The regulatory mechanism of motilin induced gastric acid and pepsinogen secretion in *Suncus murinus*

(スンクスにおけるモチリン誘導性胃酸及びペプシノーゲン分泌調節機構)

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ABSTRACT

Motilin and ghrelin constitute a peptide family, and these hormones are important for the regulation of gastrointestinal motility. In this study, we examined the effect of motilin and ghrelin on gastric acid secretion in anesthetized suncus (house musk shrew, *Suncus murinus*), a ghrelin- and motilin-producing mammal. We first established a gastric lumen-perfusion system in the suncus and confirmed that intravenous (i.v.) administration of histamine (1 mg/kg BW) stimulated acid secretion. Motilin (0.1, 1.0, and 10 μg/kg BW) stimulated the acid output in a dose-dependent manner in suncus, whereas ghrelin (0.1, 1.0, and 10 μg/kg BW) alone did not induce acid output. Furthermore, in comparison with the vehicle administration, the co-administration of low-dose (1 μg/kg BW) motilin and ghrelin significantly stimulated gastric acid secretion, whereas either motilin (1 μg/kg BW) or ghrelin (1 μg/kg BW) alone did not significantly induce gastric acid secretion. This indicates an additive role of ghrelin in motilin-induced gastric acid secretion. We then investigated the pathways of motilin/motilin and ghrelin-stimulated acid secretion using receptor antagonists. Treatment with YM 022 (a CCK-B receptor antagonist) and atropine (a muscarinic acetylcholine receptor antagonist) had no effect on motilin or motilin-ghrelin co-administration-induced acid output. In contrast, famotidine (a histamine H_2 receptor antagonist) completely inhibited motilin-stimulated acid secretion and co-administration of motilin and ghrelin induced gastric acid output. This is the first report demonstrating that motilin stimulates gastric secretion in mammals. By using the above mentioned gastric lumen-perfusion system, we also found that the intravenous administration of carbachol and motilin (0.1, 1.0, and 10 μg/kg BW) stimulated pepsinogen secretion, the latter in a dose-dependent manner, whereas ghrelin had no

effect. We then investigated the pathways of motilin-induced pepsinogen secretion using acetylcholine receptor antagonists. Treatment with atropine, a muscarinic acetylcholine receptor antagonist, completely inhibited both carbachol and motilin-induced pepsinogen secretion. Motilin-induced pepsinogen secretion was also observed in the vagotomized suncus. Our results suggest that motilin stimulates gastric acid secretion via the histamine-mediated pathway and also stimulates pepsinogen secretion through a cholinergic pathway in suncus.

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1.1. Research background

1.1.1. Gastric acid secretion and its role in gastrointestinal tract

Gastric acid secretion by parietal cells in the fundus and body of the stomach facilitates protein digestion, absorption of iron, calcium, and vitamin B12, and decreases the risk of gastrointestinal infection by *Helicobacter pylori*, *Vibrio cholera*, *Salmonella sp.*, and many others [1]. Insufficient acid secretion may result in malabsorption and increase susceptibility to intestinal infection, whereas increased production of gastric acid may cause severe mucosal damage that leads to intestinal bleeding or perforation [2]. Gastrointestinal peptides play an important role in regulating gastric acid secretion.

1.1.2. Pepsinogen secretion and its role in gastrointestinal tract

On the other hand, pepsinogens are mainly synthesized and secreted by the gastric chief cells of the stomach before being converted into the proteolytic enzyme pepsin, which is important for the digestion of foods in the stomach [3]. Pepsinogens are stored in granules during resting state, which inhibit further synthesis of pepsinogens. After getting suitable physiological or external chemical stimuli, pepsinogens are secreted in the stomach lumen where hydrochloric acid, released from the parietal cells, converts them into the corresponding active enzyme pepsins [3]. Along with protein digestion, recent findings also suggested the implication of pepsinogens in ulcerogenesis. *In vivo* and *in vitro* studies have confirmed the involvement of different gastrointestinal peptides in the regulation of pepsinogen secretion [3].

1.1.3. Motilin

Motilin was originally purified from porcine intestinal mucosa in the 1970s, and its molecular structure was determined to be a 22-amino-acid polypeptide [4,5]. Motilin is mainly produced in the duodenum and secreted into the blood stream in the fasting state. It has been demonstrated that plasma motilin is released at ~100-min intervals at the interdigestive state [6,7]. During the interdigestive state, it was found that plasma motilin concentration increased in complete accordance with the cyclical interdigestive contractions of the stomach in dogs [6]. Physiological study of motilin *in vivo* has been mainly performed by using dogs and humans, and endogenous motilin is thought to be physiologically important to induce phase III contraction as peaked plasma motilin levels was always observed in the period of gastric phase III contractions [8]. Also, exogenous administration of motilin has been shown to induce phase III-like contractions through the cholinergic pathway because atropine pretreatment completely abolished the motilin-induced contractions [6,9].

1.1.4. Ghrelin

Ghrelin, a 28 amino acid octanoylated peptide, is a peptide hormone that was first identified in rat and human stomach in 1999 as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) [10]. Ghrelin is also released during the fasting state. Several studies have demonstrated gastroprokinetic effects of ghrelin. The major physiological actions of ghrelin include regulation of growth hormone (GH) secretion [10-12], food intake [13,14], energy metabolism [15,16], gastrointestinal motility [17-19], gastric acid secretion [20-22], cardiovascular function [23], and cell proliferation [24,25]. Ghrelin stimulated gastric contraction was observed in rats [21]

and mice [19], suggesting that ghrelin serves as an alternative to motilin with regard to gastrointestinal motility in motilin-lacking rodents [26]. Although ghrelin does not induce gastric phase III contractions in dogs [27], it has been shown that ghrelin induces premature phase III contractions in the human stomach [28]. Also, the plasma peak of ghrelin is correlated with phase-III like contractions in rats [29].

1.1.5. Similar properties between motilin and ghrelin

In humans, the ghrelin and motilin ligands share about 21% amino acid identity, and about 50 % of their precursor mRNAs are identical [26]. Specific receptors for ghrelin and motilin are growth hormone secretagogue receptor (GHS-R) [30] and motilin receptor (GPR38) [31], respectively. Both motilin and ghrelin receptors belong to the class A rhodopsin-like G protein-coupled seven-transmembrane receptor family, and they have a similar structure [32]. In addition, their receptors also show a marked sequence homology with an overall identity of 44% which is 87% in the transmembrane regions. In suncus, it was observed that coordination of motilin and ghrelin are necessary to initiate phase III contraction of migrating motor complex. Moreover, previous study also demonstrated the synergistic effect of motilin and ghrelin on gastric contractions *in vitro* and in anesthetized suncus *in vivo* [33]. Therefore, there it is reasonable to assume additional or synergistic relationship between these family peptides on other physiological functions. However, detailed functions of motilin other than stimulation of gastric contraction are not well known.

1.1.6. House musk shrew (*Suncus murinus*)

One of the reasons for the slow progression of motilin study is that rodents such as rats and mice cannot be used for motilin study because the motilin gene is inactivated in the common ancestor of mice and rats [34]. For this reason, rodents, the most classical and widely used laboratory animals, are not available for motilin experiments because the motilin gene has not been cloned and the amino acid sequence has not been determined. Therefore, motilin has been studied using relatively large animals, such as dogs and rabbits, which has limitations to study detailed mechanisms for the actions of motilin. On the other hand, ghrelin can only induce gastric contraction in the rats, mice and human but not in the dogs [27] and rabbits. Therefore, it is necessary to find a new animal model comparable to human or dog. So, we looked for a suitable animal that could be used as a new model for motilin study. We focused on suncus (house musk shrew, *Suncus murinus*). Suncus belongs to the order Insectivora, family Soricidae, genus Suncus, and this order of animals has traditionally been considered as one of the key groups for understanding the origin of mammals [35,36]. In the recent years, suncus has been used for studying the mechanism of vomiting and development of antiemetic drugs [37-40]. The visceral system of suncus is very similar to that of humans and is a useful model of physiology and pathophysiology in the latter [41-44]. The general appearance of the suncus gastric mucosa and that of the human is similar, and differs from some other widely used experimental animals, like hamsters, rats and mice [45]. For example, the gastric mucosa of suncus consists of a glandular mucosa with well-developed luminal folds and suncus has no forestomach as observed in mouse and hamster. We already identified the cDNA sequence of suncus motilin and ghrelin in suncus [46,47]. We also identified the ghrelin receptor, GHS-R and motilin

receptor, GPR38 in suncus [48]. We found that suncus has almost identical GI motility and motilin responses as those found in humans and dogs [47,49]. We believe that suncus is the only small animal that can be used to study the effects of both motilin and ghrelin on gastric secretion and their mechanism.

1.2. Hypothesis

We hypothesized that motilin and ghrelin may regulate gastric secretion during interdigestive state. S. murinus has the both motilin and ghrelin and their receptors throughout the GI tract as discussed above. Firstly, we examine the role of motilin and ghrelin on gastric acid secretion using above mentioned animal. In second experiment, we study the effect of motilin and ghrelin on pepsinogen secretion. Hence, this study also investigates the regulatory mechanism of these family peptides on the gastric secretion and their physiological importance.

1.3. General objectives

Therefore, the general objective of this study is to investigate the role of motilin and ghrelin on gastric secretion and their mechanisms using suncus, a motilin- and ghrelin-producing model animal.

Chapter 2. **Motilin stimulates gastric acid secretion in co-ordination with ghrelin in *Suncus murinus***

2.1. Introduction

2.1.1. Gastric acid secretion mechanism

It is well established that gastric acid secretion is regulated by neurocrine, endocrine, and paracrine signals [50]. Histamine, acetylcholine, and gastrin are the major direct peripheral stimuli acting on the parietal cells for gastric acid secretion [50], and histamine is the most effective stimulating factor to these cells. Recently, several reports have demonstrated that ghrelin and other gut hormones also stimulate gastric acid secretion through histamine mediated pathways [51,52].

2.1.2. Motilin and ghrelin, and their functions in relation to gastric acid secretion

Ghrelin was originally isolated from rat and human stomachs [10] and its central or peripheral administration causes the initiation of food intake in many species [13,53-56]. In addition, it is well known that ghrelin is a multifunctional hormone; many studies revealed that ghrelin is important for the regulation of gastrointestinal tract [10,13-15,57-59], in particular, it stimulates gastric acid secretion in the rat [60], and also induces gastric motility by peripheral or central administration [21,61]. Motilin, produced mainly in the duodenum, is considered to be of the same peptide family as ghrelin [4,26]. It has been well documented that gastric contraction is strongly associated with plasma motilin levels and that the intravenous administration of motilin induces gastric phase III-like contractions in humans [7,9] and dogs [6,62,63]. Recently,

we reported that motilin and ghrelin synergistically stimulated gastric contraction in suncus (house musk shrew, *Suncus murinus*), suggesting that focusing on the coordination of motilin and ghrelin is important for understanding their physiological role [33]. Previous studies have shown that cyclic release of motilin in an interdigestive period induces phase III contraction of the migrating motor complex (MMC) in the stomach. However, the causes and mechanism for the ending of phase III contractions are not clearly understood. We hypothesized that the peaked concentration of motilin may be involved in the extinction of phase III contractions, and thus a return to the basal level of phase I contraction. Studies have also shown that interdigestive acid secretion in the stomach disrupts the regular occurrence of phase III contractions [64,65]. Therefore, we studied the effect of motilin on gastric acid secretion.

2.1.3. Advantages of suncus for studying gastrointestinal physiology including gastric acid experiment

Although motilin was discovered decades ago, there have been limited studies on the physiological functions of motilin, other than gastrointestinal motility, because the study of the biological action of motilin beset with several problems. The greatest difficulty in study of motilin is the lack of a suitable small laboratory animal for experimentation. Since rodents lack the genes for motilin and its receptors [34], these commonly used experimental animals cannot be employed for studies on motilin. Recently, we focused on the suncus as a laboratory animal for gastrointestinal studies using motilin-ghrelin family peptides. Under the fasting condition, the repeated occurrence of MMCs was observed in suncus and motilin stimulated gastric contraction in a dose-dependent manner in humans and dogs alike [47,49]. In addition, complete

cDNA sequences and the tissue distribution of motilin and ghrelin were identified in suncus [46,47]. These results suggest that suncus is a useful animal for studying gastrointestinal physiology, including gastric motility.

In the present study, we established an experimental procedure for acid secretion using the stomach perfusion system in anesthetized suncus, and studied the effect of ghrelin and motilin on acid secretion. In addition, we also examined the acid secretion mechanism by motilin and co-administration of motilin and ghrelin, using receptor antagonists.

2.2. Materials and Methods

2.2.1. Animals

Experiments were performed using adult male (10–30 weeks of age) and female (5–30 weeks of age) suncus of an outbred KAT strain established from a wild population in Kathmandu, Nepal [66], weighing between 50 and 100 g. Animals were housed individually in plastic cages equipped with an empty can for a nest box under controlled conditions (23 ± 2°C, lights on from 8:00 to 20:00) with free access to water and commercial feeding pellets (number 5P; Nippon Formula Feed Manufacturing, Yokohama, Japan). The metabolizable energy content of the pellets was 344 kcal/100 g. The pellets consisted of 54.1% protein, 30.1% carbohydrates, and 15.8% fat. All procedures were approved and performed in accordance with the Committee on Animal Research of Saitama University (Saitama, Japan). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiment.

2.2.2. Drugs

Urethane (Sigma Aldrich, St. Louis, MO) was used to anesthetize the animals. Gastric acid secretion was stimulated by intravenous (i.v.) bolus infusion of histamine dihydrochloride (Nakarai Chemicals Co., Ltd., Kyoto, Japan), pentagastrin (Sigma Aldrich, USA), carbachol (Tocris Bioscience, Ellisville, MO), suncus motilin (Scrum Inc., Tokyo, Japan), and human acylated ghrelin (Asubio Pharma Co., Ltd., Hyogo, Japan). Famotidine (Wako Pure Chemical Industries Ltd.), YM 022 (Sigma-Aldrich), and atropine sulfate (Mylan Pharma, Osaka, Japan) were used as H_2 receptor, CCK-B receptor, and muscarinic cholinergic receptor antagonist, respectively. Motilin, ghrelin,

and pentagastrin were dissolved in 0.1% BSA in PBS (phosphate-buffered saline), while histamine, carbachol, and atropine were dissolved in 0.9% saline. Famotidine was dissolved in 0.5 N HCl, and i.v. administration was performed after dilution in 0.1% BSA in PBS. YM 022 was dissolved in dimethylsulfoxide (DMSO) and subsequent dilutions were made in saline containing DMSO. All solutions were prepared immediately before each experiment. The dosage of each agent was administered at a rate of 100 μL per 100 g BW.

2.2.3. Determination of gastric acid output by an intragastric perfusion experimental system

Overnight-fasted animals were anesthetized with an intraperitoneal injection of 15% urethane solution at a dose of 1 ml/100 g body weight, and prepared for the gastric acid output study by using intragastric perfusion system (Fig. 1). After anesthesia, the animals' tracheas were exposed, cannulated, and exteriorized to avoid choking due to catheter insertion through esophagus. Then, a catheter (polyethylene tube) was inserted into the stomach from the mouth and the tip of the catheter was placed about 3 mm from the cardia inside the stomach; the tube was fixed to the esophagus with a suture. Then, the abdomen was opened through the linea alba to expose the stomach. The pyloroduodenal junction was then exposed, and another polyethylene tube was introduced into the stomach via an incision in the duodenum, and was secured firmly with a ligature around the pylorus. Then, we exposed the jugular vein to insert a cannula for administration of reagents/drugs. The stomach lumen was washed with saline until the effluent was clear and then perfused with saline solution at 37°C at a rate of 0.25 ml/min using a peristaltic pump (Micro tube pump MP-3EYELA; Tokyo Rikakikai Co., Ltd., Tokyo). The stomach was perfused with saline through the liquid

discharge tube and gastric output was collected through the perfusion catheter. In order to stabilize the amount of acid secretion, the pH of the collected solution was allowed to stabilize for 60 minutes from the start of the perfusion of saline. Effluents were collected in the tube continuously at 10-min intervals by a DF-2000 fraction collector (Tokyo Rikakikai Co., Ltd., Tokyo, JAPAN).

