Effects of Z-338, a novel gastroprokinetic agent, on the actions of excitatory and inhibitory neurotransmitters on neurons in area postrema

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Abstract

We investigated the effects of the novel gastroprokinetic agent Z-338 on the actions of excitatory and inhibitory neurotransmitters on neurons in area postrema (AP). Iontophoretic applications of acetylcholine (ACh), AMPA and NMDA increased, while GABA suppressed the firing rates of AP neurons recorded by extracellular electrodes. Z-338 (10 µM) suppressed the ACh-induced acceleratory and GABA-induced inhibitory actions without affecting the excitatory actions of AMPA and NMDA. Under voltage-clamp conditions, nicotine, NMDA, kainic acid (KA) and ATP evoked inward currents in dissociated single AP neurons recorded by whole-cell patch clamp technique, and GABA produced outward currents, at holding potentials (VH) of −60 or 0 mV. Z-338 (>3 µM) specifically suppressed the nicotine- and GABA-induced currents without affecting the currents induced by NMDA, KA and ATP. In addition, we found that Z-338 (30 µM) suppressed the spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from AP neurons in slice preparations. Experiments with microelectrode and histochemical methods revealed the presence of direct excitatory and di-synaptic inhibitory neural connections from AP to dorsal motor nucleus of the vagus (DMV). In some AP neurons, Z-338 (10 µM) enhanced the spontaneous firing rates recorded by extracellular electrode. The excitatory or inhibitory effects of Z-338 on the firing rates or actions of nicotine and GABA on AP neurons observed in the present study may explain the postmeal relaxation induced by Z-338 in patients with functional dyspepsia.

Key words: Z-338, gastric motility, AP, excitatory synapses, inhibitory synapses

Introduction

Z-338 (N-[(N′-N′-diisopropyl-aminoethyl)-[2-(2-hydroxy-4,5-dimethoxy benzoylamino)-1,3-thiazole-4-yl]carboxyamide monohydrochloride trihydrate) is a novel gastroprokinetic agent. It
has recently been synthesized to obtain a more potent and clinically safer agent which acts
differently from existing gastroprokinetic agents including cisapride and domperidone, that
have been reported to have unfavourable adverse actions on the central nervous and
cardiovascular systems (Ueki et al., 1998). It was reported that Z-338 enhances gastric motor
activity in conscious dogs with chronically implanted force transducers, and restores gastric
emptying suppressed by clonidine treatment in both dog and rat gastroparesis models (Ueki et
al., 1998).

The mechanisms involved in these actions of Z-338 have been investigated in more detail by
a number of techniques. In guinea pig stomach strips preincubated with [3H]-labelled choline,
Z-338 enhanced the electrically stimulated [3H]-ACh release. This is likely to occur through the
inhibition of pre-synaptic M1 and M2 receptors, since Z-338 binds to M1- and M2-mAChR
prepared from rat tissues and effectively blocks membrane currents associated with activation of
recombinantly expressed rat M1- and M2-mAChRs in Xenopus oocytes (Ogishima et al., 2000). It
was also shown that Z-338 enhances spontaneous contraction as well as electrically evoked
excitatory junction potentials and concomitant contractions in guinea pig stomach with no
significant changes in inhibitory neuro-effector transmission, basal muscle tone or resting
membrane potential of the smooth muscles (Nakajima et al., 2000). In isolated paratrabecular
ganglion cells, ACh induces slow and fast inward currents through activation of M1-mAChR and
neuronal nicotinic ACh receptors (nAChR). Z-338 inhibits slow and fast inward currents
induced by ACh in a competitive and noncompetitive manner, respectively (Kanemoto et al.,
2002). All these findings are consistent with the view that Z-338 facilitates excitatory neuro-
effector transmission in the stomach by increasing ACh release from cholinergic nerve
terminals via inhibition of pre-junctional M1- and M2-mAChR or nAChR.

In addition to these pre-junctional actions, Z-338 potentiates L-type voltage-dependent Ca2+
currents (I_Ca) possibly through activation of M5-like-mAChR in guinea pig gastric myocytes
(Morita et al., 2002). These pre- and post-synaptic effects taken together would explain the
actions of Z-338 to enhance spontaneous contractions without changing the basal muscle tone of
the stomach, and suggest that Z-338 could improve gastric emptying in patients with functional
dyspepsia.

