5-Hydroxytryptamine (5-HT; serotonin) receptors

5-Hydroxytryptamine (5-HT; serotonin) is a neurotransmitter that is essential for a large number of physiological processes, including the regulation of vascular and non-vascular smooth muscle contraction, modulation of platelet aggregation, and the regulation of appetite, mood, anxiety, wakefulness, and perception [37, 38]. To mediate this astonishing array of functions, no fewer than 15 separate receptors have evolved, of which all but two (5-HT_{3A} and 5-HT_{3B}) are GPCRs [37, 38].

Although seven different families of 5-HT receptors (5-HTR) have been identified, there is little information on the effects of anesthetics on G-protein-coupled 5-HTRs. Several investigators recently studied the effects of anesthetics on two types of metabotropic 5HTR. Enflurane inhibited the function of phosphatidylinositol-linked acetylcholine and 5-HTR [19]. We previously reported the inhibitory effects of anesthetics on 5-HT_{2A}R in detail. Halothane decreased 5-HT_{2A}R-mediated responses in a concentration-dependent manner, and the inhibitory effects of halothane were attenuated by treatment with the protein kinase C (PKC) inhibitor GF109203X. These findings imply that metabotropic 5-HTRs are affected by halothane, and that these actions may be dependent on the activity of PKC.

By contrast, the intravenous anesthetics propofol, ketamine, pentobarbital, and etomidate did not affect the functions of 5-HT_{2A}R. Dexmedetomidine has little effect on the 5-HT_{2C} receptors function expressing in *Xenopus* oocytes [7]. Tramadol inhibited 5-HT-induced Cl^- currents at pharmacologically relevant concentrations, and the mechanism of this inhibitory effect seems to involve competitive displacement of the 5-HT binding to the 5-HT_{2C}R, rather than via activation of the PKC pathway. ODT is a more potent analgesic than tramadol. ODT, at pharmacologically relevant concentrations, inhibited 5-HT-evoked Ca^{2+}-activated Cl^- currents in oocytes that expressed 5-HT_{2C}R. ODT inhibited the specific binding of [^{3}H]5-HT by 5-HT_{2C}R expressed in oocytes. ODT altered the apparent dissociation constant for binding of [^{3}H]5-HT by 5-HT_{2C}R without changing maximum binding, which indicated competitive inhibition [39].

There have been several findings with evidence that 5-HTR is a one of the targets of volatile anesthetics, but intravenous anesthetics do not seem to have an effect on them. By contrast, a recent report pointed out that tramadol and metabolite ODT would have inhibition.

Substance P receptors

Substance P receptors (SPR) are widely distributed in the CNS and peripheral nerves. SP is a neurotransmitter that is released from C-fibers within nociceptive primary afferent neurons to the spinal cord and mediates part of the excitatory synaptic input to nociceptive neurons at this level [40–42]. A recent study of mice lacking the gene encoding SPR showed that the mice had altered pain sensitivity; nociceptive responses to certain somatic and visceral noxious stimuli are reduced in SPR knockout mice [43–45]. Accordingly, much attention has been paid to the role of SPR in anesthetic mechanisms.

Recently, we reported the effects of halothane, isoflurane, enflurane, and diethyl ether on substance P-induced currents mediated by SPR expressed in *Xenopus* oocytes [9]. All of the volatile anesthetics tested inhibited SPR-induced Ca^{2+}-activated Cl^- currents at pharmacologically relevant concentrations. The PKC inhibitor GF109203X enhanced the substance P-induced Cl^- currents. However, GF109203X abolished the inhibitory effects of the volatile anesthetics examined on SPR. These results demonstrate that halothane, isoflurane, enflurane and diethyl ether inhibit the function of SPR and suggest that activation of PKC is involved in the mechanism of action of anesthetics and ethanol on the inhibitory effects of SPR.

The intravenous anesthetics ketamine and pentobarbital inhibit SPR-induced currents at pharmacologically relevant concentrations, while propofol has little effect on the currents [46]. By contrast, GF109203X did not abolish the inhibitory effects of ketamine and pentobarbital on substance P-induced Ca^{2+}-activated Cl^- currents. Moreover, ketamine and pentobarbital inhibited the specific binding of [^{3}H]-substance P to SPR expressed in *Xenopus* oocytes. Scatchard analysis of [^{3}H]SP binding revealed that ketamine and pentobarbital decreased the apparent dissociation constant for binding and maximal binding, indicating noncompetitive inhibition. The results suggest that ketamine and pentobarbital inhibit SPR function.

In contrast to anesthetics, there has been little information about the effects of analgesics on SPR. Tramadol has little effect on SPR expressed in *Xenopus* oocytes [46]. On the other hand, we recently reported that inhibitory effects of ODT have much greater analgesic potency than tramadol itself on SPR [47]. In this study, we investigated the effects of ODT on SPR expressed in *Xenopus* oocytes by examining substance P-induced Ca^{2+}-activated Cl^- currents. ODT inhibited the SPR-induced Cl^- currents at pharmacologically relevant concentrations, however, GF109203X did not abolish the inhibitory effects of ODT on SP-induced Ca^{2+}-activated Cl^- currents. The results suggest that the ODT inhibits the SPR functions, which may be independent of activation of PKC-mediated pathways.

These findings imply that SPR are affected by most volatile anesthetics and some intravenous anesthetics. Propofol and tramadol have little effect on the currents.

However, ketamine, pentobarbital and ODT have inhibitory effects. The mechanisms of inhibition by intravenous anesthetics are different from those of volatile anesthetics.

Metabotropic glutamate receptors

The metabotropic glutamate receptors (mGluRs) are distinct from the other metabotropic receptors in that they are much larger proteins and show little sequence similarity to most members of the GPCR family, although there is appreciable homology with the γ-aminobutyric acid B ($GABA_B$) receptors [48]. The mGluRs are important modulators of synaptic transmission in the mammalian CNS, and are believed to play roles in memory and learning. Therefore, it was of interest to determine whether anesthetics and analgesics affect the function of these receptors. The mGluRs form a family of receptors with eight different subtypes (mGluR1-8 [49, 50]). Based on their pharmacology, second messenger coupling, and sequence differences, these receptors can be divided into Classes I (mGluR1 and mGluR5), II (mGluR2 and mGluR3), and III (mGluR4 and mGluR6-8) [51]. The Class I receptors are linked to learning and memory [51] in pharmacological studies showing that an agonist of mGluR1 and mGluR5 enhances memory and that mutant mice lacking mGluR1 show deficits in learning and memory and reduced hippocampal LTP [51–53]. Mice lacking mGluR1 also display poor motor coordination. More recently, it was reported that mice lacking mGluR5 show impaired learning and reduced CA1 LTP, but normal CA3 LTP [54]. In view of the effects of ethanol and anesthetics on learning, memory, and motor function, it is of interest to consider the effects of these drugs on Class I mGluRs.

