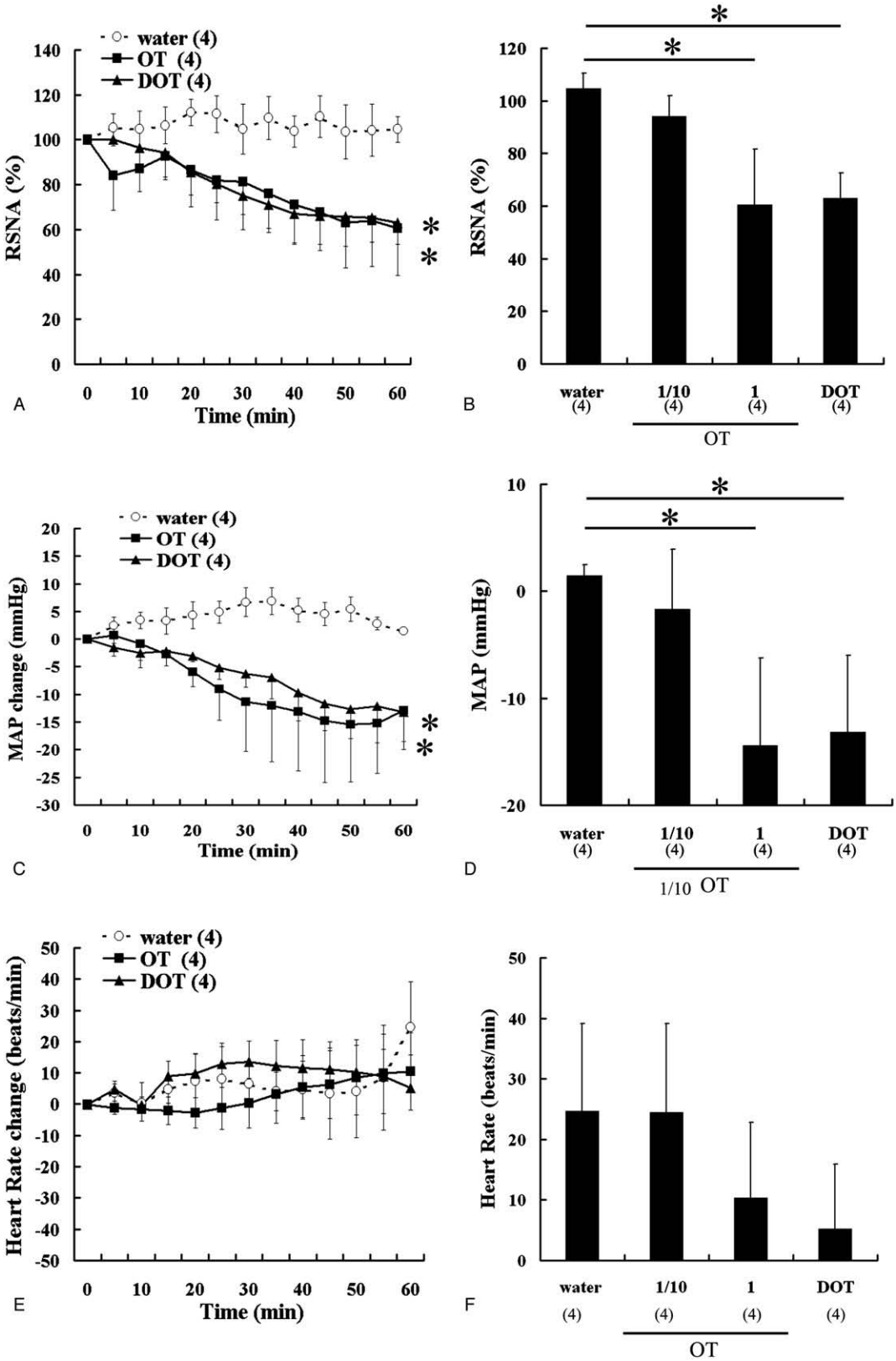

Fig. 2. Effects of ID injection of OT or DOT on RSNA and BP. Time changes of RSNA (A), MAP (C), and HR (E) after ID injection of water, OT, or DOT are expressed as means ± SEM of the percentage of the value at 0 minute (RSNA) or of absolute value change from the value at 0 minute (MAP and HR). Bars show RSNA (B), MAP (D), and HR (F) change values 60 minutes after injection of water, 2 doses (1/10 and 1) of OT, and DOT. The numbers of animals used are shown in parentheses. The significant difference between values after water and OT injections was analyzed as a group by ANOVA ($P < .05$).