Recently, a pilot study to assess the effects of Z-338 on meal accommodation, gastric
sensitivity and gastric emptying, was performed on 62 patients with functional dyspepsia (Tack et
al., 2002). This study revealed that Z-338 (300 mg t.i.d.) has significant benefit over placebo
for global symptom score including postprandial fullness and mean and maximum post-meal
relaxation. It is puzzling, however, how Z-338 is able to enhance the post-meal relaxation, since
several lines of evidence obtained so far indicate that Z-338 facilitates excitatory neuro-effector
transmission through pre- and post-junctional mechanisms, whereas there are no significant
changes in peripheral inhibitory neuro-effector transmission and basal muscle tone of the
stomach (Nakajima et al., 2000; Ogishima et al., 2000; Morita et al., 2002).

In an attempt to understand the actions of Z-338 on patients with functional dyspepsia, we
observed the effects of Z-338 on neurons in the area postrema (AP), located at the fourth
ventricle and known as the chemoreceptor zone. The AP is outside the blood brain barrier and
the cells within the AP are exposed to endogenous and exogenous substances in the blood
circulation. In addition, our recent study indicated that rat stomach receives efferent neural input from AP, and electrical stimulation of AP relaxes the stomach (Kawachi et al., 2008). Furthermore it is well documented that the motility of fundus and antrum of the stomach is mainly controlled by neurons projecting from dorsocaudal medulla oblongata, specifically in the dorsal motor nucleus of the vagus (DMV) (Gillis et al., 1989). Concerning neural circuitry of AP, the reciprocal connections between AP and the solitary complex was reported in ferrets by use of horseradish peroxidase (Strominger et al., 1994). However, the precise neural connections between AP and DMV are not well known. In the present experiments, firstly we studied the neural link between AP and DMV in the rat. Then we observed the effects of Z-338 on the spontaneous firing rates of AP neuron, and on the actions of excitatory and inhibitory neurotransmitters on AP neurons by use of intra- and extra-cellular recording and whole-cell patch clamp techniques with slice preparations and single neurons isolated from the AP.

**Materials and Methods**

*Preparations*

All experiments were carried out according to the guiding principles for the care and use of animals approved by the Council of the Physiological Society of Japan and all efforts were made to minimize both the number of animals used and their suffering.

We prepared slice preparations from young (12–15 days old) for the patch clamp experiments and from adult male Wistar rats (70–80 days old) for the intra- and extra-cellular recordings. Under ether anesthesia, each rat was euthanized by cervical dislocation, and the brain stem and cerebellum were rapidly removed to cold Krebs-Ringer solution (see below) and the brain was transected at the inferior collicular level. The cut caudal part was fixed on a stage in the chamber of a slicer with cyanoacrylate glue, and coronal sections including AP, NTS and DMV at a thickness of 400 µM were sliced. Before the start of the experiment, each slice was incubated for at least 2 hours in Krebs-Ringer solution bubbled with 95% O₂ and 5% CO₂ at 33°C. The slices were then placed on a plexiglas mesh in a submerged recording chamber perfused with oxygenated Krebs-Ringer solution at 33°C at a flow rate of 3 ml/min.

To prepare slice preparations for the patch clamp experiments, the brain stem from young rats was dissected and transversely sliced at a thickness of 230 µM in a cold low-Na⁺ medium (see below) using a microslicer (VT 1000S, Leica, Nussloch, Germany). The slices, including the AP, were kept in the incubation medium (see below) saturated with 95% O₂ and 5% CO₂ at 34–35°C for at least 1 h before recording. Thereafter the slices were transferred into a recording chamber, and the AP was identified under an upright microscope (DMLFSA, Leica). Once in the recording chamber, slices were continuously perfused at a rate of 3–4 ml/min.