There are few studies of the effects of anesthetic agents on mGluRs. Ethanol inhibits quisqualate-induced burst activity in rat cultured cerebellar Purkinje neurons, which are mediated by mGluRs [55], and quisqualate-induced currents in *Xenopus* oocytes expressing mRNA from rat cerebellum [56], but does not affect the glutamate-stimulated phospholipase C activity of brain astrocytes [57]. These results suggest that ethanol inhibits some, but not all, mGluR subtypes, and encouraged us to test the effects of ethanol and anesthetics on specific mGluR subtypes expressed in *Xenopus* oocytes.

The effects of ethanol and halothane, on the function of mGluR1 and mGluR5 expressed in *Xenopus* oocytes have been reported [8]. Halothane and ethanol inhibited mGluR5-induced Ca^{2+}-activated Cl^- currents, yet pharmacologically relevant concentrations of these compounds had little effect on the glutamate-induced currents in the oocytes expressing mGluR1. The GF109203X abolished the inhibitory effects of halothane and ethanol on mGluR5s. Conversely, the phosphatase inhibitor calyculin A prolonged the actions of halothane and ethanol. Furthermore, mutation of a PKC consensus site (Ser890) of mGluR5 abolished the inhibitory effects of halothane and ethanol. These results suggest that ethanol and volatile anesthetics inhibit mGluR5 because they promote PKC-mediated phosphorylation.

Orexin A receptor

Neurons in the hypothalamus containing the neuropeptide orexin have been implicated in the control of sleep and wakefulness and in the pathology of narcolepsy. We have investigated the effects of volatile anesthetics, ethanol and intravenous anesthetics on orexin-A-induced Ca^{2+}-activated Cl^- currents using *Xenopus* oocytes expressing orexin-1 receptors (OX1Rs) [58]. The volatile anesthetics isoflurane, enflurane and halothane inhibited Cl^- currents elicited by 1-micromol/l orexin-A. Pentobarbital and ketamine also inhibited the action of orexin-A. Dexmedetomidine had little inhibition on the orexin A-induced current in oocytes expressing the OX1Rs [26]. Although more study would be necessary, these results may, at least in part, explain the hypnotic effects of these anesthetics.

The effect of anesthetics and analgesics on G_i protein coupled receptors

The targets of anesthetics and analgesics are not only G_q coupled receptors; other GPCRs can be targets as well. There has been little information regarding G_s- and G_i-coupled receptors, especially. Especially, the G_i coupled receptors, have been thought of as one of the sites of the anesthetics and analgesics. However, historically there have not been convenient assay systems to study the effects of anesthetics and analgesics on G_i coupled receptors. Because stimulation of G_i coupled receptors results in inhibition of cyclic AMP in cells and dose does not affect the Ca^{2+} elevation; the system has been well characterized, and has proven useful for studying the effects of intravenous anesthetics on GPCRs.

Recently, several investigators reported assay systems using chimera G proteins between G_i and G_q for investigation of G_i coupled receptors (Table 4). Coward et al. [59], have reported that the chimeric G proteins alter

Table 4 The effects of anesthetics on Gi coupled receptor function

	Halothane	Sevoflurane	Ketamine	Propofol
M_2	↓			
μ opioid	↓	↓	↓	→

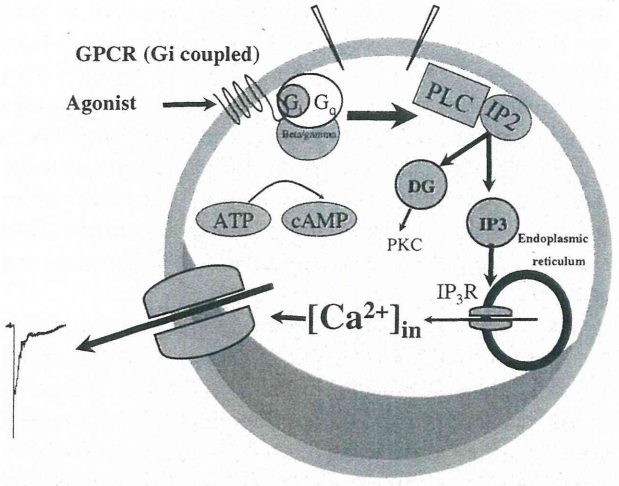

Fig. 3 Intracellular signaling in *Xenopus* oocytes expressing a Gi coupled receptor fused to chimeric Gα protein Gq_{i5}. Stimulation of $G_{i/o}$ coupled receptor fused to Gq_{i5} leads to Gq_{i5} protein-dependent activation of phospholipase C, resulting in the formation of IP_3 and diacylglycerol. The IP_3 causes the release of Ca^{2+} from the endoplasmic reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes

receptor coupling so that signaling can occur through G_q and result in mobilization of intracellular calcium stores in CHO cells. From this evidence, we drew a hypothesis that it may be able to used for the analysis by injecting both cRNA the $G_{i/q}$ chimaeric G proteins ($G_{i/q}$) and M2R into the *Xenopus* oocytes as well as G_q coupled receptors. To establish the system, we investigated whether we could measure if the acetylcholine stimulation leads to activation of phospholipase C, resulting in the formation of IP_3 and diacylglycerol and the release of Ca^{2+} from the endoplasmic reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes which were injected both cRNA and the $G_{i/q}$ chimera G proteins and G_i coupled receptor, M2R [60] (Table 4). However, regarding the established analysis of injecting both cRNA the $G_{i/q}$ chimaeric G proteins and M2R into the *Xenopus* oocytes as well as G_q coupled receptors, there were some problems for assay, i.e, low expression rates and low evoking currents. Recently, in order to improve the $G_{i/o}$-coupled receptor assay system, we made a $G_{i/o}$-coupled receptor fused to Gq_{i5} (μOR-G_{qi5}) and expressed it in *Xenopus* oocytes [61, 62] (Fig. 3). By using this assay system, we examined the effects of anesthetics on the function of μOR (Table 4).

The effects of anesthetics and on M2R

We examined ACh-induced Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes coexpressing G_i-coupled M2R with the chimeric $G\alpha_{qi5}$ [60]. In oocytes coexpressing M2R and $G\alpha_{qi5}$, halothane inhibited M2R -induced Cl^- currents in a concentration-dependent manner, suggesting that halothane inhibits M_2R-induced cellular responses at clinically relevant concentrations. Treatment with the PKC inhibitor GF109203X produced a 3.5-fold enhancement of the initial Cl^- currents induced by ACh in oocytes expressing M2R and G_{qi5}. The rate of halothane-induced inhibition of Cl^- currents elicited by ACh, however, was not changed in such oocytes pretreated with GF109203X. These findings suggest that halothane inhibits the M2R-induced signaling by acting at sites other than PKC activity.

