Molecular and Cellular Endocrinology 439 (2017) 444–456

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Sex steroids effects on guinea pig airway smooth muscle tone and intracellular Ca^{2+} basal levels

Edgar Flores-Soto [a], Jorge Reyes-García [a], Abril Carbajal-García [a], Elías Campuzano-González [a], Mercedes Perusquía [c], Bettina Sommer [b], Luis M. Montaño [a, *]

[a] Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México, 04510, Ciudad de México, Mexico
[b] Departamento de Investigación en Hiperreactividad Bronquial, Instituto Nacional de Enfermedades Respiratorias, 14080, Ciudad de México, Mexico
[c] Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, 04510, Ciudad de México, Mexico

ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form
6 September 2016
Accepted 3 October 2016
Available online 4 October 2016

Keywords:
L-type Ca^{2+} channels
Store operated Ca^{2+} channels
Canonical transient receptor potential 3 channel
Testosterone
Dehydroepiandrosterone
17β-estradiol

ABSTRACT

Testosterone (TES), other androgens and female sex steroids induce non-genomic rapid relaxing effects in airway smooth muscle (ASM). In guinea pig ASM, basal tension was relaxed by dehydroepiandrosterone (DHEA) and TES; 17β-estradiol (E2) had a small effect. Blockers of L-type voltage dependent Ca^{2+} channel (L-VDCC, D-600) and store operated Ca^{2+} channel (SOC, 2-APB) also relaxed the basal tone. In tracheal myocytes, DHEA and TES diminished intracellular basal Ca^{2+} concentrations ($_b[Ca^{2+}]_i$) as D-600+2-APB but to a higher extend. TES after D-600+2APB or Pyr3, a blocker of canonical transient receptor potential 3 (TRPC3), further decreased $_b[Ca^{2+}]_i$ rendering this response equal to TES alone. With indomethacin, the $_b[Ca^{2+}]_i$ decrease induced by the blockade of L-VDCC and TRPC3 was not changed by the addition of TES. PGE_2 or forskolin addition after D600+2-APB, decreased $_b[Ca^{2+}]_i$ resembling TES response. An adenylate cyclase inhibitor followed by D-600+2-APB lowered $_b[Ca^{2+}]_i$, TES showed no further effect. Carbachol-induced $[Ca^{2+}]i$ increment was reduced by TES or DHEA. 17β-estradiol diminished KCl-induced contraction and, in tracheal myocytes, the voltage-dependent inward Ca^{2+} current. Conclusion: DHEA and TES diminish ASM tone and $_b[Ca^{2+}]i$ by blocking L-VDCC and probably a constitutively active TRPC3, and by PGE_2 synthesis. E2 lowers ASM basal tone by blocking only L-VDCC.

1. Introduction

Testosterone (TES) and 17β-estradiol (E2) are both derived from dehydroepiandrosterone (DHEA). It has been widely documented that TES and other androgens are able to induce non-genomic rapid effects like vasorelaxation (Costarella et al., 1996; Malkin et al., 2006; Perusquía, 2003; Perusquía et al., 2012; Perusquía et al., 1996; Perusquía and Stallone, 2010) through the blockade of L-voltage dependent Ca^{2+} channels (L-VDCC) (Jones et al., 2004; Montaño et al., 2008; Scragg et al., 2004). More recently, the effects of some androgens, including the main androgen TES in airway smooth muscle, were characterized. DHEA, TES and its 5-reduced metabolites induce relaxation of KCl, acetylcholine or carbachol-precontracted tissues and, its addition during the carbachol induced intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) plateau, completely abolished this response (Bordallo et al., 2008; Espinoza et al., 2013; Kouloumenta et al., 2006; Montaño et al., 2014; Perusquía et al., 2015); these effects are due to the blockade of L-VDCC, store operated Ca^{2+} channels (SOC) and by a PGE_2 related mechanism (Perusquía et al., 2015). Regarding E2, it has also been demonstrated that it induces non-genomic effects in vascular smooth muscle, cardiomyocytes and neurons by blocking L-VDCC (Kitazawa et al., 1997; Kurata et al., 2001; Montaño et al., 2008; Nakajima et al., 1999). In airway smooth muscle, one of the first studies of E2 effects was done in 1983 (Foster et al., 1983) reporting a potentiating effect on isoproterenol-induced relaxation. Later on, E2 response was characterized as a rapid non-genomic effect mediated by reducing histamine-induced intracellular Ca^{2+}

* Corresponding author. Departamento de Farmacología, Edificio de Investigación, sexto piso, laboratorio de Investigación en Asma, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, CP 04510, México DF, Mexico.
E-mail address: lmmr@unam.mx (L.M. Montaño).

http://dx.doi.org/10.1016/j.mce.2016.10.004
0303-7207/

concentration ($[Ca^{2+}]i$) levels (Townsend et al., 2010, 2012b); and it was proposed that this effect was due to the blockade of L-VDCC and an increase in cAMP production.

The scant studies in airway smooth muscle regarding male and female sex steroids' effects i.e., androgens, progestins and estrogens, postulate that these hormones relaxed pre-contracted tissues by blocking Ca^{2+} entrance (Bordallo et al., 2008; Espinoza et al., 2013; Perusquía et al., 1997) and, this acute effect, was even more potent for androgens than it was for estrogens (Espinoza et al., 2013; Montaño et al., 2014; Townsend et al., 2012b). However, few studies actually measure $[Ca^{2+}]i$ changes induced by TES or E2 (Montaño et al., 2014; Perusquía et al., 2015; Townsend et al., 2010, 2012b). By contrast, published studies suggest that estrogens correlate with asthma symptom severity and therefore women seem to have a greater incidence and severity of this illness than man (Keselman and Heller, 2015; Townsend et al., 2012a). In fact, available data show that TES as well DHEA might be beneficial in asthma as they have been associated with high levels during puberty or deficiency in the aging male (Osman, 2003; Townsend et al., 2012a; Traish et al., 2011). To clearly understand androgen protective effects on airway smooth muscle, further research is needed.

In contrast, it has been reported that, in women, asthma exacerbations are closely related to life period and this ailment's severity to their hormonal status, i.e., puberty, menstrual cycle, pregnancy or menopause (Bellia and Augugliaro, 2007; Chandler et al., 1997; Hanley, 1981; Juniper et al., 1991). Some studies have demonstrated that asthma is more frequent in boys than in girls, but after puberty it prevails and is more severe in adult women (Bonner, 1984; de Marco et al., 2000; Kjellman and Gustafsson, 2000; Townsend et al., 2012a; Zannolli and Morgese, 1997). In accordance to this, a study in Ontario, Canada, found that, from the asthmatic patients between 18 and 55 years of age who visited the emergency department between April 1, 2003 to March 31, 2004, women over the age of 25 accounted for >62% of hospitalizations due to acute asthma attacks (Baibergenova et al., 2006). Additionally, it has been established that about 50% of women hospitalized for asthma symptoms were premenstrual (Skobeloff et al., 1996). Moreover, about 70% of the asthmatic women, report symptoms improvement during pregnancy and symptom severity seem to worsen within ~3 months after parturition (Keselman and Heller, 2015; Schatz et al., 1988). Furthermore, menopause generally exacerbates asthma symptoms (Foschino Barbaro et al., 2010). Thus, estrogen effects on airways seem to be related to its plasmatic concentration. Contrastingly, many of the experimental data in mice clearly show that estrogens play a protective role by diminishing airway hyperresponsiveness in this species (Carey et al., 2007; Dimitropoulou et al., 2009; Matsubara et al., 2008). All these data in human and animals clearly point out a biphasic effect of estrogens in asthma and require further research.