We also used mechanically isolated single cells for whole cell patch clamp experiments. Wistar rats (12–15 days old) were decapitated under pentobarbital anesthesia (50 mg kg, i.p.) and the brain stem was dissected and transversely sliced at a thickness of 350 µm with a microslicer (VT1000S; Leica). Slices containing the AP were kept in the incubation medium (see below) saturated with 95% O₂ and 5% CO₂ at room temperature (23–25°C) for at least 1 hour. For isolation of single neurons, slices were transferred into a 35 mm culture dish.
(Primaria 3801, Becton Dickinson, Rutherford, NJ, USA) containing the standard external solution (see below), and the region of the AP was identified under a binocular microscope (SMZ-1; Nikon, Tokyo, Japan). Details of the mechanical dissociation have been described previously (Rhee et al., 1999; Akaike and Moorhouse, 2003). Briefly, mechanical dissociation was accomplished using a custom-built vibration device. A fire-polished glass pipette was lightly placed on the surface of the AP with a micromanipulator and the tip of the glass pipette was vibrated horizontally for about 2 min. The slices were removed and the mechanically dissociated neurons allowed to settle and adhere to the bottom of the dish for 15 min. Such neurons undergoing dissociation retained a short portion of their proximal dendrites.

Electrical measurements

Spontaneous single unit discharges were recorded extracellularly with glass microelectrodes filled with Krebs-Ringer solution (see below) from AP neurons in the slice preparations. Ionophoretic pulses of ACh, NMDA, AMPA or GABA were delivered for 1 s through an independent multi-barreled micropipette placed in proximity to the recording neuron (Strominger et al., 2001). Intracellular membrane potentials were also recorded with microelectrodes from DMV neurons in response to electrical field stimulation (EFS) applied to the AP area. The glass microelectrodes for intracellular recording from DMV neurons were made from borosilicate capillary glass (1.2 mm outer diameter, 0.6 mm inner diameter) and filled with 3 M K-acetate and had an input resistance of about 100 MΩ. Concentric tungsten stimulating electrodes were positioned within the AP area.

For whole-cell patch clamp recording, membrane potential was held at a holding potential (V_H) of 0 or −60 mV (CEZ-2300, Nihon Kohden, Tokyo, Japan). Patch pipettes were made from borosilicate capillary glass (1.5 mm outer diameter, 0.9 mm inner diameter) in two stages on a vertical pipette puller (PB-7; Narishige). The resistance of the recording pipettes filled with internal solution was 5–6 MΩ. Electrode capacitance was compensated, but series resistance was not. Neurons were viewed under phase contrast on an inverted microscope (Diaphot, Nikon for isolated neurons, DM-LFSA, Leica for slice preparation). Current and voltage were continuously monitored on an oscilloscope (VC-6023, Hitachi, Japan), a pen recorder (RECTI-HORIT-8K, Sanei, Tokyo, Japan) and recorded on a digital-audio tape recorder (RD-120TE, TEAC). Membrane currents were filtered at 1 kHz (E-3201A Decade Filter, NF Electronic Instruments, Tokyo, Japan), digitized at 4 kHz, and stored on a computer equipped with pCLAMP 8.0 (Axon Instruments, USA). When recording, 10 mV hyperpolarizing step pulses (30 ms in duration) were periodically delivered to monitor the access resistance. All experiments were performed at room temperature (23–25°C).

For morphological studies, Lucifer yellow CH (Sigma, 10% in distilled water) was injected into a cell through a special intracellular recording electrode with input resistance of 30–50 MΩ by application of 2 nA negative current pulses of 250 ms duration at 2 Hz for 1.5–2 m. The recording chamber was perfused with Krebs-Ringer solution (33°C) saturated with 95% O_2 and 5% CO_2 at 3 ml/min.
Data analysis

Spontaneous IPSCs (sIPSCs) were counted and analyzed using Mini Analysis program (Synaptosoft, Inc., Decatur, GA, USA) as described previously (Jang et al., 2002). Briefly, sIPSCs were screened automatically with an amplitude threshold of 10 pA, and then visually accepted or rejected based upon the rise and decay times. The average values of sIPSC frequency and amplitude during the control period (5–10 min) were calculated, and the frequency and amplitude of all the events during Z-338 application (5 min) were normalized to these values. The effect of Z-338 was quantified as a percentage decrease in sIPSC amplitude compared to the control values.