2.2.4. Measurement of pH and amount of gastric acid

We measured the pH of the gastric output with a pH meter (HORIBA Scientific, LAQUA ELECTRODE, HORIBA Ltd., JAPAN). We determined the acid output by a neutralization titration using 0.01 N NaOH solution. The amount of acid secretion was expressed per 10 minutes as H^+ μEq. The amount of change in gastric secretion was measured by deducting the area under the curve (AUC) of the gastric acid secretion at 50 min before and after the administration of each drug. The values are expressed as ΔμEq/50 min.

2.2.5. Experimental protocols

Intravenous (i.v.) bolus infusion of histamine dihydrochloride at the dose of 1 mg/kg BW [67], human acylated ghrelin (0.1, 1, and 10 μg/kg BW), and suncus motilin (0.1, 1, and 10 μg/kg BW) was done to study their stimulatory effect on acid secretion. A previous study confirmed that seven amino acids with acyl modification on the third Ser residue in the N-terminal region of ghrelin showed full biological activity [68]. In addition, the first 10 amino acids of mature suncus ghrelin sequence are identical among other mammals, including humans [46], indicating that human ghrelin is enough to exert full biological activity in suncus. Therefore, we used human ghrelin in this experiment. Combined effects of motilin and ghrelin were studied by the co-

administration of motilin and ghrelin at doses of 0.1, 1, and 10 µg/kg BW (each). Based on the dose response, motilin and co-administration of motilin and ghrelin at 10 µg/kg BW were selected for all further studies. Famotidine (0.33 mg/kg BW) [69] was used to evaluate the role of the histamine (H_2) receptor on motilin and co-administration of motilin and ghrelin stimulating gastric acid secretion. After confirming that pretreatment of famotidine (0.33 mg/kg BW) completely inhibited histamine-stimulated (1 mg/kg BW) acid secretion, famotidine at the pretreatment dose was administered 30 min before administration of motilin (10 µg/kg BW) and co-administration of motilin (10 µg/kg BW) and ghrelin (10 µg/kg BW). To examine the involvement of gastrin (CCK-B) receptors in motilin-stimulated gastric acid secretion, we also administered YM 022 (0.2 mg/kg BW) [70,71]. Vehicle or YM 022 (0.2 mg/kg BW) was administered 30 min before each drug injection. Similarly, an mACh receptor antagonist, atropine (30 µg/kg BW) was also administered 30 min before motilin or motilin/ghrelin co-administration to examine the influence of these receptors on gastric acid secretion.

2.2.6. Statistical analyses

We repeated the recording experiments individually at least three times. All data are indicated as mean ± S.E.M. We used GraphPad Prism 5 software (GraphPad Software Inc., CA, USA) to analyze the data. Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparisons Test or Student's *t* test. $p < 0.05$ was considered statistically significant.

2.3. Results

2.3.1. Effect of ghrelin, motilin, and co-administration of motilin and ghrelin on gastric acid secretion

To establish the perfusion system for gastric acid experiment in suncus, we first examined the effect of histamine (1 mg/kg) on acid secretion (Fig. 2A). Intravenous (i.v.) administration of histamine started to increase the acid output after 10 min and reached its maximum at 20 min, after which the acid output reduced to baseline around 30–40 min after histamine administration (Fig. 2A). The pH of the gastric output had maximally decreased 20 min after histamine administration and restored to its previous values at 40 min after administration (Fig. 2A). The amount of acid secretion induced by 1 mg/kg histamine was 4.22 ± 0.32 μEq/50 min and the acid output significantly increased compared to the vehicle (control: 0.52 ± 0.27 μEq/50 min) (Fig. 2F). Vehicle administration (0.1% BSA in PBS) did not change the basal gastric acid secretion (Fig. 2B). The i.v. administration of ghrelin at the doses of 0.1, 1.0, and 10 μg/kg did not increase the acid output and the pH was slightly decreased, but not significantly so (Fig. 2C). In contrast, i.v. administration of motilin started to increase acid secretion after 10 min and reached its maximum at 20 min after administration (Fig. 2D). The motilin-induced acid secretion returned to baseline 40 min after administration and the pH value of the gastric output decreased within 30–40 min of motilin administration (Fig. 2D). The amount of gastric acid secretion 50 min after motilin administration at the doses of 0.1, 1.0, and 10 μg/kg increased dose-dependently (Fig. 2F). Co-administration of motilin and ghrelin (0.1, 1.0, and 10 μg/kg BW each) also induced the secretion of gastric acid and it reached its maximum in 20 min after administration, and

decayed gradually before 50 min had elapsed (Fig. 2E). A maximum decline of gastric pH was observed within 20 min and returned to baseline 50 min after the administration of each dose (Fig. 1E). The amount of gastric acid secretion 50 min after co-administration of motilin and ghrelin increased in a dose-dependent manner (Fig. 2F). Statistical analysis showed that 10 μg/kg motilin, and co-administration of motilin and ghrelin (1 and 10 μg/kg, each) significantly increased acid output compared to vehicle administration (Fig. 2F).

2.3.2. Effect of famotidine on motilin, and co-administration of motilin and ghrelin-stimulated gastric acid secretion

We next examined the effect of famotidine (an H_2 receptor antagonist) on motilin, and co-administration of motilin and ghrelin-stimulated gastric acid secretion. Famotidine (0.33 mg/kg) significantly decreased histamine-induced acid output and also inhibited the decrease in pH by histamine treatment (Fig. 3A–C). In order to examine whether the H_2 receptor is the mediator of motilin and combined motilin and ghrelin-stimulated acid secretion, i.v. administration of vehicle and famotidine (0.33 mg/kg) was done 30 min before the administration of motilin (10 μg/kg) and co-administration of motilin (10 μg/kg) and ghrelin (10 μg/kg). Famotidine treatment gradually decreased baseline acid secretion; however, no peak was observed after motilin administration. The stimulatory effect of motilin on gastric acid secretion was completely inhibited by famotidine treatment (Fig. 4A–C). Similarly, the co-administration of motilin and ghrelin was almost completely eliminated by famotidine treatment (Fig. 5A–C).

2.3.3. Effect of YM 022 on gastric acid secretion stimulated by motilin, and co-administration of motilin and ghrelin

To examine the involvement of gastrin mediated pathways for motilin-stimulated gastric acid secretion, we used YM 022 (a CCK-B receptor antagonist). Pentagastrin (1 μg/kg) stimulated acid secretion (8.95 ± 0.73 μEq/50 min) continued for 30 min, but this increase was significantly and almost completely attenuated (0.82 ± 0.62 μEq/50 min) by pretreatment with YM 022 (0.2 mg/kg) (Fig. 6A–C). In contrast, gastric acid secretion stimulated by the motilin treatment did not change with pretreatment of YM 022 (Fig. 7A, B). The stimulatory effect of motilin (10 μg/kg BW) on gastric acid secretion after vehicle/YM 022 pre-administration was statistically insignificant over 50 min (Fig. 7C). Furthermore, we also checked the effect of YM 022 on gastric acid secretion stimulated by motilin and ghrelin co-administration. Co-administration of motilin and ghrelin showed remarkable change in gastric acid output which was found unaffected by the pretreatment of YM 022 (Fig. 8A, B). The change in motilin-induced acid output for 50-min with vehicle or YM 022 pretreatment was not statistically significant suggesting no effect was found for YM 022 pretreatment (Fig. 8C).

2.3.4. Effect of atropine on motilin, and co-administration of motilin and ghrelin-stimulated gastric acid secretion

Thirty minutes of pre-administration with atropine (30 μg/kg BW) almost completely inhibited the carbachol (5 μg/kg BW) stimulated acid secretion (Fig. 9A–C). Vehicle pretreatment had no effect on motilin (10 μg/kg BW) stimulated gastric acid secretion (Fig. 10A). However, pretreatment with atropine could not inhibit the motilin-stimulated gastric acid output (Fig. 10B). Furthermore, net change in cumulative gastric

acid output by motilin for 50 min was not changed by atropine pretreatment (Fig. 10C). Similarly, atropine pretreatment before motilin and ghrelin co-administration did not suppress gastric acid secretion which was observed with vehicle pretreatment (Fig. 11A, B). The net change of acid output for 50-min after co-administration of motilin and ghrelin was not significantly different as compared between vehicle and atropine pretreated group (Fig. 11C). From this figure, it is clear that cholinergic pathway was not involved for co-administration of motilin and ghrelin stimulated gastric acid secretion.

2.4. Discussion

2.4.1. Suncus for gastric acid secretion study

We used suncus as a model animal to study the effect of motilin and/or ghrelin on gastric acid secretion. Suncus, which belongs to the order Insectivora, was first established as a model animal for research on emesis and vomiting [72,73]. Recently, we found that this animal produced not only motilin but also its receptor [48]. Considering that the motilin gene in rodents, the most widely used small laboratory animal, is converted into a pseudogene and lacks gastric contraction in response to motilin treatment [26,34], suncus might be a unique small animal model for studies on motilin [47]. The mucosa of suncus is similar to that of humans and dogs [45], and also has an almost identical MMC of gastrointestinal motility, and motilin responses, as that found in humans and dogs [49]. In addition, the coordinating effect of motilin and ghrelin was also observed in regulating the MMC of gastrointestinal motility in suncus [33]. In this study, we focused on the effect of motilin on gastric acid secretion by using suncus.

2.4.2. Motilin but not ghrelin stimulate gastric acid secretion

In previous studies, it has been demonstrated that intravenous administration of ghrelin stimulates acid secretion in a dose-dependent manner in the rat through vagal afferent nerve [21,60,61]. However, in this study, intravenous administration of ghrelin showed no significant increase in acid secretion even at high concentrations (10 μg/kg). On the basis of our results that motilin stimulated gastric acid output in a dose-dependent manner, ghrelin might act to compensate for the function of motilin in rodents because a similar example is found in rat gastric motility [21]. It has been reported that ghrelin

is a strong prokinetic hormone in rats and mice [19,21]. However, in the suncus and humans, ghrelin has less potent effects on gastric contraction compared to that of motilin [9,33,49], suggesting that ghrelin stimulates gastric contraction instead of motilin. It is interesting to study the effect of motilin and/or ghrelin on gastric acid output in motilin-producing animals such as humans and dogs.

2.4.3. Physiological importance of motilin-induced gastric acid secretion

We first found a novel physiological function for motilin as a stimulator of gastric acid secretion in suncus. In previous studies, plasma motilin concentration was found to increase at the start of gastric phase II in fasted dogs, and gradually reached a peak just after the termination of phase III in an interdigestive state [6,74]; further, these motilin peaks were found at an interval of about 100 min, and induced phase III contractions of the MMC in human [9] and in dogs [6]. Moreover, Gielkens *et al.* reported that, in humans, the amount of gastric acid output during phase I and early phase II was low, but increased significantly during late phase II and reached its highest point during phase III of the MMC [75]. Taken together with our present results, this suggests that peak concentration of motilin may not only stimulate the phase III contraction but also gastric acid secretion. Further, many studies have clearly shown that stomach acidification attenuated spontaneous phase III and motilin-induced contractions. For example, Yamamoto *et al.* reported that antral acidification inhibits motilin-induced phase III contractions in the stomach of dogs through vagovagal reflux [76], and, similarly, intragastric acidification also inhibits the effect of motilin on antroduodenal motility and causes a delay in the occurrence of phase III in humans [65]. Furthermore, pentagastrin independently inhibits motilin-induced phase III-like contraction by gastric acidification via a CCK receptor-dependent mechanism [77]. Additionally, studies have

also shown that motilin can induce gastric contraction after inhibition of gastric acid secretion by famotidine (an H_2 receptor antagonist) in a patient with duodenal ulcers [78]. According to these studies, motilin-induced phase III contractions are suppressed by motilin-induced gastric acidification, and this may stop the phase III period even at high concentrations of motilin.

2.4.4. Regulatory mechanism of motilin-induced gastric acid secretion

In this study, the effect of the co-administration of low-dose (1 μg/kg) motilin and ghrelin, but not motilin (1 μg/kg) or ghrelin (1 μg/kg) alone, was found to significantly stimulate gastric acid secretion compared with the vehicle and was almost comparable with that of 10 μg/kg motilin alone. This suggests that the additive role of ghrelin in motilin-induced gastric acid secretion is close to the physiological concentration. Moreover, further studies are required to elucidate the detailed mechanism of ghrelin, especially the target site of ghrelin. Meanwhile, we demonstrated the mechanism of action of motilin on gastric acid secretion. We observed that gastric acid secretion by motilin administration was completely eliminated by pretreatment with famotidine (an H_2 receptor antagonist), but not atropine or gastric/CCK-B receptor antagonists, indicating that motilin-stimulated acid secretion was finally mediated by the H_2 receptor in the stomach. In addition, the suncus genome database confirmed that the predicted mRNA sequence of the H2 receptor, which is highly conserved in mammals, shows high homology with that in humans (data not shown). This suggests that histamine and famotidine are a specific agonist and antagonist, respectively, for H2 receptor in suncus. Hormone stimulating gastric acid secretion through histaminergic neural pathway is not a rare occurrence; for example, famotidine can inhibit ghrelin-stimulated gastric acid secretion in the rat [51] and PACAP (pituitary adenylate cyclase

activating polypeptide) has also induced histamine release via a PACAP type 1 receptor on enterochromaffin-like (ECL) cells [52]. Motilin also stimulates gastric acid secretion via a histaminergic pathway, as was found in the case of ghrelin and PACAP. However, details regarding the specific target site of motilin for histamine release is unknown to date. There are three possible pathways that can be considered. One candidate is the central pathway. This possibility is supported by our findings that GPR38 mRNA is expressed in the nodose ganglion and the medulla oblongata [48], suggesting motilin affects vagus afferents and may stimulate acid output at its cephalic phase. Peripheral neural stimulation is the second candidate, as we showed that motilin excited cholinergic neural pathways in the myenteric plexus in an *in vitro* study of suncus; this enteric neural pathway may affect ECL cells. As a third candidate, the direct action of motilin on ECL cells cannot be ruled out. Further studies are needed for complete understanding of the target site of motilin, as well as the precise mechanism of motilin-induced gastric acid secretion.

2.5. Summary

In summary, the present study provides new findings that ghrelin alone has no effect on gastric acid secretion, but that motilin stimulates gastric acid secretion in a dose-dependent manner in suncus. Further studies using different animals, including humans, are needed to clarify the effect of motilin on gastric acid secretion. This may lead to useful knowledge in the clinical realm.

Chapter 3. **Motilin stimulates pepsinogen secretion in *Suncus murinus***

3.1. Introduction

3.1.1. Regulatory mechanism of pepsinogen secretion

Pepsinogen is a zymogen secreted by gastric chief cells that is activated by hydrochloric acid produced by parietal cells in the stomach mucosa to form the protein-digesting enzyme pepsin [3]. Pepsinogen is secreted in response to a meal but also occurs during the interdigestive state [79]. In vivo and in vitro studies on pepsinogen secretion have demonstrated that acetylcholine and cholinergic agents stimulate pepsinogen secretion and this response is inhibited by cholinergic antagonists [80,81], suggesting that muscarinic cholinergic receptors are directly and strongly associated with the stimulation of chief cells for pepsinogen secretion [3]. In addition, pepsinogen secretion appears to be regulated by several hormones. Some studies have shown that although histamine can stimulate pepsinogen secretion in vivo, it has no effect in isolated peptic cells from animal stomachs, suggesting that histamine acts as an indirect regulator of pepsinogen secretion by chief cells [3,82]. Similarly, gastrin and secretin stimulate pepsinogen secretion in vivo, but show weak effects in vitro [80]. Moreover, bombesin, glucagon, and somatostatin reportedly regulate pepsinogen release, while cholinergic agonists and cholecystokinin (CCK)-like peptides stimulate pepsinogen secretion by increasing intracellular calcium concentrations in gastric gland and chief cells [3]. Other studies have also reported the involvement of the vagus nerve in the stimulation of pepsinogen secretion [3].

