

STUDY OF OESTRADIOL EFFECT UPON ATP RELEASE IN PRIMARY PIG UROTHELIAL CELL CULTURE: A POSSIBLE MECHANISM OF ACTION FOR TOPICAL VAGINAL OESTRADIOL?

Hypothesis / aims of study

Reduction of estrogens after menopause leads to significant atrophic changes in the genital tract and bladder dysfunction, and is associated with increased prevalence of urinary frequency and urgency. The recent Cochrane review [1] concluded that topical vaginal oestradiol therapy provides benefit for urgency and urge incontinence, but the mechanism of estrogen effect remains unknown.

Recent studies demonstrated that ATP release by urothelial cells plays an important role in bladder function and dysfunction (urgency / urge incontinence) through purinergic receptors on the cells in bladder lumen. Therefore, we hypothesized that the beneficial effect of oestradiol on bladder urgency is through inhibiting ATP release of bladder.

The aim of this study is to investigate whether ATP release from bladder cells is altered by oestradiol treatment.

Study design, materials and methods

Because pig urothelial primary cell culture has been previously shown to be as a good *in vitro* model for human bladder studies [2], we have employed the pig urothelial primary cell cultures to investigate the effect of oestradiol on ATP release.

Fresh adult female pig bladders were obtained from the abattoir. The luminal surface of the bladder was exposed and digested with trypsin before the urothelium was scraped off and plated in a 48-well plate. Urothelial cells were cultured in RPMI supplemented with 10% FBS, antibiotics and epidermal growth factor (25 ng/ml) at 37°C with 5% CO₂ until nearly confluent (10 days). Fresh RPMI 1640 medium with or without 17- β -estradiol (20ng/ml) was applied to the cultured cells, 8 experiments (8 individual pigs) using 6 replicates per treatment were performed (n = 48 experiments). ATP release experiments were performed after short exposure (overnight) or after long term exposure (5 days) to 17- β -estradiol. Before ATP measurement, cultured cells were equilibrated with carbogenated Krebs solution. Basal ATP release was determined by exposure to fresh Krebs (pH 7.4), or hypotonic Krebs (50%) was used to mimic stretch in the cell culture. ATP release was then measured using Bio-luminescence ATP Assay kit (Sigma) and a luminometer (GloMax 20/20), and expressed as median, IQR. Individual treatment groups were compared using a non-parametric Mann-Whitney test.

Results

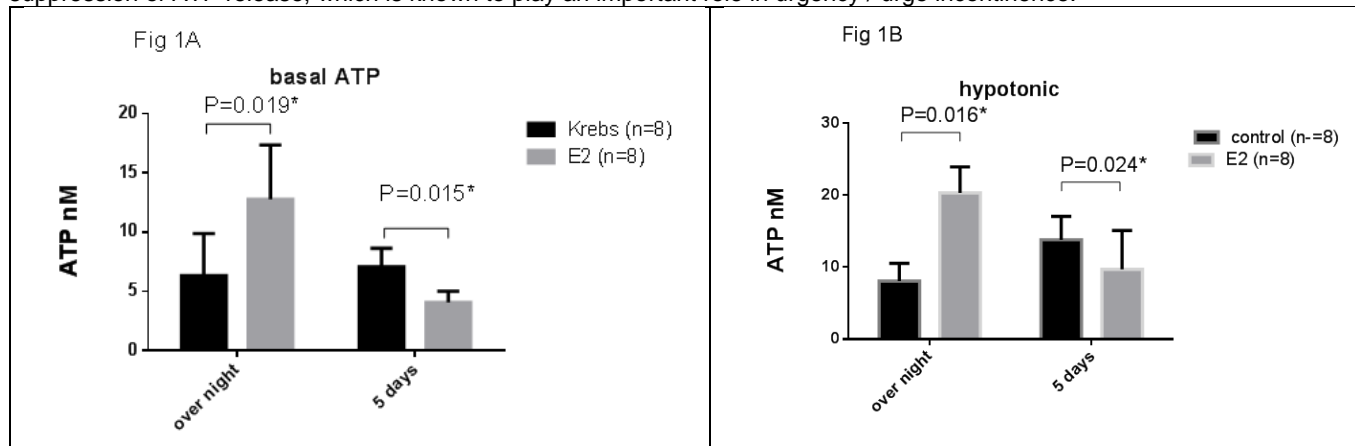
The results show that ATP release was significantly induced by 17- β -estradiol with short term (overnight) treatment in both basal (without stretch) (Fig 1A) (p=0.019) and stretched groups (p=0.016) (Fig 1B); however, after long term exposure (5 days) to 17- β -estradiol treatment, ATP release was significantly reduced in both basal (p=0.015) (Fig 1A) and stretched groups (p=0.024) (Fig 1B).

Interpretation of results

Our results show that although oestradiol accelerated ATP release after initial over night treatment, however the long term (5 days) effect is to significantly inhibit ATP release in either stretched or non-stretched bladder cells. This agrees with the hypothesis that topical vaginal oestradiol, which are absorbed locally into the lower bladder / trigone, may help maintain normal bladder function through inhibiting ATP release by urothelial cells.

Concluding message

To our knowledge, this is the first study to reveal the effect of topical oestradiol on urgency/urge incontinence may act via suppression of ATP release, which is known to play an important role in urgency / urge incontinence.



References

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2. Cheng Y1, Mansfield KJ, Sandow SL, Sadananda P, Burcher E, Moore KH. Porcine bladder urothelial, myofibroblast, and detrusor muscle cells: characterization and ATP release. *Front Pharmacol.* 2011 Jun 7;2:27. doi: 10.3389/fphar.2011.00027. eCollection 2011.

Disclosures

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