Table 1
Basal levels of RSNA, MAP, and HR in experimental groups

Groups	RSNA (spikes/5 s) (no. of rats)	MAP (mm Hg) (no. of rats)	HR (beats/min) (no. of rats)
Experiment I			
Water	159.0 ± 32.1 (4)	91 ± 4 (4)	354 ± 25 (4)
OT (1/10)	139.1 ± 20.7 (4)	79 ± 15 (4)	376 ± 15 (4)
OT (1)	128.6 ± 23.9 (4)	85 ± 10 (4)	388 ± 21 (4)
DOT	182.9 ± 58.9 (4)	87 ± 13 (4)	403 ± 19 (4)
Experiment II			
Water, intragastric injection	140.4 ± 32.9 (4)	92 ± 3 (4)	386 ± 28 (4)
OT, intragastric injection	211.7 ± 97.9 (4)	94 ± 5 (4)	398 ± 26 (4)
Experiment III			
aCSF + water	142.6 ± 16.9 (4)	82 ± 7 (4)	374 ± 31 (4)
aCSF + OT	152.3 ± 40.6 (4)	84 ± 12 (4)	399 ± 15 (4)
Thioperamide + OT	178.4 ± 46.7 (4)	92 ± 8 (4)	394 ± 23 (4)
Experiment IV			
Sham, OT	176.9 ± 40.1 (4)	96 ± 4 (4)	386 ± 28 (4)
Vagotomy, OT	110.1 ± 14.3 (4)	86 ± 5 (4)	328 ± 18 (4)

Data are shown as means ± SEM.

weight of OT-treated group was lower than that of the water group.

4. Discussion

Some evidences that OT is beneficial for antiobesity have previously been reported [2,12]. Because obesity is closely related to hypertension [3], we investigated in this study the validity of the hypothesis that OT can prevent hypertension by using urethane-anesthetized rats and SHR. We found new evidence that ID injection of OT suppresses RSNA and BP and that long-term OT ingestion significantly inhibits BP elevation in SHR. These findings suggest that OT may exert its hypotensive action via suppression of RSNA.

Oolong tea contains various substances; and it is generally thought that the effects of OT are due to the action of caffeine, one of the bioactive components of OT [2]. To determine whether caffeine functions as a potential suppressor of BP, we examined the effects of DOT on RSNA and BP and found that ID injection of DOT suppressed RSNA and BP as well as OT. Thus, some as-yet-unidentified substances besides caffeine may affect autonomic and cardiovascular functions. With respect to this point, ID injection of OT from another company did not affect RSNA and BP (unpublished observation).Thus, it should be noted that the OT used in the present study (from SUNTORY) contains tea tannins such as epigallocatechin and epicatechin gallate or original effective substance (data not shown). The actions of these substances may well cause autonomic and cardiovascular changes, but further studies are needed to substantiate this notion. On the other hand, we determined the effects of intragastric injection of OT on RSNA and BP because most of the tea is absorbed in the stomach; and the various

