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Afferent fibers involved in the bradykinin-induced cardiovascular reflexes from the ovary in rats

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ABSTRACT

Bleeding or rupture of the ovary often accompanies ovarian cysts and causes severe pain and autonomic responses such as hypotension. It would be expected that ovarian afferents contribute to cardiovascular responses induced by ovarian failure. The present study examined cardiovascular responses to noxious chemical stimulation of the ovary by bradykinin, an algesic substance released by tissue damage, and explored the role of ovarian afferents in the ovarian–cardiovascular responses in anesthetized rats. Non-pregnant adult rats were anesthetized with pentobarbital and artificially ventilated. The carotid artery was cannulated to monitor blood pressure and heart rate. Noxious chemical stimulation was achieved by applying a small piece of cotton soaked with bradykinin to the surface of the ovary for 30 s. Application of bradykinin (10^{-4} M) to the ovary decreased heart rate and blood pressure. These cardiovascular responses were not significantly influenced by severance of the vagal nerves or the superior ovarian nerve, but were abolished by severance of the ovarian nerve plexus (ONP). Application of bradykinin (10^{-4} M) to the ovary evoked afferent activity of the ONP both in vivo and in vitro preparations. These results indicate that the decreases in heart rate and blood pressure following chemical noxious stimulation of the ovary with bradykinin are reflex responses, whose afferent nerve pathway is mainly through afferent fibers in the ONP.

1. Introduction

Clinically, nociceptive information from visceral organs is conveyed through the visceral autonomic afferent fibers (Cervero, 1994; Jänig, 2006; Bielefeldt and Gebhart, 2013), and produces pain or discomfort as well as autonomic responses such as hypotension and nausea (Procacci et al., 1986; Cavun et al., 2004). Bleeding or rupture of the ovarian cysts and ovarian cancer causes severe pain or tenderness of the lower abdomen, as well as autonomic responses such as hypotension, anorexia, and dyspepsia (Samraj and Curry, 2004; Hammond, 2008; Kruszka and Kruszka, 2010). Therefore, nociceptive information from the ovary may travel through the autonomic sensory fibers innervating the ovary, and may evoke pain as well as cardiovascular reflexes. However, the physiological role of ovarian afferents has not yet been elucidated.

There have been many studies regarding the role of visceral afferents as an afferent limb of the cardiovascular reflexes evoked by noxious chemical stimulation of visceral organs, using the algesic substance bradykinin in anesthetized animals (Gorman et al., 1983; Longhurst and Ordway, 1983; Niitani et al., 1988). Noxious chemical stimulation of the heart by bradykinin elicits excitations of both cardiac vagal afferent activity (Kaufman et al., 1980; Hisata et al., 2006) and cardiac spinal afferent activity (Uchida and Murao, 1974; Baker et al., 1980). However, the afferent limb of the cardiovascular reflex responses produced by noxious chemical stimulation of the heart has been shown to be the cardiac spinal afferents but not the vagal afferents, since vagal denervation does not affect those cardiovascular responses in anesthetized and artificially ventilated animals (Gorman et al., 1983; Niitani et al., 1988). Similarly, cardiovascular reflex responses to bradykinin stimulation of abdominal visceral organs such as gallbladder are mediated by the splanchnic afferent nerves, but not by the vagal afferent nerves (Longhurst and Ordway, 1983).

With regard to the female reproductive organs, there have been studies on the uterine afferents (Berkley et al., 1987, 1988, 1990, 1993; Robbins et al., 1990, 1992; Robbins and Sato, 1991). The uterus is innervated by afferent fibers in the hypogastric and pelvic nerves (Baljet and Drukker, 1980; Gabella, 1995). Algesic chemical stimulation by bradykinin, or mechanical stimulation by uterine distension, particularly in the noxious range, result in an increase in activity of both the hypogastric and pelvic afferent nerves in rats (Berkley et al., 1987, 1988, 1990, 1993; Robbins et al., 1990). Robbins and Sato (1991) reported that the afferent limb for the decreases in heart rate and blood pressure produced by noxious distension of the uterine horn is the hypogastric nerve but not the pelvic nerve.

The ovary is innervated by autonomic nerves. Histological studies have demonstrated the presence and distribution of sympathetic

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(splanchnic) and parasympathetic (vagal) nerves in the rat ovary (Burden, 1985; Papka and Traurig, 1993; Gerendai et al., 1998, 2000). These autonomic nerves reach the ovary via two routes: the ovarian nerve plexus (ONP) along the ovarian artery and the superior ovarian nerve (SON) in the suspensory ligament (Baljet and Drukker, 1979; Lawrence and Burden, 1980). These ovarian innervations include both afferent and efferent fibers. Burden et al. (1983) reported the existence of sensory innervation of the ovary by histological studies that showed that injections of horseradish peroxidase (HRP) into the right or left ovary of the rat produced labeling of perikarya in both nodose ganglia and ipsilateral dorsal root ganglia (DRGs) from T10 to L2. Klein and Burden (1988) reported that both the SON and the ONP contain afferent fibers, observing labeled afferent perikarya located in lower thoracic–upper lumbar DRG following direct application of true blue to either the SON or the ONP. These histological studies suggest that ovarian afferents may carry noxious sensory information from the ovary and trigger cardiovascular responses. However, no study has investigated the physiological role of ovarian afferents.

