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Centrally administered ghrelin activates cardiac vagal nerve in anesthetized rabbits

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ABSTRACT

Although central ghrelin has cardioprotective effect through inhibiting sympathetic nerve activity, the effects of central ghrelin on cardiac vagal nerve remain unknown. We investigated the effects of centrally administered ghrelin on cardiac autonomic nerve activities using microdialysis technique. A microdialysis probe was implanted in the right atrial wall adjacent to the sinoatrial node of an anesthetized rabbit and was perfused with Ringer's solution containing a cholinesterase inhibitor, eserine. After injection of ghrelin (1 nmol) into the right lateral cerebral ventricle, norepinephrine (NE) and acetylcholine (ACh) concentrations in the dialysate samples were measured as indices of NE and ACh release from nerve endings to the sinoatrial node using high-performance liquid chromatography. Heart rate was 270 ± 4 bpm at baseline and decreased gradually after ghrelin injection to 234 ± 9 bpm ($P<0.01$) at 60–80 min, followed by gradual recovery. Dialysate ACh concentration was 5.5 ± 0.8 nM at baseline and increased gradually after ghrelin injection to 8.8 ± 1.2 nM ($P<0.01$) at 60–80 min; the concentration started to decrease gradually from 100 to 120 min after injection reaching 5.6 ± 0.8 nM at 160–180 min. Central ghrelin did not change mean arterial pressure or dialysate NE concentration. The elevated dialysate ACh concentration declined rapidly after transection of cervical vagal nerves. These results indicate that centrally administered ghrelin activates cardiac vagal nerve.

1. Introduction

Ghrelin, a growth-hormone-releasing acylated peptide, was originally isolated from rat stomach (Kojima et al., 1999). Immunohistochemical studies have revealed that ghrelin-immunoreactive neurons are also present in the central nervous system including the hypothalamic arcuate nucleus (ARC) (Date et al., 2000) and that growth hormone secretagogue receptors (GHS-R) are expressed in hypothalamic nucleus including the ARC (Guan et al., 1997). Several studies have demonstrated that centrally administered ghrelin inhibits sympathetic nerve activity. Matsumura et al. (2002) reported that intracerebroventricular (icv) injection of ghrelin decreased renal sympathetic nerve activity in conscious rabbits. Lin et al. (2004) showed that microinjection of ghrelin into the nucleus of the solitary tract (NTS) also suppressed the renal sympathetic nerve activity in rats. However, whether central ghrelin affects cardiac vagal nerve activity remains unknown. Recently we have developed a microdialysis technique that allows direct monitoring of norepinephrine (NE) and acetylcholine (ACh) released into the sinoatrial (SA) node (Shimizu et al., 2009, 2010). Dialysate NE or ACh concentration monitored by this technique significantly correlates with heart rate and the frequencies of electrical stimulation of sympathetic or vagal nerve. In the present study, we used this technique to investigate the effect of centrally administered ghrelin on cardiac vagal nerve activity as well as sympathetic nerve activity in anesthetized rabbits.

2. Materials and Methods

2.1. Surgical Preparation

Animal care was provided in 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 approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center. Twenty four Japanese white rabbits weighing 2.3 to 3.1 kg were used in this study. Anesthesia was initiated by an intravenous injection of pentobarbital sodium (50 mg/kg) via the marginal ear vein, and then maintained at an appropriate level by continuous intravenous infusion of α-chloralose (16 mg/kg/h) and urethane (100 mg/kg/h) through a catheter inserted into the femoral vein. Since the duration of this experiment was projected to be over 8 h, the animals were intubated and ventilated mechanically with room air mixed with oxygen. Respiratory rate and tidal volume were set at

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30 cycles/min and 15 ml/kg, respectively. Systemic arterial pressure was monitored by a catheter inserted into the femoral artery. Body temperature was measured in the esophagus by a thermometer (CTM-303, Terumo, Japan), and was maintained between 38 and 39 °C using a heating pad. For icv injection of ghrelin, a polyethylene tube (500 μm outer diameter) was stereotactically inserted into the right lateral cerebral ventricle using a guiding needle (900 μm outer diameter, 600 μm inner diameter) and was perfused continuously with artificial cerebrospinal fluid (CSF) solution (ARTCEREB®, Otsuka, Japan) at a rate of 2 μl/min using a microinjection pump (CMA/102, Carnegie Medicin, Sweden).

With the animal in the lateral position, right lateral thoracotomy was performed and the right 3rd to 5th ribs were partially resected to expose the heart. Three stainless electrodes were placed around the thoracotomy incision to record the body surface electrocardiogram. The heart rate was determined from the electrocardiogram using a cardiotachometer. Heparin sodium (100 IU/kg) was administered intravenously to prevent blood coagulation. A dialysis probe was implanted and dialysis was conducted as described in *Dialysis Technique* below. At the end of the experiment, the animal was euthanized by injecting an overdose of pentobarbital sodium. In the postmortem examination, the right atrial wall with the implanted dialysis fiber was resected. The endocardial side of atrial wall was examined macroscopically to confirm that the dialysis membrane was not exposed to the right atrial lumen.