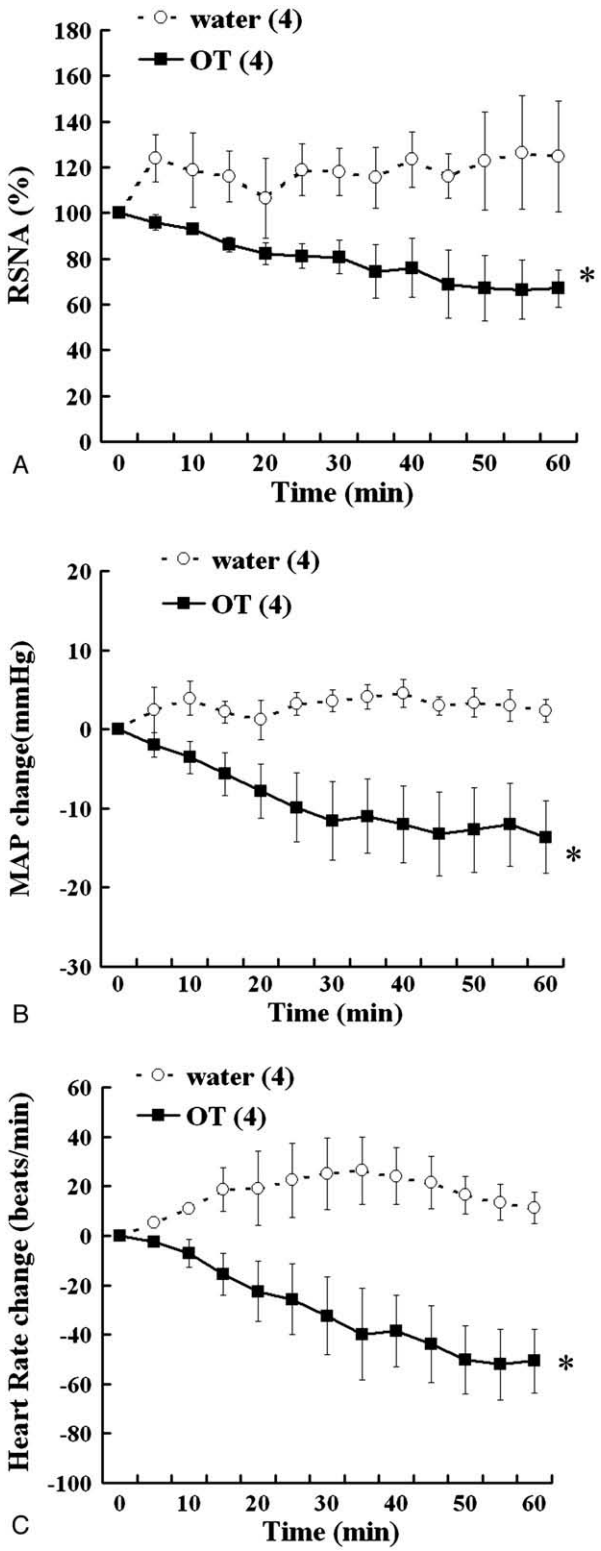

Fig. 3. Effects of intragastric injection of OT on RSNA and BP. Time changes of RSNA (A), MAP (C), and HR (E) after intragastric injection of water or OT are expressed as means ± SEM of the percentage of their values at 0 minute (RSNA) or of absolute value change from the value at 0 minute (MAP and HR). The numbers of animals used are shown in parentheses. The significant difference between values after water and OT injections was analyzed as a group by ANOVA ($P < .05$).

