Comparative Biochemistry and Physiology, Part C xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Serotonin regulates contractile activity of the uterus in non-pregnant rabbits

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ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 15 May 2014
Accepted 22 May 2014
Available online xxxx

Keywords:
Serotonin
Serotonin receptors
Uterus
Contraction
Cholinergic system

ABSTRACT

Serotonin (5-HT) can stimulate the cholinergic system of the uterus by indirect actions on the modulation of reflexes and a direct action on smooth muscles. This study aimed to investigate the role of 5-HT in the regulation of the cholinergic activity in the uterine parts of non-pregnant rabbits. The right vagus or pelvic nerve and the left sympathetic trunk were stimulated by an electrical field, and the uterine contractile activity was evaluated by measuring the amplitude and frequency of slow wave electromyogram (EMG), with the surface of microelectrodes applied to the uterus bottom, body, and cervix, respectively. Double stimulation of the vagus or pelvic nerve and the serotoninergic fibers of the sympathetic trunk increased the frequency and the amplitude of the slow wave EMG in all the uterine parts. Furthermore, the administration of exogenous 5-HT increased the vagus or pelvic induced EMG activity in all parts of the uterus. Overall our results demonstrate that 5-HT enhances the vagus contractile activity with a magnitude of the effect decreasing from the bottom to the cervix, whereas 5-HT enhances the pelvic nerve contractile functions with a magnitude of the response increasing from the bottom to the cervix. The administration of droperidol, a 5-HT3 and 4 receptor inhibitor, and spiperone, a 5-HT2 receptor antagonist, inhibited the effect of the serotoninergic fibers of the sympathetic trunk to increase the vagus and pelvic nerve EMG activity. These data suggest that 5-HT stimulation of the parasympathetic nerves results in the induction of uterine contraction via the activation of 5-HT2, 3, and 4 receptor subfamilies.

1. Introduction

The uterus is a smooth muscle hollow organ composed of three portions: the bottom, the body, and the cervix. The bottom is the convex upper part of the uterus protruding above the confluence of the fallopian tubes to the uterus; the body is the middle cone-shaped part of the organ; and the cervix is the narrowed portion of the uterus between the body and the neck. The uterus is composed with a well-differentiated lining layer (endometrium), a thick muscular coat (myometrium) and a serosal outer layer. An important characteristic of the uterus is its contractile activity. The dominant cell population of the uterine wall consists of smooth muscle cells that contain the contractile apparatus responsible for the generation of the contractile force (Hutchings et al., 2009). Although there is considerable knowledge about uterine smooth muscle reactivity to both contractile and relaxant stimuli (López Bernal, 2007; Darios et al., 2012), less is known about the contractile function of smooth muscles separately in the different uterine parts.

The smooth muscle of the uterus is influenced by various neuronal, hormonal, metabolic, and mechanical factors, including intramural acetylcholine and serotonin (5-hydroxytryptamine, 5-HT) (Gnanamanickam and Llewellyn-Smith, 2011; Darios et al., 2012). In particular, 5-HT is a regulatory and biologically active neurotransmitter in the CNS and peripheral tissues (Pavone et al., 2007; Narboux-Nème et al., 2008; Pavone et al., 2009; Spina et al., 2011): 5-HT, derived from L-tryptophan and degraded by monoamine oxidases, together with its receptors (divided into seven distinct classes, from 5-HT1 to 5-HT7), and its transporter SERT constitutes the serotonergic system. The biogenic amine, primarily produced and secreted by enterochromaffin cells of the intestine, induces different mechanical responses (i.e., contraction, relaxation or both) on vascular and non-vascular smooth muscles (gastrointestinal tract, heart, urethra, prostate, uterus) of several mammalian species. The tissue- and species-related variations in 5-HT-induced mechanical responses are due to the multiple 5-HT receptor subtypes and their heterogeneous expression in the different organs.

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http://dx.doi.org/10.1016/j.cbpc.2014.05.006
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Human uterine smooth muscle cells express the 5-HT_{2B} receptor (Kelly and Sharif, 2006). In the uterus of rodents and humans, 5-HT found in mast cells has been suggested to be an endogenous regulator of myometrial contractility (Garfield et al., 2000; Bytautiene et al., 2008). It caused the contraction of uterine strips through the activation of the 5-HT_{2A} receptor subtype in rat (Minosyan et al., 2007), through 5-HT_{2B} (Kelly and Sharif, 2006), 5-HT_{2A}, and at lesser extent, $5HT_{1A}$ receptors in isolated human uterine strips (Cordeaux et al., 2009), and in mouse uterine strips (Rudolph et al., 1992). In porcine uterine cornu (horn), 5-HT inhibited contraction through the activation of the 5-HT7 receptor (Kitazawa et al., 2000), but the expression of excitatory (contractile) 5-HT_{2A} receptor in the porcine myometrium was also demonstrated (Nakamura et al., 2008). Cells with 5-HT immunereactivity, different from mast cells, were scattered in the endometrium of uterine glands of pig uterus. Despite these findings, the mechanisms of serotonergic regulation of the different parts of the uterus are still not fully understood, and, at the same time, the nature of the 5-HT receptors mediating myometrial contraction has not yet been definitely classified.

Cholinergic innervation of the uterus is accomplished via the vagus, pelvic, and sacral nerves (Papka et al., 1999). The parasympathetic innervation of the uterus is mainly represented by nerve fibers extending from the pelvic plexus. Afferent impulses derive from the afferent fibers of the vagus nerve. The sympathetic innervation of the uterus mainly involves the hypogastric nerves (Houdeau et al., 1998). Innervation density increases from the inner layer to the outer. Pelvic ganglions of rodents contain sympathetic and parasympathetic neurons, which are innervated by lumbar or sacral preganglionic axons, respectively. Uterine innervation changes throughout the estrous cycle (Zoubina et al., 1998). The mucous of the uterus body in the secretion stage is regulated by cholinergic nerve fibers (Hammarström and Sjöstrand, 1979). The marked region-specific innervation suggests that nerve control of the myometrial activity may be functionally different between the oviduct and the cervical ends of the uterus.

In this study, we evaluated the effect of the stimulation of the sympathetic trunk (e.g., serotoninergic fibers in it) on the electromyogram (EMG) induced by parasympathetic nerves (vagus and pelvic) in the bottom, the body, and the cervix of the rabbit uterus. The involvement of specific 5-HT receptor subfamilies in the contractile function of rabbit uterus was also investigated using the 5-HT receptor antagonists, droperidol and spiperone. Droperidol is a known inhibitor of 5-HT3 and 5-HT4 receptors (Castillo et al., 1995), whereas spiperone is a 5-HT2 receptor antagonist (Shum et al., 2002).

2. Materials and methods

2.1. Animals

The electrophysiological experiments were performed on 24 female Standard Chinchilla rabbits (*Oryctolagus cuniculus*), weighing 2.5–3.5 kg, between 5 and 6 months of age. The animals were provided from the Animal Facility of the Russian National Research Medical University, Moscow, Russian Federation. Experiments were carried out in accordance with the national ethical guidelines, and the animals were handled in a manner approved by the Institutional Animal Use and Care Committee of the Russian National Research Medical University.

2.2. Surgery

Animals were placed under the conditions of the surgical stage of Nembutal narcosis (40 mg/kg, intraperitoneally), and inferior-medial laparotomy was performed. After accessing to the uterus, paired electrodes were placed on the surface of the following parts of the uterus: the bottom, the body, and the cervix. Contact between the electrode tips and the uterus surface was achieved. Control experiments confirming the absence of instrument-derived artifacts were carried out following standard procedures (Ballaro, 2008).

2.3. Drugs

Serotonin adipinate (PubChem CID: 27644), administered at the dose of 50–100 mg/kg body mass, was purchased from Vecton. Spiperone hydrochloride (PubChem CID: 11957687), used at the dose of 2 mg/kg body mass, was purchased from Tocris. Droperidol (PubChem CID: 3168) (Droleptan), used at the dose of 1.0 mg/kg body mass, was purchased from Gedeon Richter Ltd. Sumatriptan (PubChem CID: 5358) (Imigran), used at doses of 0.5–1.0 mg/kg body mass, was purchased from GlaxoSmithKline. All drugs were dissolved in physiological 0.9% NaCl solution immediately before use. Q7

2.4. Electrical stimulation of nerves

The EM-42 Medicor (Hungary) electro-stimulator was used to stimulate cholinergic and serotonergic nerve fibers (as part of the sympathetic trunk). Electric field stimulation was applied to the peripheral cervical segment of the right vagus nerve. The level of parasympathetic nerve stimulation was sufficiently low (2 ms, 1.5–7.0 V, 10 Hz), so that the uterus contraction rate remained stable during 60–90 s in each experiment. Electrical stimulation was also applied to the serotonergic fibers contained in the peripheral cervical segment of the left sympathetic trunk.

2.5. Measurements of uterus EMG

The uterus EMG was measured by using surface bipolar silver electrodes (contact area 1.5–2.0 mm^2, distance between electrodes 1.5 mm) for extracellular recordings. EMG recording was performed with a 21-channel electroencephalograph (Nihon-Kohden, Neurofax, EEG 4400 series, Washington, DC). EMG reflects the transmembrane electrical activity associated with the contractile function of smooth muscles.

2.6. Statistical analysis

Data are expressed as means ± standard error (S.E.). Student's *t*-test was used for statistical comparisons when appropriate, and differences were considered significant at $P < 0.05$.

3. Results

3.1. The uterus bottom

3.1.1. Vagus nerve stimulation of the uterus bottom

The stimulation of the vagus nerve led to an increase of 32.5% and 19% of the frequency and amplitude, respectively, of slow wave EMG of the bottom of the uterus (Table 1). The joint stimulation of the vagus nerve and the sympathetic trunk enhanced vagal stimulatory effect on slow wave EMG (Fig. 1): the frequency increased by 41.1% and amplitude up to 92% (Table 1).

After direct intravenous (i.v.) injection of exogenous 5-HT, the stimulation of the only vagus nerve increased both the EMG frequency and amplitude by 35.1% and 33.3%, respectively (Table 1). Exogenous 5-HT administration combined with double stimulation of the vagus nerve and sympathetic trunk increased the stimulatory effect: slow wave EMG frequency further increased by 22%, with a stable amplitude (Table 1).

The administration of droperidol associated with the stimulation of the vagus nerve increased the frequency of the slow wave EMG by 27.3%, while the amplitude remained stable (Table 1). However, as a result of droperidol administration, the joint stimulation of the vagus nerve and the sympathetic trunk didn't further enhance the EMG activity (Fig. 1), the frequency and the amplitude of slow wave EMG remaining stable (Table 1).

Please cite this article as: Lychkova, A.E., et al., Serotonin regulates contractile activity of the uterus in non-pregnant rabbits, Comp. Biochem. Physiol., C (2014), http://dx.doi.org/10.1016/j.cbpc.2014.05.006