3.1.2. Motilin and ghrelin, and their functions in gastrointestinal tract

Motilin, a gastrointestinal hormone consisting of 22 amino acids produced mainly in the duodenum [26], plays an important role in gastric contractions. Previous studies demonstrated that gastric contractions are strongly associated with plasma motilin levels and that gastric phase III-like contractions were induced by the intravenous administration of motilin in humans [9], dogs [6,63], and suncus [45,49]. Ghrelin, a well-known multifunctional hormone from the same peptide family as motilin, and originally isolated from rat and human stomachs [10], causes the initiation of food intake in many species after central or peripheral administration [13,53,56]. In addition, many studies revealed that ghrelin is an important regulator of the gastrointestinal tract [10,13,57]and its administration stimulates gastric acid secretion and gastric motility in the rat [21,61].

3.1.3. Advantages of suncus as an experimental animal for pepsinogen secretion study

Although motilin was discovered decades ago, limited studies on its physiological functions, other than those on gastrointestinal motility, have been conducted. The lack of a suitable small laboratory animal model is the most important hindrance to study the biological actions of motilin, as most commonly used experimental animals, i.e., mice and rats, lack the genes for motilin and its receptors [34]. Therefore, we have recently been using suncus as a laboratory animal to study the physiological actions of motilin and the role of the motilin-ghrelin peptide family on gastrointestinal function. Using this animal model, we showed that the migrating motor complex (MMC) repeatedly occurred at 90–120 min intervals in the interdigestive state, and motilin stimulated

gastric contractions in a dose-dependent manner, with a gastrointestinal motility pattern similar to that of humans and dogs [47,49]. The general appearance of the gastric mucosa in suncus is similar to that of humans, but differs from that of other widely used experimental animals, such as hamsters, rats, and mice [3]. For example, the suncus has no forestomach (stratified squamous epithelial region) such as that found in mouse and hamsters, and its gastric mucosa consists of glandular mucosa with well-developed luminal folds [3].

3.1.4. Gastric contraction and pepsinogen secretion

Recently, we found that motilin but not ghrelin stimulate gastric acid secretion in a dose dependent manner (manuscript preparation), and the intravenous administration of motilin causes gastric phase-III like contractions in suncus [61]. Gastric acid and pepsinogen secretion increase during the late phase II and phase III [83], and the higher luminal acid concentration leads to an increase in pepsinogen secretion in humans [84]. Considering these findings, we hypothesized that motilin may be involved in the regulation of pepsinogen secretion.

In this study, we established an experimental procedure for the measurement of pepsinogen secretion using a stomach perfusion system in anesthetized suncus to investigate the effect of ghrelin and motilin on pepsinogen secretion. In addition, we also examined the mechanism of motilin-induced pepsinogen secretion using receptor antagonists and vagotomized suncus.

3.2. Materials and Methods

3.2.1. Animals

As described in materials and methods of chapter 2 (Section 2.2.1.).

3.2.2. Drugs

The following drugs were used: Urethane (Sigma Aldrich, St. Louis, MO), histamine dihydrochloride (Nakarai Chemicals Co., Ltd., Kyoto, Japan), carbachol (Tocris Bioscience, Ellisville, MO), suncus motilin (Scrum Inc., Tokyo, Japan), and human acylated ghrelin (Asubio Pharma Co., Ltd., Hyogo, Japan). Atropine sulfate (Mylan Pharma, Osaka, Japan) was used as a muscarinic cholinergic receptor antagonist. Motilin and ghrelin were dissolved in 0.1 % BSA in PBS (phosphate-buffered saline), while histamine, carbachol, and atropine were dissolved in 0.9 % saline. All solutions were prepared immediately before each experiment. Each agent was administered in a volume of 100 μL per 100 g body weight (BW).

3.2.3. Determination of pepsin output using an intragastric perfusion experimental system

Prior to pepsinogen secretion experiments, overnight-fasted animals were anesthetized intraperitoneally with an injection of 15 % urethane solution at a dose of 1 mL/100 g body weight. After anesthesia, the animals' tracheas were exposed, cannulated, and exteriorized, and a catheter (polyethylene tube) was inserted through the mouth into the stomach, the tip was placed at approximately 3 mm from the cardia, and the tube was fixed to the esophagus with a suture. The stomach was exposed through an incision through the linea alba of the abdomen, and after exposing the pyloroduodenal junction,

another polyethylene tube was introduced into the stomach via an incision in the duodenum. The inserted polythene tube was secured firmly with a ligature around the pylorus, and the abdominal cavity was closed with sutures. Reagents/drugs were administered via a cannula inserted into the jugular vein. The stomach lumen was washed with saline solution until the effluent was clear, perfused at 37 ºC at a rate of 0.25 mL/min through the perfusion catheter using a peristaltic pump (Micro tube pump MP-3EYELA; Tokyo Rikakikai Co., Ltd., Tokyo), and the gastric output was collected through the liquid discharge tube. The body temperature of anesthetized suncus was monitored using a midi logger GL220 (Graphtec, Japan) and maintained at 35–38 ºC. In order to stabilize the amount of pepsinogen secreted, we allowed 60 min from the start of the saline perfusion before commencing effluent tube collection continuously at 10-min intervals using a DF-2000 fraction collector (Tokyo Rikakikai Co., Ltd., Tokyo, JAPAN).

3.2.4. Vagotomy

For experiments using vagotomized suncus, a truncal subdiaphragmatic vagotomy was performed as described previously [61]. In brief, the lower part of the esophagus was exposed to isolate the dorsal and ventral vagus nerves. Both branches of the vagus nerves were cut, and segments of about 3 mm in length were resected. All the neural connections in the resected area were completely peeled using a wiping tissue (Kimwipes; Nippon Paper Crecia, Tokyo, Japan). A stereomicroscope was used to confirm the complete nerve disconnection. After performing the experiments, the suncus were anatomized and completness of vagotomy was confirmed.

3.2.5. Pepsin measurement

Pepsin (pepsinogen) content in the gastric output was measured according to a previously described method [85]. Briefly, 100 µL of gastric content were incubated with 1 mL of 2 % hemoglobin (Sigma Aldrich, St. Louis, MO) for 10 min at 37 ºC, and 5 mL of 5 % trichloroacetic acid were added to stop the reaction. Following centrifugation (6000 g, 30 min), supernatants containing the soluble hydrolysis products were collected. The absorbance of the supernatants was measured at 280 nm, and the amount of pepsinogen secreted was calculated from a standard curve of authentic pepsin (Sigma Aldrich, St. Louis, MO), and expressed as µg/10 min. The magnitude of the change in gastric pepsinogen secretion was calculated by deducting the area under the curve (AUC) of gastric pepsin/pepsinogen secretion 50 min before and after the administration of each drug. The values were expressed as Δ µg/50 min.

3.2.6. Experimental protocols

All drugs/agents were administered by an intravenous (i.v.) bolus infusion. Carbachol was administered at a dose of 10 µg/kg of BW, while human acylated ghrelin and suncus motilin were both administered at a dose of 0.1, 1, and 10 µg/kg BW to study their stimulatory effect on pepsinogen secretion. Based on the dose response obtained, a motilin dose of 10 µg/kg BW was selected for subsequent experiments. Atropine (100 µg/kg BW) was used to evaluate the role of muscarinic acetylcholine (mACh) receptors on motilin-induced pepsinogen secretion. After confirming that pretreatment of atropine (100 µg/kg BW) completely inhibited carbachol-induced (10 µg/kg BW) pepsinogen secretion, pretreatment with vehicle or atropine was carried out 30 min before motilin administration (10 µg/kg BW).

3.2.7. Statistical analyses

Individual experiments were repeated and recorded at least three times. All data are shown as mean ± S.E.M. GraphPad Prism 5 software (GraphPad Software Inc., CA, USA) was used to analyze the data. Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons or Student’s t- test. $p < 0.05$ was considered statistically significant.

3.3. Results

3.3.1. Establishment of a perfusion system for pepsinogen measurement in suncus

We first examined the effect of carbachol (10 μg/kg BW) on pepsinogen secretion to establish a perfusion system for pepsinogen measurement in suncus. We found that i.v. administration of carbachol to urethane-anesthetized suncus increased pepsinogen secretion (488.1 ± 96.3 μg/50 min), in a manner comparable to that observed in other experimental animals (Fig. 12A). Pretreatment with atropine (100 μg/kg BW), a muscarinic acetylcholine receptor antagonist, abolished carbachol (10 μg/kg BW)-induced pepsinogen secretion (Fig. 12B). Compared to vehicle pretreatment, the amount of carbachol-induced pepsinogen secreted in 50 min was significantly reduced in atropine pretreated animals (Fig. 12C).

3.3.2. Effect of motilin on pepsinogen secretion

Although i.v. administration of vehicle (0.1% BSA in PBS) did not change basal pepsinogen (68.5 ± 10.9 μg/10 min) secretion, motilin caused a gradual increase after 10 min that reached a maximum within 20 min (Fig. 13A), and a return to baseline levels 40 min after administration (Fig. 13A). Motilin administration caused a dose-dependent increase in the levels of pepsinogen secretion of 56.1 ± 22.9, 172.3 ± 24.0 and 320.3 ± 49.2 μg/50 min at the doses of 0.1, 1.0, and 10 μg/kg BW, respectively (Fig. 13B).

3.3.3. Effect of ghrelin on pepsinogen secretion

By contrast, i.v. administration of vehicle and ghrelin at doses of 0.1, 1.0, and 10 μg/kg BW caused an unremarkable increase in pepsinogen secretion (Fig. 14A). The levels of

pepsinogen secreted in 50 min were 10.8 ± 13.8, 32.2 ± 9.8, and 49.5 ± 16.3 μg after administration of ghrelin at 0.1, 1.0, and 10 μg/kg BW, respectively. However, compared to vehicle, this increase was not statistically significant (Fig. 14B).

3.3.4. Effect of atropine on motilin-induced pepsinogen secretion

In order to examine whether the muscarinic acetylcholine receptor is the mediator of motilin-induced pepsinogen secretion, we next studied the effect of atropine on this response. Vehicle or atropine (100 μg/kg BW) was administered i.v. 30 min before the administration of motilin (10 μg/kg BW). Atropine treatment gradually decreased the baseline levels of pepsinogen secretion, which did not peak after motilin administration (Fig. 15B). The stimulatory effect of motilin on the 50-min cumulative pepsinogen secretion was completely inhibited (427.9 ± 61.5 μg/50 min vs 22.5 ± 26.1 μg/50 min) by atropine pretreatment, and this effect was significant compared to that of vehicle (Fig. 15C).

3.3.5. Effect of vagotomy on motilin-induced pepsinogen secretion

To clarify the role of the vagus nerve on motilin-induced pepsinogen secretion, we administered motilin to vagotomized and anesthetized suncus. However, pepsinogen secretion did not differ between vagotomized and control animals (Fig. 16A, B). Moreover, the net change in the 50-min cumulative pepsin output between control and vagotomized suncus after motilin administration was not statistically significant (Fig. 16C).

3.3.6. Effect of motilin and histamine on both pH and pepsinogen secretion

Measurement of pH and amount of pepsinogen secretion was done simultaneously after intravenous administration of motilin (10 µg/kg BW) and histamine (1 mg/kg BW). Motilin administration lowered the pH of the gastric output and increased the amount of pepsinogen secretion (Fig. 17A, B). But administration of histamine only decreased the pH without affecting the pepsinogen secretion. The pepsin output for 50-min that was changed after administration of motilin and histamine was statistically significant (Fig. 17C).

3.3.7. Effect of co-administration of motilin and ghrelin on pepsinogen secretion

Co-administration of motilin and ghrelin (10 µg/kg BW, each) caused a gradual increase after 10 min that reached a maximum within 20 min, and picked concentration of pepsin returned to baseline levels 40 min after administration (Fig. 18A). Motilin and ghrelin co-administration caused a dose-dependent increase in the levels of pepsinogen secretion of 66.8 ± 52.7, 268.9 ± 40.6 and 397.1 ± 76.3 µg/50 min at the doses of 0.1, 1.0, and 10 µg/kg BW, respectively (Fig. 18B). Co-administration of motilin and ghrelin at a dose of 1 and 10 µg/kg were significantly higher as compared with vehicle administration (Fig. 18B).

3.4. Discussion

3.4.1. Suncus as a suitable model animal for pepsinogen secretion experiments

As common laboratory rodents, such as rats and mice, lack genes for motilin and its receptor (GPR38) [34], these animals unsuitable for motilin studies. Thus, in this study we used suncus, a motilin and ghrelin-producing mammal, to study the physiological roles of motilin. While suncus was initially established as an animal model for emesis and vomiting research [72], we found that it not only produced motilin, but also expressed the motilin receptor [48]. In addition, the general appearance of the gastric mucosa and gastrointestinal motility, including motilin-induced gastric contractions during MMC in these animals appeared to be similar to those of humans and dogs [3,47,49]. Furthermore, the coordinating effect of suncus motilin and ghrelin on the regulation of the MMC of gastrointestinal motility was elucidated [45], and it was also found that motilin stimulate gastric acid release via the histaminergic pathway as described in part I. Considering the close relationship between acid and pepsinogen secretion, we hypothesized that motilin might be involved in the mechanism of pepsinogen secretion.

3.4.2. Motilin-induced pepsinogen secretion and its physiological importance

In this study, we found that intravenous administration of motilin but not ghrelin significantly stimulated pepsinogen secretion in a dose-dependent manner. Our results are consistent with a previous study which reported that exogenous administration of motilin increases pepsin output in dogs, with cyclical peaks in motilin plasma concentration coinciding with maximal pepsin secretory activity during the interdigestive state [79]. The cyclic release of motilin during the interdigestive period

induces MMC phase III gastric contractions in humans [9] and dogs [6,63], and its exogenous administration induces gastric phase III-like contractions in dogs [63], humans [9], and suncus [49]. The physiological importance of phase III MMC activity in the gastrointestinal tract is related to the mechanical and chemical cleansing of the empty stomach in preparation for the next meal [78]. Previously, we found that motilin stimulates gastric acid secretion and this study showed that it can also stimulate pepsinogen secretion in a dose dependent manner. As previous study showed that increasing acid concentrations led to an increase in pepsin output [84], we also investigated whether motilin-induced gastric acid production stimulated pepsinogen secretion. However, intravenous administration of histamine (1 mg/kg BW) did not show any effect on pepsinogen secretion, despite causing a dramatic reduction in gastric pH. As pepsinogen is converted to active pepsin in the presence of gastric acid to enable protein digestion [3], our findings suggest that motilin-induced gastric acid and pepsinogen secretion may facilitate the complete digestion and food removal from the stomach. During the interdigestive MMC, motilin-induced gastric phase III activity and pepsinogen secretion lead to mechanical food removal and chemical gastric cleansing by breaking down proteins into smaller peptides, respectively, thus enhancing gastric emptying. Hence, impaired gastric phase III activity may cause the retention of gastric content for a longer period and bacterial overgrowth.

Acid and pepsinogen are considered the two major factors involved in the development of gastric ulcers, with pepsinogen being considered a pathogenic factor. Furthermore, duodenal ulcerogenesis in rats was found to be related to the combined effect of acid and endogenous rat pepsin [86]. Another study also showed that pepsin plays an important role in the pathogenesis of ischemia/reperfusion-induced gastric lesions [87].

Cyclical increases in gastric contractile activity and increased plasma immunoreactive motilin concentrations were associated with an intermittent increase in pepsin output during the interdigestive state [79]. During fasting, cyclic release of gastric acid and pepsinogen for a long period may contribute to the development of gastric lesions. To the best of our knowledge, studies investigating the relationship between motilin and peptic ulcer have not been conducted. Thus, further studies are necessary to achieve a full understanding of the physiological role of motilin-induced pepsinogen secretion.