The effects of anesthetics and analgesics on opioid receptors

Opioids are commonly used analgesics in clinical practice, however, the actual role of opioid receptors (OR) in anesthetic action have been unclear. It has been reported that OR antagonist naloxone does not affect the anesthetic potency of halothane in animals [63, 64]. Moreover, naloxone dose does not antagonize the analgesic effects of inhalation anesthetics [65]. On the other hand, Sarton et al. [66], reported that S(+) ketamine interacts with the μ-opioid system at supraspinal sites. In order to make clear the role of ORs in anesthetic action, it would be necessary to study the direct effects on OR functions.

The ORs belong to the GPCR family and three types of opioid receptors, μ, δ and κ, have been identified by molecular cloning [67]. Within three subtypes of these receptors, μORs are the major receptor to mediate the analgesic effects of opioids [67]. On the basis of second messenger signaling, μOR couple to the $G\alpha_{i/o}$ protein to cause inhibition of adenylate cyclase, inhibition of voltage-dependent Ca^{2+} channels or activation of G protein-coupled inwardly rectifying K^+ channels (GIRKs) [67]. Functions of G_q coupled receptors have been reported to be modified by some anesthetics and analgesics [5, 68]; as far as the functions of $G_{i/o}$-coupled receptors including μOR are concerned, much less is known about the direct effects of anesthetics and analgesics.

We and others have previously used oocytes expressing GIRK channels for the analysis of the function of $G_{i/o}$ protein-coupled receptors such as μOR, $5HT_{1A}R$, $GABA_BR$ or cannabinoid CB_1 receptors [62, 69–71]; GIRKs has been demonstrated as being reporter channels for assay of the activity of $G_{i/o}$-coupled receptors [69]. However, recent reports have revealed that GIRKs are possible targets for several anesthetics including halothane [72, 73]. Also, GIRKs have been reported to be possible targets for alcohol [74]. In such situations, it should be taken into consideration that functions of either $G_{i/o}$-coupled receptors or GIRKs, or both, could be affected by

anesthetics if GIRKs are used as reporters [72–74]. Thus, we employed a μOR fused to G_{qi5} (μOR-G_{qi5}) in the *Xenopus* oocyte expression assay system. Accordingly, this system makes possible the study of the direct effects of anesthetics on μOR functions.

In our recent report, halothane, ketamine, propofol and ethanol themselves had no effects on μOR-G_{qi5} in oocytes expressing μOR-G_{qi5} [61]. In contrast, ketamine and ethanol inhibited the [D-Ala2,N-Me-Phe4,Gly5-ol] enkephalin (DAMGO)-induced Cl^- currents at clinically equivalent concentrations. Halothane and propofol only inhibited the DAMGO-induced Cl^- currents at higher but clinically used concentrations. These findings suggest that ketamine and ethanol inhibit the μOR function directly in clinical practice.

Sevoflurane is commonly used together with opioids in clinical practice. However, the effects of sevoflurane on μOR functions are still unclear. Our recent study reveals that the effects of sevoflurane on the μOR functions were analyzed by using *Xenopus* oocytes expressing a μOR fused to chimeric Gα protein Gq_{i5} (μOR-Gq_{i5}) [75]. Sevoflurane inhibited the DAMGO-induced Cl^- currents at clinically used concentrations by PKC activation. These findings suggest that sevoflurane would inhibit the μOR function in clinical practice. The same as with sevoflurane, propofol has been commonly used together with opioids clinically. From the viewpoint of the effects of the opioid receptors function, propofol may be a better choice than sevoflurane.

Future directions

Several lines of study have shown that GPCRs are also targets for them. It has been reported that some anesthetics inhibit the functions of G_q-coupled receptors, such as M1R. Although GPCRs are the most numerous therapeutic targets known, the ligands for approximately two-thirds of these receptors remain unknown. The challenge in the post-genomic era is to evaluate the role of these orphan GPCRs (oGPCRs) in normal physiology and disease, and to develop new therapies based on this information. Many oGPCRs are expressed in the brain, suggesting the existence of unidentified neurotransmitters. Nearly 160 GPCRs have been identified based on their gene sequence and ability to interact with known endogenous ligands. However, an estimated 500–800 additional GPCRs have been classified as "orphan" receptors (oGPCRs) because their endogenous ligands have not yet been identified. Given that known GPCRs are targets for anesthetics and analgesics, these oGPCRs represent a rich group of receptor targets for them.

Previous reports show that anesthetics affect the function of GPCRs, and this suggests that some oGPCRs are the targets of anesthetics. Several oGPCRs involved in pain and nociception have been reported [76]. Majane and Yang [76] reported that neuropeptide FF (NPFF) modulated pain sensation and morphine analgesia under normal and pathological conditions, via both spinal and brain mechanisms [77]. It would be interesting to study the effects of anesthetics on oGPCRs, which modulate pain like NPFF. More information about orphan GPCRs might help to elucidate the role of GPCRs in the mechanisms of anesthetics and analgesics.

The G_s- and G_i-coupled receptors might also be targets for anesthetics. Compared with Gq coupled GPCRs, there has been less information which shows the effects of anesthetics on G_s- and G_i-coupled receptors. The new assay systems using chimeric G protein [61] could be helpful to study them and make a role to help clarify their mechanisms .

Conclusion

Until today, ligand-gated ion channels, such as GABA, have been believed to be the site of action of the anesthetic. The mechanisms of anesthetics on GPCRs has become more evident in recent years. The action of anesthetics on GPCRs could also play an important role for anesthetic mechanisms. In particular, the effect of anesthetics on Gq protein coupled receptors has become apparent. The effects of anesthetics on Gs and Gi protein-coupled receptors are expected to be studied more in the future. In addition, the effect of anesthetics on the orphan receptor is not clear in how it works. It would be interesting to make clear the effects of anesthetics on these receptors. More time and more research on the effects of anesthetics for GPCRs, could make clear the mechanism of action of anesthetic agents in vivo.

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Efficacy of Ghrelin in Cancer Cachexia: Clinical Trials and a Novel Treatment by Rikkunshito

Naoki Fujitsuka[1,2], Akihiro Asakawa[1], Haruka Amitani[1], Tomohisa Hattori[2], & Akio Inui[1,*]

[1]Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8520, Japan, [2]Tsumura Research Laboratories, Ibaraki 300-1192, Japan

*Address all correspondence to: Akio Inui, Professor, Kagoshima University Graduate School of Medical and Dental Sciences, Department of Psychosomatic Internal Medicine, 8-35-1 Sakuragaoka, Kagoshima, 890-8520, Japan; Tel. & Fax: +81-99-275-5748; inui@m.kufm.kagoshima-u.ac.jp

ABSTRACT: Cachexia is characterized by decreased food intake, increased energy expenditure, and muscle wasting. It is observed in 80% of patients with advanced-stage cancer and is a major source of decreased quality of life and increased morbidity and mortality in cancer patients. Ghrelin plays an important role in stimulating hunger and maintaining energy homeostasis and is the first-line treatment option for cancer cachexia. Several studies in rodent models and clinical trials have demonstrated that ghrelin or ghrelin receptor (GHS-R) agonists are effective in the treatment of cancer cachexia; however, further large-scale long-term clinical trials are needed to confirm sustained effects. Recently, the traditional Japanese medicine rikkunshito has been shown to increase food intake in rats with cancer or administered chemotherapeutics. The orexigenic effect of rikkunshito is involved in the stimulation of endogenous ghrelin secretion by blocking the serotonin (5-HT) 2b/2c receptor pathway and the enhancement of GHS-R activity. A potentiator of ghrelin signaling such as rikkunshito may represent a novel approach for the treatment of cancer cachexia.