The present study examined cardiovascular responses to chemical noxious afferent stimulation of the ovary in anesthetized, non-pregnant adult rats. Noxious chemical stimulation was applied using bradykinin, an algesic substance, produced by tissue damage. We further examined the involvement of the vagal nerve, the SON and the ONP in the afferent limb of the cardiovascular response to ovarian stimulation.

2. Materials and methods

2.1. Animals

Adult virgin female Wistar rats (3–7 months old; 155–210 g body weight) were maintained in a 12 h:12 h light–dark schedule. Rat chow and water were provided ad libitum. Fourteen animals with a regular 5-day estrous cycle, established by examining daily vaginal smears, were used on the day of estrus. All procedures were approved by the Animal Committee of the Tokyo Metropolitan Institute of Gerontology.

Eleven rats were used for measuring cardiovascular response. ONP afferent activity was recorded in the in vivo preparation in one of 11 rats and in three other rats. In another 4 of the 11 rats used for cardiovascular response, ONP afferent activity was recorded in the in vitro preparation.

2.2. Surgical procedures

Animals were anesthetized initially with pentobarbital (60 mg/kg i.p.), maintained using a continuous infusion of pentobarbital in saline (10 mg pentobarbital/ml saline infused via a jugular venous catheter at 0.2–0.4 ml/h; 11–26 mg/kg/h), in a manner similar to the previous reports (Klein et al., 2014; Premilovac et al., 2013; Uchida et al., 2008). Whenever heart rate and blood pressure were unstable during the experiment, additional doses (about 5 mg/kg) of pentobarbital were administered by bolus i.v. injection. The animals were artificially ventilated using a Harvard rodent ventilator (model 683, Harvard, Massachusetts, USA): and end-tidal CO_2 concentrations measured using a gas monitor (Microcap, Oridion Medical, Jerusalem, Israel) were kept at about 3.0% by controlling respiratory rate and tidal volume. Body temperature, monitored by a probe in the rectum, was maintained at approximately 37.5 °C using a body temperature control system consisting of a thermostatically-regulated DC current heating pad and an infrared lamp (ATB-1100, Nihon Kohden, Tokyo, Japan). Systemic blood pressure was continuously recorded from a cannula inserted into the common carotid artery. Heart rate was measured with a pulse rate tachometer (AT-601G, Nihon Kohden, Tokyo), which was triggered by systolic blood pressure waves.

2.3. Chemical stimulation of the ovary by bradykinin

After ventral exposure of the ovary at the left or right side with a wide abdominal wall opening, the bursa around the ovary was peeled. Bradykinin acetate (Sigma, St. Louis, USA) dissolved in saline (10^{-8}–10^{-4} M) was applied topically to the surface of the ovary with a thin piece of cotton (7×7 mm square) soaked with the solution. After application for 30 s, the cotton was removed. Each of the stimuli was delivered to the animal after observing stabilization of heart rate and mean arterial pressure. We waited more than 10 min between stimulations to avoid development of tachyphylaxis of bradykinin (Mizumura et al., 2009). Usually, the ovary was stimulated unilaterally in each rat. However, in some rats, each side of the ovary (left and right) was stimulated in the same animal to record cardiovascular responses ($n = 1$) or ONP afferent activities ($n = 2$).

2.4. Denervation of the vagus nerve

In five rats, the cardiovascular responses were tested in the intact rat, and the vagus nerves were then cut bilaterally at the level of cervix to examine the contribution of the vagal afferent nerve from the ovary on the ovarian–cardiovascular reflex. In one rat, the vagi were cut bilaterally at the beginning of the experiment.

2.5. Denervation of the SON and the ONP

To examine the contribution of the afferent nerves in the SON and ONP to the ovarian–cardiovascular reflex, either the SON or the ONP ipsilateral to the ovary that was used for bradykinin application was cut. After the cardiovascular responses to bradykinin application were measured in rats with intact nerves or vagal denervation, denervation of the SON was performed by cutting the suspensory ligament and the accompanying blood vessels in five rats. In another four rats, denervation of the ONP was performed by transection of the ONP together with the ovarian vessels after ligation, or by topical application of the local anesthetic procaine (1.0%) to the ovarian artery.

2.6. Recording of afferent nerve activity from the ONP during in vivo preparation

The left or right ONP running along the ovarian artery was cut in four rats approximately 20 mm from the ovary and covered with warm liquid paraffin. Mass action potentials were amplified (S-0476, Nihon Kohden, 0.001 s time constant), audibly monitored through a connection to a speaker, visually displayed on a digital oscilloscope (DS-5312, Iwatsu, Tokyo), and digitized (Micro1401, Cambridge Electronic Design, UK). Nerve discharges were counted in 5-s intervals using software (Spike 2 software, Cambridge Electronic Design, UK). Activity of the ONP afferent nerve was recorded from both sides of the ovaries in two rats, and recorded unilaterally from the ovary another two rats.