3.4.3. Motilin but not ghrelin stimulates pepsinogen secretion through cholinergic pathway

We observed that motilin-induced pepsinogen secretion was completely inhibited by atropine pretreatment (a muscarinic acetylcholine receptor antagonist), suggesting a cholinergic receptor-mediated response. In addition, results from a previous study also showed that atropine suppressed motilin-induced pepsin output, indicating that pepsinogen secretion was regulated by motilin through an intramural cholinergic pathway. However, the specific pathway for motilin-induced cholinergic receptor activation and pepsinogen secretion is unknown to date. In this study, we did not observe a significant change in motilin-induced pepsinogen secretion in vagotomized suncus compared to control animals, indicating that this response was not mediated by the vagus nerve. Thus, there is a possibility that motilin may induce cholinergic stimulation of pepsinogen secretion through the enteric nervous system, as we found GPR38 mRNA expressed in both gastric mucosa and muscle layers in these animals. Further studies are required to elucidate whether motilin directly stimulates pepsinogen secretion by binding to GPR38 expressed in chief cells or through an indirect pathway.

3.5. Summary

In summary, this study demonstrated that unlike ghrelin, which had no effect on pepsinogen secretion, motilin stimulated pepsinogen secretion in a dose dependent manner through a cholinergic pathway in suncus. Detailed future studies on the mechanism of motilin-induced pepsinogen secretion are important to understand the pathophysiology of gastrointestinal diseases and facilitate drug development.

Conclusion

Motilin and ghrelin are gastrointestinal hormones that stimulate the migrating motor complex (MMC) of gastrointestinal motility during the fasting state. In this study, we examined the effect of motilin and ghrelin on gastric acid and pepsinogen secretion in anesthetized suncus (house musk shrew, Suncus murinus), a ghrelin- and motilin-producing mammal. I observed that motilin but not ghrelin can stimulate gastric acid and pepsinogen secretion in suncus. Acid output stimulated by motilin and by the co-administration of motilin and ghrelin were mediated through a histamine receptor, whereas the pepsinogen secretion stimulated by motilin involved the cholinergic pathway. Gastric acidity and pepsinogen secretion caused by motilin in the interdigestive state may be involved in the regulation of the intragastric environment.

Abbreviations

CCK, cholecystokinin;

CCK-B, cholecystokinin type B;

MMC, migrating motor complex;

BSA, bovine serum albumin;

PBS, phosphate-buffered saline;

DMSO, dimethyl sulfoxide;

AUC, area under curve;

mACh, muscarinic acetylcholine;

ANOVA, analysis of variance;

PACAP, pituitary adenylate cyclase activating polypeptide;

ECL, Enterochromaffin-like;

GPR38, G protein-coupled receptor 38;

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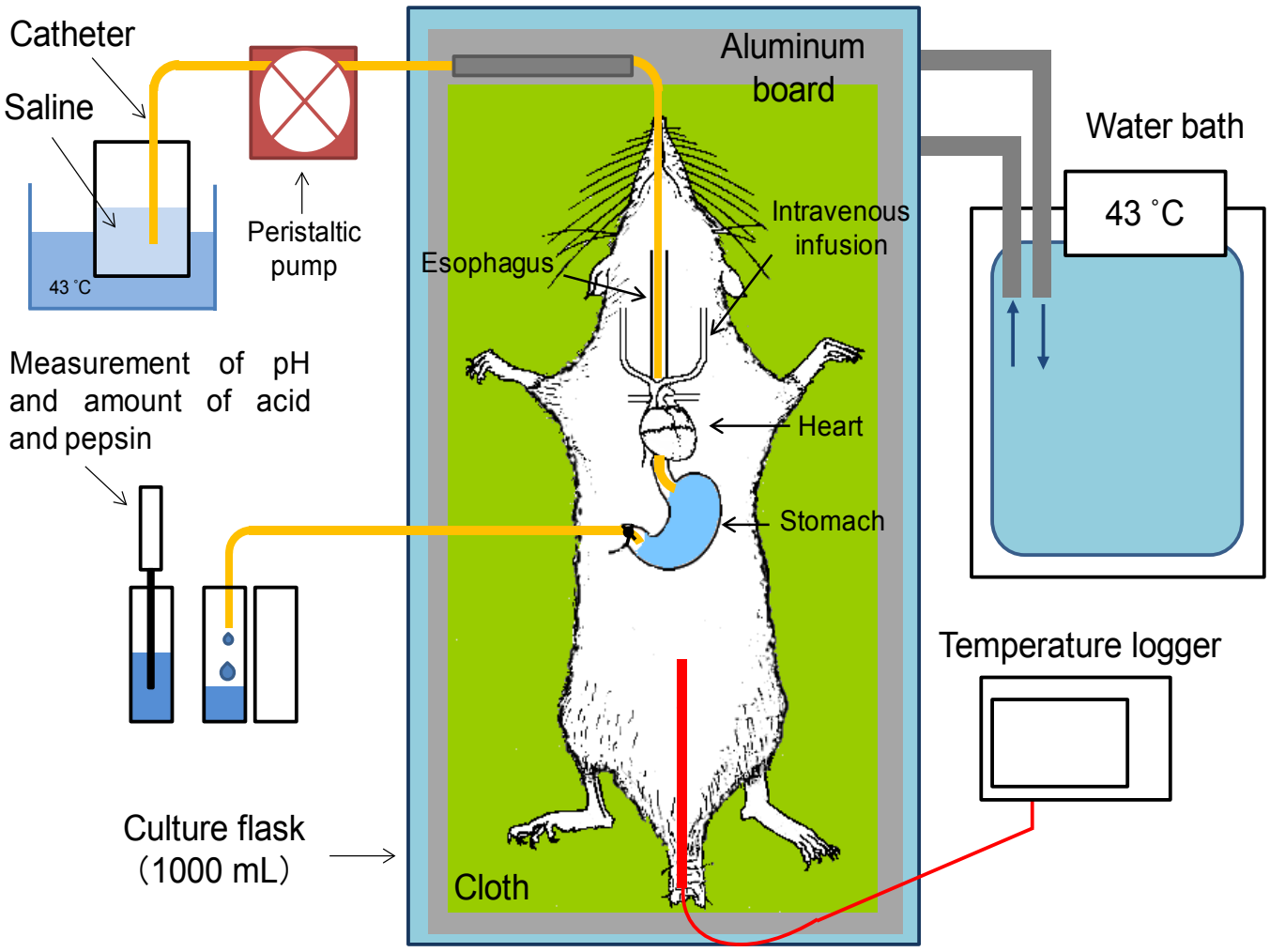

Figure 1: Schematic representation of perfusion setup for gastric acid and pepsin secretion experiment in suncus. After anesthesia, a suncus was placed on a culture flask containing circulating hot water from a water bath. A logger was used to monitor body temperature during the experiment. Perfusion of saline solution was done by a peristaltic pump, and gastric output was collected through a catheter from the stomach at intervals of 10 minutes. Effluents were used to measure pH and acid output. Intravenous drug administration was done through the jugular vein.

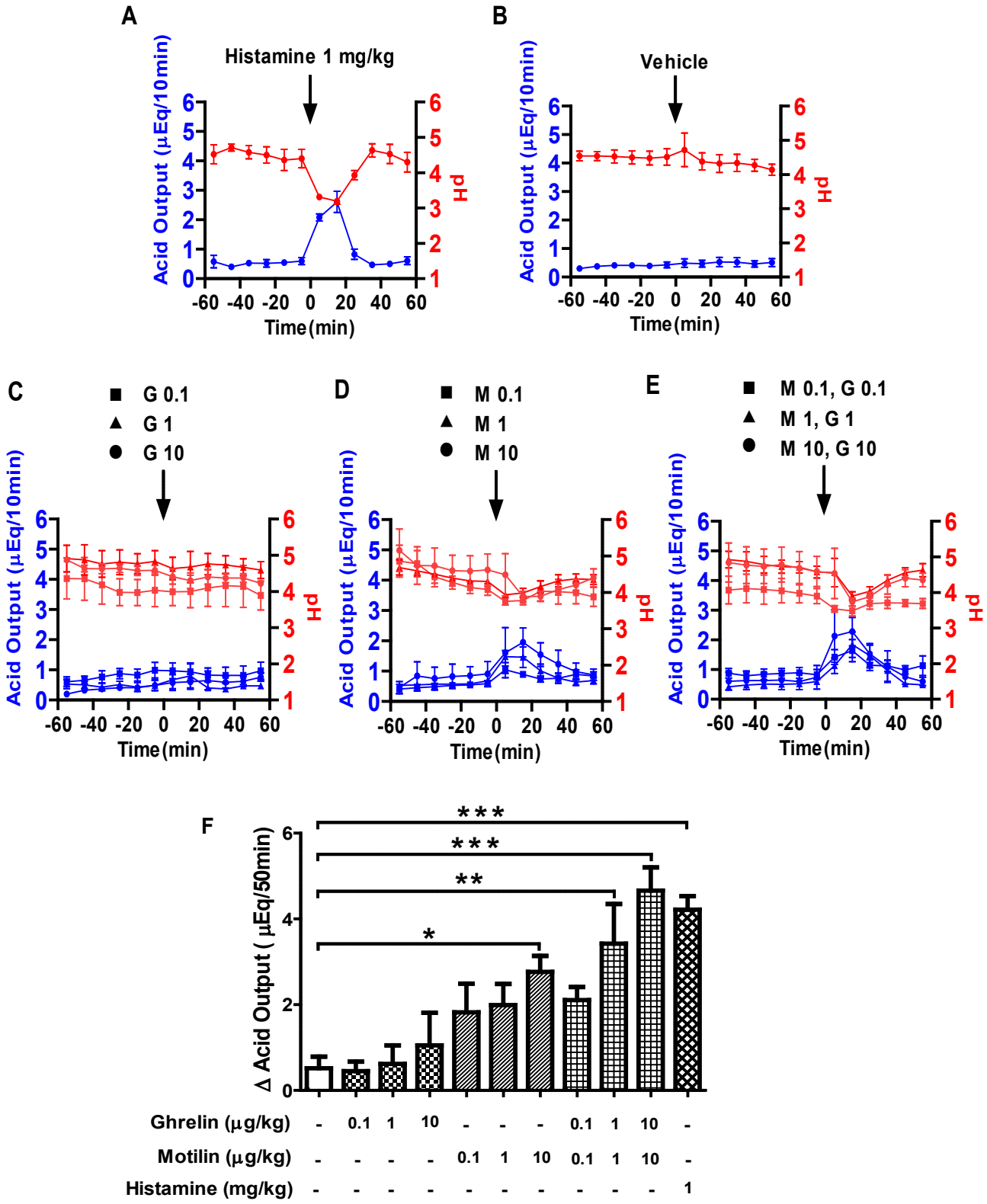

Figure 2: Effects of motilin and ghrelin on gastric acid secretion. In urethane-anaesthetized suncus, after a 60-min basal period, gastric acid secretion was measured after administration of histamine (1 mg/kg BW) (A), vehicle (B), ghrelin (0.1, 1.0, and 10 µg/kg BW) (C), motilin (0.1, 1.0, and 10 µg/kg BW) (D), and co-administration of motilin and ghrelin (E). Gastric acid secretion (blue line) and pH (red line) changes were monitored at 10-min intervals throughout the experiment. The net change in cumulative acid output for 50 min after each administration was also calculated (F). Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. G: ghrelin; M: motilin, figures after the abbreviations denote concentration in µg/kg. n = 3-7.

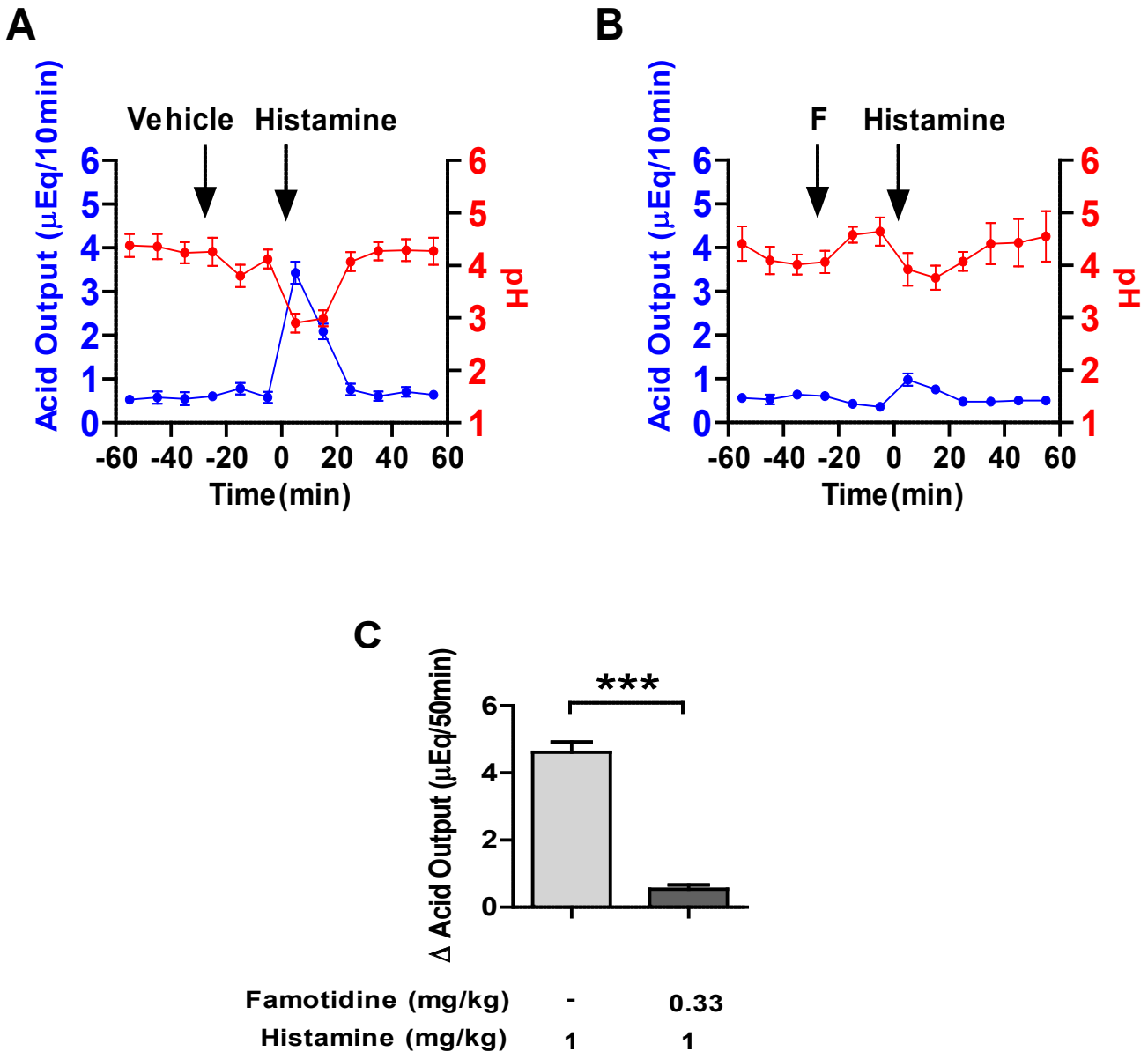

Figure 3: Effects of famotidine on histamine-stimulated gastric acid secretion. Gastric acid secretion was stimulated with histamine (1 mg/kg BW) with or without famotidine (0.33 mg/kg BW). Vehicle or famotidine was administrated intravenously 30 min before histamine treatment as shown in figure A and B, respectively. Gastric acid secretion (blue line) and pH (red line) changes were monitored at 10-min intervals throughout the experiment. The net change in cumulative acid output for 50 min after administration of histamine with pre-administration of vehicle or famotidine. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. F: famotidine. n = 4.