The concentration-response data for GABA and nicotine were fitted to the following modified Michaelis-Menten equation, using a least-squares fitting routine:

\[ I = \frac{I_{\text{max}} \times C^n H}{(C^n H + EC_{50}^n H)} \text{ or } I = 1 - \frac{I_{\text{max}} \times C^n H}{(C^n H + IC_{50}^n H)} \]

where \( I \) is the amplitude of the agonist-induced postsynaptic currents and \( C \) is the corresponding agonist concentration. \( EC_{50}, IC_{50} \) and \( nH \) denote the half-effective, half-inhibitory concentration and the Hill coefficient, respectively. Numerical values are given as means ± standard error of the mean (SEM) from values normalized to the control, except where indicated. Possible differences in amplitude and frequency distribution were tested by Student’s paired two-tailed \( t \)-test using their absolute values rather than normalized ones. Values of \( P<0.05 \) were considered significant.

Solutions

For the intra- and extra-cellular recordings, we used Krebs-Ringer solution containing (in mM), 126 NaCl, 5 KCl, 2.4 CaCl₂, 1.3 MgSO₄, 1.26 KH₂PO₄, 26 NaHCO₃ and 10 D-glucose saturated with 95% O₂ and 5% CO₂.

For the patch clamp experiments, we used the following solutions. The ionic composition of the incubation medium consisted of (in mM) 124 NaCl, 5 KCl, 2.4 CaCl₂, 1.3 MgSO₄, 1.5 KH₂PO₄, 24 NaHCO₃ and 10 glucose saturated with 95% O₂ and 5% CO₂. The low-Na⁺-medium consisted of (in mM) 230 sucrose, 5 KCl, 2.4 CaCl₂, 1.3 MgSO₄, 1.5 KH₂PO₄, 24 NaHCO₃ and 10 glucose saturated with 95% O₂ and 5% CO₂. The pH was about 7.45. For isolated neurons, the standard external solution was (in mM) 150 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂ and 10 glucose and 10 HEPES. For recording NMDA receptor-mediated currents, MgCl₂ was simply deleted from the standard external solution. These external solutions were adjusted to pH 7.4 with Tris-base. For recording sIPSCs, external solution routinely contained 500 nM tetrodotoxin (TTX) to block voltage-dependent Na⁺ channels. The ionic composition of the internal (patch-pipette) solution for whole-cell patch-clamp recording consisted of (in mM) 135 Cs-methanesulfonate, 5 TEA-Cl, 5 CsCl, 2 EGTA, 4 ATP-Mg and 10 HEPES with pH adjusted to 7.2 with Tris-base. For slice preparations, the external bath solution consisted of (in mM) 124 NaCl, 3 KCl, 2 CaCl₂, 1 MgCl₂, 1.5 KH₂PO₄, 24 NaHCO₃ and 10 glucose saturated with 95% O₂ and 5% CO₂.

Drugs

The drugs used in the present study were tetrodotoxin (TTX), kainic acid (KA), N-methyl-D-
aspartate (NMDA), gamma amino butyric acid (GABA), glycine, bicuculline and ATP-Mg (from Sigma, St. Louis, MO). Z-338 was kindly donated by Zeria Pharmaceutical Co., Ltd. For isolated neurons, external solutions containing drugs were applied with the ‘Y-tube system’ for rapid solution exchange (Akaike and Harata, 1994). For slice preparations, drugs were applied to the bath perfusion, except where indicated.

Results

The neural links between AP and DMV

To study the neural link between AP and DMV, we injected Lucifer Yellow CH into neurons in AP or DMV that allows visualization of the cell body and its dendrites. Figure 1 shows representative neurons injected with Lucifer Yellow CH. Axon-like processes from the small neuron in AP are extending into DMV through nucleus tractus solitarius (NTS) (Fig. 1, A and B), and relatively luxuriant dendritic arborization radiates from larger somata in DMV into AP and also into the contralateral DMV (Fig. 1, C and D). These observations indicate that the

Fig. 1. Photomicrographs of coronal sections at the level of obex with Lucifer Yellow CH injected neurons. A & B: The axon (arrows) projects from relatively small soma in AP to DMV. C & D: Relatively luxuriant dendritic arborizations radiate from larger somas in DMV to AP. E: Schematic representation of neural link from AP to DMV. AP: area postrema, CC: central canal, DMV: dorsal motor nucleus of the vagus nerve, IVth: fourth ventricle. Each somata was impaled by a microelectrode filled with Lucifer Yellow CH.
reciprocal connections exist between AP and DMV in the rat (Fig. 1E). To obtain more direct evidence for the neural links between AP and DMV, we applied electrical field stimulation (EFS) to AP and made intracellular recordings from neurons in DMV. In about 40–50% of the examined cells, EFS evoked excitatory post synaptic potentials (EPSPs). These triggered action potentials with increased stimulus intensity in the DMV neuron (Fig. 2A). In contrast, roughly 30–40% of the impaled neurons in DMV responded with inhibitory post synaptic potentials (IPSPs) with a longer delay than EPSPs (Fig. 2B). The reversal potential was –60 to –70 mV. The EPSPs and IPSPs were sensitive to TTX (1 µM), indicating that there are direct excitatory and inhibitory neural connections from AP to DMV neurons (Fig. 2C).

**Fig. 2.** Intra-cellular recordings from neurons in DMV showing response to electrical field stimulation (EFS) applied to AP. A: Excitatory postsynaptic potentials with and without action potential. B: Inhibitory postsynaptic potentials recorded at resting membrane potential (middle trace) or after injection of 500 pA current into the neuron (the lowest trace), upper trace indicates 0 mV. Note the polarity of the IPSP reversed after the injection of the current. C: Schematic representation of neural link between AP and DMV.

**Effects of Z-338 on AP neurons in slice preparations**

To observe the effects of Z-338 on the spontaneous activity and actions of excitatory and inhibitory neurotransmitters, we recorded single unit discharges from AP neurons by
extracellular recording from slice preparations. Single unit discharges had firing rates ranging between 0.7 and 5 unit discharges/sec. As shown in Fig. 3A, Z-338 (10 µM) markedly enhanced the frequency of spontaneous unit discharges in AP neurons with relatively low (<1~2 unit discharges/sec) firing rates. The acceleratory effect of Z-338 was reversible, although the effects lasted for prolonged period (more than 1 hour) after wash out of Z-338. In neurons with relatively higher firing rates (>3~5 unit discharges/sec), on the other hand, Z-338 showed little effect on the firing rates (Fig. 3B).

More than 60% of neurons did not respond to iontophoretically applied ACh, AMPA, NMDA or GABA. The lack of response to these chemicals may reflect difficulty in positioning the iontophoretic electrode close enough to the small neuron, or the particularly rich vascular supply in this structure may interfere with iontophoresis by dispersing the effluent. However, some neurons exhibited brief excitation to ACh, AMPA, NMDA and inhibition to GABA, in a concentration dependent manner (Fig. 4). GABA completely suppressed spontaneous action potentials, and induced a ‘silent period’, the duration of which was prolonged with increase in the current intensity used to apply GABA (Fig. 4). The excitatory actions of ACh, AMPA and NMDA were unchanged when modified Krebs-Ringer solution containing low Ca²⁺ (0.1 mM)
and high Mg$^{2+}$ (4.3 mM) was used to block synaptic transmission (data not shown).

Figure 5 shows the effects of Z-338 (10 µM) on the excitatory and inhibitory actions of ACh, AMPA, NMDA and GABA. Z-338 suppressed the excitatory action of ACh but not those of AMPA or NMDA, and shortened the GABA-induced 'silent period'. These observations indicate that Z-338 specifically antagonized the excitatory and inhibitory actions of ACh and GABA. To study further the inhibitory effect of Z-338 on ACh-induced excitation, we applied ACh at three different current intensities and observed the effects of Z-338. As shown in Fig. 6, the number of spikes just after the application of ACh increased with increase in the current intensities used to apply ACh. Z-338 significantly suppressed the ACh-induced increase in the number of spikes from 34/s and 51/s to 23/s and 19/s at stimulus intensities of 15 and 20 nA, respectively. The inhibitory effect of Z-338 on the ACh-induced excitation was reversible (Fig. 6).

**Effects of Z-338 on the actions of nicotine, KA, NMDA and ATP on mechanically isolated AP neurons**

To study the mechanisms involved in the inhibitory effect of Z-338 on ACh-induced
Fig. 5. Effects of Z-338 (10 µM) on the excitatory and inhibitory actions of ACh, AMPA, NMDA, and GABA. Current intensity to apply the drugs was 10 nA. A and B were recorded before and during application of Z-338 (10 µM for 1sec), and C after wash out of Z-338. Z-338 inhibited the excitatory action of ACh but not AMPA and NMDA, and shortened the ‘silent period’ induced by GABA.

