gene expression was evaluated by both semiquantitative and quantitative RT-PCR. The cell proliferation rate was evaluated by the [^{3}H]thymidine incorporation. The pharmacological characterization was conducted using selective and non-selective muscarinic receptor agonists and antagonists.
Results: mRNA encoding M1-M4 subytpe of the known muscarinic receptors are expressed in HDSMC. The highest mRNA expression was observed for M2 and M3 receptors, however, their expression is reduced by succeeded passages. Carbachol (1-100 mM) caused a dose-dependent increase in ^{3}H-thymidine incorporation (up to 46 ± 3.7%), that was prevented by muscarinic receptor antagonists. M1 family agonist McN-343 (0.01-100 mM) induced as well a concentration-dependent increase (up to 53.6 ± 5%), that was prevented by pre-incubation of cells with M1 selective antagonists.
Conclusions: HDSMC express muscarinic receptors that mediate proliferative stimulus; and this phenomenon seems to be mediated by M3 and M4 subtypes. Based on present results, the use of muscarinic receptor antagonists in the therapy of urinary bladder obstructions may suggest effects not only in the regulation of contractility but also in the modulation of urinary bladder wall thickness. Indeed, prolonged cholinergic stimulation may initially be protective, but over time may trigger pathologic remodelling of the bladder wall, due to the muscarinic-mediated hyperplasia of the HDSMC.

264 DETRUSOR CONTRACTILE RESPONSE RESISTANT TO CHOLINERGIC AND PURINERGIC PATHWAY BLOCKADE

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Introduction & Objectives: Excitatory innervation of the bladder is supposed to be mediated by 2 neurotransmitters: acetilcholine and ATP. The aim of this study is to evaluate the contractile response to electrical field stimulation (EFS) after blocking both pathways in a porcine model of detrusor contraction.
Material & Methods: Healthy pig detrusor strips were removed from the slaughterhouse and placed in organ baths with Krebs solution. The strips were connected to an electrode on one end and to a tension transducer on the other. EFS trains were applied from 0,3 to 30Hz, evaluating the intensity of the contractile response. 5 groups of 6 stripes were designed. Different blockers of the excitatory pathway of the bladder were added to each group in order to evaluate the contractile response to EFS: 1.- Atropine; 2.- Atropine + NF 279 (P2X1 inhibitor); 3.- Atropine + suramine (non-selective P2X inhibitor); 4.- Atropine + apamine (Ca2+ activated K channels blocker); 5.- Atropine + suramine + desensitization with alfa-beta-methylene-ATP.
Results: After EFS, an increase in contraction intensity is observed, with a maximum contraction at 20Hz. This response was homogeneous and reproducible in >90% of the strips. After adding atropine, a decrease in EFS contraction was observed. However, adding NF-279, suramin or apamin did not cause any change in EFS response. In group 5, a residual detrusor contraction was objectivated after desensitizing ATP pathway with alfa-beta-methylene-ATP. Tetrodotoxin addition in the bath completely abolished the EFS response, confirming the neurogenic action of the EFS.
Conclusions: Porcine detrusor organ bath is a useful model to study bladder contractility. We observed a neurogenic EFS contractile response which was resistant to cholinergic and purinergic pathways blockade.

265 BETA-NAD+ AND CYCLIC ADP-RIBOSE INDUCE RELAXATION OF THE HUMAN DETRUSOR SMOOTH MUSCLE

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Introduction & Objectives: β-NAD$^+$ and its metabolite cyclic ADP-ribose (cADPR) are novel nucleotides released together with ATP from postganglionic nerve terminals in the human detrusor muscle (Sciencesignaling 2009, vol 2 (57):1-5). We investigated the effects of exogenous administered β-NAD$^+$ and cADPR on spontaneous developed- and ATP- induced contractions in human detrusor smooth muscle strips.
Material & Methods: Human detrusor biopsies were obtained from macroscopic normal areas of side walls of the urinary bladder from male patients undergoing cystectomy due to bladder cancer. Longitudinal detrusor strips (2-3 mg) were mounted in a myograph and the developed tension was recorded during experiments. Small conductance Ca^{2+}-activated K^+- (SK) channels expression in human detrusor muscle was determined using real-time quantitative PCR
Results: β-NAD$^+$ (100 µM-1mM) and cADPR (10-100 µM) induced a concentration-dependent relaxation of detrusor muscle resting tone in 95 % of the detrusor preparations (n=71). Spontaneously developed contractions (>70% of the biopsies) were completely inhibited by β-NAD$^+$ (1 mM) and cADPR (100 µM). On exposure to ATP (0.1-5 mM) detrusor strips exhibited a dose-dependent biphasic force response characterized by a rapid and transient contraction, followed by a slow and sustained relaxation. β-NAD$^+$ (0.1-1 mM) and cADPR (10-100 µM) inhibited significantly the transient contractions but not the relaxations induced by ATP (1-5 mM). Furthermore, both β-NAD$^+$ and cADPR induced dose dependent relaxations of carbachol-induced tonic contractions. β-NAD$^+$ induced relaxations were not affected by treatment with 8-sPT (5 µM), an adenosine (P1) receptor antagonist. UTP mimicked the effects of β-NAD$^+$ by inducing relaxation and by inhibiting ATP induced contractions. Apamin (1 µM), a selective blocker of SK channels, effectively increased the spontaneous contractions and inhibited β-NAD$^+$ induced relaxation. Human detrusor expressed mRNA transcripts for SK2 and SK3 channels.
Conclusions: These results show, for the first time, that exogenous β-NAD$^+$ and cyclic ADP-ribose induce relaxation of the human detrusor smooth muscle tone and inhibit ATP induced phasic contractions. These effects are probably mediated through a pathway that may involve definable P2Y receptors subtypes and apamin-sensitive SK channels. Thus, the β-NAD$^+$/cADPR system may be a novel extracellular player in the regulation of detrusor muscle tension of the human urinary bladder. Furthermore, we also show for the first time the presence of SK channels in the human detrusor muscle.