2.7. Recording of afferent nerve activity from the ONP during in vitro preparation

In four rats, recording of afferent nerve activity from the ONP was also performed during in vitro organ preparation of the ovary. After completion of the experiment to measure cardiovascular responses to bradykinin application in vivo, the right side of the ovary, together with the ONP, ovarian vessels, abdominal vein, and cannulated abdominal aorta was dissected from the surrounding tissues. Immediately, before removing the dissected tissue from the abdominal cavity, the rat was killed with an overdose of anesthetic. Then, the ovary, together with the dissected tissue, was placed in a warm mineral oil bath and perfused with oxygenated Krebs solution (pH 7.4) at a constant flow rate of 0.5 ml/min through the abdominal aorta and ovarian artery (Berkley et al., 1988). Multiunit or single unit afferent

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activity was recorded from dissected strands of the ONP using the same recording procedures as in the in vivo experiments. Nerve discharges were counted in 1-s intervals using software (Spike 2 software, Cambridge Electronic Design, UK). Fig. 4B illustrates the preparation.

Chemical stimulation with bradykinin was delivered via pulse injections through the abdominal aorta and ovarian artery. Bradykinin at a dose of 10^{-4} M in Krebs solution was injected in a 0.3 ml bolus, and the ovary was flushed with 0.3 ml of Krebs solution immediately after the injection of the bradykinin. To confirm that the bradykinin was perfusing the tissue adequately, a bolus of Evans Blue dye was injected before and/or after injecting the bradykinin. All of the preparations for which data are reported here were adequately perused.

2.8. Statistics

Data were expressed as the mean ± standard error of the mean (SEM). Statistical comparisons were carried out by means of one-way factorial ANOVA or repeated-measures ANOVA followed by a Dunnett's multiple comparison test, two-way repeated-measures ANOVA followed by a Bonferroni correction, or paired *t*-test. A *p*-value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Cardiovascular responses to bradykinin stimulation of the ovary

The resting heart rate and mean arterial pressure, measured at the beginning of an experiment, before any nerve manipulation or stimulation, were 399 ± 10 beats/min and 139 ± 3 mm Hg, respectively (n = 10 rats). Fig. 1A shows sample recordings of heart rate and mean arterial pressure in a single rat, and Fig. 1B summarizes the time course of those cardiovascular responses measured every 30 s in 11 ovaries in 10 rats. Application of 10^{-4} M bradykinin to the ovary produced significant decreases in heart rate and mean arterial pressure. However, the magnitudes and time courses of these decreases varied in each stimulation trial. Both the heart rate and the mean arterial pressure started to decrease at around 10 s after the onset of bradykinin application (Fig. 1A). The maximum reduction of heart rate was 13.7 ± 2.3 beats/min at 60 s after the end of stimulation, and that of mean arterial pressure was 13.2 ± 3.5 mm Hg at 30 s after the end of stimulation (Fig. 1B). The significant decreases in heart rate and mean arterial pressure lasted until 2–2.5 min after the end of stimulation before returning to the basal level. Application of saline to the ovary produced no changes in heart rate or mean arterial pressure (n = 7 ovaries in 7 rats).

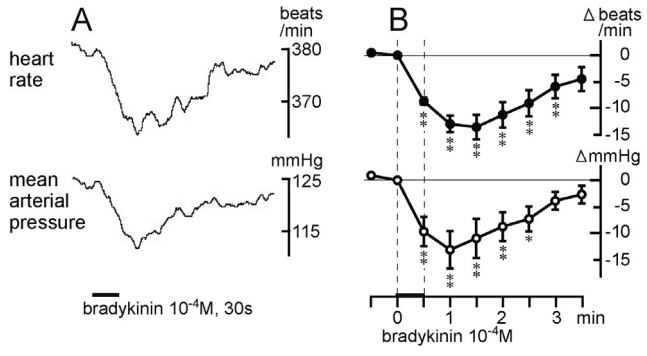

Fig. 1. Cardiovascular responses to bradykinin stimulation of the ovary. A: Sample recordings of heart rate and mean arterial pressure. B: Summary of heart rate and mean arterial pressure responses. Changes in heart rate and mean arterial pressure compared with the prestimulus basal values (at 0 min) were plotted every 30 s. The heavy bar on the abscissa indicates the period of topical bradykinin application (10^{-4} M) to the ovary (30 s). Each point and vertical bar represents a mean ± SEM (n = 11). For each ovary, averaged data obtained from 1 to 2 trials were summarized. *p < 0.05; **p < 0.01; statistically significant differences from the prestimulus basal values using one-way repeated ANOVA followed by Dunnett's multiple comparison test.

3.2. Cardiovascular responses after denervation of autonomic nerves to the ovary

We explored the afferent limb of the reflex decreases in heart rate and mean arterial pressure by lesioning the vagal nerve, the SON, or the ONP. In vagotomized animals, application of 10^{-4} M bradykinin to the ovary produced bradycardia and hypotension (Fig. 2B) similar to the responses evoked when vagal innervation was intact (Fig. 2A). There were no significant differences in magnitudes and time courses of the heart rate and blood pressure responses between conditions of intact nerves and vagal denervation (tested by two-way repeated ANOVA with Bonferroni correction).

After lesioning the SON ipsilateral to the ovary used for bradykinin stimulation (keeping the ONP intact), application of 10^{-4} M bradykinin to the ovary produced bradycardia and hypotension (Fig. 2C) as observed with intact nerves (Fig. 2A). There were no significant differences in magnitudes and time courses of the heart rate and blood pressure responses between conditions of intact nerves and SON denervation (tested by two-way repeated ANOVA with Bonferroni correction).

