European Journal of Pharmacology 723 (2014) 246–252

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Pulmonary, gastrointestinal and urogenital pharmacology

Underlying mechanisms involved in progesterone-induced relaxation to the pig bladder neck ☆

Vítor S. Fernandes [a], Ana S.F. Ribeiro [a], Ana Martínez-Sáenz [b], Igor Blaha [c], Daniel Serrano-Margüello [a], Paz Recio [a], Ana Cristina Martínez [a], Salvador Bustamante [d], David Vázquez-Alba [d], Joaquín Carballido [d], Albino García-Sacristán [a], Medardo Hernández [a,*]

[a] Departamento de Fisiología, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain
[b] Unidad Experimental, Fundación de Investigación Biomédica, Hospital Universitario Puerta de Hierro-Majadahonda, 28222 Madrid, Spain
[c] Departamento de Urología, Hospital General Universitario Gregorio Marañón, 28007 Madrid, Spain
[d] Departamento de Urología, Hospital Universitario Puerta de Hierro-Majadahonda, 28222 Madrid, Spain

ARTICLE INFO

Article history:
Received 1 October 2013
Received in revised form
12 November 2013
Accepted 22 November 2013
Available online 1 December 2013

Keywords:
Pig bladder neck
Smooth muscle relaxation
Progesterone
K^+ channels
VOC channels

ABSTRACT

Progesterone increases bladder capacity and improves the bladder compliance by its relaxant action on the detrusor. A poor information, however, exists concerning to the role of this steroid hormone on the bladder outflow region contractility. This study investigates the progesterone-induced action on the smooth muscle tension of the pig bladder neck. To this aim, urothelium-denuded bladder neck strips were mounted in myographs for isometric force recordings and for simultaneous measurements of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and tension. On phenylephrine (PhE)-precontracted strips, progesterone produced concentration-dependent relaxations only at high pharmacological concentrations. The blockade of progesterone receptors, nitric oxide (NO) synthase, guanylyl cyclase, large conductance Ca^{2+}-activated K^+ (BK_{Ca}) or ATP-dependent K^+ (K_{ATP}) channels reduced the progesterone relaxations. The presence of the urothelium and the inhibition of cyclooxygenase (COX), intermediate- and small-conductance Ca^{2+}-activated K^+ channels failed to modify these responses. In Ca^{2+}-free potassium rich physiological saline solution, progesterone inhibited the contraction to $CaCl_2$ and to the L-type voltage-operated Ca^{2+} (VOC) channel activator BAY-K 8644. Relaxation induced by progesterone was accompanied by simultaneous decreases in smooth muscle $[Ca^{2+}]_i$. These results suggest that progesterone promotes relaxation of pig bladder neck through smooth muscle progesterone receptors via cGMP/NO pathway and involving the activation of BK_{Ca} and K_{ATP} channels and inhibition of the extracellular Ca^{2+} entry through L-type VOC channels.

1. Introduction

Progesterone is a steroid hormone that produces smooth muscle relaxation in the urinary tract and low circulating levels of this steroid have been associated with the etiology of lower urinary tract symptoms (Tincello et al., 2009). In fact, progesterone promotes an increase in bladder capacity, improves the bladder's compliance by direct relaxation of the detrusor (Tincello et al., 2009; Tong et al., 1995) and relaxes rat ureter and rabbit urethra smooth muscle (Raz et al., 1972, 1973; Rodríguez et al., 2004).