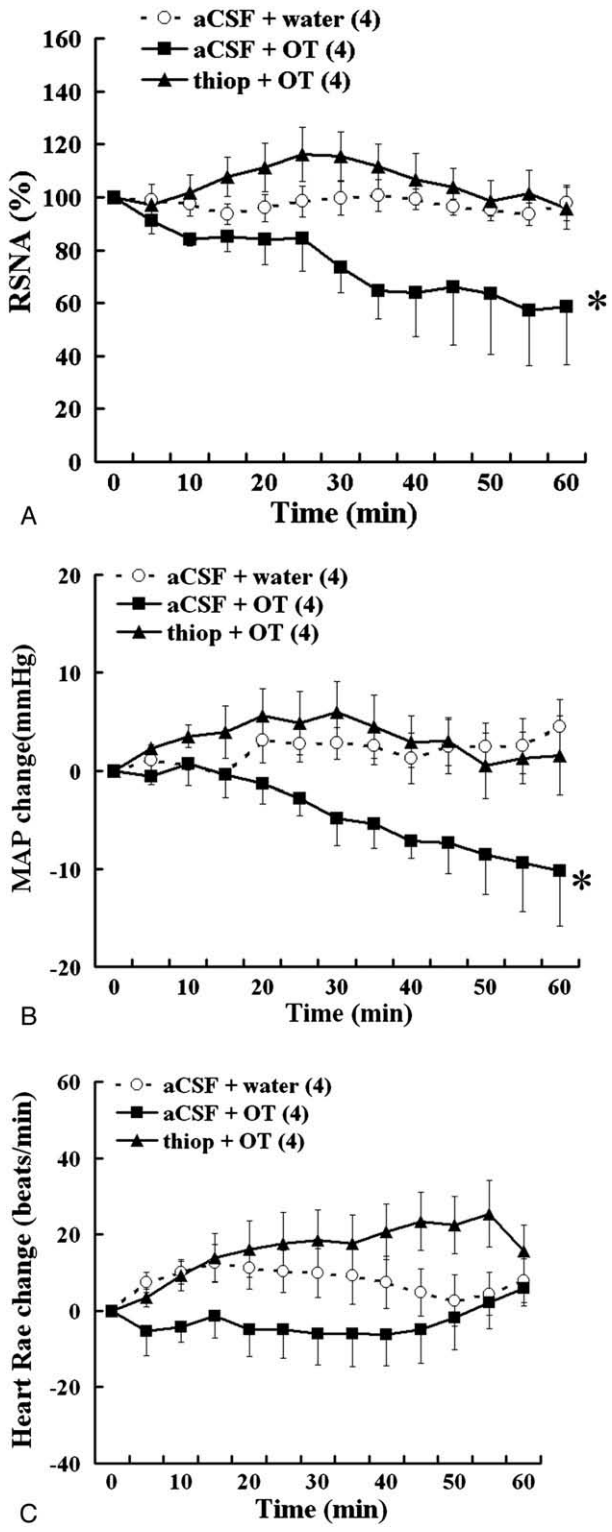

Fig. 4. Effects of thioperamide on changes in RSNA and MAP after ID injection of OT. Time changes of RSNA (A), MAP (B), and HR (C) after ID injection of OT are expressed as means ± SEM of percentage of their values at 0 minute (RSNA) or of absolute value change from the value at 0 minute (MAP and HR). An ICV injection of aCSF or thioperamide was given 15 minutes before ID injection of OT. The significance of the difference between the values after water and OT from 5 to 60 minutes was analyzed as a group by ANOVA ($P < .05$). Thiop indicates thioperamide.

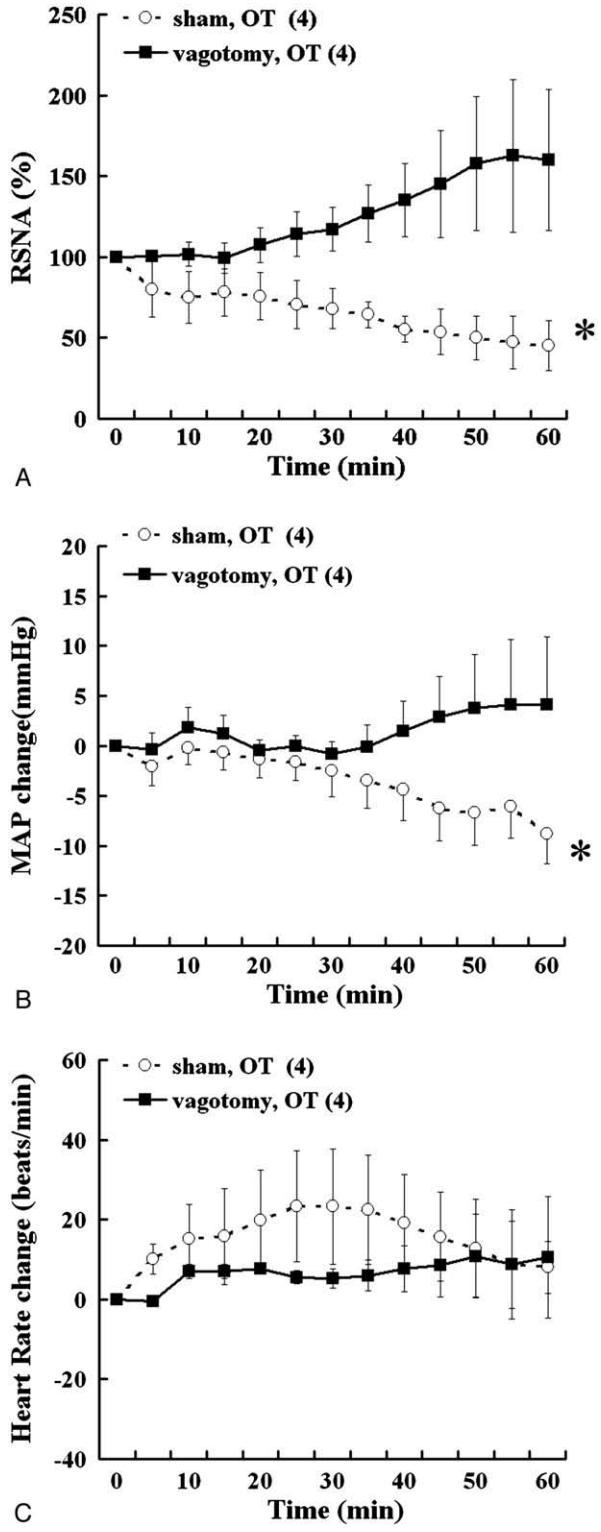

Fig. 5. Effects of bilateral subdiaphragmatic vagotomy on changes in RSNA and MAP after ID injection of OT. Time changes of RSNA (A), MAP (B), and HR (C) after ID injection of OT are expressed as means ± SEM of percentage of their values at 0 minute (RSNA) or of absolute value change from the value at 0 minute (MAP and HR). Data from sham-operated (sham) and vagus nerves–cut (vagotomy) rats are shown. The significance of the differences between the values after water and OT from 5 to 60 minutes was analyzed as a group by ANOVA ($P < .05$).