In contrast, after lesioning the ONP ipsilateral to the ovary used for bradykinin stimulation (keeping the SON intact), application of 10^{-4} M bradykinin to the ovary produced no changes in heart rate and mean arterial pressure (Fig. 2D). The basal heart rate and mean arterial pressure before bradykinin stimulation were not significantly influenced by any nerve manipulation (tested by one-way factorial ANOVA with Dunnett's multiple comparison test).

3.3. Response of ONP afferent fibers to bradykinin application to the ovary

We made direct recordings of ONP afferent nerves to clarify that a significant increase in ONP afferent activity was the neural basis for the afferent limb of the reflex bradycardia and hypotension produced by bradykinin application to the ovary. When ONP afferent activity was recorded during in vivo preparation (Fig. 3A), application of 10^{-4} M bradykinin to the ovary produced a significant increase in ONP afferent activity (Fig. 3B). In general, ONP afferent activity started to increase at 5–10 s after the onset of bradykinin stimulation and reached its maximum during the 30-s stimulation period. After 2 min or later after the end of stimulation, the elevated level of ONP afferent activity gradually returned to the control level before stimulation. The peak (maximum) responses of ONP afferent nerve activity after the application of different doses of bradykinin or saline to the ovary are summarized in Fig. 3C. ONP afferent activity was increased by bradykinin application to the ovary in a dose-dependent manner. The threshold dose of bradykinin to produce a significant increase in ONP afferent activity was 10^{-7} M.

In addition, mass or multiunit responses of ONP afferent fibers to bradykinin stimulation of the ovary were obtained in four in vitro preparations to confirm the existence of ovarian receptors responsive to bradykinin (Fig. 4). As shown in Fig. 4B, bradykinin was delivered directly to the ovary via intra-arterial injection through the ovarian artery. Fig. 4A shows that single unit activity responded an injection of 10^{-4} M bradykinin. A single unit was identified by height and shape. This single unit showed no spontaneous activity, but was excited by injection of 10^{-4} M bradykinin but not by injection of Krebs solution. Similar excitation of ONP afferent fibers following injection of 10^{-4} M bradykinin was observed in another single unit and in another three mass recordings that were tested.

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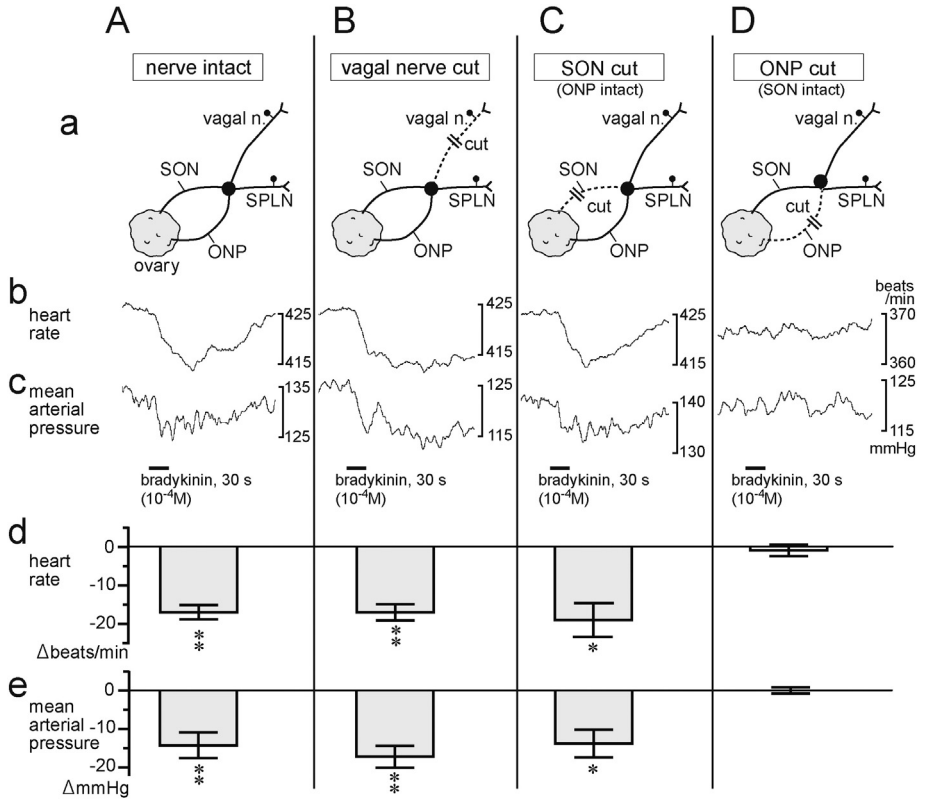

Fig. 2. Effects of denervation of autonomic nerves to the ovary on the cardiovascular responses to bradykinin stimulation of the ovary. A: Nerve intact [vagi, superior ovarian nerve (SON) and ovarian nerve plexus (ONP) intact], B: Vagi cut (SON and ONP intact), C: SON cut (ONP intact, vagi is either intact or cut), D: ONP cut (SON intact, vagi is either intact or cut). a: Representation of the experimental conditions of denervation. b, c: Sample recordings of heart rate and mean arterial pressure, respectively. d, e: Summary of heart rate and mean arterial pressure responses, respectively, for each condition. The maximum changes in heart rate and mean arterial pressure within 2 min after the end of stimulation are compared with the prestimulus basal values. Each column and vertical bar represent a mean ± SEM (A: n = 11, B: n = 6, C: n = 5, D: n = 4). *$p < 0.05$; **$p < 0.01$; statistically significant differences from prestimulus basal values using paired-*t* tests.