KEY WORDS: Ghrelin, cancer, cachexia, GHS-R, Rikkunshito

ABBREVIATIONS

AgRP: agouti-related peptide; BMI: body mass index; CCK: cholecystokinin; GH: growth hormone; GHS-R: growth hormone secretagogue receptor; GI: gastrointestinal; GOAT: ghrelin O-acyltransferase; IGF-1: insulin-like growth factor-1; IL-1β: interleukin-1β; NPY: neuropeptide Y; QOL: quality of life; TNF-α: tumor necrosis factor-α.

I. INTRODUCTION

Cachexia is characterized by decreased food intake, weight loss, and muscle tissue wasting and is observed in 80% of patients with advanced-stage cancer.[1, 2] Cancer cachexia not only is a major source of decreased quality of life (QOL) but also increases morbidity and mortality in cancer patients. A persistent loss of appetite leads to a progressive depletion of body energy stores; accordingly, the development of anorexia is frequently associated with cachexia. Cancer cachexia is predominantly dependent on an imbalance between anorexigenic and orexigenic signals induced by proinflammatory cytokines that are either produced by cancer cells or released by the host immune system in response to the cancer.[3] Weight loss is a potent stimulus of food intake in healthy humans and animals, but not in individuals with cancer. Consequently, the improvement of neurochemical mechanisms regulating appetite and energy homeostasis is critically important for the treatment of cancer cachexia.

The ghrelin system is involved in eliciting feeding, inducing adiposity, and regulating energy expenditure and body weight.[4,5] Ghrelin plays an important role in triggering the adaptive response to starvation. In addition, ghrelin has much broader physiologic functions, including roles in growth hormone secretion,[6] gastrointestinal (GI) motility,[7] and suppressing inflammation.[8] Thus, ghrelin is expected be an

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effective therapy for lean patients with cachexia. Recent reports[9–11] have indicated that the traditional Japanese medicine rikkunshito, which stimulates the secretion of endogenous ghrelin by blocking 5-HT2b/2c receptors and enhances GHS-R activity in rats, increases food intake in rats with cancer or undergoing chemotherapy. The purpose of this article is to review the current medical treatment of cancer cachexia, in particular focusing on ghrelin and ongoing research.

II. GHRELIN PATHOPHYSIOLOGY

Ghrelin is a 28-amino-acid peptide, first isolated from the stomachs of humans and rats, that acts as a natural ligand for the growth hormone secretagogue receptor (GHS-R).[6] Ghrelin is mainly produced by the P/D1 cells lining the fundus of the stomach in humans and the X/A-like cells in rodents. Ghrelin mRNA is predominantly expressed in the stomach, but small amounts are seen in several tissues.[12] Acylation of Ser-3 by the addition of *n*-octanoic acid is essential for the biological activity of ghrelin via the GHS-R. Acyl modification of ghrelin is performed by the polytopic membrane-bound enzyme ghrelin O-acyltransferase (GOAT).[13] Once released, acyl ghrelin has a short half-life of approximately 10 min in the general circulation before being converted to desacyl ghrelin.[14]

Ghrelin enhances growth hormone secretion, but it has much broader physiologic functions, including appetite, GI motility, inflammation, circulation, and cell proliferation.[4–8,15] Plasma ghrelin levels increase in response to prolonged fasting and decrease rapidly after feeding. Weight loss is a potent stimulus of food intake in healthy humans and animals, and ghrelin secretion increases under conditions of negative energy balance such as starvation. Plasma ghrelin levels are higher in subjects with a low body mass index (BMI) compared with normal- or high-BMI subjects.[16] Accordingly, ghrelin is thought to be an orexigenic peptide that maintains energy homeostasis and provides a defense against starvation.

Administration of ghrelin has been shown to increase the gene expression of the orexigenic neuropeptides, namely, neuropeptide Y (NPY) and agouti-related peptide (AgRP) and to decrease the expression of the anorexigenic neuropeptide pro-opiomelanocortin.[17] Central or peripheral administration of ghrelin strongly stimulates food intake in animals.[4,5] Continuous intracerebroventricular administration of ghrelin induces food intake and increases fat mass, leading to weight gain.[18] Intravenous administration of ghrelin to healthy humans increased energy intake from a buffet lunch by 28%, and visual analog scores for appetite also increased under these conditions.[19] Administration of a single dose of GHS-R agonists to healthy volunteers has resulted in an increase in food intake.[14] These results suggest the possible clinical applications of ghrelin as a potent stimulator of appetite.

III. EFFICACY OF GHRELIN ON CANCER CACHEXIA

Increased circulating ghrelin levels have been observed in underweight patients and rodents with malignancy-associated cachexia.[20] Garcia et al. reported that ghrelin levels were significantly elevated in cachectic subjects compared with noncachectic cancer controls and noncancer controls (141, 91, and 78 pg/mL, respectively).[21] These elevations may be a compensatory response reflecting the state of negative energy balance. However, this phenomenon has been called "ghrelin resistance" due to a failure of the adaptive feeding response by ghrelin, which is robust in normal animals and subjects.[22–24] Cancer cachexia is associated with high concentrations of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α).[25] Proinflammatory cytokines induce the release of 5-HT, leptin, cholecystokinin (CCK), peptides derived from the glucagon precursor, and insulin, all of which are hormones that act as satiety signals.[26–28] In particular, the 5-HT concentration in the hypothalamus is increased in humans and animals with cancer.[29,30] Makarenko et al. demonstrated that NPY and dopamine concentrations decrease while serotonin concentration increases in the paraventricular nucleus at the onset of anorexia in tumor-bearing rats.[31]