Fig. 6. Effects of Z-338 (10 µM) on the ACh-induced increase in the unit discharges. Three different current intensities (10 nA (A), 15 nA (B) and 20 nA (C)) were used to apply ACh iontophoretically. The number of unit discharges in a second, just after the application of ACh, are shown in parenthesis. Bars indicate the application of ACh.
excitation of AP neurons, we used single neurons isolated from AP and applied conventional whole-cell patch clamp recording. Since it is known that AP neurons do not possess mAChR (Strominger et al., 2001), we used nicotine in the following experiments. As shown in Fig. 7, nicotine (30 µM) evoked inward current with an initial peak followed by a steady current. Upon repetitive applications of nicotine, peak currents showed strong fading, and therefore it was not technically feasible to observe the effects of Z-338 on the peak current induced by nicotine. Therefore we observed the effects of Z-338 on the steady current induced by nicotine (30 µM), and found that Z-338 suppressed the steady current in a dose-dependent manner (Fig. 7B). Interestingly, when a higher concentration of nicotine (100 µM) was used, Z-338 showed a greater tendency to suppress the steady current, although the effects were not statistically significant (Fig. 7B). Z-338 had no effect on the inward currents induced by KA, NMDA or ATP (Fig. 8).

Effects of Z-338 on the action of GABA on single AP neurons

We also observed the effects of Z-338 on the action of GABA by use of single AP neurons. As shown in Figure 9, exogenously applied GABA (>1 µM) induced outward currents at a holding potential of 0 mV, and Z-338 (30 or 100 µM) suppressed the GABA-induced current in a concentration-dependent manner. Z-338 shifted the concentration-response relationship of the GABA-induced current to the right in a parallel manner (Fig. 9B), and Lineweaver-Burk plots
differ in slope and shared a common intercept on the I/R axis, thereby indicating that Z-338 inhibits the action of GABA in a competitive manner (Fig. 9C).

Effects of Z-338 on sIPSCs in slice preparation

We recorded spontaneous inhibitory postsynaptic currents (sIPSCs) in slice preparations to observe the effects of Z-338 on the action of endogenous GABA. Figure 10 shows sIPSCs recorded from an AP neuron. This was sensitive to bicuculline (10 µM, data not shown), thereby indicating that these spontaneous events are GABAergic sIPSCs. Z-338 (>30 µM) reduced the amplitude of sIPSCs in a concentration-dependent manner, and 300 µM Z-338 almost completely abolished the sIPSCs.

Discussion

In the rat paratracheal ganglion, we reported that activation of nAChRs and mAChRs induced inward currents with rapid and slow time courses, and that Z-338 inhibited mAChR- and nAChR-mediated inward currents competitively and uncompetitively, respectively (Kanemoto et al., 2002). AP neurons are considered to possess nAChRs but not mAChRs (Strominger et al.,
and in the present experiments Z-338 inhibited nicotine-induced inward currents as in the case of paratracheal ganglion cells. However, it was not technically feasible to study the detailed mechanisms involved in the inhibitory action of Z-338 on nicotine-induced current in the present experiments, since it was not possible to record stable nicotine-induced currents in AP neurons. It is well documented, however, that Z-338 acts antagonistically on mAChRs (M₁ and M₂) and nAChRs (Ogishima et al., 2000; Kanemoto et al., 2002), and the present results support this.

In addition to the inhibitory action of Z-338 on nAChRs, the present study revealed that Z-338 also inhibits GABA-induced current in a competitive manner in AP neurons. In slice preparations, iontophoretically applied GABA completely inhibited the generation of spontaneous action potentials, thereby inducing ‘silent periods’. Z-338 (10 μM) shortened these GABA-induced ‘silent period’. Furthermore, Z-338 suppressed the amplitude of sIPSCs, suggesting that Z-338 antagonises the action of endogenously released GABA. These observations strongly indicate that Z-338 suppresses the GABAergic inhibitory neurotransmission within the AP, in addition to the inhibitory action on nAChR. However, it should be pointed out that the minimum concentrations of Z-338 to suppress the nicotine- and GABA-induced currents were about 3 and 30 μM, respectively. The peak concentration of GABA within the synaptic cleft after single vesicle release has been estimated to be ~300 μM (Perrais 2001), and in the present experiments Z-338 inhibited nicotine-induced inward currents as in the case of paratracheal ganglion cells. However, it was not technically feasible to study the detailed mechanisms involved in the inhibitory action of Z-338 on nicotine-induced current in the present experiments, since it was not possible to record stable nicotine-induced currents in AP neurons.
and Ropert, 1999), and thus Z-338 at concentrations less than 30 µM would not efficiently antagonize the action of GABA at GABA<sub>A</sub> receptors (GABA<sub>A</sub>R), although Z-338 competitively inhibited GABA<sub>A</sub>R (Fig. 7). Taken together, it seems that Z-338 would exert its effects mainly through the action on nAChRs rather than GABA<sub>A</sub>R in AP.