266 MIRABEGRON (YM178), A NOVEL β3-ADRENOCEPTOR (AR) AGONIST, INCREASED FUNCTIONAL BLADDER CAPACITY AND DECREASED MICTURITION FREQUENCY IN CONSCIOUS WATER-LOADED CYNOMOLGUS MONKEYS

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Introduction & Objectives: It is known that β-AR subtypes involved in bladder relaxation varies among species. For example, the predominant subtype is β3-AR in humans and monkeys whereas both β2- and β3-ARs in rats. Mirabegron is a novel β3-AR agonist and the relaxant effects were demonstrated with human and rat bladder strips as well as in vivo efficacy in rats (J Pharmacol Exp Ther 2007;321:642; J Urol 2009;181 Suppl:10). However, no in vivo data on bladder function have been demonstrated in subhuman species. In the present study the agonistic activities of mirabegron to monkey β-AR subtypes were examined. In addition, the effects on the voided volume per micturition and micturition frequency were examined in conscious water-loaded monkeys.
Material & Methods: Agonistic activity was determined with Chinese hamster ovary cells expressing monkey β1-, β2- or β3-AR by measuring cAMP accumulation induced by mirabegron or isoproterenol, a non-selective β-AR agonist. The 50% effective concentration (EC50) of the maximal response by isoproterenol and intrinsic activity (IA) were calculated, when the maximum response by isoproterenol was taken as 1.0. In the in vivo study, mirabegron (0.3, 1 or 3 mg/kg) or 0.5% methylcellulose (MC) was orally administered to male cynomolgus monkeys (2.4 to 4.1 kg). Fifteen min thereafter, 50 mL/kg of water was orally administered to induce water diuresis. Each monkey was individually housed in a metabolic cage and voided volume and micturition frequency were continuously measured for 8 h. The same monkeys were used repeatedly in each experimental group with a withdrawal period of over a week.
Results: Mirabegron and isoproterenol showed agonistic activity to β3-AR with EC50 values of 32 and 170 nmol/L, respectively. The IA value of mirabegron was 0.8. Mirabegron showed little agonistic activity to β1- and β2-ARs with IA values of 0.2 and 0.1, respectively, while isoproterenol showed agonistic activities with EC50 values of 84 and 77 nmol/L, respectively. In the in vivo study, micturition frequency and voided volume per micturition in 0.5% MC-treated group were 8.6±1.1 times and 22.5±3.7 mL, respectively. Mirabegron significantly decreased micturition frequency at 1 and 3 mg/kg p.o. (6.0±0.6 and 5.9±0.8 times, respectively) and increased voided volume per micturition at 3 mg/kg p.o. (30.4±4.5 mL). Mirabegron did not affect total voided volume.
Conclusions: The bladder functional enhancement observed in cynomolgus monkeys, that have a similar β-AR expression pattern in human bladder, would be caused by bladder relaxation through the β3-AR stimulation with mirabegron. These data suggest that mirabegron enhances human bladder function and, therefore, could be a new therapeutic agent for the treatment of overactive bladder.

267 OBSTRUCTION ENHANCES RHO-KINASE PATHWAY AND DIMINISHES PROTEIN KINASE C PATHWAY IN CARBACHOL-INDUCED CALCIUM SENSITIZATION IN ALPHA-TOXIN PERMEABILIZED GUINEA-PIG DETRUSOR SMOOTH MUSCLE

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Introduction & Objectives: The aim of this study was to investigate the role of Rho-kinase (ROK) pathway and protein kinase C (PKC) pathway in carbachol (CCh)-induced Ca^{2+} sensitization in α-toxin permeabilized detrusor smooth muscle (DSM) fibber obtained from guinea-pig DSM following bladder outlet obstruction (BOO).
Material & Methods: Partial BOO was created by the placement of silver jump rings