Nevertheless, ghrelin administration in rodent models of cancer cachexia led to a significant increase in food intake. Hanada et al.[32] and Wang et al.[33] demonstrated that twice-daily intraperitoneal administration of ghrelin (800 nmol/kg/day) to melanoma-bearing nude mice improved food intake and weight gain 5 or 6 days after treatment. DeBoer et al. demonstrated that the administration of ghrelin and a GHS-R agonist (BIM-28131) to tumor-bearing rats with cachexia as a continuous infusion (500 nmol/kg/day) via an osmotic minipump for 5 days of treatment resulted in increased food intake and weight gain and inhibited the loss of lean body mass.[34] These findings suggest that high plasma concentrations of ghrelin may overcome resistance to the appetite-stimulating effects of the endogenous peptide in the short term. In addition, ghrelin inhibits the production of anorectic proinflammatory cytokines, including IL-1β, IL-6, and TNF-α.[35] DeBoer et al. demonstrated that ghrelin-treated animals exhibited a significant decrease in the expression of IL-1 receptor-I transcript in the hypothalamus and brainstem. The combination of these actions suggests that ghrelin has benefits for the treatment of cachexia.[34]

IV. CLINICAL TRIALS

Several randomized, double-blinded, placebo-controlled trials have demonstrated that ghrelin or an GHS-R agonist effectively increases food intake and lean body mass in cachectic patients with cancer. Neary et al. demonstrated that energy intake from a buffet lunch was increased by 31% during ghrelin infusion (5 pmol/kg/min for 180 min) compared with the saline control in seven cancer patients.[36] Analysis of the visual analog score revealed a significant increase of 23% in meal appreciation on the ghrelin administration day. No adverse effects were observed. There was no evidence of a compensatory decrease in food intake after ghrelin treatment as assessed by a 24-h food diary. Strasser et al.[37] studied 21 cancer patients who were randomized to receive ghrelin on days 1 and 8 and placebo on days 4 and 11 or vice versa, given intravenously over a 60-min period before lunch. Ten patients received 2 mg/kg of ghrelin (lower dose) ; 11 received 8 mg/kg of ghrelin (higher dose). At day 8, 81% of patients preferred ghrelin to placebo against 63% at the end of study. Nutritional intake and eating-related symptoms were not significantly different between ghrelin and placebo. Ghrelin was well tolerated and safe in patients with advanced cancer. For safety, tolerance, and patients' preference for treatment, no difference was observed between the lower- and higher-dose groups. Garcia et al.[38] demonstrated that the GHS-R agonist RC-1291 orally administered over a 12-week period to 81 patients with a variety of cancers resulted in an increase in total body mass, lean body mass, and handgrip strength. No significant differences were found in QOL.

An important concern regarding the use of ghrelin in cancer cachexia is that ghrelin may stimulate tumor growth via GHS-Rs expressed in cancer cells or via growth factors such as growth hormone (GH) and insulin-like growth factor-1 (IGF-1).[39] However, there are conflicting data about the possible role of ghrelin in oncogenesis,[40] and no *in vivo* studies have examined the differences in tumor growth after ghrelin or GHS treatment. Further large-scale, long-term clinical trials are required to determine the efficacy and safety of ghrelin or GHS-R agonists on cancer cachexia.

V. A NOVEL APPROACH FOR THE TREATMENT OF CANCER CACHEXIA

The traditional Japanese medicine *rikkunshito* is widely prescribed in patients exhibiting upper GI symptoms such as functional dyspepsia and gastroesophageal reflux.[41–45] Several reports have demonstrated that the oral administration of rikkunshito increases plasma ghrelin levels in humans and rodents and effectively improves food intake and modulates GI motility.[9,10,46] Takeda et al. demonstrated that rikkunshito ameliorated cisplatin-induced anorexia in rats by inhibiting the decreased concentration of circulating ghrelin.[9] Cisplatin is widely used in clinical practice; however, adverse reactions to the production of excess 5-HT lead to the discontinuation of chemotherapy in cancer patients. The 5-HT produced during treatment

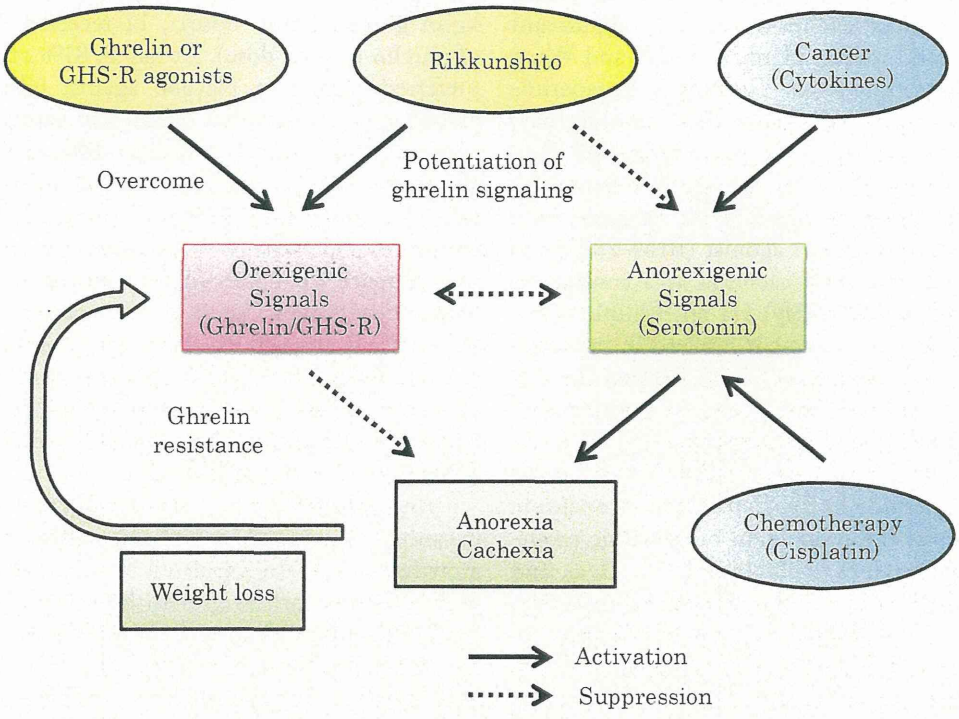

FIGURE 1. Pathophysiological role of ghrelin signaling in cancer cachexia. Cancer anorexia-cachexia is predominantly dependent on an imbalance between anorexigenic and orexigenic signals. In the hypothalamus, increased levels of proinflammatory cytokines produced by cancer cells play a role in activating the anorexigenic signals and inhibiting the orexigenic signals. Serotonin (5-HT) produced during treatment with cisplatin stimulates the 5-HT2b/2c receptors, resulting in decreased hypothalamic and peripheral ghrelin secretion. In patients and rodents with cancer cachexia, circulating ghrelin levels increase due to the adaptive response to weight loss; however, ghrelin resistance is induced by excessive hypothalamic anorexigenic activity. Treatment with ghrelin or GHS-R agonists can overcome resistance to the appetite-stimulating effect of the endogenous ghrelin. Rikkunshito increases food intake in rats with cancer or chemotherapy by the stimulation of endogenous ghrelin secretion by blocking the 5-HT 2b/2c receptor pathway and the enhancement of GHS-R activity. A potentiator of ghrelin signaling such as rikkunshito may represent an additional novel approach for the treatment of cancer cachexia.