4. Discussion

The present study is the first to demonstrate the functional role of ovarian afferents in the cardiovascular responses to noxious chemical stimulation of the ovary in anesthetized animals. The results of the nerve transection and recording experiments indicate that the afferent

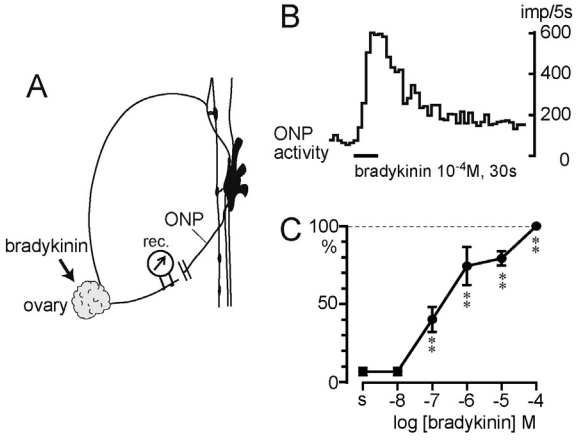

Fig. 3. Activity of ovarian afferents in the ovarian nerve plexus (ONP) in response to bradykinin stimulation of the ovary. A: Illustration showing the recording (rec.) of afferent activity of the ONP. B: Sample histogram of mass activity of ovarian afferents in the ONP in response to topical 10^{-4} M bradykinin application to the ovary. C: Sensitivity of ONP afferents to different doses of bradykinin and saline. The maximum changes in spike rate (Δ imp/5 s) of ONP afferents activity are plotted at each dose of bradykinin and saline. The data has been normalized: the change in spike rate recorded at 10^{-4} M is taken as 100%. Each point and vertical bar represents a mean ± SEM (n = 6). *$p < 0.05$; **$p < 0.01$; statistically significant differences from the saline control values using one-way repeated ANOVA followed by Dunnett's multiple comparison test.

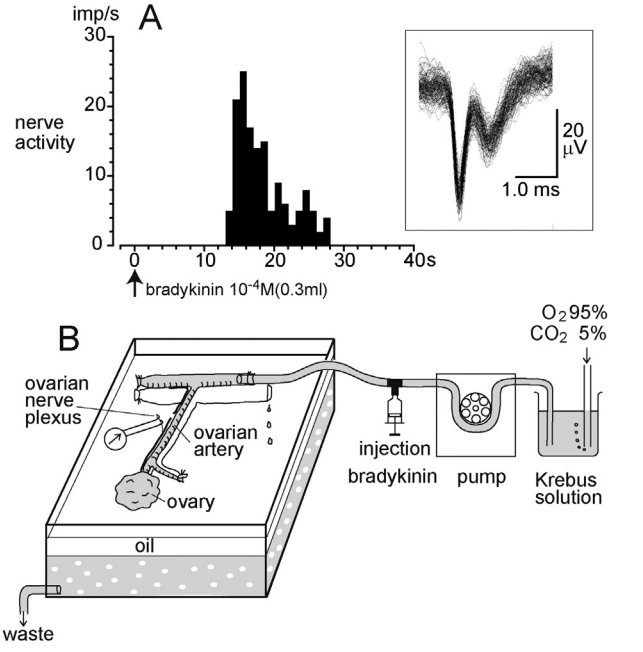

Fig. 4. Activity of ovarian nerve plexus (ONP) afferent fibers in the isolated perfused ovary. A: Sample histogram of single unit activity in ONP afferent fibers following intra-arterial injection of bradykinin. B: Diagram illustrating the in vitro preparation.

limb of the cardiovascular reflex, which was induced by the stimulation of the ovary by the bradykinin, is the ONP, but not the vagus or the SON.

Bradykinin is a potent algesic substance released by damaged tissue. From the present results, ONP afferents may transmit nociceptive information to the central nervous system from the ovary, such as pain during ovulation, or pathological ovarian pain associated with an ovarian cyst, ovarian cancer, etc. Clinically, ovarian failure, such as bleeding or rupture of the ovary, is known to cause severe pain and autonomic dysfunction such as hypotension (Samraj and Curry, 2004; Hammond, 2008; Kruszka and Kruzka, 2010). The present findings of the functional role of ovarian afferents in the ovarian–cardiovascular reflex may be involved in the neural mechanism of cardiovascular responses clinically reported during ovarian failure.