2.2. Dialysis Technique

The materials and properties of the dialysis probe have been described previously (Akiyama et al., 1991; Shimizu et al., 2009, 2010). A dialysis fiber composed of semipermeable membrane (4 mm length, 310 μm outer diameter, 200 μm inner diameter; PAN-1200, 50,000 molecular weight cutoff; Asahi Chemical, Tokyo, Japan) was attached at both ends to polyethylene tubes (25 cm length, 500 μm outer diameter, 200 μm inner diameter). A fine guiding needle (30 mm length, 510 μm outer diameter, 250 μm inner diameter) with a stainless steel rod (5 mm length, 250 μm outer diameter) was used for implantation. A dialysis probe was implanted into the right atrial myocardium near the junction between the superior vena cava and the right atrium. After implantation, the dialysis probe was perfused with Ringer's solution (NaCl 147 mM, KCl 4 mM, and $CaCl_2$ 3 mM) containing the cholinesterase inhibitor, eserine (100 μM), at a rate of 2 μl/min using a microinjection pump (CMA/102). Experimental protocols were started 120 min after implantation of the dialysis probe. We took account of the dead space between the dialysis membrane and the sample tube at the start of each dialysate sampling. Eight microliters of phosphate buffer (pH 3.5) was added to each sample tube before dialysate sampling. The duration of dialysate sampling was fixed at 20 min (1 sample volume = 40 μl). Half of the dialysate sample was used for ACh measurement and the other half for NE. Dialysate NE and ACh concentrations were measured separately using two high-performance liquid chromatographs with electrochemical detection as previously described (Akiyama et al., 1991, 1994).

2.3. Experimental Protocols

2.3.1. Protocol 1

We investigated the time courses of heart rate, mean arterial pressure, and dialysate NE and ACh concentrations following icv injection of ghrelin. One hundred microliters of artificial CSF containing 1 nmol of human ghrelin (Peptide Institute, Osaka, Japan) or 100 μl of artificial CSF alone (vehicle) was injected into the lateral cerebral ventricle of a rabbit. Baseline dialysate sample was collected before injection and then 20-min dialysate samples were collected consecutively up to 180 min after injection.

2.3.2. Protocol 2

We investigated the effect of vagotomy on heart rate and cardiac vagal ACh release after icv injection of ghrelin (1 nmol). Baseline dialysate sample was collected before icv injection of ghrelin and another sample was collected when heart rate reached a trough after ghrelin injection. Immediately after this sampling, bilateral cervical vagal nerves were transected and dialysate was sampled for a 20-min duration.

2.3.3. Protocol 3

As a supplemental protocol, we investigated the dose-dependent effects of ghrelin on heart rate and dialysate ACh concentration using icv injection of 0.2 nmol (n = 3) or 5 nmol (n = 4) of human ghrelin into the right lateral cerebral ventricle. Baseline dialysate sample was collected before injection and then 20-min dialysate samples were collected consecutively up to 180 min after injection.

2.4. Statistical analysis

Heart rate and mean arterial pressure were averaged over each 20-min duration of dialysate sampling. All data are presented as mean ± SE. In Protocols 1 and 2, heart rate and mean arterial pressure were compared by one-way repeated measures analysis of variance (ANOVA) followed by a Dunnett's test against baseline. Our previous studies demonstrated that dialysate NE or ACh concentration exponentially increased in response to electrical stimulation of sympathetic or vagal nerve. Then, heart rate linearly correlated with logarithms of dialysate NE or ACh concentration (Shimizu et al., 2009, 2010). Thus, after logarithmic transformation, dialysate NE and ACh concentrations were compared by one-way repeated measures ANOVA followed by a Dunnett's test against baseline. The differences between ghrelin and vehicle groups were compared using unpaired *t*-test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Protocol 1

In the ghrelin-treated rabbits, the heart rate was 270 ± 4 bpm at baseline and decreased gradually after icv ghrelin injection reaching a trough of 233 ± 9 bpm at 80–100 min ($P<0.01$ vs. baseline), followed by gradual recovery (271 ± 8 bpm at 160–180 min). In the vehicle control group, heart rate was 270 ± 6 bpm at baseline and increased slightly to 278 ± 6 bpm at 40–60 min after injection ($P<0.05$ vs. baseline), and was maintained until the end of the protocol (284 ± 7 bpm at the 160–180 min, $P<0.01$ vs. baseline) (Fig. 1A).

Although mean arterial pressure did not change after icv injection of ghrelin and remained constant throughout the experiment, mean arterial pressure decreased gradually from 80 ± 4 mm Hg at baseline to 72 ± 2 mm Hg at 160–180 min ($P<0.01$ vs. baseline) after icv injection of vehicle (Fig. 1B).

Dialysate ACh concentration did not change after icv injection of vehicle. In the ghrelin-treated rabbits, the dialysate ACh concentration was 5.5 ± 0.8 nM at baseline and increased gradually after ghrelin injection, reaching a plateau of 8.8 ± 1.2 nM at 60–80 min ($P<0.01$ vs. baseline). The concentration appeared to decline after 100 min and returned to 5.6 ± 0.8 nM at 160–180 min (N.S. vs. baseline) (Fig. 2A).

Dialysate NE concentration did not change after icv injection of ghrelin or vehicle, and did not vary significantly throughout the experiment (Fig. 2B).

3.2. Protocol 2

Heart rate decreased significantly from 283 ± 5 bpm at baseline to a trough of 249 ± 5 bpm after icv injection of ghrelin ($P<0.01$ vs. baseline) (Table 1). Dialysate ACh concentration increased from 5.3 ± 1.3 nM at