with cisplatin stimulates the 5-HT2b receptor in gastric smooth muscle and the 5-HT2c receptor in the central nervous system, resulting in decreased plasma ghrelin. Heptamethoxyflavone, hesperidin, and isoliquiritigenin (components of rikkunshito) have been shown to antagonize 5-HT2b/2c receptors and stimulate ghrelin secretion in cisplatin-treated rats, suggesting that these molecules play an important role in the improvement of appetite by rikkunshito. Our previous study[10] reported that the oral administration of rikkunshito to fenfluramine-treated rats increased plasma ghrelin levels, food intake, and gastric emptying time and restored GI dysmotility. Fenfluramine altered the fasted motor activities to become fed-like motor activities in the antrum and duodenum via the activation of the central 5-HT2c receptor, mediated by the ghrelin-NPY signaling pathway. These effects of rikkunshito in fenfluramine-treated rats were blocked by the GHS-R antagonist (D-Lys3)-GHRP-6, suggesting

that the increase in circulating ghrelin induced by rikkunshito leads to the improvement of anorexia and gastric function. Yakabi et al. also demonstrated that urocortin-induced reduction of food intake was restored by rikkunshito.[47]

A recent report has demonstrated that rikkunshito enhances hypothalamic ghrelin secretion.[48] In cisplatin-treated rats, hypothalamic ghrelin secretion was markedly reduced 24 and 48 h after cisplatin treatment, although plasma ghrelin levels were higher than in saline-treated rats due to the adaptive response to weight loss. Cisplatin-induced anorexia in the late phase is mediated through reduced hypothalamic ghrelin secretion. Cerebral 5-HT2c receptor activation partially induces decreases in hypothalamic ghrelin secretion, and rikkunshito suppresses cisplatin-induced anorexia by enhancing this secretion. In addition, rikkunshito and 5-HT2c receptor antagonists suppress cisplatin-induced anorexia by inhibiting the reduction of GHS-R1a gene expression in the hypothalamus.[49] The efficacy of rikkunshito in cisplatin-induced anorexia may reduce the risk of discontinuation of chemotherapy in cancer patients. In addition, Takeda et al. reported that rikkunshito improved aging-associated anorexia by inhibiting the reduced reactivity of the hypothalamic ghrelin receptor, which is caused by an increase in plasma leptin level.[50] The components of rikkunshito that inhibit phosphodiesterase type 3 mediation downstream of the leptin receptor have been identified.

Cancer cachexia is caused by multiple underlying mechanisms. Anorexigenic neurochemical mediators such as 5-HT, which increase in the hypothalamus in humans and animals with cancer, are predominantly involved.[25] The excessive hypothalamic anorexigenic activity may induce ghrelin resistance, which attenuates the adaptive feeding response by endogenous ghrelin in cancer cachexia. More recently, we found that rikkunshito improved anorexia, GI dysmotility, muscle wasting, and anxiety-related behavior and prolonged survival in tumor-bearing rats. This effect is mediated by the stimulation of ghrelin secretion and the enhancement of GHS-R activity.[11] These findings suggest that rikkunshito may be effective for ghrelin resistance such as cancer cachexia (Figure 1).

VI. CONCLUSION

Several rodent models and short-term clinical studies have demonstrated that ghrelin or GHS-R agonists are effective in the treatment of cancer cachexia. However, further large-scale long-term clinical trials are required to determine the efficacy and safety of ghrelin or GHS-R agonists in cancer cachexia. A potentiator of ghrelin signaling such as rikkunshito may represent an additional novel approach for the treatment of cancer cachexia.

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CHAPTER EIGHTEEN

Ghrelin and Gastrointestinal Movement

Naoki Fujitsuka*,†, Akihiro Asakawa*, Haruka Amitani*, Mineko Fujimiya‡, Akio Inui*,1

*Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
†Tsumura Research Laboratories, Ibaraki, Japan
‡Department of Anatomy, Sapporo Medical University School of Medicine, Hokkaido, Japan
1Corresponding author: e-mail address: inui@m.kufm.kagoshima-u.ac.jp

Contents

Abstract

Ghrelin is a potent stimulant for gastric emptying and gastrointestinal (GI) movement. Clinically, it has been reported that the intravenous administration of ghrelin accelerates the rate of gastric emptying and induces gastric phase III contractions of the migrating motor complex in healthy volunteers. Recent technical advances in the measurement of GI motility in conscious small animals, including rats, mice, and the house musk shrew (*Suncus murinus*), have helped to elucidate the precise mechanism of action of ghrelin. Intravenous administration of ghrelin induces fasted motor activities with phase III-like contractions of the migrating motor complex in the antrum and duodenum in animals. These effects of ghrelin are mediated by activating the hypothalamic orexigenic neuropeptide Y neuron through ghrelin receptors located at the vagal afferent terminal. Stress hormone and anorexigenic peptides cause the disruption of fasted motor activity and induce fed-like motor activity. Ghrelin and

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the ghrelin signal potentiator rikkunshito successfully restore fed-like motor activities to fasted activities in fenfluramine-treated rats and in a cancer anorexia–cachexia animal model. These findings suggest that ghrelin can be expected to be a therapeutic target for GI disorders.

1. INTRODUCTION

Ghrelin is a potent stimulant of gastric emptying and gastrointestinal (GI) motility. In humans in the fasted state, cyclic changes in contraction waves known as the migrating motor complex (MMC) are observed in the GI tract (Vantrappen et al., 1977). The MMC consists of three phases: a period of motor quiescence (phase I), a period of irregular contractions (phase II), and a period of clustered potent contractions (phase III). These phases are observed at regular intervals of 90–120 min in humans. Clinically, ghrelin accelerates the rate of gastric emptying (Levin et al., 2006) and induces gastric phase III contractions in healthy volunteers (Bisschops, 2008; Tack et al., 2006). The same findings regarding GI motility in animal models have been reported following free-moving, conscious animal experiments. These experiments provide more physiological information than other approaches to estimating GI motility, which is regulated by the brain–gut interaction (Inui et al., 2004). Notably, the dog is a popular model for GI motility research. An early study (Itoh et al., 1976) showed that motilin induces gastric phase III contractions in dogs. However, there have been few reports showing that ghrelin, which has a structural resemblance to motilin, has an effect on the digestive tract in dogs (Ohno et al., 2010).

