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EFFECTS OF A B3-ADRENOCEPTOR AGONIST ON RELAXATION OF THE HUMAN PROSTATE IN VITRO

Hypothesis / aims of study
Little is known about β_3-adrenoceptor (AR) expression and function in human prostate. We examined the expression and distribution of β-AR subtypes in normal prostate and benign prostatic hyperplasia (BPH) tissues, and investigated which selective β-AR subtype agonist was most involved in the relaxation of isolated human prostate strips.

Study design, materials and methods
Prostate tissue specimens were obtained from patients undergoing either radical cystectomy for bladder carcinoma or transurethral/open prostatectomy for BPH. Specimens collected from radical cystectomy patients with no evidence of either lower urinary tract symptoms (LUTS) or benign prostatic enlargement were defined as normal prostate tissues. Specimens collected from transurethral or open prostatectomy for LUTS/BPH were defined as BPH tissues. The expression of messenger RNA (mRNA) for β_1-, β_2-, and β_3-AR was investigated using reverse transcriptase-polymerase chain reactions (RT-PCR) in 35 patients (19 normal prostate and 16 BPH specimens, mean age: 68.5 ± 8.5 years, age range: 55-88). Quantitative analysis of mRNA expression of β-AR subtypes between normal prostate and BPH tissues was performed using quantitative RT-PCR. Distributions were examined by immunohistochemistry (IHC). Paraffin sections of surgical resection specimens of human prostate were obtained from 15 patients (nine normal prostate and six BPH specimens; mean age, 69.1 ± 9.4 years; age range: 52-88). Human prostate strips obtained from 36 patients
(25 normal prostate and 11 BPH specimens, mean age: 67.8 ± 9.6 years, age range: 44-88) were suspended in organ baths and exposed to isoproterenol (a non-selective β-AR agonist), dobutamine (a selective β_1-AR agonist), procaterol (a selective β_2-AR agonist), and TRK-380 (a selective β_3-AR agonist) to investigate their relaxant effects on KCl-induced contractions, and their inhibitory effects on electrical field stimulation (EFS)-induced contractions.

Results
We confirmed the presence of mRNA for β_1-, β_2-, and β_3-ARs both in normal prostate and in BPH tissues. For β_3-AR, mRNA expression in BPH tissues was significantly higher than in normal prostate tissues, but there was no significant difference in β_1- and β_2-AR expression between normal and BPH tissues. IHC revealed differences in staining intensity between smooth muscle cells and glandular cells, with different proportions for different β-AR subtypes. Staining of β_3-AR was particularly intense in smooth muscle cells as opposed to glandular cells. Isoproterenol and TRK-380 significantly decreased the tone of KCl-induced contractions of the normal prostate strips (Fig. 1a). The rank order of relaxant effects was isoproterenol > TRK-380 > procaterol > dobutamine. All selective β-AR agonists significantly decreased the amplitude of EFS-induced contractions of the normal prostate strips (Fig. 2a). The rank order of inhibitory effects was isoproterenol > dobutamine >TRK-380 > procaterol. In BPH strips, all selective β-AR agonists showed no significant relaxant or inhibitory effects on KCl- or EFS-induced contractions (Figs.1b and 2b).

Interpretation of results
Present study demonstrates that all β-AR subtypes are expressed in both normal prostate and BPH. The expression of β_3-AR is predominant in prostate smooth muscle cells. Relaxation of human prostate is mainly mediated via β_1- and β_3-ARs. Previous study demonstrated that the β_3-AR agonist mirabegron produced concentration-dependent relaxation against phenylephrine-induced contraction in human and rabbit prostate [1]. Present study is the first report confirming the function of β_3-AR compared to other β-AR subtypes, β_3-AR agonists may have the potential to relax normal human prostate in clinical settings. Our results support the idea that β_3-AR agonists may have clinical application in non-BPH patients with voiding symptoms such as weak stream, intermittency, and straining, and in neurogenic bladder patients with similar symptoms.