Noxious stimulation of visceral organs and deep somatic tissues such as skeletal muscle is known to produce reflex responses in heart rate and blood pressure, which are either excitatory or inhibitory. These response patterns are considered to be dependent on the type of algesic substances used for stimulation, the species of animal, and the type and depth of anesthesia. Sato et al. (1982) showed that changes in heart rate and blood pressure upon stimulation of skeletal muscle by algesic agents in anesthetized cats depend on the type of algesic chemicals used. Potassium ions always increase heart rate and blood pressure, while bradykinin induces increase and decrease in both heart rate and blood pressure. Gorman et al. (1983, 1984) showed that changes in heart rate and blood pressure upon epicardial bradykinin stimulation are dependent on the species of animal, with an excitatory response in cats, an insignificant response in dogs, and an inhibitory response in monkeys. Ness and Gebhart (1988) reported that cardiovascular responses to noxious colorectal stimulation are affected by the type of anesthetic used or depth of anesthesia. In the present study, to mimic the hypotensive response clinically shown in ovarian failure, we used pentobarbital anesthesia, which is known to produce stable inhibitory cardiovascular responses to noxious colorectal stimulation in rats (Ness and Gebhart, 1988; Li et al., 2006; Li and Suzuki, 2006). Under pentobarbital anesthesia, the present study showed that bradykinin stimulation of the ovary significantly reduced heart rate and blood pressure.

Burden et al. (1983) clarified sensory innervation of the ovary with a histological study that showed that retrograde labeling from the ovary could be traced to both nodose ganglia and ipsilateral dorsal root ganglia from T10 to L2. However, the function of ovarian afferents has remained unresolved. The present study demonstrated that chemical stimulation of the ovary by bradykinin, an algesic substance released by tissue damage, produced decreases in heart rate and blood pressure in anesthetized rats. Furthermore, we explored the afferent pathway for this ovarian–cardiovascular reflex by lesioning vagal nerves, the SON, or the ONP. The decreased heart rate and blood pressure induced by ovarian bradykinin stimulation was not affected by severing the bilateral vagal nerves or the SON, but was abolished by severing the ONP. Furthermore, application of bradykinin to the ovary stimulated afferent activity of the ONP. Therefore, the ONP is the main afferent pathway for the ovarian–cardiovascular reflexes (Fig. 5).

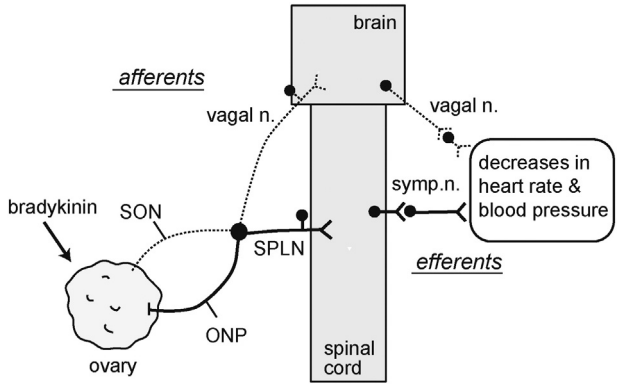

Fig. 5. Schematic diagram of the reflex pathway for cardiovascular responses to bradykinin stimulation of the ovary.

In the present study, the vagal nerve was not involved in the afferent pathway of the ovarian–cardiovascular reflexes. This result is in accord with other reports that showed that bilateral vagotomy does not affect cardiovascular responses induced by bradykinin application to the heart (Gorman et al., 1983; Gorman and Zucker, 1984; Niitani et al., 1988; Veelken et al., 1996) or abdominal visceral organ such as the gallbladder (Longhurst and Ordway, 1983). In contrast bradykinin applied to the heart is known to stimulate cardiac vagal afferents (Kaufman et al., 1980; Hisata et al., 2006). It has been suggested that vagal afferents mediate symptoms of nausea and discomfort related to visceral pain, rather than cardiovascular responses (Beyak and Grundy, 2005; Babic and Browning, 2014).

Klein and Burden (1988) reported that both the SON and the ONP contain afferent neurons, after observing labeled afferent perikarya located in lower thoracic–upper lumber DRG following application of true blue, an antidromic marker, directly to either the SON or the ONP. However, in the present study, the afferent pathway of the ovarian–cardiovascular reflex response was mainly afferent fibers in the ONP but not in the SON. Afferent fibers in the SON may transmit information about noxious mechanical stimulation, such as torsion of the ovary, rather than noxious chemical stimulation by bradykinin.

The present study demonstrated that afferent activity of the ONP is stimulated by bradykinin application to the ovary. There have been few studies on pain from female reproductive organs. Responsiveness of afferent fibers in the pelvic and hypogastric nerves innervating the uterus to algesic substances, including bradykinin, has been demonstrated in rats (Berkley et al., 1987, 1988, 1990, 1993). The present study is the first to demonstrate that the ONP afferents convey chemical nociceptive information from the ovary in response to bradykinin. In this study, the threshold dose of bradykinin to produce a significant increase in ONP afferent activity was 10^{-7} M, which is in accord with the threshold dose to produce a significant increase in afferent activity from the testis (Kumazawa and Mizumura, 1980). Bradykinin is known to increase the activity of both A and C afferent fibers in the splanchnic nerve innervating abdominal visceral organs, such as the stomach, duodenum, and gallbladder (Longhurst et al., 1984). Therefore, both A and C afferent fibers are suggested to be responsible for the reflex cardiovascular responses caused by bradykinin stimulation to abdominal visceral organs (Longhurst et al., 1984). In contrast, our previous electrophysiological study showed an evoked potential of primarily unmyelinated C fibers in the ONP (Kagitani et al., 2008; Hanada et al., 2011; Uchida, 2015). From this evidence, unmyelinated C fibers in the ONP may be the main afferents in the cardiovascular responses produced by bradykinin stimulation of the ovary shown in the present study.

