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Functional evidence for GABA as modulator of the contractility of the longitudinal muscle in mouse duodenum: Role of $GABA_A$ and $GABA_C$ receptors

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Abstract

We investigated, in vitro, the effects of γ-aminobutyric acid (GABA) on the spontaneous mechanical activity of the longitudinal smooth muscle in mouse duodenum. GABA induced an excitatory effect, consisting in an increase in the basal tone, which was antagonized by the $GABA_A$-receptor antagonist, bicuculline, potentiated by (1,2,5,6-Tetrahydropyridin-4-yl)methylphosphinic acid hydrate (TPMPA), a $GABA_C$-receptor antagonist and it was not affected by phaclofen, a $GABA_B$-receptor antagonist. Muscimol, $GABA_A$ receptor agonist, induced a contractile effect markedly reduced by bicuculline, tetrodotoxin (TTX), hexamethonium and atropine. Cis-4-aminocrotonic acid (CACA), a specific $GABA_C$ receptor agonist, induced an inhibitory effect, consisting in the reduction of the amplitude of the spontaneous contractions and muscular relaxation, which was antagonised by TPMPA, $GABA_C$-receptor antagonist, TTX or N^{ω}-nitro-L-arginine methyl ester (L-NAME), nitric oxide (NO) synthase inhibitor, but not affected by hexamethonium. In conclusion, our study indicates that GABA is a modulator of mechanical activity of longitudinal muscle in mouse duodenum. GABA may act through neuronal presynaptic receptors, namely $GABA_A$ receptors, leading to the release of ACh from excitatory cholinergic neurons, and $GABA_C$ receptors increasing the release of NO from non-adrenergic, non-cholinergic inhibitory neurons.

Keywords: GABA; Mouse duodenum; Intestinal motility; Cholinergic excitatory nerves; NANC inhibitory nerves; GABA receptors

1. Introduction

GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the central nervous system (CNS) acting through three different receptors identified as: $GABA_A$, $GABA_B$ and $GABA_C$ (Mody et al., 1994; Johnston, 1996; Couve et al., 2000). In the last years evidence has been provided that GABAergic neurons and GABA receptors are also present in the enteric nervous system of vertebrates (Zeiter et al., 1996; Krantis et al., 1998; Akinci and Schofield, 1999; Castelli et al., 1999; Fletcher et al., 2002) and may play a physiological role, modulating and controlling the gastrointestinal motility (Bertolino et al., 1997; Greenwood-Van Meerveld and Barron, 1998; Sivarao et al., 1998). Enteric GABAergic neurons appear to be exclusively interneurons and activation of the $GABA_A$ and $GABA_B$ receptors can lead to variable effects depending on the part of the gut or the animal species studied. It is generally accepted that activation of the ionotropic $GABA_A$ receptors may stimulate the enteric cholinergic excitatory and the non-adrenergic, non-cholinergic (NANC) inhibitory motor neurons, leading to either contraction or relaxation of the intestinal smooth muscle (Frigo et al., 1987; Krantis and Harding, 1987; Boeckxstaens et al., 1990; Minocha and Galligan, 1993; Kaputlu and Sadan, 1996; Pencheva, 1997). The effects of metabotropic $GABA_B$-receptor activation are generally related to presynaptic inhibition of acetylcholine release via the

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blockade of voltage-sensitive calcium channels (Cherubini and North, 1984; Marcoli et al., 2000). Moreover, Hebeiss and Kilbinger (1999) reported that, in longitudinal muscle-myenteric plexus preparations from guinea pig, activation of both $GABA_A$- and $GABA_B$-receptors inhibited nitric oxide (NO) release from NANC inhibitory neurons. Furthermore, recent evidence has indicated that the ionotropic $GABA_C$-receptors, believed to be expressed only in the retina (Johnston, 1996), are also present by neurons in the rat gastrointestinal tract (more than 50% were also nitric oxide synthase immunoreactive neurons), suggesting that $GABA_C$ receptors may play a role in the gastrointestinal functions such as intestinal motility (Fletcher et al., 2001). Anyway, the role of GABA on gastrointestinal motility remains rather complex and not fully understood.

The aim of the present work was to investigate the effects of GABA on the spontaneous mechanical activity of the longitudinal smooth muscle in mouse duodenum. More specifically, we examined the subtypes of GABA receptors involved in the response and the possible site(s) of action of GABA. We chose this preparation because, although mice are becoming increasingly important models for studying gastrointestinal functions, there are few studies about the role of GABA in the enteric neurotransmission. In particular, baclofen, $GABA_B$ receptor agonist, has been shown to accelerate gastric emptying in vivo (Symonds et al., 2003) and $GABA_B$ receptors have been reported to mediate the constipating effects of γ-hydroxybutyric acid in the small intestine (Carai et al., 2002).

