INTRODUCTION

In cardiomyocytes and blood vessels, beta 3-adrenoceptor (β-3AR) is coupled to Gs and/or Gi, thus leading to cAMP accumulation and nitric oxide synthase activation respectively⁷. Mirabegron (trade name Mybetrix) is a new β-3AR agonist approved by US Food and Drug Administration (FDA) in 2012 for the treatment of overactive bladder (OAB). Benign prostatic hyperplasia is an underlying cause of OAB and with less evidence erectile dysfunction⁸.

OBJECTIVE

-To evaluate by means of functional and immunohistochemical assays the role of β3-AR activation in isolated prostate from rabbit and human.

METHODS

Patients

Human tissue prostate were obtained from seven patients (65 ± 3 years), who had undergone transurethral resection or open prostatectomy. All experimental protocols were approved by the Human Ethics Committee (protocol number: 294.927) from State University of Campinas.

Animals

Male New Zealand rabbits (weighing 3–4 kg) were provided by Anilab Company (Paulinia, São Paulo, Brazil). All experimental protocols were approved by Institutional Committee for Ethics in Animal Research (protocol number: 2720-1) from State University of Campinas.

Functional assays

Tissues segments excised from the transition zone of human or rabbit prostates were placed immediately in Krebs-Henseleit solution. Tissues were processed within one hour after prostate removal. Four to six longitudinal strips were obtained, mounted in organ bath and continuously bubbled with a mixture of 95%O₂ and 5% CO₂ (pH 7.4) at 37°C. After the equilibration period, tissues were challenged with 80 mM KCl to confirm tissue viability. In human prostate, cumulative concentration-response curve to the β₂-AR agonist phenylephrine (0.00001 - 1 mM) were carried out in the absence (control) and in the presence of mirabegron (1 or 10 μM, 30 min).

In rabbit prostate, concentration-response curves to mirabegron (0.000001-1 mM) was carried out in tissues pre-contraction with phenylephrine (10 μM) in the absence (control) and presence of β₁-, β₂, and β₃ AR antagonists atenolol (3 μM), ICI 118,551 (1 μM) and L 748,337 (300 μM), respectively. Inhibitors of NO synthase (L-NAME, 100 μM) and of soluble guanylate cyclase (ODQ, 10 μM), as well as a cocktail of potassium channel inhibitors consisting of tetraethylammonium (1 mM), charybdoxtoxin (100 nM), apamin (1 μM) and glybenclamide (10 μM) were also used. Electrical field stimulation (EFS; 50 V, 1-32 Hz, 10 sec of stimulation)-induced contractions were carried out in the absence (control) and presence of mirabegron (1 or 10 μM). The potency (pEC₅₀) and maximal response (Eₘₐₓ) values were determined.

Immunohistochemistry

Immunofluorescence

Human prostate was isolated and fixated in paraformaldehyde 4%. Primary antibody for beta-3 receptor (rabbit, 1:100) and anti-alpha actin (clone1A4-mouse, 1:100) were used and observed in a fluorescence microscope.

RESULTS

Immunofluorescence for beta-3 receptor in human prostate

Figure 1: Immunohistochemical analysis reveals the presence of beta-3-adrenoceptor (β₃-AR, red) in smooth muscle (alpha-actin, green) layer from human prostate. Blue color indicates staining for nuclei.

Figures 2-5: Concentration-dependent rabbit prostate relaxations to mirabegron (0.000001-1 mM) in the absence (Control) and in the presence of atenolol (1 μM), ICI 118,551 (1 μM) and L 748,334 (0.3 μM), ODQ, L-NAME and inhibitor K₁-channel.

Figure 5. Concentration-dependent rabbit prostate relaxations to mirabegron (0.000001-1 mM) in the absence (Control) and in the presence of L-NAME, 100 μM, n=4, ODQ, 10 μM, n=4 and tetraethylammonium [1 mM], charybdoxtoxin [100 nM], apamin [1 μM] and glybenclamide [10 μM], n=4.

Figure 2. (A) Concentration-response curve to mirabegron (0.000001-1 mM, n=4) in in rabbit prostate pre-contraction with PE (10 μM). (B) Concentration response curve to PE (0.000001-1 mM) in human prostate in the absence (control) and in the presence of mirabegron (1 and 10 μM, n=5).

Figure 3. Rabbit prostate contractions induced by electrical-field stimulation (1-32 Hz, 50 V, 10 sec stimulation) in the absence (Control, n=7) and in the presence of mirabegron (1 and 10 μM, n=5-6).

CONCLUSION

Clinical studies have shown the efficacy of pharmacological treatment to reduce BPH complications, the need for surgery and the progression of prostate cancer. Mirabegron has been approved for the OAB treatment and may constitute a new therapeutic option for the treatment of LUTS secondary to BPH. However, larger scale, randomized placebo-controlled trials are needed to ascertain its safety, efficacy and cost-effectiveness in the treatment of prostatic disorders.

Reference:


Financial Support: