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K_V7 channels regulate muscle tone and nonadrenergic noncholinergic relaxation of the rat gastric fundus

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ABSTRACT

Voltage-dependent type 7 K^+ (K_V7) channels play important physiological roles in neurons and muscle cells. The aims of the present study were to investigate the motor effects of K_V7 channel modulators in the rat gastric fundus and the expression of K_V7 channels in this tissue.

Muscle tone and electrical field stimulation (EFS)-evoked relaxations of precontracted longitudinal muscle strips of the rat gastric fundus were investigated under nonadrenergic noncholinergic conditions by organ bath studies. Gene expression was studied by real-time PCR and tissue localization of channels was investigated by immunohistochemistry.

The K_V7 channel blocker XE-991 induced concentration-dependent contractions, with mean pD_2 and E_{max} of 5.4 and 48% of the maximal U46619-induced contraction, respectively. The K_V7 channel activators retigabine and flupirtine concentration-dependently relaxed U46619-precontracted strips, with pD_2s of 4.7 and 4.4 and E_{max} of 93% and 91% of the maximal relaxation induced by papaverine, respectively. XE-991 concentration-dependently inhibited retigabine-induced relaxation with a pIC_{50} of 6.2. XE-991 and DMP-543, another K_V7 channel blocker, increased by 13–25% or reduced by 11–21% the relaxations evoked by low- or high-frequency EFS, respectively. XE-991 also reduced the relaxation induced by vasoactive intestinal polypeptide (VIP) by 33% of controls. Transcripts encoded by all K_V7 genes were detected in the fundus, with 7.4 and 7.5 showing the highest expression levels. $K_V7.4$ and 7.5 channels were visualized by confocal immunofluorescence in both circular and longitudinal muscle layers.

In conclusion, in the rat proximal stomach, K_V7 channels appear to contribute to the resting muscle tone and to VIP- and high-frequency EFS-induced relaxation. K_V7 channel activators could be useful relaxant agents of the gastric smooth muscle.

1. Introduction

In mammalian cells, K^+ channels are involved in several physiological processes, such as neurotransmitter and hormone release, motor activity of muscle cells, regulation of water-electrolyte balance and epithelial secretion [1]. Opening of K^+ channels hyperpolarizes the resting membrane potential and decreases cell excitability. Many K^+ channel subtypes in different cell types of the gastrointestinal tract (epithelial, smooth muscle cells [SMC], interstitial cells of Cajal [ICC], fibroblast-like cells [FLC] and neurons) are involved in gut secretory and motor activities. By differentially controlling the membrane resting potential of SMC–ICC–FLC syncytial apparatus in the various gut segments, K^+ channels participate to the region-dependent differences in basal muscle tone levels [2]. Opening of K^+ channels is among the signal transduction mechanisms activated by the neurotransmitters released from the inhibitory motor neurons [2]. Voltage-dependent K^+ (K_V) channels are particularly expressed in the GI tract; indeed, $K_V1.2$, 1.5, 1.6, 2.2, 4.1, 4.3, 7, 11.1 and 12.1 transcripts and/or proteins have been shown in the GI tract, with $K_V1.2$ and 2.2 playing a dominant role in regulating SMC contractility [2].

The K_V7 channel subfamily includes 5 members ($K_V7.1$–7.5), each with distinct expression pattern and functional roles. $K_V7.1$ channels mediate the slowly activating K^+ current (I_{Ks}) involved in the late repolarizing phase of the action potential in cardiomyocytes [3]. In neuronal cells, $K_V7.2$, 7.3 and 7.5 represent the molecular basis of the M current (I_{KM}), a slowly activating and deactivating current inhibited by muscarinic receptors stimulation, that modulates cell excitability and firing pattern [4,5]. $K_V7.4$ channels have been first described in the inner ear and auditory neurons [6] and more recently in skeletal muscle cells [7]. K_V7 channels have been shown to regulate vascular, gastrointestinal and genitourinary

Abbreviations: TTX, tetrodotoxin; CTX GVIA, ω-conotoxin GVIA; ICC, interstitial cells of Cajal; FLC, fibroblast-like cells; SMC, smooth muscle cells; TP, thromboxane prostanoid; VIP, vasoactive intestinal polypeptide.

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smooth muscle activity [8–11]. In the gastrointestinal tract, acetylcholine has been long known to increase the membrane excitability of toad [12] and guinea-pig [13] gastric SMC through the inhibition of a voltage-dependent K^+ current active at resting membrane potential, having properties similar to neuronal I_{KM}. This evidence might suggest that K_V7 channels are expressed in the SMC–ICC–FLC syncytial apparatus of the stomach and their inhibition mediate acetylcholine-induced electrophysiological effects. The expression of K_V7 channels and the motor effects of K_V7 channel modulators have been investigated in the mouse colon [9]. In the same study, all K_V7 channel gene transcripts have been shown to be expressed in both gastric fundus and antrum in the mouse, with $K_V7.4$ and 7.5 showing the highest levels [9]. Only the expression of $K_V7.1$–7.3 channel genes has been investigated in the rat stomach [14]. $K_V7.1$ and 7.3 channel mRNAs were detected in the whole stomach and their levels were relatively high in the antrum but very low in the fundus [14].