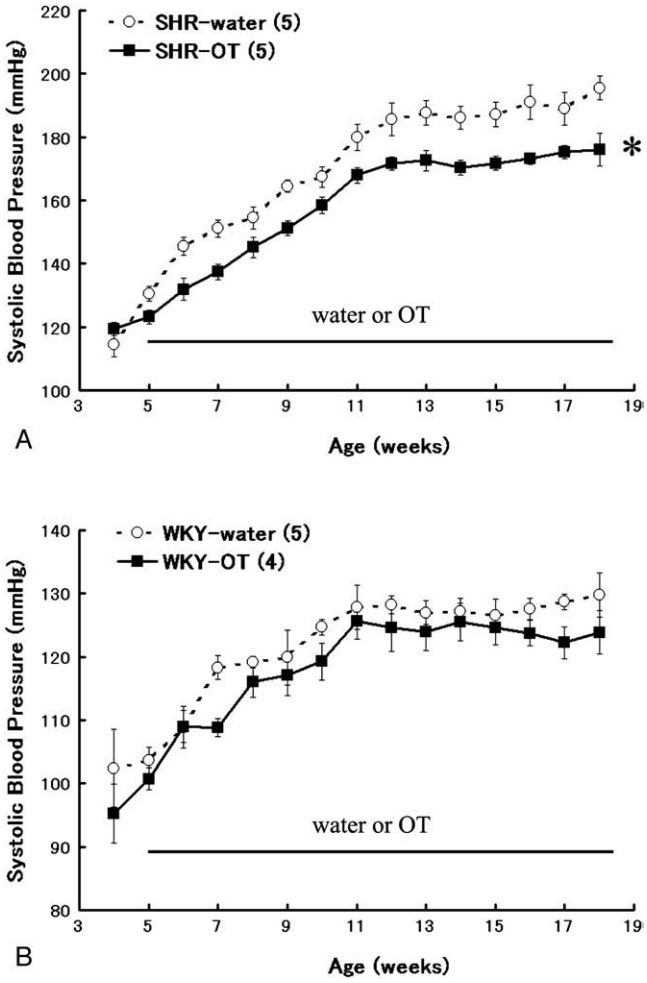

Fig. 6. Effects of OT drinking on SBP in SHR and WKY. Time changes in SBP of SHR (A) and WKY (B) with water or OT treatment (14 weeks) are expressed as means ± SEM. The significance of the differences between the values from weeks 5 to 18 was analyzed as a group by ANOVA ($P < .05$).

substances of the tea will be destroyed because of acid and mucous in the stomach. However, significant suppressions of RSNA, MAP, and HR in urethane-anesthetized rats were caused by intragastric injection of OT (Fig. 3). Moreover, long-term OT ingestion significantly inhibits BP elevation in SHR (Fig. 6). These findings suggest that the effective substance of OT might affect RSNA and BP without it being broken in the stomach and intestines.

It is well known that histaminergic neurons play an important role in the regulation of cardiovascular functions [5,6]. We were recently able to demonstrate that central injection of low-dose histamine lowered, and that of high-dose histamine elevated, RSNA and BP [6]. In addition, the effects of low- or high-dose histamine were blocked by thioperamide or diphenhydramine, respectively [6]. These findings suggest that central histamine may affect RSNA and BP through the various histaminergic receptors. In support of this notion, a previous study provided evidence that the inhibitory effects of ID injection of *L johnsonii* La1 on RSNA and BP were attenuated by thioperamide [7]. In the study presented here, we found new supporting evidence, namely, that the suppression of RSNA and BP due to OT was undone by thioperamide (Fig. 4). It is thus possible that OT may affect RSNA and BP through mediation by central histaminergic H3-receptors. As for the role of histaminergic receptors in synaptic neurotransmission, it is known that H3-receptors localized in the presynaptic cleft bind with small amounts of histamine released from the cleft, causing the inhibition of histamine release in the presynaptic histamine neurons [13]. The effect of OT via H3-receptors may therefore trigger a decrease in histamine release. However, we could not determine the validity of this hypothesis within the constraints of this study; therefore, further investigation into this possible effect of OT is needed.

