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# IS THE UROTHELIUM-DERIVED INHIBITORY FACTOR AN ENDOGENOUS STEROID?

Hypothesis / aims of study

The presence of the urothelium reduces the maximal contraction of detrusor smooth muscle in response to carbachol by approximately 50% in pig and human [1]. This phenomenon is attributed to the release of urothelium derived inhibitory factor (UDIF). The UDIF may be of great clinical importance in understanding the pathology of detrusor overactivity and may aid the development of novel treatments.
The aim of this study was to discover whether the UDIF could be an endogenous steroid, such as 17-β estradiol, progesterone or testosterone. 17-β estradiol and progesterone have been shown to inhibit detrusor maximal contractions in response to carbachol [2] and clinical studies show that oestrogen treatment decrease OAB symptoms [3].
Steroids are produced from the common precursor cholesterol (figure 1). The rate-limiting step for steroid biosynthesis is the production of pregnenolone from cholesterol by the enzyme cytochrome P450scc. Steroid receptor antagonists and an inhibitor of both cytochrome P450scc and aromatase were used. The inhibitory effect of the urothelium was observed in the presence and absence of oestrogen and progesterone and both male and female bladders were investigated.

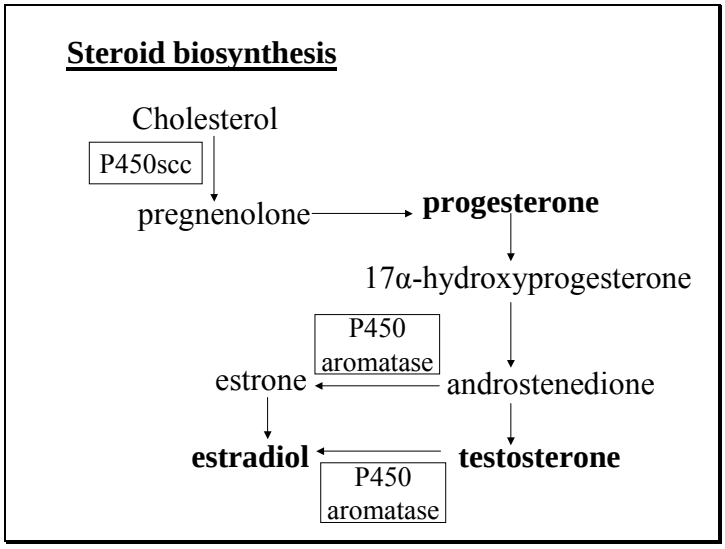


Figure 1.

Study design, materials and methods
Paired strips of pig bladder dome with and without urothelium were set up under 1g tensions in gassed Krebs bicarbonate solution at 37°C. Cumulative carbachol concentration response curves were obtained in the absence and presence of various steroids, inhibitors of steroid synthesis or receptor antagonists. Responses were expressed as mean percentage inhibition ± sem. Paired Student's t-test was used for statistical analysis.

Results
The presence of the urothelium significantly inhibited detrusor responses to carbachol in both male and female pig detrusor by 46.7 ± 9.4% (n=6, p=0.016) and 41.1 ± 2.4% (n=74, p=<0.0001) respectively.
The steroid synthesis inhibitor and receptor antagonists did not significantly alter the urothelial inhibition of female detrusor responses nor did they have any effect on tissue sensitivity to carbachol. (fig 2).

Figure 2.

| Drug | Action | Urothelial inhibition (%) | | n |
|---|---|---|---|---|
| | | control | With drug | |
| 20μM Tamoxifen | Oestrogen receptor antagonist | 44.9 ± 9.5 | 48.4 ± 6.6 | 8 |
| 30μM Mifepristone | Progesterone receptor antagonist | 31.3 ± 6.8 | 32.6 ± 7.4 | 6 |
| 100μM Aminoglutethimide | Cytochrome P450scc and aromatase inhibitor | 57.0 ± 6.8 | 51.1 ± 7.8 | 8 |

17-β estradiol and progesterone exogenously applied to female detrusor (both denuded and intact) caused inhibition of carbachol responses in a concentration-dependant manner (fig 3). The degree of urothelial inhibition did not significantly alter in the presence or absence of 17-β estradiol or progesterone. In the presence of a high concentration

(1mM) of 17-β estradiol and progesterone the urothelium further inhibited the maximum contraction by 57.4 ± 9.5% and 55.9 ± 9.2% respectively (n=4 and 6). Testosterone (10μM) did not significantly inhibit detrusor maximal contractions to carbachol in female denuded or intact detrusor strips.

Figure 3.

| Steroid concentration | 17-β estradiol percentage inhibition (%) | | | | Progesterone percentage inhibition (%) | | |
|---|---|---|---|---|---|---|---|
| | Denuded strips | n | Intact strips | n | Denuded strips | Intact strips | n |
| 100nM | 28.1 ± 5.6** | 3 | 23.2 ± 13.9 | 6 | 7.5 ± 7.7 | 10.4 ± 2.2 | 7 |
| 10μM | 37.4 ± 6.0* | 4 | 22.1 ± 9.2 | 4 | 22.2 ± 9.6 | 19.6 ± 7.5 | 7 |
| 1mM | 48.1 ± 4.0* | 4 | 33.4 ± 8.2* | 4 | 45.8 ± 5.1 | 54.1 ± 8.6 | 6 |

*p=<0.05 and **p=<0.01 indicates a significant difference from the control contraction without steroid.

In male bladders, as in female, 10μM 17-β estradiol significantly inhibited maximal responses to carbachol of intact and denuded strips by 13.1 ± 4.6% and 22.7 ± 12.9% respectively (p=0.03 and 0.04, n=4). Progesterone did not significantly inhibit male detrusor contractility (n=4).

Interpretation of results

Female detrusor contractility was inhibited by oestrogen and progesterone and was further inhibited by approximately 50% by the presence of the urothelium. This suggests that the UDIF is likely to be a different compound to either oestrogen or progesterone and inhibits via an alternate mechanism. As the urothelial inhibition is observed in female bladders and testosterone did not significantly inhibit female detrusor contractility, the UDIF is unlikely to be testosterone. The urothelial inhibition was not reduced by the application of tamoxifen or mifipristone suggesting that the UDIF is unlikely to be oestrogen or progesterone acting via their classical genomic receptors. However, there is evidence that steroids can have alternate non-genomic actions not requiring their classical receptors.The UDIF inhibition could not be reduced by aminoglutethimide, therefore the UDIF is unlikely to be an endogenous steroid produced *de novo* via the conventional steroid biosynthesis pathway.

Concluding message

The UDIF is unlikely to be a steroid and remains unidentified. However this study confirms that steroids may play a role in bladder functioning.

The search for the UDIF's identity will continue as it may have a major impact on the understanding and treatment of detrusor overactivity.


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**ANIMAL SUBJECTS: This study did not follow the guidelines for care and use of laboratory animals because the tissue was obtained from an abattoir**