In conclusion, this study showed for the first time the functional role of ovarian afferents in cardiovascular reflexes evoked by noxious chemical stimulation of the ovary by bradykinin in anesthetized rats. These findings will help to expand our knowledge regarding the functional role of ovarian autonomic innervation in both physiological and pathological conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

Babic, T., Browning, K.N., 2014. The role of vagal neurocircuits in the regulation of nausea and vomiting. Eur. J. Pharmacol. 722, 38–47.

Baker, D.G., Coleridge, H.M., Coleridge, J.C.G., Nerdrum, T., 1980. Search for a cardiac nociceptor: stimulation by bradykinin of sympathetic afferent nerve endings in the heart of the cat. J. Physiol. 306, 519–536.

Baljet, B., Drukker, J., 1979. The extrinsic innervation of the abdominal organs in the female rat. Acta Anat. (Basel) 104, 243–267.

Baljet, B., Drukker, J., 1980. The extrinsic innervation of the pelvic organs in the female rat. Acta Anat. (Basel) 107, 241–267.

Berkley, K.J., Robbins, A., Sato, Y., 1987. Uterine afferent fibers in the rat. In: Schmidt, R.F., Schaible, H.-G., Vahle-Hinz, C. (Eds.), Fine Afferent Nerve Fibers and Pain. VMH, Weinheim, pp. 128–136.

Berkley, K.J., Robbins, A., Sato, Y., 1988. Afferent fibers supplying the uterus in the rat. J. Neurophysiol. 59, 142–163.

Berkley, K.J., Hotta, H., Robbins, A., Sato, Y., 1990. Functional properties of afferent fibers supplying reproductive and other pelvic organs in pelvic nerve of female rat. J. Neurophysiol. 63, 256–272.

Berkley, K.J., Robbins, A., Sato, Y., 1993. Functional differences between afferent fibers in the hypogastric and pelvic nerves innervating female reproductive organs in the rat. J. Neurophysiol. 69, 533–544.

Beyak, M.J., Grundy, D., 2005. Vagal afferents innervating the gastrointestinal tract. In: Undem, B.J., Weinreich, D. (Eds.), Advances in Vagal Afferent Neurobiology. Taylor & Francis, Boca Raton, pp. 315–350.

Bielefeldt, K., Gebhart, G.F., 2013. Visceral pain: basic mechanisms. In: McMahon, S.B., Koltzenburg, M., Tracey, I., Turk, D.C. (Eds.), Wall and Melzack's Textbook of Pain, 6th ed. Elsevier Sanders, pp. 703–717.

Burden, H.W., 1985. The adrenergic innervation of mammalian ovaries. In: Ben-Jonathan, N., Bahr, J.M., Weiner, R.I. (Eds.), Catecholamines as Hormone RegulatorsSerono Symposia Publications from Raven Press Vol. 18. Raven Press, New York, pp. 261–278.

Burden, H.W., Leonard, M., Smith, C.P., Lawrence Jr., I.E., 1983. The sensory innervation of the ovary: a horseradish peroxidase study in the rat. Anat. Rec. 207, 623–627.

Cavun, S., Goktalay, G., Millington, W.R., 2004. The hypotension evoked by visceral nociception is mediated by delta opioid receptors in the periaqueductal gray. Brain Res. 1019, 237–245.

Cervero, F., 1994. Sensory innervation of the viscera: peripheral basis of visceral pain. Physiol. Rev. 74, 95–138.

Gabella, G., 1995. Autonomic nervous system. In: Paxinos, G. (Ed.), The Rat Nervous System, 2nd ed. Academic Press, San Diego, pp. 81–103.

Gerendai, I., Tóth, I.E., Boldogköi, Z., Medveczky, I., Halász, B., 1998. Neuronal labeling in the rat brain and spinal cord from the ovary using viral transneuronal tracing technique. Neuroendocrinol. 68, 244–256.

Gerendai, I., Tóth, I.E., Boldogköi, Z., Medveczky, I., Halász, B., 2000. CNS structures presumably involved in vagal control of ovarian function. J. Auton. Nerv. Syst. 80, 40–45.

Gorman, A.J., Zucker, I.H., 1984. Renal nerve and blood pressure responses to stimulation of cardiac receptors in dogs and cats by bradykinin. Basic Res. Cardiol. 79, 142–154.

Gorman, A.J., Zucker, I.H., Gilmore, J.P., 1983. Renal nerve responses to cardiac receptor stimulation with bradykinin in monkeys. Am. J. Physiol. 244, F659–F665.

Hammond, R., 2008. Gynaecological causes of abdominal pain. Surgery 26, 467–470.

Hanada, T., Uchida, S., Hotta, H., Aikawa, Y., 2011. Number, size, conduction, and vasoconstrictor ability of unmyelinated fibers of the ovarian nerve in adult and aged rats. Auton. Neurosci. 164, 6–12.

Hisata, Y., Zeredo, J.L., Eishi, K., Toda, K., 2006. Cardiac nociceptors innervated by vagal afferents in rats. Auton. Neurosci. 126–127, 174–178.

Jänig, W., 2006. Visceral afferent neurons and autonomic regulations. The Integrative Action of the Autonomic Nervous System. Cambridge University Press, Cambridge, pp. 35–84.