Recent technical advances have permitted the measurement of GI motility in conscious small animals, including rats, mice, and house musk shrews (*S. murinus*), using manometric methods (Ataka et al., 2008; Fujino et al., 2003; Tanaka et al., 2009) or force-transducer implantation (Ariga et al., 2007; Fujitsuka et al., 2009; Sakahara et al., 2010; Zheng et al., 2009a). These studies have demonstrated that ghrelin induces a fasted motor pattern and augments the motility of the antrum and duodenum in the fed or fasted state of healthy animals through brain–gut interactions. Moreover, ghrelin and rikkunshito have been shown to improve gastric emptying and GI motility in animal models of GI disorder. Rikkunshito, a traditional Japanese herbal (Kampo) medicine, potentiates ghrelin

signaling (Fujitsuka et al., 2011; Takeda et al., 2008, 2010; Yakabi et al., 2010) and is widely prescribed for patients exhibiting functional dyspepsia (Kusunoki et al., 2010). In this section, the role of ghrelin in GI motility and the methods for the measurement of GI motility in experimental animals are introduced.

2. THE ROLE OF GHRELIN IN GASTRODUODENAL MOTILITY

2.1. The strain-gauge force-transducer measurement of gastroduodenal motility in conscious rats and mice

The role of ghrelin in the control of gastroduodenal motility was evaluated in free-moving, conscious rats using a strain-gauge force-transducer method (Fig. 18.1A). In fasted rats, cyclic changes in contraction waves were detected in both the antrum and duodenum; these waves included a quiescent period (phase I-like contractions) followed by a group of contractions (phase III-like contractions) (Fig. 18.1B). Phase III-like contractions of the antrum occur periodically at intervals of approximately 10 min, and most of these contractions appear to occur in conjunction with phase III-like contractions of the duodenum. Circulating ghrelin levels in fasted rats fluctuate; the peaks of these fluctuations are highly associated with phase III-like contractions in the antrum (Ariga et al., 2007; Fujitsuka et al., 2009).

Intravenous administration of ghrelin to fasted rats immediately potentiates the fasted motor activity and increases the motility index (MI) and the frequency of phase III-like contractions in the antrum and duodenum (Fig. 18.1C). The physiological fasted motor activity decreases with the administration of the growth-hormone secretagogue receptor (ghrelin receptor) antagonist (D-Lys^3) GHRP-6. Exogenous ghrelin eliminates the fed motor pattern, which is irregular contractions of high frequency caused by feeding, and produces a fasted motor pattern (Fig. 18.1B and D) (Fujitsuka et al., 2009). Gastric motility in the physiological fed and fasted states of conscious mice has also been measured successfully by a method involving the implantation of a transducer in the mouse stomach (Zheng et al., 2009a). The protocol presented below has been used to measure gastroduodenal motility in conscious rats using the strain-gauge force-transducer method (Fig. 18.1A).

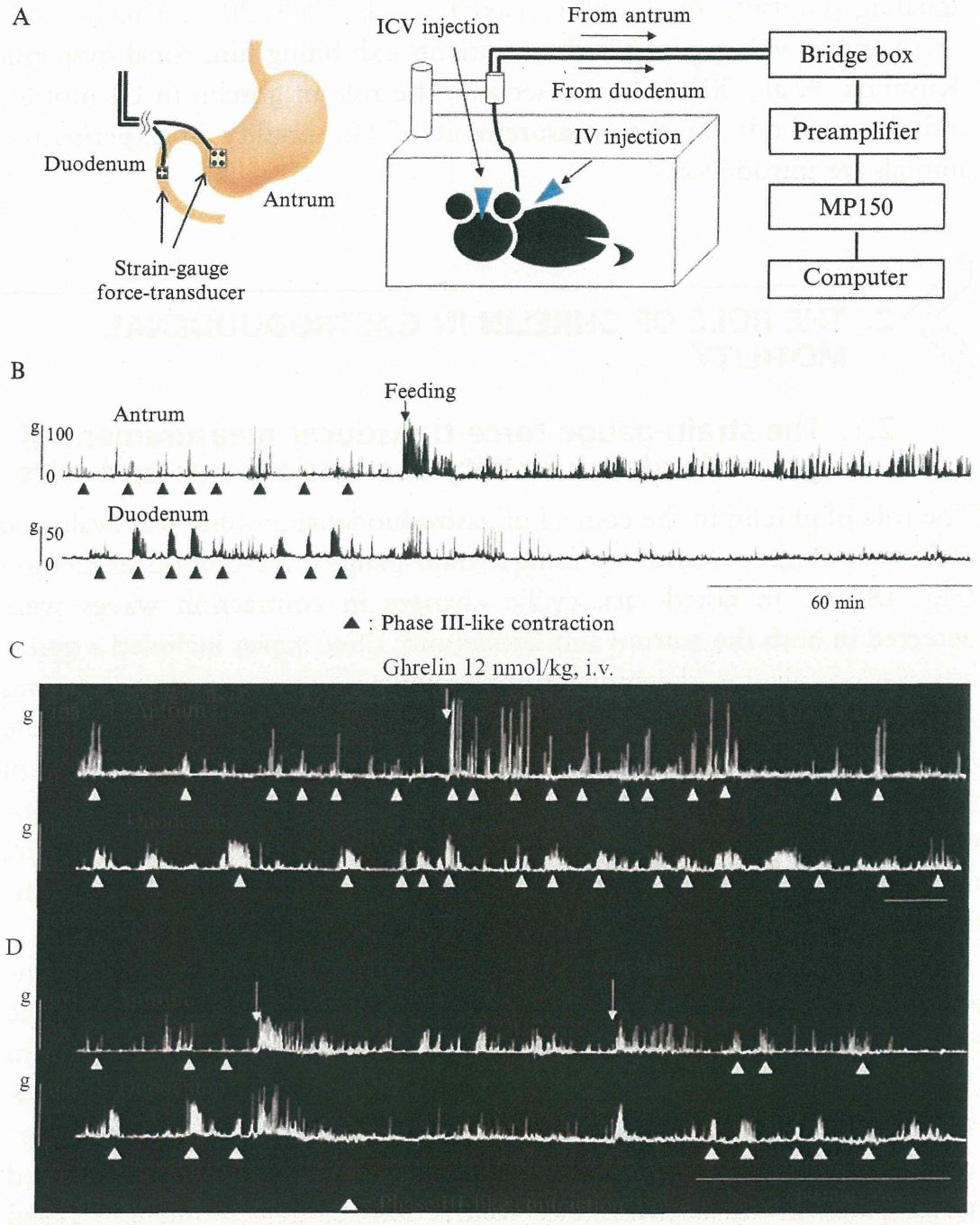

Figure 18.1 Method for strain-gauge force-transducer measurements of gastrointestinal motility in conscious rats. (A) Strain-gauge force transducers were placed on the serosal surface of the antrum and duodenum. The wires of the transducers were drawn out from the back of the neck and connected to a preamplifier via a bridge box. Data were recorded using an MP150. (B) The fasted patterns were replaced by the fed patterns in antrum and duodenum after feeding. (C) Intravenous administration of ghrelin to fasted rats immediately potentiated the frequency of phase III-like contractions in antrum and duodenum. (D) Intravenous administration of ghrelin eliminated the fed motor activities and induced phase III-like contractions in the antrum and duodenum of fed rats (from Fujitsuka et al., 2009).