2. Methods

2.1. Tissue preparation

Experiments, authorised by the Ministero della Sanità (Rome, Italy), were performed on adult male mice (C57BL/10SnJ, Charles River, Calco LC, Italia), killed by cervical dislocation. The abdomen was immediately opened and segments of duodenum, just distal to the pylorus, were removed and placed in Krebs solution (mM: NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.1). The contents of the excised segments were gently flushed out with Krebs solution. Segments (20 mm in length) were suspended in 10 ml organ baths containing oxygenated (95% O_2 and 5% CO_2) Krebs solution maintained to 37 °C. Silk ligatures closed up each end of the segment. The distal end was then tied to an organ holder and the proximal end was secured to an isometric force transducer (FORT 10, Ugo Basile, Biological Research Apparatus, Comerio VA, Italy). Longitudinal preparations were subjected to an initial tension of 200 mg and were allowed to equilibrate for at least 30 min in the Krebs solution before starting the experiments. Krebs solution contained neostigmine (10 μM) to increase the reactivity of the muscle to contractile agents. Mechanical activity was digitised on an A/D converter, visualised, recorded and analysed on a personal computer using the PowerLab/400 system (Ugo Basile, Italy).

2.2. Experimental protocols

At the beginning of each experiment the preparation was challenged either with 10 μM carbachol (CCh) or with 100 μM sodium nitroprusside (SNP) for 2 min until reproducible responses were obtained. The amplitude of the contractile response to CCh was 341.9 ± 24.6 mg ($n = 21$) and the amplitude of the relaxant response to SNP was 161.0 ± 21.3 mg ($n = 10$).

In a first set of experiments, concentration-dependent curves for the responses to GABAergic receptor agonists were constructed by non-cumulative addition of the drug before and after the selective GABA receptor antagonists used. Agonists were applied for 3 min at 20 min intervals. All the antagonists were allowed to maintain contact with the tissue for at least 30 min before repeating the curve of the agonist. The interval between the two assays was at least 1 h. Each preparation was tested with a single antagonist. Time control experiments showed that a second curve to the agonist was reproducible.

In a second set of experiments, a submaximal dose of GABA receptor agonists was tested in the presence or in the absence of tetrodotoxin, hexamethonium, atropine, L-NAME (30 min pretreatment). Each preparation was tested with a single antagonist. Concentrations of the drugs used were determined from literature.

2.3. Drugs

Drugs used: atropine sulphate, baclofen, bicuculline, cis-4-aminocrotonic acid (CACA), gamma amino butyric acid (GABA), hexamethonium bromide, neostigmine bromide, N^{ω}-nitro-L-arginine methyl ester (L-NAME), sodium nitroprusside (SNP), (1,2,5,6-Tetrahydropyridin-4-yl)methylphosphinic acid hydrate (TPMPA), tetrodotoxin (TTX), all purchased from Sigma (Sigma-Aldrich, Inc., St. Louis, USA). Phaclofen was from Tocris (Tocris Cookson LtD., Avonmouth, UK). Bicuculline was dissolved in dimethyl sulphoxide (DMSO) and phaclofen in 0.1 N NaOH. All the other drugs were dissolved in distilled water. The working solutions were prepared fresh on the day of the experiment by diluting the stock solutions in Krebs. Drugs were added to the organ bath in volumes of less than 1.0% of the bathing solution. Control experiments using DMSO or NaOH alone showed that they have no effect on the spontaneous contractile activity or on the concentration-response curve to GABAergic drugs.

2.4. Statistical analysis

All data are given as means ± SEM, "n" indicates the number of animals from which intestinal segment was taken. The contractile responses to GABAergic receptor agonists were expressed as a percentage of the response to CCh (10 μM). The relaxant responses were expressed as a percentage of the response to SNP (100 μM). Agonist responses were fitted to sigmoid curves (Prism 4.0, GraphPad, San Diego, CA, USA), and EC_{50} values (with 95% Cls) were determined from these curves. Statistical analysis was performed by means by Student's t-test. A probability value of less than 0.05 was regarded as significant.

3. Results

Isolated segments of mouse duodenum displayed spontaneous activity with an amplitude of 259.3 ± 25.5 mg ($n = 28$) and a frequency of 39.2 ± 1.6 cpm ($n = 28$).

Exogenous GABA induced concentration-dependent monophasic effects consisting in an increase in the basal tone of the longitudinal muscle, lasting from 15 to 30 s. The contractile effect appeared in a concentration dose range from 0.3 μM to 1 mM, with an EC_{50} of 16 μM (95% CLs 12–22 μM, $n = 12$) and the maximal response reached about 70% of the response to CCh (Figs. 1 and 2). Pre-incubation with the selective $GABA_A$-receptor antagonist, bicuculline (10 μM), which per se did not modify the amplitude or the frequency of spontaneous activity, markedly reduced GABA-induced contractile effects (Fig. 2). Phaclofen (10 μM), selective $GABA_B$-receptor antagonist did not modify the response to GABA (Fig. 2). Interestingly, pre-treatment with TPMPA (10 μM) a selective $GABA_C$-receptor antagonist, increased the contractile response to GABA (Fig. 2).