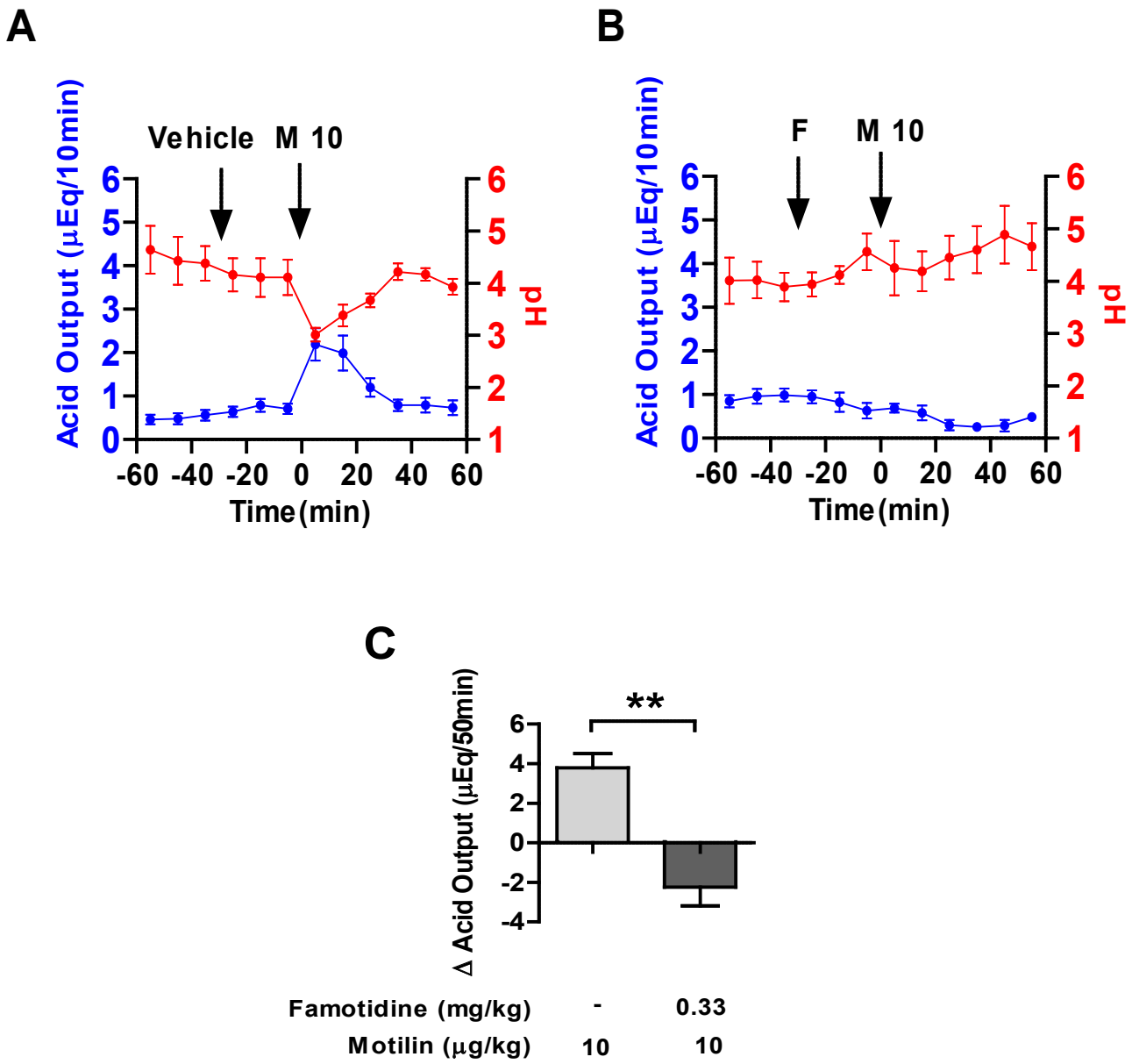

Figure 4: Effects of famotidine on motilin-induced gastric acid secretion. Intravenous administration of vehicle or famotidine was done 30 min before motilin (A, B) administration. Blue lines represent gastric acid secretion and red lines for pH changes which were monitored in an interval of 10-min throughout the experiment. The net change in cumulative acid output for 50 min after administration of motilin (C) with pre-administration of vehicle or famotidine. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. F: famotidine; M: motilin, figures after the abbreviations denote concentration in μg/kg. n = 3.

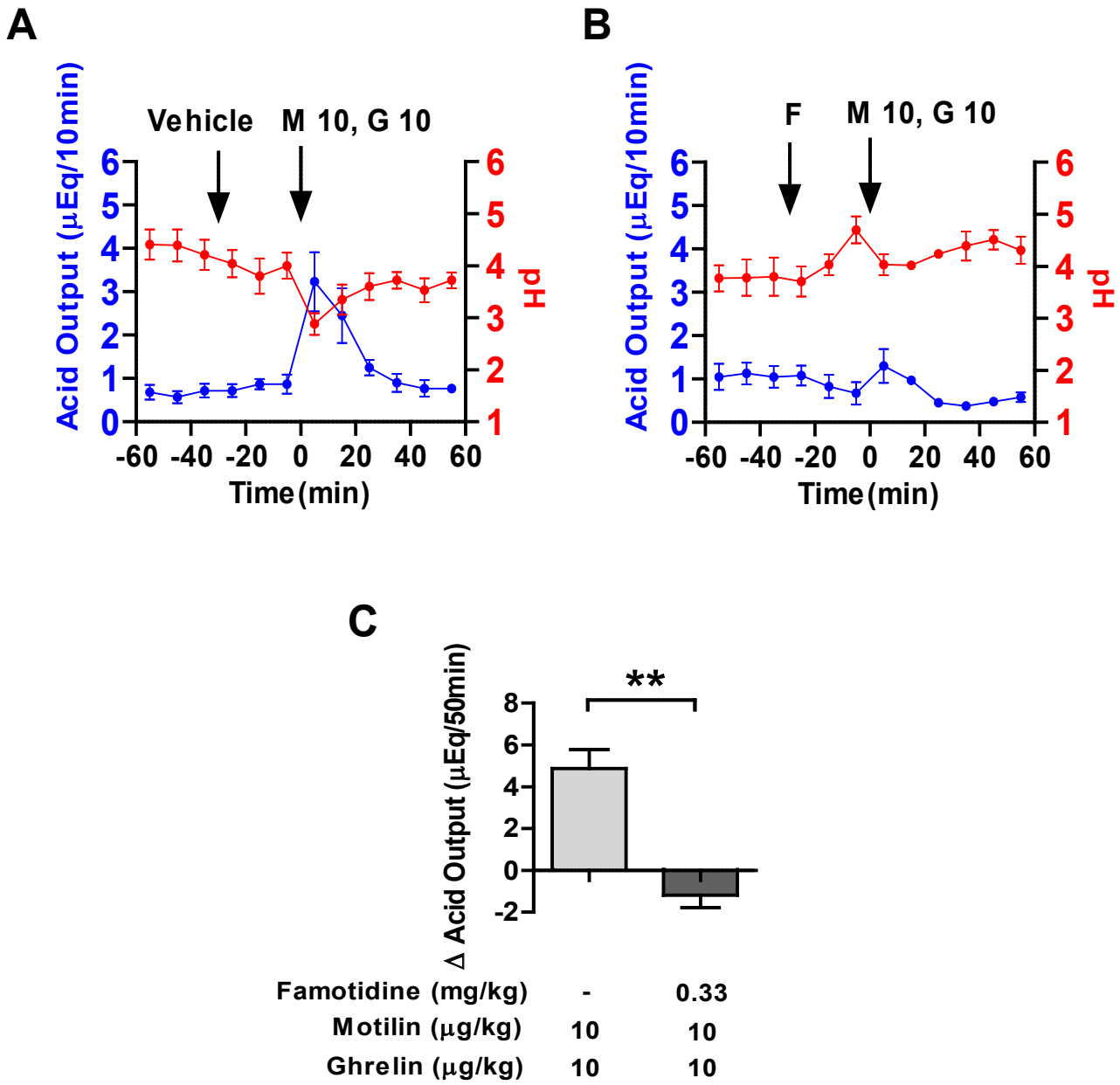

Figure 5: Effects of famotidine on gastric acid secretion stimulated by co-administration of motilin and ghrelin. Gastric acid secretion was stimulated with co-administration of motilin (10 μg/kg BW) and ghrelin (10 μg/kg BW) with or without famotidine (0.33 mg/kg BW). Intravenous pretreatment of vehicle or famotidine was performed 30 min before co-administration of motilin and ghrelin as shown in A and B, respectively. Gastric acid secretion (blue line) and pH (red line) changes were observed at 10-min intervals. The net changes in cumulative acid output were calculated for 50 min after co-administration of motilin and ghrelin (I) with pre-treatment of vehicle or famotidine. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. F: famotidine; G: ghrelin; M: motilin, figures after the abbreviations denote concentration in μg/kg. n = 3.

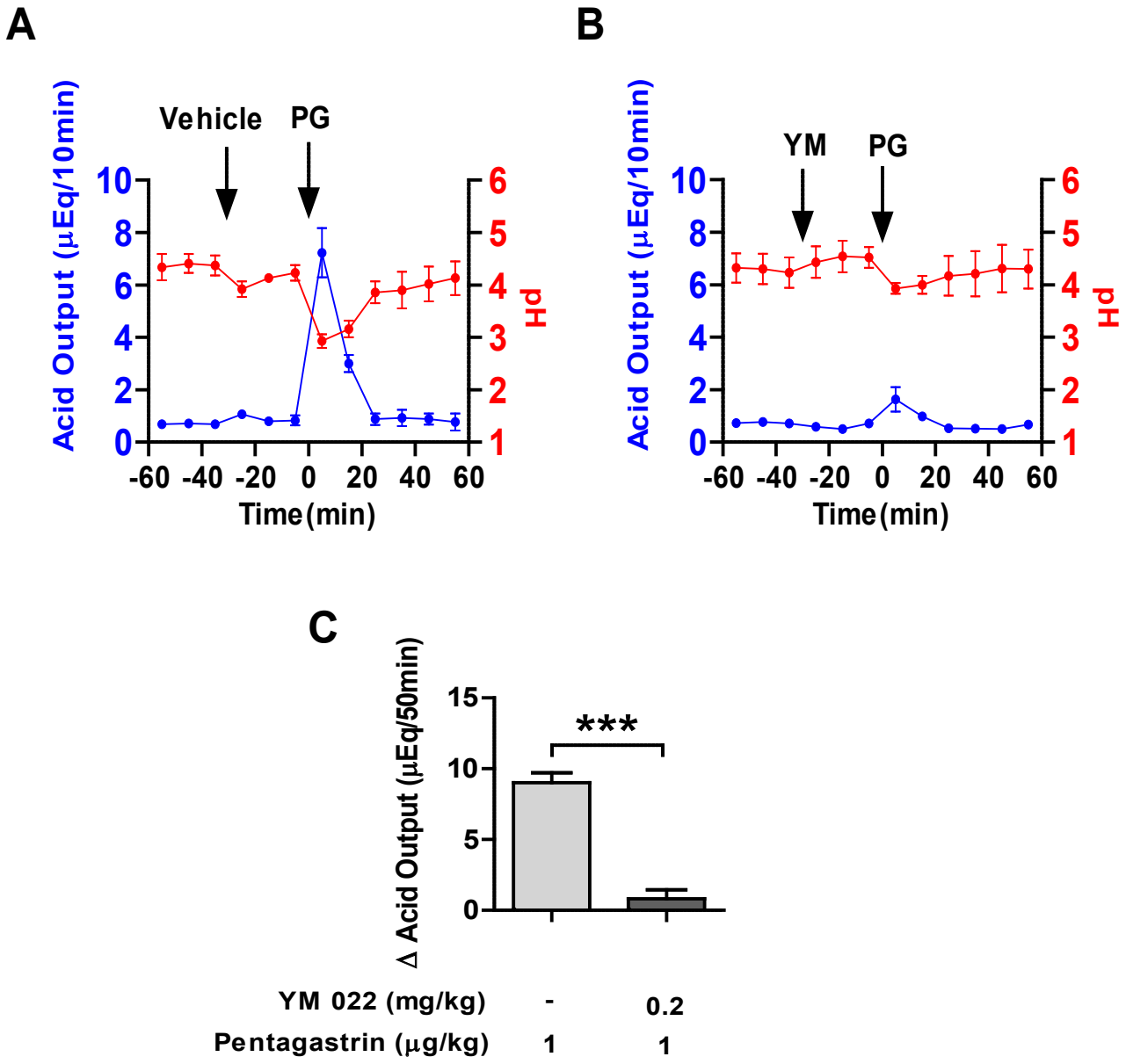

Figure 6: Effects of YM 022 on pentagastrin-stimulated gastric acid secretion. Gastric acid secretion (blue line) and pH (red line) changes at 10-min intervals after intravenous administration of vehicle or YM 022 (0.2 mg/kg BW) followed by pentagastrin (1 μg/kg BW) (A, B) throughout the experiment. The net change in cumulative acid output for 50 min after administration of pentagastrin (C) with or without YM 022 pretreatment. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. YM: YM 022; PG: pentagastrin. n = 3.

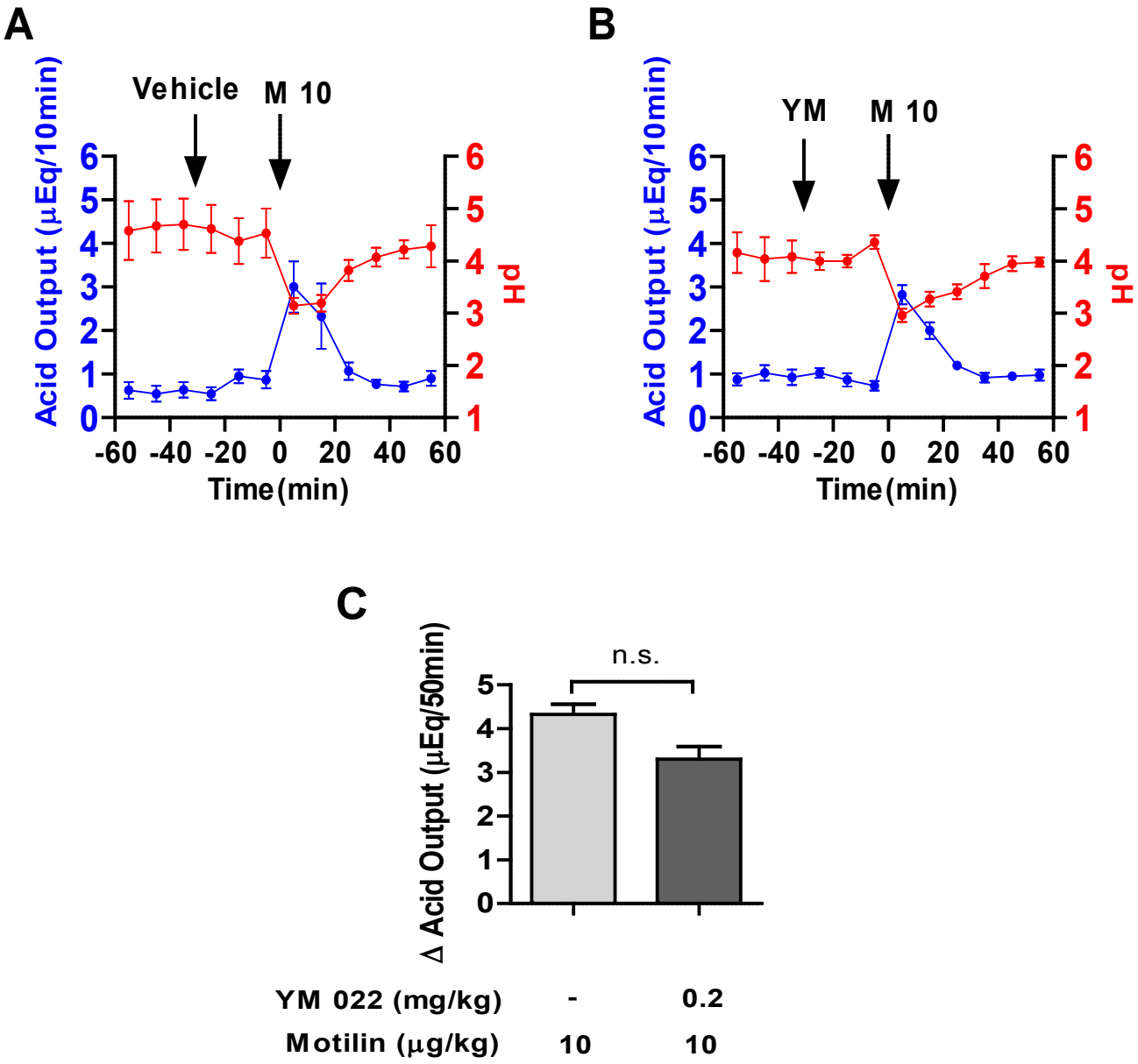

Figure 7: Effects of YM 022 on motilin-induced gastric acid secretion. Gastric acid secretion as represented in blue line and pH changes in red line were monitored at 10-min intervals after intravenous administration of vehicle/YM 022 (0.2 mg/kg BW) followed by motilin (10 μg/kg BW) (A, B) throughout the experiment. The net change in cumulative acid output for 50 min after administration of motilin with vehicle or YM 022 pretreatment was also compared as shown in C. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. YM: YM 022; M: motilin, figures after the abbreviations denote concentration in μg/kg. n = 3. n.s.: non-significant.