2.1.1 Animal preparation involving the strain-gauge force-transducer method

1. Rats deprived of food overnight and weighing 200–250 g are anesthetized with intraperitoneal injections of pentobarbital sodium (50 mg/kg body weight).
2. After laparotomy, strain-gauge force transducers (F-08IS, Star Medical, Tokyo, Japan) are placed on the serosal surface of the antrum and duodenum.
3. The wires of the transducers are drawn out through a protective coil from the back of the neck via the subcutaneous part of the back.
4. Measurements are made with the animals in a free-moving condition system (Sugiyana-gen Co., LTD, Tokyo, Japan) in individual cages after a 5-day postoperative period for recovery.

2.1.2 Measurement of gastroduodenal motility

1. Rats are deprived of food but not water for 16 h before the experiment.
2. The strain-gauge force transducer placed in rats is connected to a preamplifier via a bridge box (Star Medical).
3. Data are recorded using an MP150 (BIOPAC Systems, Goleta, California).
4. The experiment is started when the fasted gastric contraction is stabilized, 2 h after the initial measurement.
5. The frequency of the fasted pattern is obtained from the average of the onset of phase III-like activities for each hour of the experiment.
6. The area under the wave (MI) per minute in the antrum and duodenum is measured and is shown as a percentage (%MI) relative to control data.

2.2. The manometric measurement of gastroduodenal motility in conscious rats and mice

Gastroduodenal motility in the physiological fed and fasted states of conscious rats has also been measured using a manometric method (Fig. 18.2A) (Fujimiya et al., 2000; Fujino et al., 2003). The frequency of phase III-like contractions in the antrum was 5.3 ± 0.5/h and that in the duodenum was 5.6 ± 0.8/h in fasted rats. This fasted pattern was disrupted and replaced by the fed pattern after feeding (Fig. 18.2B). The intravenous injection of ghrelin induced the fasted pattern in the duodenum when rats in the fed state were injected, increasing their %MI in the antrum (Fig. 18.2C). Recent advances in transgenic and knockout technologies have provided tools to investigate the pathogenesis of disease models, and these technologies have typically been applied to mice.

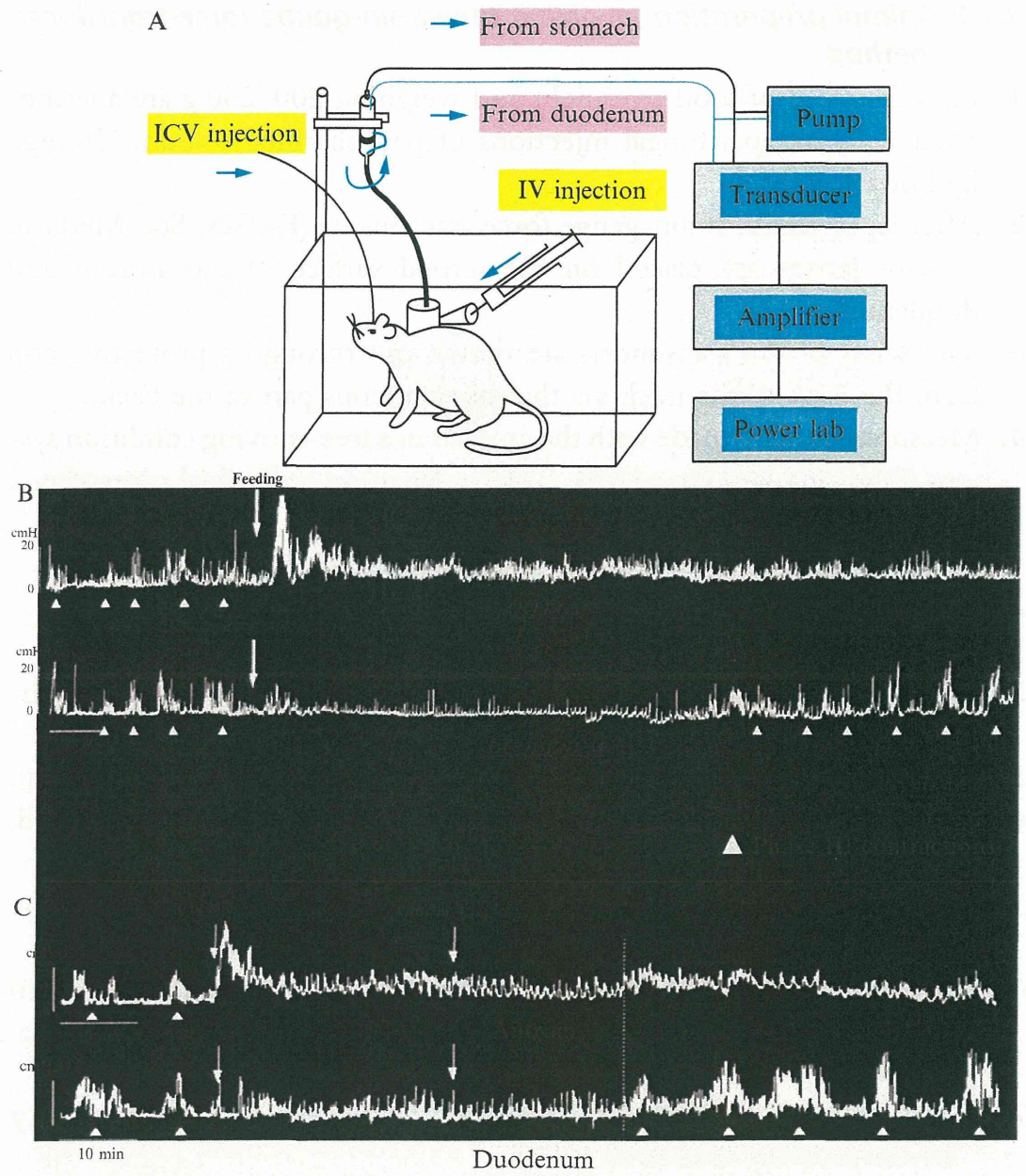

Figure 18.2 Method for manometric measurements of gastrointestinal motility in conscious rats. (A) Catheters for motility recordings are inserted into the antrum and duodenum, connected to the infusion swivel to allow free movement and then connected to a pressure transducer. The data are recorded and stored in a PowerLab. (B) Phase III-like contractions in fasted rats were disrupted and replaced by the fed pattern, which is irregular contraction of high frequency, after feeding. From Kihara et al. (2001). (C) Intravenous administration of ghrelin eliminated the fed motor pattern and produced a fasted motor pattern in duodenum (from Fujino et al., 2003).

Manometric methods allow dual monitoring of the motility of the stomach and duodenum in conscious mice (Tanaka et al., 2009). In fasted mice, the frequency of phase III-like contractions in the antrum was 7.8 ± 0.5/h and that in the duodenum was 6.6 ± 0.7/h. However, the frequency of phase