The bladder neck is part of the urine bladder outflow region in which nitric oxide (NO) (Bustamante et al., 2010; Hernández et al., 2007, 2008) and several non-NO mediators, such as adenosine 5′-triphosphate (ATP) (Hernández et al., 2009), 5-hydroxytryptamine (5-HT) (Recio et al., 2009), peptides, such as calcitonin gene-related peptide (CGRP) (Martínez-Sáenz et al., 2011a) and pituitary adenylate cyclase-activating polypeptide 38 (PACAP 38) (Hernández et al., 2006a, 2006b), steroids as testosterone (Fernandes et al., 2012) and an unidentified nerve-dependent component (Martínez-Saénz et al., 2011b), are involved in the inhibitory transmission. Recently, a key role for H_2S, endogenously generated from L-cysteine by cystathionine γ-lyase (CSE), has been unmasked as signaling gaseous molecule in the inhibitory transmission to the pig bladder neck

Abbreviations: 4-AP, 4-aminopyridine; COX, cyclooxygenase; IbTX, iberiotoxin; TRAM 34, 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole; K_{ATP}, ATP-dependent K^+; BK_{Ca}, large conductance Ca^{2+}-activated K^+ channels; IK_{Ca}, intermediate conductance Ca^{2+}-activated K^+; SK_{Ca}, small conductance Ca^{2+}-activated K^+; K_V, voltage-gated K^+ channels; L-NOARG, N^G-nitro-L-arginine; NO, nitric oxide; ODQ, 1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one; PhE, phenylephrine; VOC, voltage-gated Ca^{2+}

☆This work was done in Departamento de Fisiología, Facultad de Farmacia, Universidad Complutense de Madrid.

* Corresponding author. Tel.: +34 91 3947192; fax: +34 91 3942267.
E-mail address: medardo@farm.ucm.es (M. Hernández).

0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.11.025

(Fernandes et al., 2013a), producing smooth muscle relaxation via activation of K_{ATP} channel and by intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) desensitization-dependent mechanisms. H_2S also promotes the release of sensory neuropeptides and of cyclooxygenase-1 (COX-1) pathway-derived prostanoids from capsaicin-sensitive primary afferents (CSPAs), through transient receptor potential A1 ($TRPA_1$), transient receptor potential vanilloid 1 ($TRPV_1$) and/or related ion channel activation (Fernandes et al., 2013b).

The knowledge of the mechanisms involved in the control of the bladder neck smooth muscle tone is essential for the treatment of urinary obstruction symptoms associated with benign prostatic hypertrophy (Fernandes et al., 2012). However, scarce information exists about the role of progesterone on the contractility of the bladder outflow region. Therefore, this study investigates the mechanisms involved in the progesterone-induced effect in the pig bladder neck.

2. Materials and methods

2.1. Dissection and mounting

Adult pigs of either sex with no lesions in their urinary tract were selected from the local slaughterhouse. Urinary bladders were removed immediately after the animals were sacrificed, and kept in chilled physiological saline solution (PSS) at 4 °C. Bladder neck strips were dissected transversely to a size of 4–6 mm long and 2–3 mm wide, and suspended horizontally with one end connected to an isometric force transducer (Grass FT03C) and the other one to a micrometer screw, in 5 ml organ baths containing PSS at 37 °C continuously gassed with carbogen (95% O_2 and 5% CO_2) to obtain a final pH of 7.4. The signal was continuously recorded on a polygraph (Graphtec Multicorder MC6621). Passive tension of 2.0 g was applied to the strips and they were allowed to equilibrate for 60 min (Hernández et al., 2008).

2.2. Experimental procedure

The contractile ability of the strips was determined by exposing them to a 124 mM potassium rich PSS (124 mM KPSS). The contractile effect produced by progesterone was assessed on basal tension of the preparations, whilst progesterone-induced relaxations were obtained in 1 μM phenylephrine (PhE)-precontracted strips. Cumulative concentration response curves (CRCs) to the agonists were obtained by increasing the organ bath concentration in half log unit steps. Relaxations elicited by progesterone were reproducible in at least two consecutive CRCs. A first control CRC to progesterone was obtained. The bath solution was then changed every 15 min for a period of 90 min, the preparations were incubated with the specific treatments for 30 min, and then a second relaxation CRC was constructed.

To evaluate a possible inhibitory effect of progesterone on membrane Ca^{2+} channels, contraction CRCs to $CaCl_2$, in Ca^{2+}-free KPSS on basal tone of the preparations in the absence or presence of 100 and 300 μM progesterone were performed (Fernandes et al., 2012).