The neuronal cells within AP are exposed to endogenous and exogenous substances in the blood circulation. For instance, intravenous administration of apomorphine, that is a non-specific dopaminergic agonist and induces emesis, evokes two distinct gastric motor responses in rat. One is the inhibition of phasic contractions that appear just after administration (Abrahamsson et al., 1973; Blancquaert et al., 1985), and the other is an increase in the frequency of small phasic contractions accompanied by increased gastric tone appearing with a relatively longer delay (Koga et al., 2003). Nicotine is also known to induce excitatory and inhibitory effects on the gastrointestinal tract, some of which may be caused by interaction of nicotine with nAChR in the medulla oblongata (Nagata and Osumi, 1991; Ferreira et al., 2000, 2001). Relatively low doses of i.v. nicotine produce dose-related decrease in intragastric pressure (IGP), and tonic contraction of the fundus without affecting the phasic contractions of the antrum. Microinjections of nicotine into DMV and medial nucleus of the tractus solitarius (mNTS) induces increase and decrease in IGP, respectively (Ferreira et al., 2000, 2001). All these observations indicate that apomorphine and nicotine induce excitatory and inhibitory

![Fig. 10. Effects of Z-338 on GABAergic sIPSCs in the slice preparation. A: Typical trace from one cell of GABAergic sIPSCs under control conditions and in the presence of Z-338 at various concentrations. B: All-points scatter plot against time of sIPSC amplitude.](image)
effects on the motility of the stomach through the central nervous system including the AP, NTS and DMV. Thus, it is reasonable to assume from the present study that Z-338 modifies the neuronal activity in AP through inhibitory actions mainly on nAChR and GABA$_A$R.

The AP has extensive connections with DMV (Hyde and Miselis, 1983; Leslie and Gwyn, 1984; Miselis et al., 1984; present observations), and efferent pathways from the DMV dominantly control the mechanical behaviour of the stomach. It appears that at least three types of efferent pathways from the DMV can influence the motility of the stomach. Firstly, there is a pathway with cholinergic motor neurons in the DMV which synapse onto postganglionic cholinergic neurons which could inhibit or accelerate gastric contractility. Secondly, another pathway has cholinergic motor neurons in the DMV which synapse onto nitriergic neurons in the stomach (Berthoud, 1995). Finally, a pathway with nitriergic motor neurons in the DMV, directly innervates gastric smooth muscle cells (Krowicki et al., 1997, 1999). Activation or inhibition of these pathways would inhibit or accelerate gastric contractility. In addition, the motility of the fundic and antral region of stomach is regulated by separate neural pathways from DMV (Fukuda et al., 1987; Ferreira et al., 2002). Therefore it seems rational that Z-338 has both facilitatory and inhibitory actions on motility and muscle tone of the stomach, and the present study indicates that these effects could be induced through the actions of Z-338 on AP neurons. Z-338 (10 $\mu$M) enhanced the firing rates in AP neurons with relatively low firing rates, although this agent did not significantly affect those with relatively higher firing rates. Furthermore, our recent study indicates that rat stomach receives efferent neural input from AP and electrical stimulation of AP neurons relaxes the stomach in rat, thereby suggesting that some functional roles of AP neurons in the regulation of gastric motility (Kawachi et al., 2008).

Z-338 has well documented excitatory but not inhibitory action on stomach motility through the peripheral autonomic nervous system (Ueki et al., 1998; Nakajima et al., 2000; Kanemoto et al., 2002; Morita et al., 2002). The actions of Z-338 on central and peripheral autonomic nervous system, taken together, would therefore explain the observations that Z-338 induces postmeal relaxation and improve postprandial fullness in patients with functional dyspepsia (Tack et al., 2002).

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