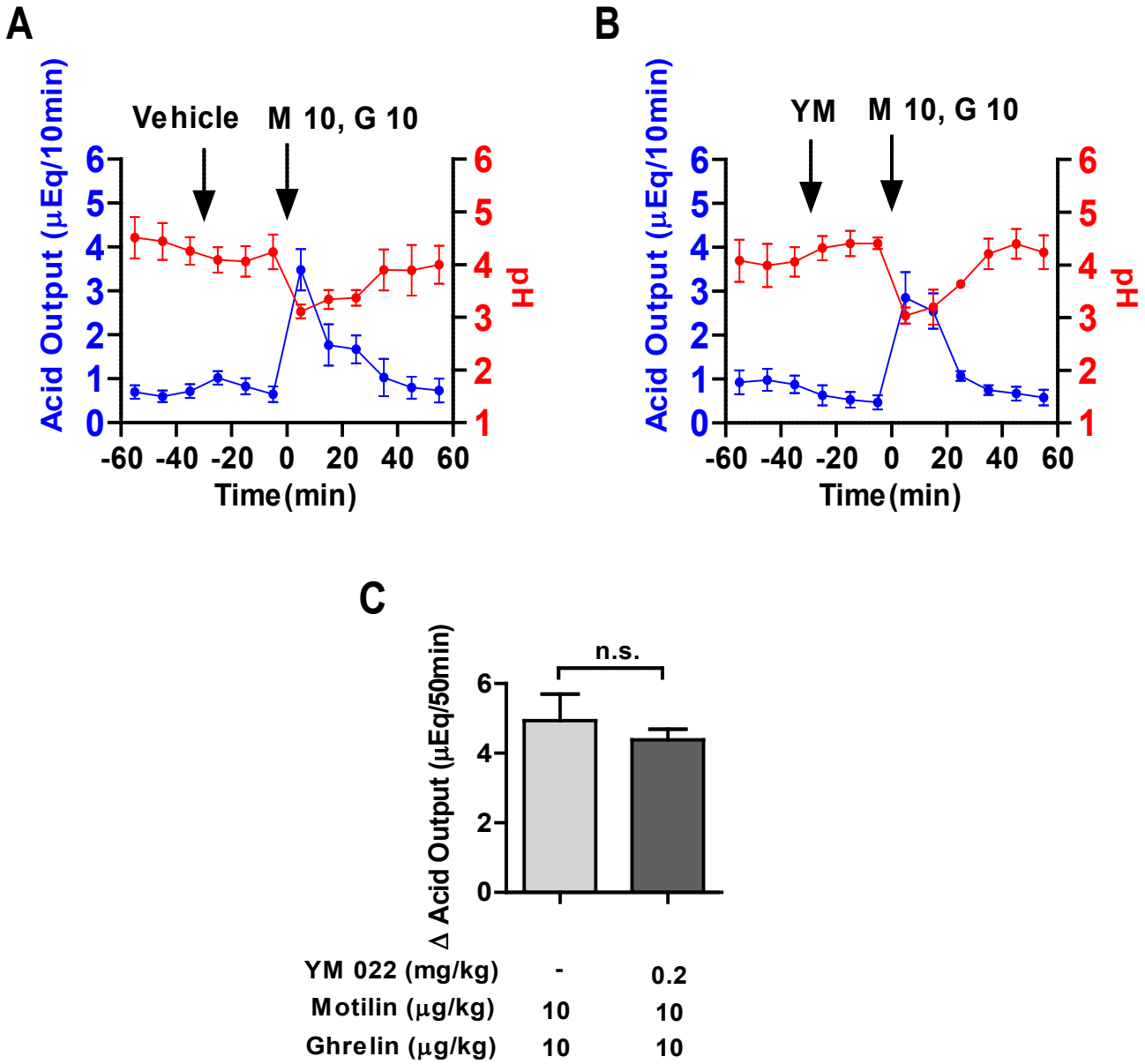

Figure 8: Effects of YM 022 on gastric acid secretion stimulated by co-administration of motilin and ghrelin. A and B represent the gastric acid secretion (blue line) and pH (red line) changes at 10-min intervals after intravenous administration of vehicle or YM 022 (0.2 mg/kg BW) followed by co-administration of motilin (10 μg/kg BW) and ghrelin (10 μg/kg BW) throughout the experiment. Effect of pretreated vehicle or YM 022 on net change in cumulative acid output for 50 min after co-administration of motilin and ghrelin (C) was also calculated. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. YM: YM 022; M: motilin; G: ghrelin, figures after the abbreviations denote concentration in μg/kg. n = 3. n.s.: non-significant.

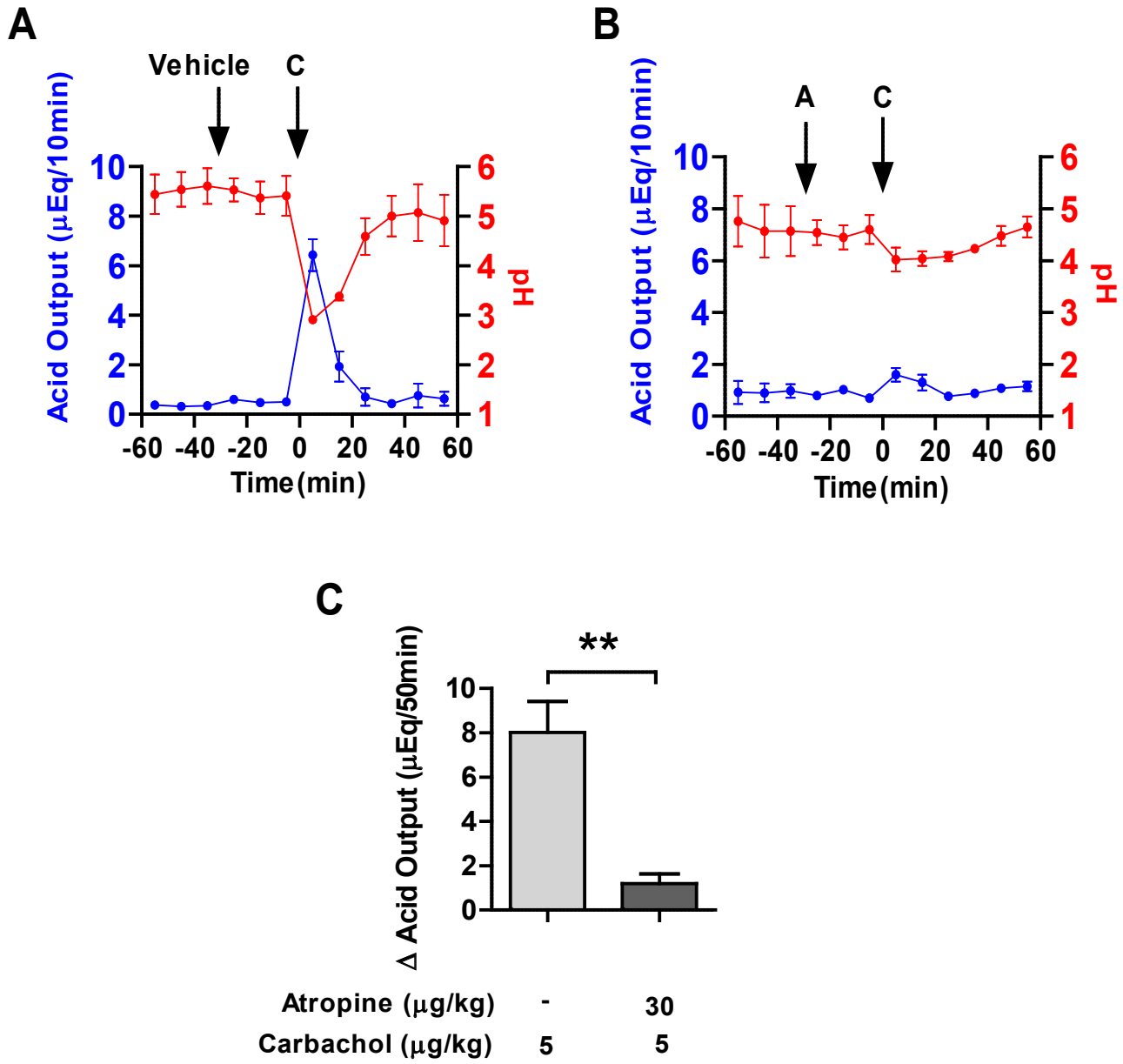

Figure 9: Effects of atropine on carbachol-stimulated gastric acid secretion. Gastric acid secretion (blue line) and pH (red line) changes were monitored in an interval of 10-min throughout the experiment. Intravenous administration of either vehicle or atropine (30 µg/kg BW) was done 30 min before carbachol (5 µg/kg BW) (A, B) treatment. Comparison was done between th net changes in cumulative acid output for 50 min after administration of carbachol (C) with vehicle or atropine pretreatment. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. A: atropine; C: carbachol. n = 3.

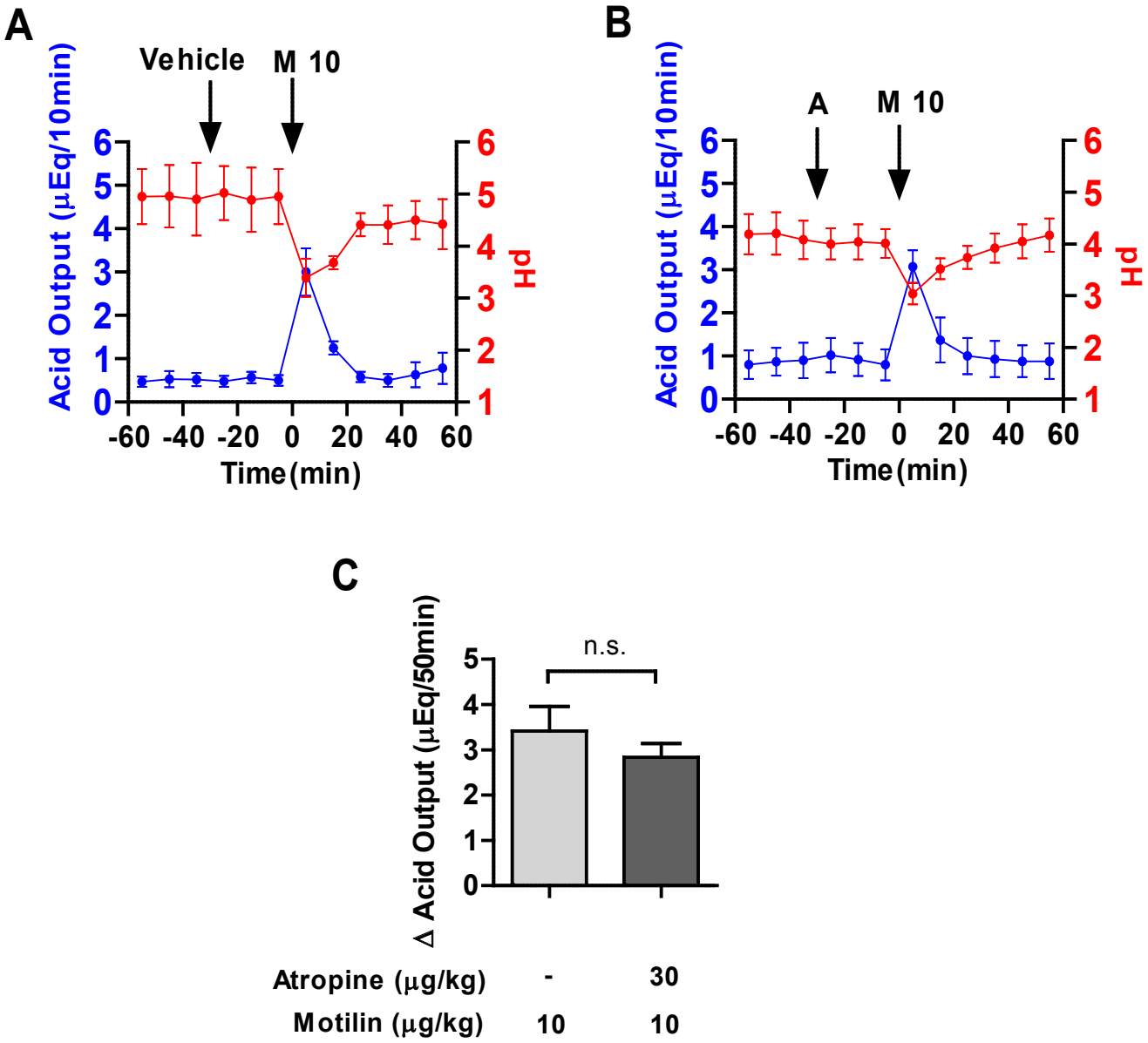

Figure 10: Effects of atropine on motilin-stimulated gastric acid secretion. In an interval of 10-min, gastric acid secretion (blue line) and pH (red line) changes were observed throughout the experiment. Intravenous administration of either vehicle or atropine (30 μg/kg BW) was done 30 min before motilin (10 μg/kg BW) (A, B) treatment. The net change in cumulative acid output for 50 min after administration of motilin (C) with vehicle or atropine pretreatment was also measured. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. A: atropine; M: motilin, figures after the abbreviations denote concentration in μg/kg. n = 3. n.s.: non-significant.

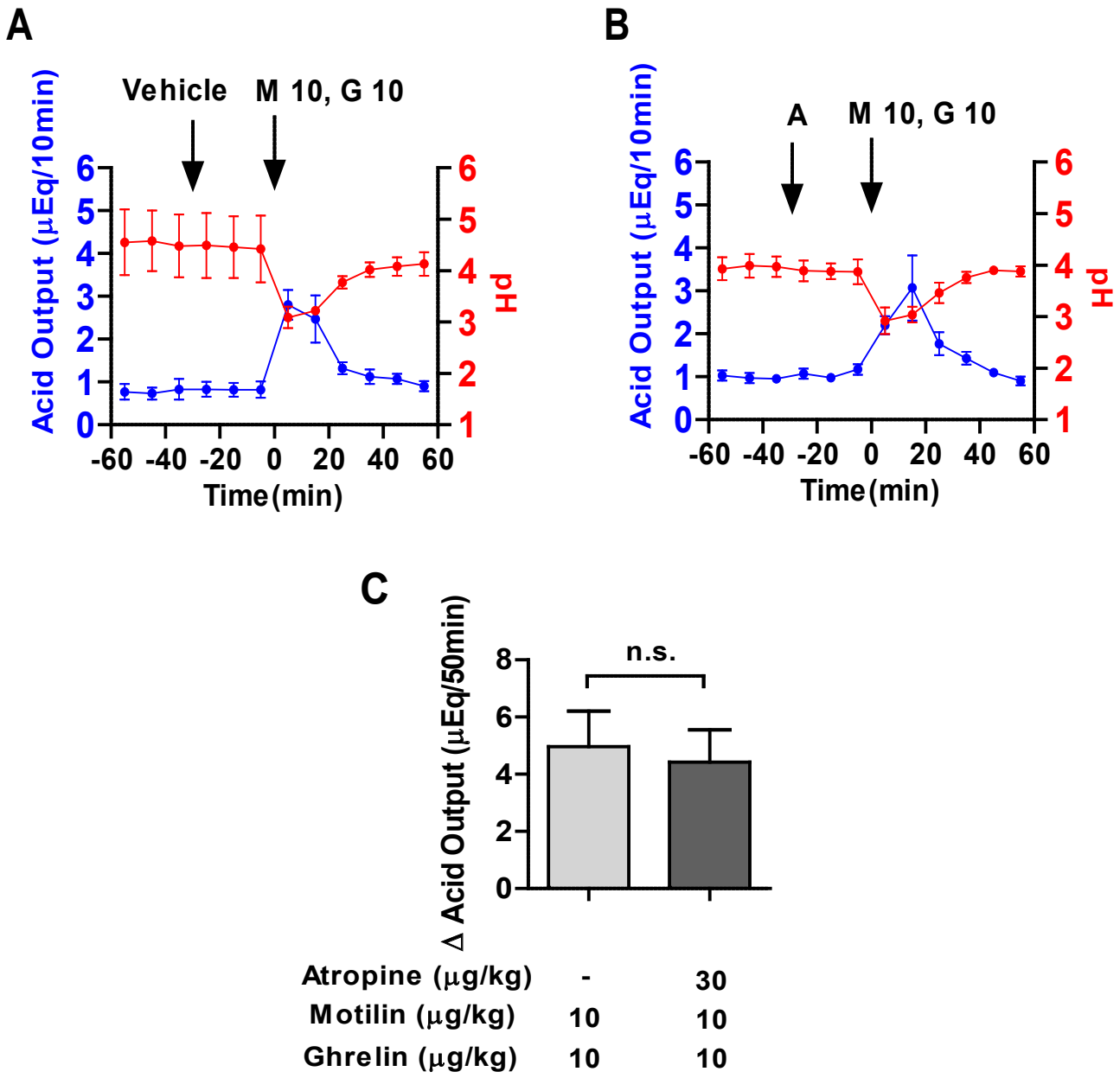

Figure 11: Effects of atropine on gastric acid secretion stimulated by co-administration of motilin and ghrelin. Gastric acid secretion (blue line) and pH (red line) changes were monitored at 10-min intervals throughout the experiment. According to A and B, Intravenous administration of either vehicle or atropine (30 μg/kg BW) was done 30 min before co-administration of motilin (10 μg/kg BW) and ghrelin (10 μg/kg BW). Statistical analysis was done between the net changes in cumulative acid output for 50 min after co-administration of motilin and ghrelin (C) for vehicle or atropine pretreatment. Each value represents the mean ± SEM. $p < 0.05$ was considered statistically significant. A: atropine; G: ghrelin; M: motilin, figures after the abbreviations denote concentration in μg/kg. n = 3. n.s.: non-significant.