2.3. Simultaneous measurements of $[Ca^{2+}]_i$ and tension

Simultaneous measurements of $[Ca^{2+}]_i$ and tension were performed in intact bladder neck strips by fura-2 AM fluorescence as previously described (Fernandes et al., 2013b). Thus, preparations were loaded in the dark in PSS containing 8 μM fura-2 AM and 0.05% Cremophor EL for 2 h at 37 °C. They were washed three times in PSS, and the solution was changed to PSS with fresh fura-2 AM after 45 min. The myograph chamber was mounted on a Zeiss inverted microscope equipped for dual-excitation wave-length fluorimetry (Deltascan, Photon Technology). Strips were illuminated with alternating 340 and 380 nm light, and the intensity of the emitted fluorescence was collected at a wavelength of 510 nm using a photomultiplier and monitored together with the tension. At the end of each experiment, Ca^{2+}-insensitive signals were determined after quenching with Mn^{2+}, and the values obtained were subtracted from those obtained during the experiment. The ratio of fluorescence at 340 and 380 nm (F_{340}/F_{380}) corrected for autofluorescence was taken as a measure of $[Ca^{2+}]_i$. Minimum (R_{min}) and maximum (R_{max}) R values were 0.56 ± 0.01 and 1.26 ± 0.09, respectively.

2.4. Data analysis and statistics

Sensitivity to progesterone is expressed in terms of pD_2, where $pD_2 = -\log EC_{50}$ and EC_{50} is the agonist concentration needed to produce half-maximal response. pD_2 was estimated by computerized non-linear regression analysis (GraphPad Prism, USA). Passive tension of the strips and the contraction induced by PhE (1 μM) or KPSS (80 mM or 124 mM) are expressed in absolute values as g of tension. Relaxation induced by progesterone is expressed as a percentage reversal of the PhE-, KPSS- or BAY-K 8644-induced contraction, and represent the mean ± S.E.M. of n (number of preparations, 1–2 strips per animal). Differences were analyzed by Student´s t-test for paired observations and by analysis of variance (ANOVA) and a posteriori Bonferroni method for multiple comparisons. The differences were considered significant with a probability level of $P < 0.05$. P values are shown in the figure legends.

2.5. Drugs and solutions

The following drugs were used: 4-aminopyridine (4-AP), apamin, 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylic acid, methyl ester ((±)-BAY-K 8644), glibenclamide, iberiotoxin (IbTX), indomethacin, N^G-nitro-L-arginine (L-NOARG), 1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), phenylephrine (PhE), progesterone, (11β,17β)-11-[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one (RU 486), 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole (TRAM 34). All drugs from Sigma (USA) except IbTX, ODQ, RU 486, TRAM 34 and progesterone from Tocris (UK). Indomethacin and progesterone were dissolved in 96% ethanol. ODQ, RU 486 and TRAM 34 were dissolved in dimethylsulfoxide. The other drugs were dissolved in distilled water. PSS composition was (mM) NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 1.5, ethylenediaminetetraacetic acid 0.027 and glucose 11. The solution was kept at 37 °C and continuously gassed with carbogen (95% O_2 and 5% CO_2), to maintain pH at 7.4. KPSS was PSS with KCl exchanged for NaCl on an equimolar basis. Stock solutions were prepared daily in distilled water.

3. Results

Urothelium-denuded bladder neck strips were allowed to equilibrate to a passive tension of 1.9 ± 0.2 g ($n = 103$ from 56 pigs). PhE (1 μM) induced a sustained contraction above basal tension of 2.5 ± 0.1 g ($n = 103$). Progesterone failed to increase the smooth muscle basal tension ($n = 6$ from 3 pigs).

3.1. Relaxation induced by progesterone

On PhE-precontracted strips, progesterone (0.1 μM–1 mM) induced concentration-dependent relaxations (pD_2 and E_{max} values of 4.2 ± 0.1 and $97 \pm 3\%$, $n = 6$ from 3 pigs). In our study, similar relaxations to progesterone were obtained in samples from