Kagitani, F., Uchida, S., Hotta, H., 2008. Effects of electrical stimulation of the superior ovarian nerve and the ovarian plexus nerve on the ovarian estradiol secretion rate in rats. J. Physiol. Sci. 58, 133–138.

Kaufman, M.P., Baker, D.G., Coleridge, H.M., Coleridge, J.C.G., 1980. Stimulation by bradykinin of afferent vagal C-fibers with chemosensitive endings in the heart and aorta of the dog. Circ. Res. 46, 476–484.

Klein, C.M., Burden, H.W., 1988. Anatomical localization of afferent and postganglionic sympathetic neurons innervating the rat ovary. Neurosci. Lett. 85, 217–222.

Klein, A.H., Joe, C.L., Davoodi, A., Takechi, K., Carstens, M.I., Carstens, E., 2014. Eugenol and carvacrol excite first- and second-order trigeminal neurons and enhance their heat-evoked responses. Neuroscience 271, 45–55.

Kruszka, P.S., Kruszka, S.J., 2010. Evaluation of acute pelvic pain in women. Am. Fam. Physician 82, 141–147.

Kumazawa, T., Mizumura, K., 1980. Chemical responses of polymodal receptors of the scrotal contents in dogs. J. Physiol. 299, 219–231.

Lawrence Jr., I.E., Burden, H.W., 1980. The origin of the extrinsic adrenergic innervation to the rat ovary. Anat. Rec. 196, 51–59.

Li, W.M., Suzuki, A., 2006. Reflex inhibition of heart rate and efferent cardiac sympathetic outflow induced by colorectal distension in anesthetized rats. J. Physiol. Sci. 56, 187–190.

Li, W.M., Suzuki, A., Cui, K.M., 2006. Responses of blood pressure and renal sympathetic nerve activity to colorectal distension in anesthetized rats. J. Physiol. Sci. 56, 153–156.

Longhurst, J.C., Ordway, G.A., 1983. Bradykinin-induced cardiovascular reflexes from the gallbladder in cats. Adv. Exp. Med. Biol. 156, 639–650.

Longhurst, J.C., Kaufman, M.P., Ordway, G.A., Musch, T.I., 1984. Effects of bradykinin and capsaicin on endings of afferent fibers from abdominal visceral organs. Am. J. Physiol. 247, R552–R559.

Mizumura, K., Sugiura, T., Katanosaka, K., Banik, R.K., Kozaki, Y., 2009. Excitation and sensitization of nociceptors by bradykinin: what do we know? Exp. Brain Res. 196, 53–65.

Ness, T.J., Gebhart, G.F., 1988. Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudaffective reflexes in the rat. Brain Res. 450, 153–169.

Niitani, S., Tomomatsu, E., Ohba, H., Yoshida, Y., Yagi, S., 1988. Renal nerve and cardiovascular responses to cardiac receptor stimulation in rabbits. Am. J. Physiol. 254, R192–R196.

Papka, R.E., Traurig, H.H., 1993. Autonomic efferent and visceral sensory innervation of the female reproductive system: special reference to neurochemical markers in nerves and ganglionic connections. In: Maggi, C.A. (Ed.), Nervous Control of the Urogenital System. Harwood Academic Publishers, Switzerland, pp. 423–466.

Premilovac, D., Bradley, E.A., Ng, H.L.H., Richards, S.M., Rattigan, S., Keske, M.A., 2013. Muscle insulin resistance resulting from impaired microvascular insulin sensitivity in Sprague Dawley rats. Cardiovasc. Res. 98, 28–36.

Procacci, P., Zoppi, M., Maresca, M., 1986. Clinical approach to visceral sensation. Prog. Brain Res. 67, 21–28.

Robbins, A., Sato, Y., 1991. Cardiovascular changes in response to uterine stimulation. J. Auton. Nerv. Syst. 33, 55–63.

Robbins, A., Sato, Y., Hotta, H., Berkley, K.J., 1990. Responses of hypogastric nerve afferent fibers to uterine distension in estrous or metestrous rats. Neurosci. Lett. 110, 82–85.

Robbins, A., Berkley, K.J., Sato, Y., 1992. Estrous cycle variation of afferent fibers supplying reproductive organs in the female rat. Brain Res. 596, 353–356.

Samraj, G.P.N., Curry Jr., R.W., 2004. Acute pelvic pain: evaluation and management. Compr. Ther. 30, 173–184.

Sato, A., Sato, Y., Schmidt, R.F., 1982. Changes in heart rate and blood pressure upon injection of algesic agents into skeletal muscle. Pflugers Arch. 393, 31–36.

Uchida, S., 2015. Sympathetic regulation of estradiol secretion from the ovary. Auton. Neurosci. 187, 27–35.

Uchida, Y., Murao, S., 1974. Bradykinin-induced excitation of afferent cardiac sympathetic nerve fibers. Jpn. Heart J. 15, 84–91.

Uchida, S., Kagitani, F., Hotta, H., 2008. Mechanism of the reflex inhibition of heart rate elicited by acupuncture-like stimulation in anesthetized rats. Auton. Neurosci. 143, 12–19.

Veelken, R., Glabasnia, A., Stetter, A., Hilgers, K.F., Mann, J.F.E., Schmieder, R.E., 1996. Epicardial bradykinin B_2 receptors elicit a sympathoexcitatory reflex in rats. Hypertension 28, 615–621.

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