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EVALUATION OF THE SITE OF ACTION OF PROSTANOIDS IN THE MICTURITION REFLEX PATHWAY OF THE RAT.

Aims of Study:

Prostanoids increase the frequency of spontaneous activity of the detrusor smooth muscle, contribute to basal tone and lower the threshold volume for micturition [1,2]. The aim of the present study was to identify the site(s) of action of prostanoids in the micturition reflex by examining the effects of the cyclo-oxygenase inhibitor, indomethacin, on responsiveness of rat urinary bladder in vitro and in vivo.

Methods:

In vitro studies: Urinary bladders were removed from rats that had been euthanized using CO_2 asphyxiation. Bladders were opened along their ventral surface and longitudinal strips of muscle (2-3 mm in diameter) were prepared. Strips were mounted in 5 ml tissue baths containing oxygenated (95% O_2: 5% CO_2) modified Krebs buffer solution (mM:NaCl, 118.2; Dextrose, 10.0; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4 7H_2O$, 1.2; $NaHCO_3$, 24.8; $CaCl_2$, 2.5; 37°C, pH of 7.4.) and allowed to equilibrate at a resting tension of ~2 g for 60 min. Indomethacin (1 μM) or vehicle (0.5 % Na_2CO_3) were applied to tissues and equilibrated for 60 min. The effects of treatment were evaluated on both basal tone and spontaneous myogenic activity of the preparations to identify potential post-junctional effects of prostanoids.

In vivo studies: Two in vivo models were utilized. The electrically-stimulated pithed rat model (ESPR) provides a means of evaluating the effects of drugs on the efferent limb of the micturition reflex [3]. The volume-induced bladder contraction model (VIBC) [4] involves both the afferent and efferent limbs of the micturition reflex and, in conjunction with the ESPR model, provides a means of evaluating the effects of drugs on the afferent limb of the micturition reflex. For the ESPR model, rats were anesthetized with pentobarbital sodium (60mg/kg, i.p) and the femoral artery and both femoral veins were cannulated for the measurement of blood pressure and administration of drug and fluid, respectively. The bladder was cannulated via the external urethral orifice and connected to a pressure transducer and infusion pump. An insulated steel pithing rod was inserted through the ocular orbit and into the spinal column, dissociating the central nervous system from the peripheral nervous system. The tip of the rod, which was not insulated, was placed 14 cm down the length of the spinal cord such that electrical stimulation of the rod activated nerves in the L6-S2 spinal regions, eliciting contraction of the detrusor smooth muscle via activation of pre-ganglionic efferent fibers. For the VIBC model, rats were anesthetized with urethane (1.5 g/kg, s.c.) and the carotid artery and femoral vein were cannulated for the recording of blood pressure and administration of drug, respectively. The bladder was cannulated via the external urethral orifice and connected to a pressure transducer and infusion pump. Rhythmic bladder contractions were induced in these animals by the infusion of 1.5 ml of warm saline into the bladder. In both

models the effects of indomethacin (1.0 mg/kg iv, bolus) were investigated on bladder contractions induced either by activation of the pre-ganglionic efferent pathway (ESPR) or through the activation of afferent and efferent pathways, induced by bladder filling (VIBC).

Results:

In vitro, indomethacin (1 μM) reduced basal tone of bladder preparations and reduced, or in some cases entirely eliminated, spontaneous myogenic activity of the smooth muscle preparations.

In vivo, indomethacin (1.0 mg/kg) had no effect on bladder contractions induced by pre-ganglionic efferent nerve stimulation (ESPR model), while in the VIBC model frequency of contraction was reduced (6.17 ± 0.75 to 2.33 ± 0.33 contractions, per 10 min interval,) with no alteration in amplitude of the contractions.

Conclusions:

These data suggest that prostanoids act at several levels in the micturition reflex. The *in vitro* studies suggest a degree of prostanoid-induced basal tone and spontaneous myogenic activity of urinary bladder smooth muscle. Data from the VIBC studies suggest that prostanoids are involved in the neuronal control of micturition. The fact that the frequency rather than the amplitude of volume-induced bladder contractions were altered by indomethacin suggests an involvement of prostanoids at the afferent level of the micturition reflex. The lack of effect of indomethacin in the spinally-stimulated pithed rat studies (ESPR model) further supports the notion that prostanoids modulate the micturition reflex at the level of the bladder afferents or the central nervous system.

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BLADDER INSTABILITIES AND ADRENERGIC SUPERSENSITIVITY OF DETRUSOR SMOOTH MUSCLE IN FEMALE AGED RAT

Disturbances of bladder function are common in the elderly. Despite the extensive use of the rat in research on aging, the effects of senescence on urinary bladder function are not yet well characterized. Particularly, there is no report on urinary bladder function in conscious aged animals and the consequences of aging on the adrenergic neurotransmission to the detrusor smooth muscle are still debated.

Aims. To characterize the consequences of aging on micturition profiles in conscious female rats and the associated modifications in adrenergic responses *in vitro*.

Methods. In vivo experiments were performed in 10 and 30 months old female Wistar/Rij rats chronically instrumented with an intra-vesical catheter. The bladder was perfused at a rate of 6ml/h and intravesical pressure and urinary volume recorded. Five reproductible micturition cycles were analyzed and means of the different cystometric parameters calculated. In vitro, two detrusor strips and the bladder neck were isolated from each animal and placed in 20 ml glass organ baths containing a modified Krebs solution with 1 μM propranolol, maintained at 37° C and aerated with 95% O_2 and 5% CO_2. After a contractile response to 80 mM KCl, cumulative concentration-response curves to noradrenaline (NA) (0.01-100 μM) were performed and results were expressed as % of the contraction to KCl.