The abdominal afferent nerves have been implicated in signal transduction from internal organs to the brain. For example, the hyperthermic response to intestinal osmotic stimulation is attenuated in vagotomized rats [14]. Moreover, afferent nerves have been shown to mediate the effects of some peptide hormones derived from internal organs, such as leptin [15], ghrelin [16], and PYY [17]. Recently, we obtained evidence that changes in RSNA and BP induced by ID injection of *L johnsonii* La1 were eliminated in vagotomized rats [7]. This could imply that the afferent nerves may be implicated in the sympathetic and cardiovascular effects of intestinal stimulation with OT. In support of this notion, the current study demonstrated for the first time that no effects of OT were detected in vagotomized rats

Table 2
Effects of 14 weeks OT drinking in SHR or WKY

Groups	Body weight (g)	Abdominal fat weight (g)	Blood glucose (mg/dL)	SBP (mm Hg)	HR (beats/min)
SHR					
Water group (n = 5)	369.0 ± 10.4	14.8 ± 1.0	144.6 ± 2.5	196 ± 4	454 ± 10
OT group (n = 5)	341.2 ± 6.9 *	11.5 ± 0.4 *	131.6 ± 5.4 *	176 ± 5 *	437 ± 23
WKY					
Water group (n = 5)	367.0 ± 11.7	16.6 ± 1.0	143.2 ± 4.5	130 ± 4	465 ± 14
OT group (n = 4)	345.0 ± 7.4	14.2 ± 0.7 *	134.3 ± 3.6	124 ± 3	441 ± 10

Data are shown as means ± SEM.
* $P < .05$ as compared with water group.

(Fig. 5), suggesting that such effects may depend on the afferent nerve pathways. On the other hand, in this study, we could not identify the exact pathway in the brain that connects hypothalamic nuclei containing the histaminergic neurons and vagal nerves. It is known that the afferent signal of vagal nerves is transmitted to the neurons in the nucleus tractus solitarius (NTS) and then conveyed to the hypothalamus via various pathways [18]. Although we did not test whether OT injection affects NTS neurons, the central pathway via the NTS, which receives signals from afferent vagal nerves and transmits them to the hypothalamus, may be implicated in autonomic and cardiovascular changes due to OT. To validate this notion, further research is needed.

We obtained evidence that long-term ingestion of OT inhibited BP elevation in SHR (Fig. 6). Because the sympathetic tone of SHR is accelerated [19], it is possible that chronic OT decreases sympathetic tone and inhibits hypertension in SHR. With respect to this point, our preliminary study confirmed that acute ID injection of OT suppressed RSNA and BP in SHR given water for 14 weeks (unpublished observation). Thus, the data support the above idea; but certain mechanism is unknown, and further study must be performed. In addition, as also shown in a previous study that demonstrated the antiobese effect of OT on animals [2,12], we observed that chronic ingestion of OT reduced body weight and abdominal fat in SHR (Table 2). It is known that body fat accumulation is implicated in the hypertensive mechanism associated with obesity [3]. It is thus possible that OT ingestion may reduce abdominal fat and cause the hypotensive action of OT via neural mechanism.

The present study observed that ID injection of OT lowered BP without reduction of HR (Fig. 2). At present, it is not clear why the effect was caused. To explore some possible reasons, we note the role of peripheral vasomotion or baroreflex in BP regulation. In regard to the vasomotion, it is generally known that BP is derived from blood flow from the heart and peripheral vasomotion and that BP reduction and the vasodilation are evoked simultaneously [20]. Thus, it is possible that rather than HR reduction, the vasodilation might be caused by OT. With respect to the baroreflex, which functions as a short-term negative feedback regulator of BP, it is generally known that acute BP depression resulted in baroreflex-mediated tachycardia. Previous study indicated that under acute suppressions of BP and HR, baroreflex sensitivity is attenuated; and changes in both BP and HR are caused in a similar manner [21]. Afterward, BP suppression without HR reduction in OT-injected rats might be derived from long-lasting preservation of the baroreflex function that modulates HR. Of course, these ideas are not certain; and further research to check this mechanism in detail, such as blood flow measurement or baroreflex control experiment, will be needed in the future.

In conclusion, we found that OT suppressed RSNA and BP and that the vagal afferent pathway is involved in the sympathetic and cardiovascular effects of OT. In addition, long-term OT ingestion significantly suppressed development of hypertension in SHR. Thus, daily OT ingestion may have a hypotensive effect via the autonomic nervous system.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.metabol.2007.11.016.

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