Airway basal tone and intracellular basal Ca^{2+} concentrations ($_b[Ca^{2+}]_i$) keep a close mutual balance to maintain an equilibrium between bronchoconstriction and bronchodilation. In airway smooth muscle cells at rest, regulation of $_b[Ca^{2+}]_i$ between 100 and 150 nM (Bazan-Perkins et al., 2003; Carbajal et al., 2005; Montaño et al., 2003), is essential to keep an optimal airway caliber. This regulation process involves Ca^{2+} entry that contributes to the $_b[Ca^{2+}]_i$, myogenic tone, resting membrane potential and sarcoplasmic reticulum (SR) Ca^{2+} refilling.

Basal Ca^{2+} entry has been explored in aortic vascular smooth muscles where, by using pharmacological channel blockers, two main basal Ca^{2+} entries were described; one, (~23–43%) corresponding to L-VDCC and another (~30%) to SOC (Poburko et al., 2004).

In this regard, studies in airway smooth muscle remain very scarce. We previously found that, in bovine tracheal myocytes, $_b[Ca^{2+}]_i$ was reduced by 1 mM Ni^{2+}, suggesting the participation of several Ca^{2+} channels in this phenomenon, including L-VDCC and SOC (Montaño and Bazan-Perkins, 2005). Since Ni^{2+} is a very unspecific drug, further research is required to confirm this hypothesis.

Therefore, because data on the role of sex steroids in the regulation of airway tone and in the $_b[Ca^{2+}]_i$ levels are currently limited, we studied the effect of DHEA, TES and E2 on these two mechanisms. Since Ca^{2+} regulation plays a key role in airway smooth muscle contraction and in airway hyperresponsiveness in asthma, it is essential to explore the effect of sex steroids on this phenomenon.

2. Materials and methods

2.1. Experimental animals

Male Hartley guinea pigs weighing 400–600 g were used. They were bred in our institutional animal facility (filtered conditioned air, 21±1 °C, 50–70% humidity, sterilized bed), fed with Harlan® pellets and sterilized water. The Scientific and Bioethics Committees of the Facultad de Medicina, Universidad Nacional Autónoma de México approved the experimental protocol. During the experiment development, the Guiding Principles for the Care and Use of Vertebrate Animals in Research and Training published by the American Physiological Society were followed. The Mexican National Protection Laws on Animal Protection and the General Health Law Related to Health Research (NOM-062-Z00-1999) were also taken into consideration.

2.2. Organ baths

Animals were anesthetized with an i.p. administration of pentobarbital sodium (35 mg/kg) and exsanguinated. Tracheas were dissected and cleaned of connective tissue, cut in eight rings and each hung in a 5 ml organ bath containing Krebs solution (in mM): 118 NaCl, 25 $NaHCO_3$, 4.6 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 11 glucose, and 2 $CaCl_2$ at 37 °C. Preparations were continuously bubbled with 5% CO_2 in oxygen to maintain pH at 7.4. Tracheal rings were tied with silk thread to an isometric force transducer (model FT03; Grass Instruments, West Warwick, RI, USA) connected to a signal conditioner (CyberAmp 380, Axon Instruments, Foster City, CA, USA) and to an analog-to-digital interface (Digidata 1440A; Axon). Data were recorded and analyzed with an acquisition and analysis software (AxoScope version 10.2; Axon). Tissues were submitted to a resting tension of 1 g during 30 min at the beginning of these experiments. To allow tissue conditioning and optimization of the contractile apparatus, three consecutive KCl (60 mM) stimulations were given. Some tissues were added at the basal tone with a non-cumulative concentration (10, 32, 100 or 178 μM) of dehydroepiandrosterone (DHEA), testosterone (TES) or 17-β estradiol (E2). In another set of experiments, tracheal rings received a non-cumulative concentration of salbutamol (10, 32, 100 or 320 nM). Some preparations were added with methoxyverapamil (D-600, 30 μM, a blocker of L-DVCC), 2-aminoethyl diphenylborinate (2-APB, 100 μM, a blocker of SOC) or the combination of both drugs. Because it has been demonstrated that at nanomolar concentrations (100 nM), flutamide abolishes testosterone genomic effects in cultured male human umbilical vein cells (Ling et al., 2002), we chose to incubate 1 μM (Sanchez Aparicio et al., 1993) in our tissues to assure a proper effect.