Concluding message
β_3-AR is abundant in human prostate smooth muscle, whose relaxation is mediated by β_1- and β_3-AR stimulation. β_3-AR agonists may have clinical use in the treatment of male non-BPH patients or neurogenic bladder patients with voiding dysfunction.

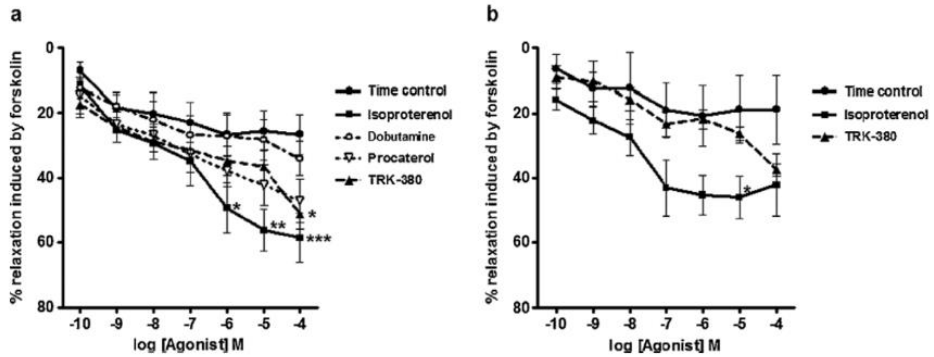

Fig. 1 Relaxant effects of isoproterenol, dobutamine, procaterol, and TRK-380 on 80 mM KCl-induced contraction of human prostate strips. Data are expressed as a percentage of the maximal relaxation induced by 10 μM forskolin. Each point represents the mean ± SEM. (**a**) normal prostate vehicle (n = 9), isoproterenol (n = 7), dobutamine (n = 9), procaterol (n = 8), TRK-380 (n = 7). (**b**) BPH vehicle (n = 4), isoproterenol (n = 6), and TRK-380 (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle (time control).

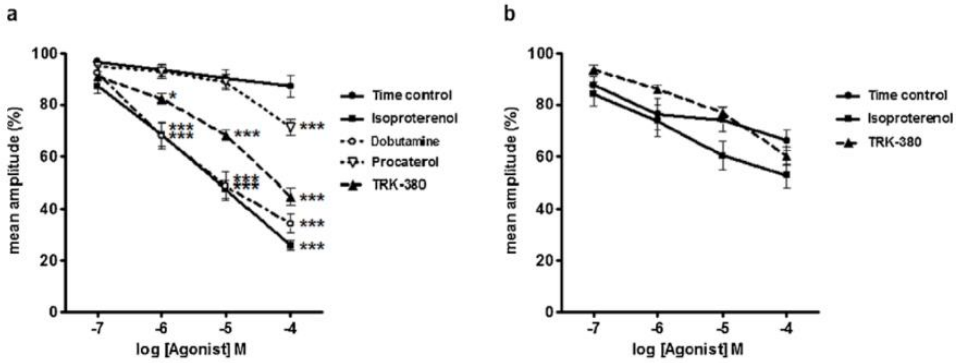

Fig. 2 Inhibitory effects of isoproterenol, dobutamine, procaterol and TRK-380 on EFS-induced contraction of human normal prostate strips. Data are expressed as a percentage of contractile amplitude before administration of each agent. Each point represents the mean ± SEM. (**a**) normal prostate vehicle (n = 9), isoproterenol (n = 9), dobutamine (n = 7), procaterol (n = 11), and TRK-380 (n = 10). (**b**) BPH vehicle (n = 4), isoproterenol (n = 3), and TRK-380 (n = 4). $^{*}P < 0.05$, $^{***}P < 0.001$ versus vehicle (time control).

References
1. Prostate 75:440–447, 2015

Disclosures
Funding: Ministry of Education, Culture, Sports, Science, and Technology; Grant numbers: 21791498; 23592365 **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** The ethics committee of Hamamatsu University School of Medicine **Helsinki:** Yes **Informed Consent:** Yes