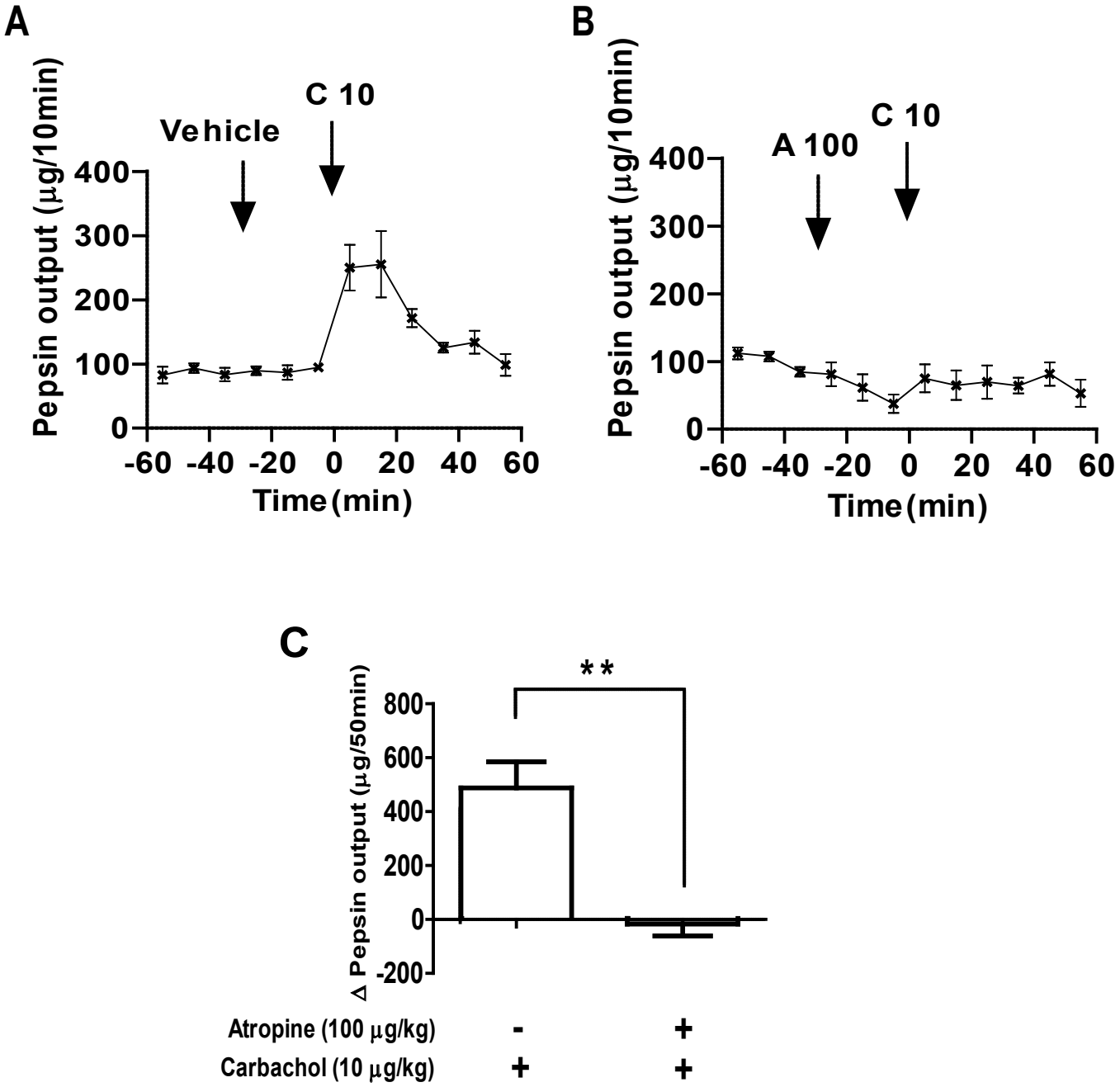

Figure 12: Effect of carbachol on pepsinogen secretion. Pepsinogen secretion was measured after a 60-min basal period following the administration of carbachol to urethane-anaesthetized suncus (10 µg/kg BW) pretreated with vehicle (A) or atropine (B), a muscarinic cholinergic receptor antagonist. Changes in pepsin output were monitored at 10-min intervals throughout the experiment. The net change in the 50-min cumulative pepsin output was calculated for each treatment (C). Pre-treatment with atropine inhibited carbachol-induced pepsinogen secretion. Each value represents the mean ± SEM (n = 3). C: carbachol; A: atropine; figures after the abbreviations denote the concentration in µg/kg. **$p < 0.01$.

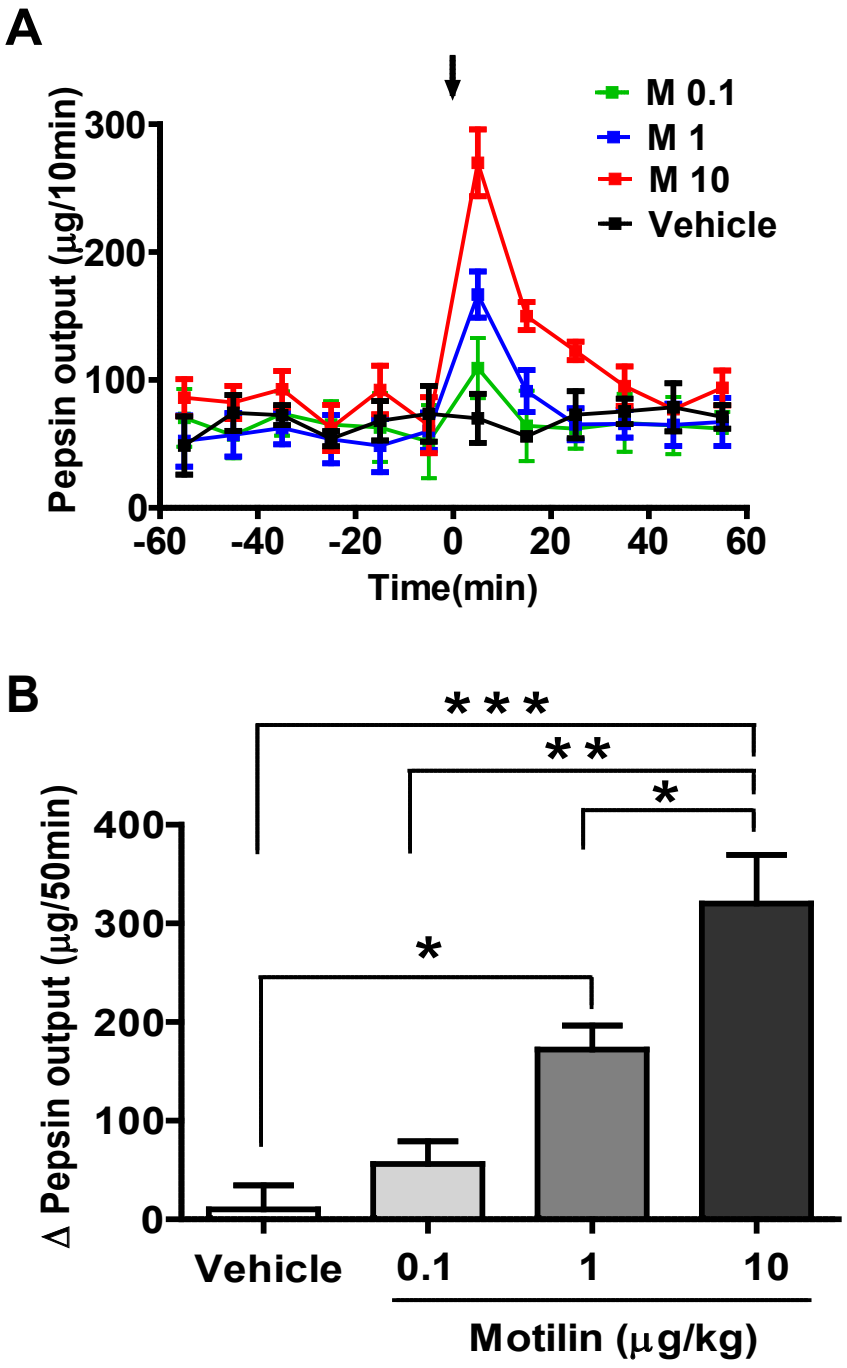

Figure 13: Effects of motilin on pepsinogen secretion. Pepsinogen secretion was measured after vehicle and motilin administration (0.1, 1 and 10 µg/kg BW), and pepsin outputs were monitored at 10-min intervals throughout the experiment (A). The net changes in the 50-min cumulative pepsin output were calculated after administration of vehicle and different motilin doses (B). Motilin stimulated pepsinogen secretion in a dose dependent manner. Each value represents the mean ± SEM (n = 3; M 1, n = 4). M: motilin; figures after the abbreviations denote concentrations in µg/kg. *p < 0.05, **p < 0.01, ***p < 0.001.

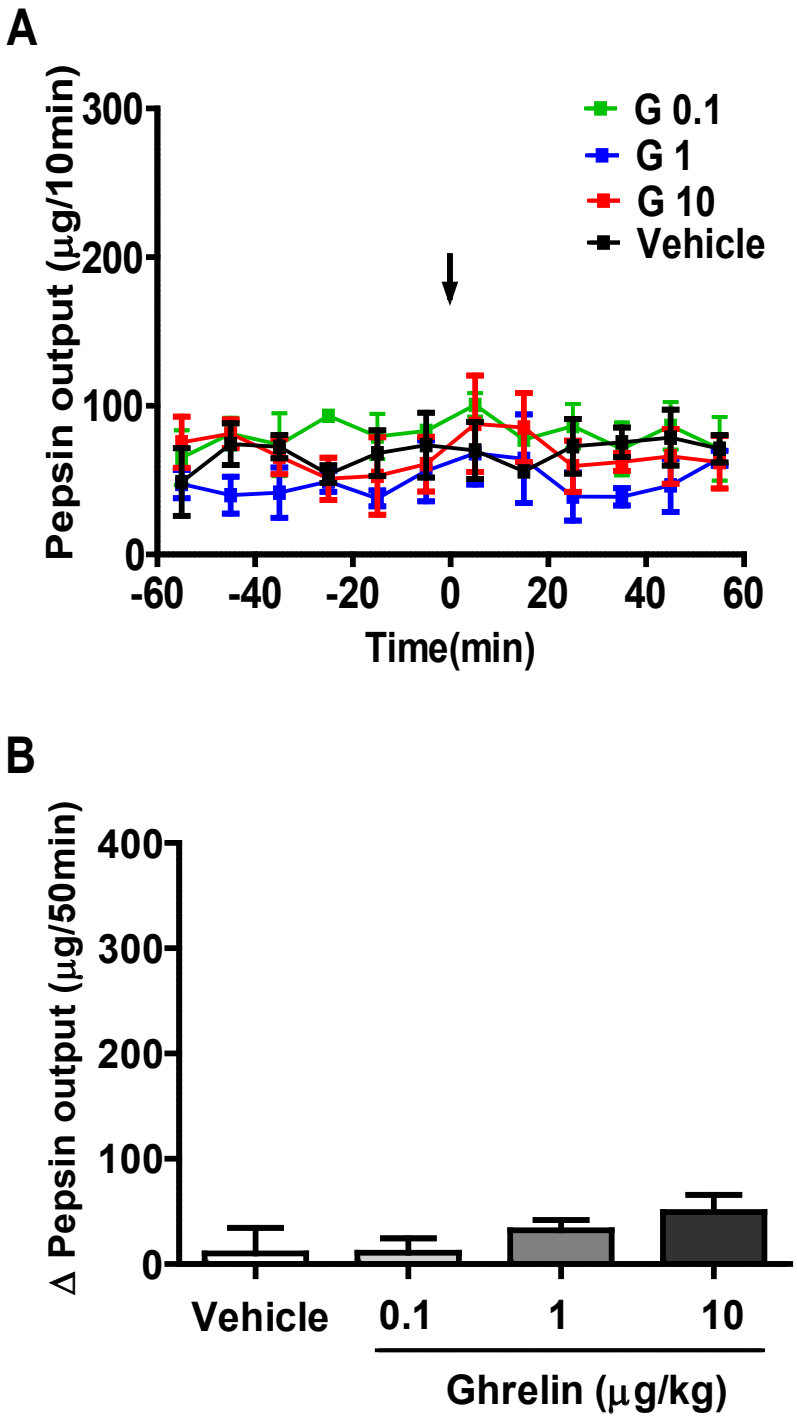

Figure 14: Effects of ghrelin on pepsinogen secretion. Changes in pepsin output at 10-min intervals after intravenous administration of vehicle and ghrelin (0.1, 1 and 10 µg/kg BW) throughout the experiment (A). After administration of vehicle and different doses of ghrelin, changes in the 50-min cumulative pepsin output were estimated (B). Ghrelin had no significant effect on pepsinogen secretion. Each value represents the mean ± SEM (n = 3). G: ghrelin; figures after the abbreviations denote concentrations in µg/kg.

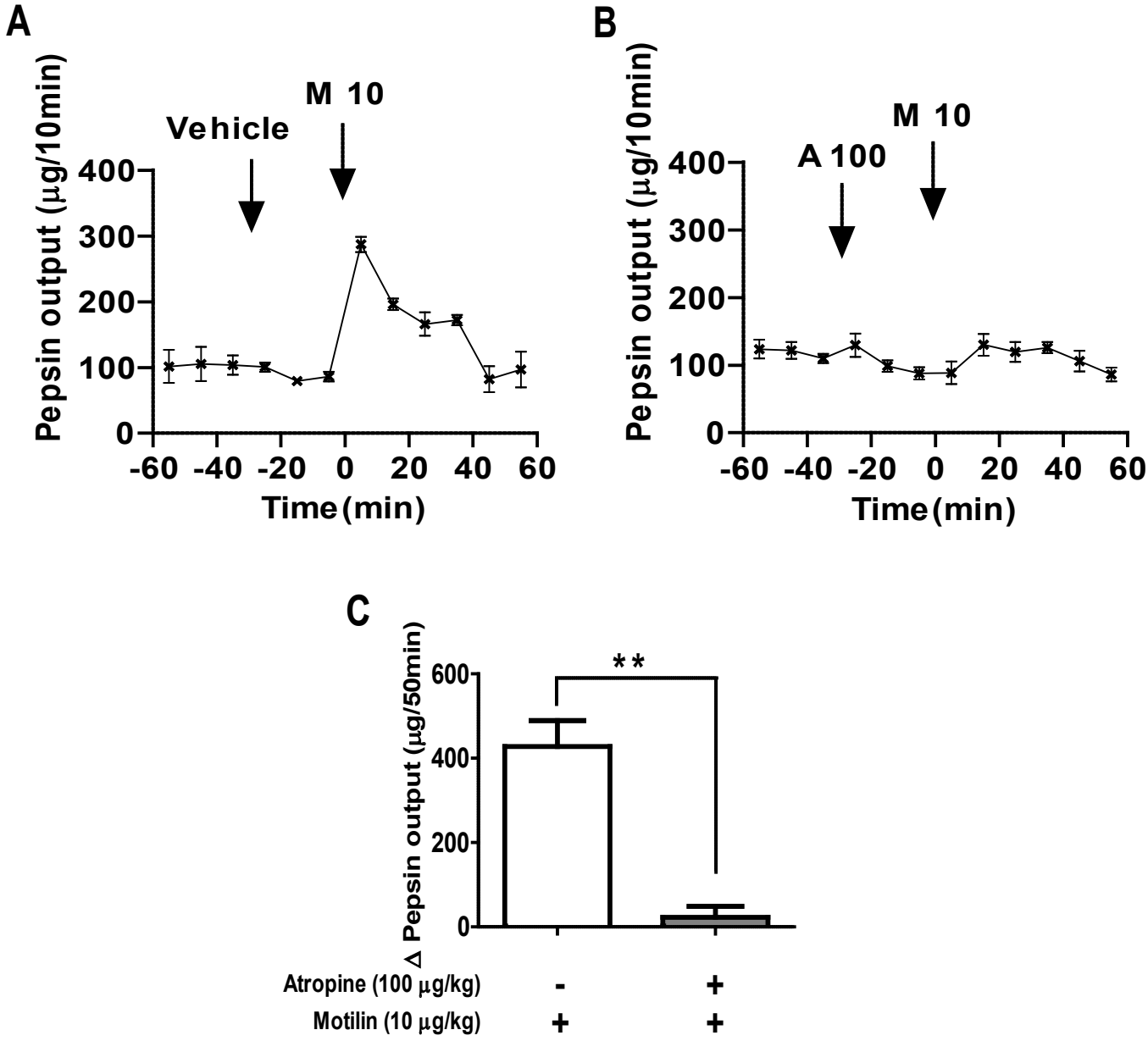

Figure 15: Effect of atropine on motilin-induced pepsinogen secretion. Vehicle or atropine (100 μg/kg BW) were administered intravenously 30 min before motilin (10 μg/kg BW) treatment (A, B). Changes in pepsin output were monitored at 10-min intervals throughout the experiment. The net change in the 50-min cumulative pepsin output after administration of motilin with or without atropine pretreatment (C) calculated. Atropine pretreatment abolished motilin-induced pepsinogen secretion. Each value represents the mean ± SEM (n = 3). A: atropine; M: motilin; figures after the abbreviations denote concentrations in μg/kg. **$p < 0.01$.

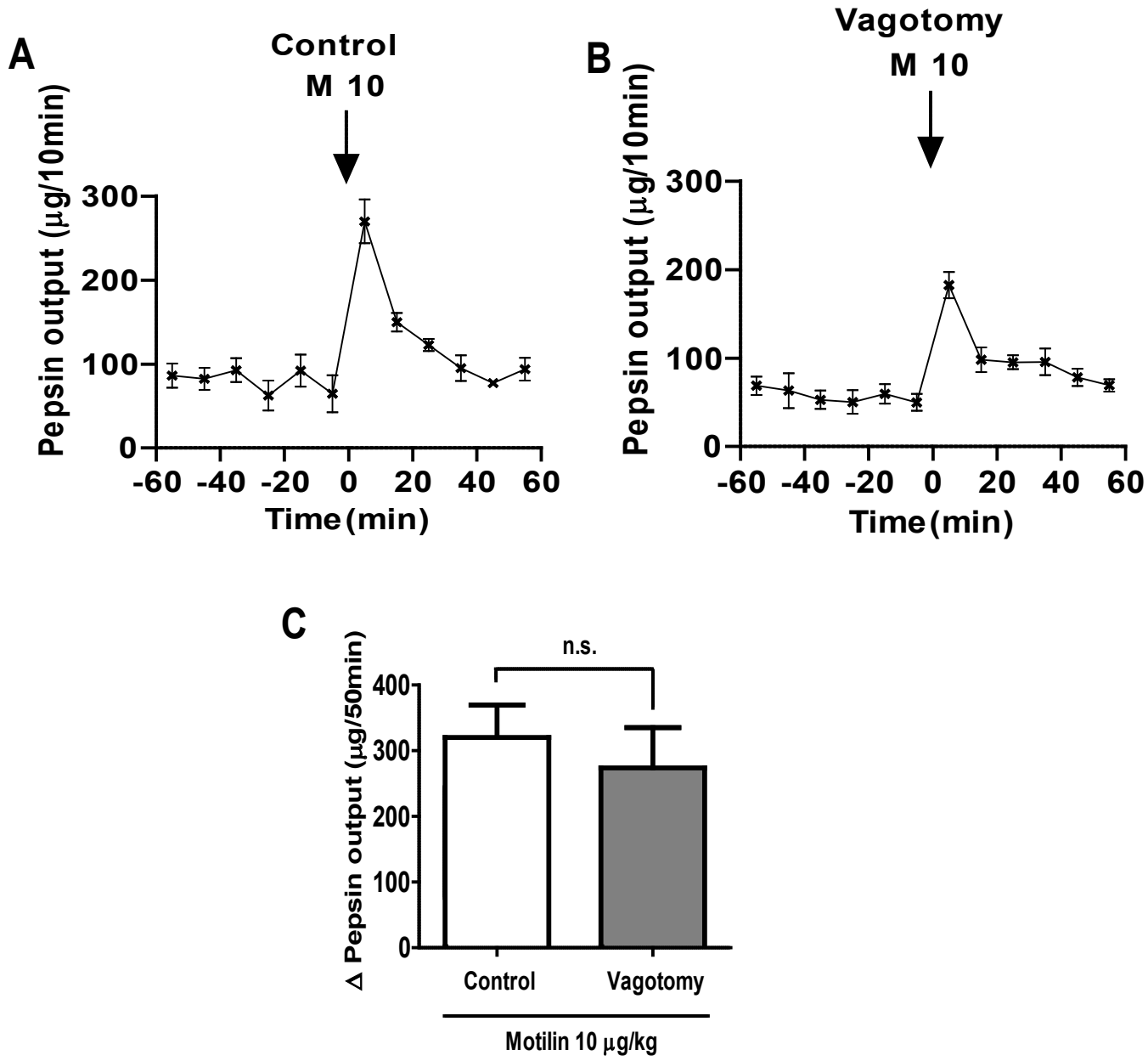

Figure 16: Effect of vagotomy on motilin-induced pepsinogen secretion. Motilin-induced pepsin output was observed in control (A) and vagotomized (B) suncus. The net changes in the 50-min cumulative pepsin output in control and vagotomized suncus were calculated. Vagotomy had no effect on motilin-induced pepsinogen secretion. Each value represents the mean ± SEM (n = 5). A: atropine; M: motilin; figures after the abbreviations denote concentrations in μg/kg. n.s.: not significant.

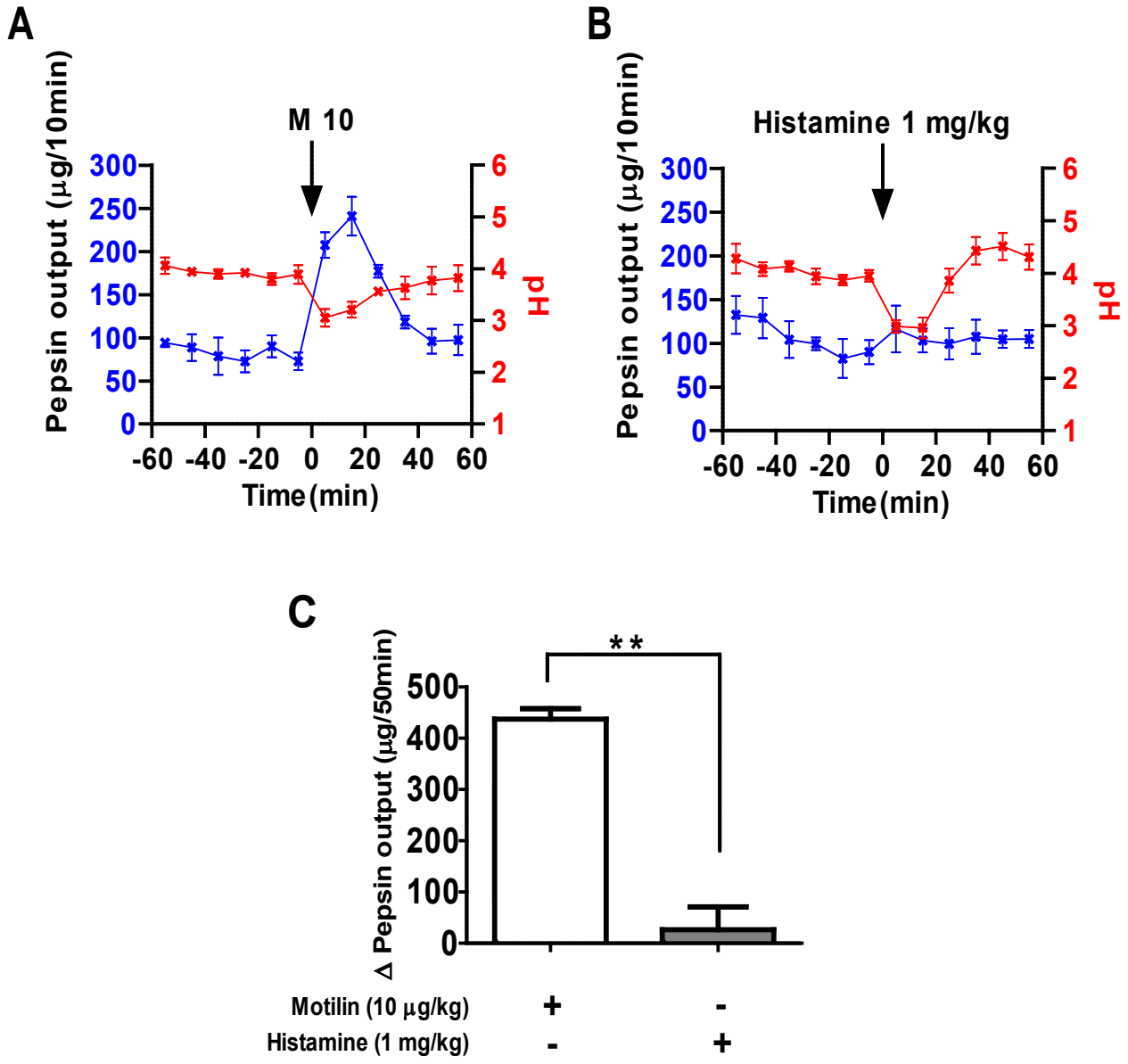

Figure 17: Effect of motilin and histamine on gastric pH and pepsinogen secretion. Pepsinogen secretion was measured in 100 µl of gastric output after motilin (10 µg/kg BW) and histamine (1 mg/kg BW) administration to urethane-anaesthetized suncus following a 60-min basal period. Pepsinogen secretion (blue line) and pH (red line) changes were monitored at 10-min intervals throughout the experiment (A, B). After administration of motilin and histamine, net changes in the 50-min total pepsin output were estimated (C). Motilin administration decreased gastric pH and increased pepsinogen secretion whereas histamine decreased gastric pH but failed to stimulate pepsinogen secretion. Each value represents the mean ± SEM (n = 3). M: motilin; figures after the abbreviations denote concentrations in µg/kg. **$p < 0.01$.

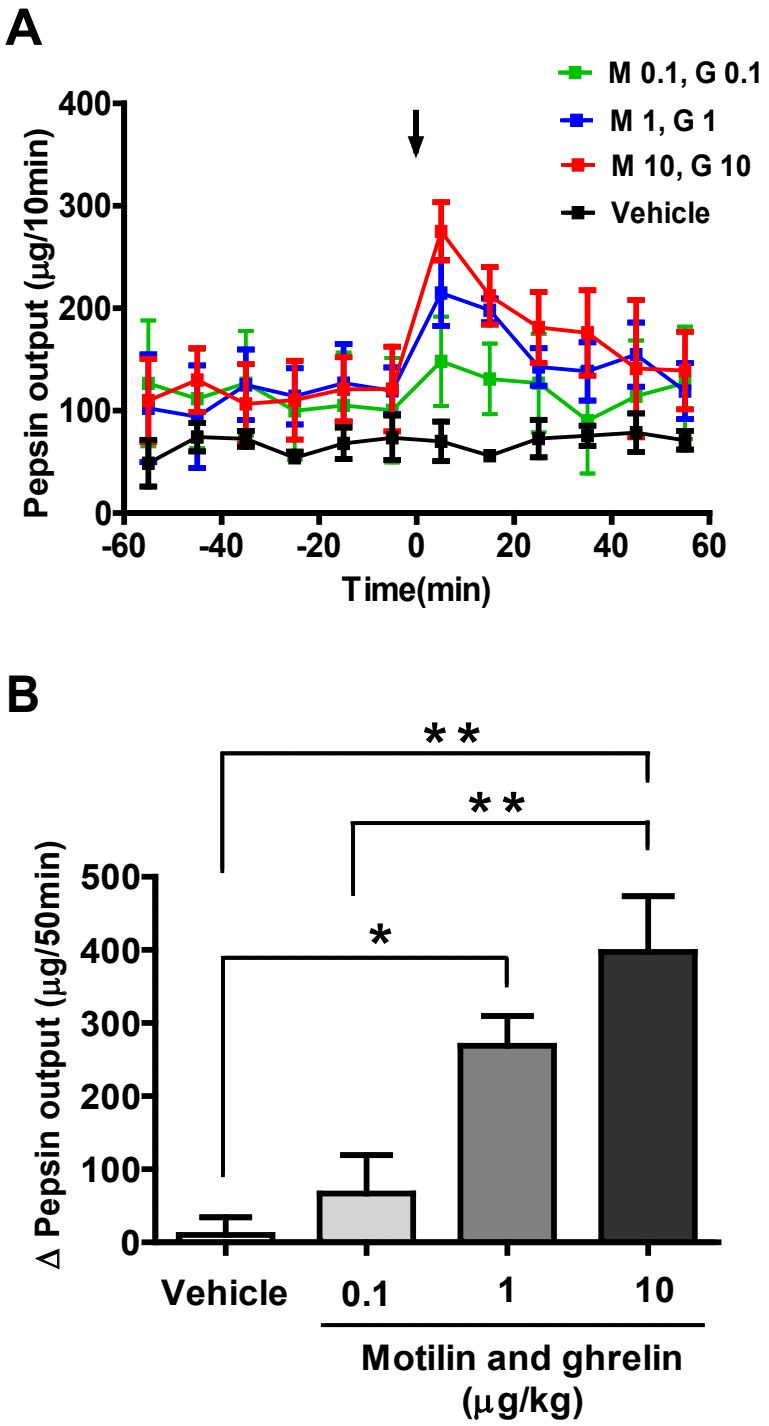

Figure 18: Effects of co-administration of motilin and ghrelin on pepsinogen secretion. After co-administration of motilin and ghrelin (10 µg/kg BW each), pepsin outputs were monitored at 10-min intervals throughout the experiment (A). The net changes in the 50-min cumulative pepsin output were calculated after administration of vehicle and different doses of motilin and ghrelin (B). Each value represents the mean ± SEM (n = 3). M :motilin; G: ghrelin; figures after the abbreviations denote concentrations in µg/kg. *$p < 0.05$, **$p < 0.01$.