The proximal stomach plays an important "reservoir" function, i.e. it accommodates high volumes of food bolus with small increases in intraluminal pressure. The accommodative gastric function occurs mostly through the active reflex neural nonadrenergic noncholinergic (NANC) relaxation of the smooth muscle. *In vitro* preparations of the rat proximal stomach passively and progressively relax under a constant load during the equilibration period and generally stabilize on a very low basal muscle tone. In addition, they show a very low phasic muscle activity. *In vivo*, gastric tone appears to be maintained by vagally mediated cholinergic input [15]. The most probable neurotransmitters released by the inhibitory motor neurons are nitric oxide (NO) and vasoactive intestinal polypeptide (VIP). NO is mainly responsible for the rapid beginning and the high speed of the initial phase of the relaxation, whereas VIP and its related peptide, peptide histidine isoleucine (PHI), are mainly involved in the long duration of the inhibitory response evoked by high-frequency neuronal activation [16]. At least a third component, probably produced by a non-purinergic neurotransmitter acting via apamin-sensitive mechanisms, seems also to be present [17]. It is well known that NO and VIP mainly act through the activation of soluble guanylate cyclase and VPAC2 receptors followed by stimulation of adenylate cyclase through G_s protein, respectively. However, the final molecular mechanisms linked to the relaxation have not been fully elucidated in the rat gastric fundus. In addition, the ion channels contributing to the membrane resting potential of the SMC–ICC–FLC syncytial apparatus of the rat gastric fundus have not been definitively characterized. In particular, a characterization of the role of K_V7 channels in the motor activity of rat proximal stomach has never been performed. In this study, we investigated the effects of K_V7 channel modulators on the resting muscle tone and on the NANC relaxation of the rat gastric fundus. The effects of K_V7 channel blockade on the relaxations induced by NO and VIP were also evaluated. The results of the present study indicate that K_V7 channels play important roles in the maintenance of the low muscle tone in resting conditions and in the VIP-induced relaxation in the rat gastric fundus. Altogether, the results obtained provide the first functional demonstration of a critical control exerted by K_V7 channels over rat proximal stomach motor activity, revealing a novel pharmacological target for therapeutic interventions against gastric motor disturbances.

2. Methods

2.1. Policy and ethics

This study was approved by the institutional Ethical Committee for the Animal Experimentation of the Catholic University. The work described in this article was carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. In addition, this paper fulfils the Uniform Requirements for Manuscripts Submitted to Biomedical Journals of ICMJE.

2.2. Motor activity studies

2.2.1. General methods

Wistar rats of either sex, weighing 180–320 g, were fasted overnight with free access to water, afterwards killed by decapitation and exsanguinated. The gastric fundus was removed and two longitudinal muscle strips (3×20 mm) were prepared according to the method of Vane [18] in a Krebs solution of the following composition (mM): NaCl 118.5, KCl 4.8, $CaCl_2$ 1.9, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 10.1 (pH 7.4). The strips were mounted between parallel platinum electrodes (22 mm long, 4 mm wide and 5.5 mm apart) and suspended in Krebs solution maintained at 37 °C and bubbled with a 95/5 O_2/CO_2 mixture inside 5-ml organ baths. The strips were connected to isotonic transducers (model 7006; Ugo Basile Biological Research Apparatus, Comerio, Italy) under a 1-g load. Smooth muscle activity was recorded on a computer using the PowerLab data acquisition system (ADInstruments, Castle Hill, Australia). Isolated EFSs, consisting of rectangular and bipolar pulses of constant duration (1 ms) and amplitude (120 mA), were performed via platinum plate electrodes by a stimulator (model 6012; Palmer Bioscience, now Harvard Apparatus Ltd., Edenbridge, UK) linked in series with a 4-channel constant-current unit (model Multiplexing Pulse Booster; Ugo Basile Biological Research Apparatus). Tissues were initially allowed to equilibrate for 40 min in Krebs solution. After this period, in all experimental series, the bath solution also contained atropine (1 μM) and guanethidine (5 μM) (to achieve NANC conditions). Strips were allowed to equilibrate for 20 more min in this bath solution. The incubation medium was always changed every 10 min (during the equilibration period and in between drug administration and/or periods of EFS).

2.2.2. Study of the motor effects induced by U46619

In a first series of experiments, the strips were exposed to consecutive 5-min incubations with increasing concentrations of 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U46619, 1 nM to 1 μM), a selective thromboxane receptor (TP) agonist, to investigate the concentration–response relationship for this muscle contracting agent and set the maximal contractile capacity of the strips. Strips were allowed to recover to basal tone prior to the subsequent U46619 concentration. Contractions were expressed as percentages of the effect produced by the maximal concentration used (1 μM).

2.2.3. Study of the motor effects induced by K_V7 channel blockers

In a second series of experiments, the preparations were first contracted by a submaximal concentration (0.1 μM) of U46619. After 10 min, U46619 was washed out from the incubation medium and the strips were allowed to return to basal tone. Then, the strips were exposed to consecutive 5-min incubations with increasing concentrations of the selective K_V7 channel blocker XE-991 (0.5–100 μM) [19]. XE-991 was added cumulatively to the incubation medium. In four strips, the effects of retigabine (1–30 μM), a substance considered to be a selective activator of neuronal K_V7 channels [4,20], added cumulatively to the bath medium, were investigated at the top of the concentration-dependent contraction induced by XE-991 (0.5–100 μM). Then, the substance/s was/were washed out from the bath and strip motor activity was recorded for 30–60 min. At this time, U46619 (0.1 μM) was added to the medium for a second time and left in the bath for 10 min.