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INVESTIGATION OF THE INHIBITORY MECHANISM OF ACTION OF ERYTHROMYCIN ON THE HUMAN BLADDER

Aims of Study

Erythromycin (EM) has been shown to produce a direct inhibitory effect on rat urinary bladder smooth muscle (1).Our earlier work has demonstrated that this antibiotic differentially inhibits human detrusor muscle contraction *in vitro* in response to agonists and activation of intrinsic nerves. Understanding the underlying mechanism of its action may lead to identification of possible targets for the suppression of detrusor overactivity, and lead to the development of new pharmacological treatments for patients with incontinence.

The aim of this work is to investigate this inhibitory mode of action by trying to separate neurogenic effects from possible myogenic effects using agents to block reponses to the transmitters (acetylcholine and ATP). To achieve this, continuous application of ATP was used to desensitise purinergic receptors, atropine was used to block cholinergic receptors, and tetrodotoxin (TTX) used to block neurogenic responses.

Methods

Human bladder tissue was retrieved at cystectomy and from cadaveric organ donors. The urothelium was removed, and 1 x 4mm smooth muscle strips were dissected and suspended by silk ligatures in 0.2ml superfusion organ baths. The strips were tensioned to 1g, and left to equilibrate for 1.5 hours in oxygenated Krebs' solution (37°C, pH7.4). After an initial stimulation with carbachol (10^{-5}M) to assess viability, the strips were exposed to 30 mins of either Krebs' or erythromycin lactobionate 10^{-3}M solutions alone or containing ATP (10^{-4}M), atropine (10^{-6}M), ATP and atropine, or ATP, atropine and TTX ($3x10^{-7}$M). The strips then received electrical field stimulation (EFS) at 1-60Hz (5sec trains, pulse duration 0.05ms, 50 volts). Time-matched controls were used, and a maximum of 3 different parameters were tested on each strip to avoid time-related tissue fatigue effects.

Results

Bladder tissue was taken from 10 subjects (8 males and 2 females) aged between 37–73 years old (mean age of 53.9 years). Where reported, patients with significant lower urinary tract symptoms were excluded.

EM (10^{-3}) alone significantly reduced contractions to EFS (eg to 40% of the Krebs' response at 50Hz). As expected, atropine caused a marked inhibitory effect in Krebs' control (50hz – 21%), and equally in EM (50Hz – 23%).

ATP did not significantly decrease responses to EFS in Krebs' alone, but the addition of ATP to tissues exposed to EM produced a further significant decrease in response (50hz - 24%). The addition of ATP had no significant effect on responses of tissues already exposed to EM and atropine. The addtion of TTX on its own blocked about 80% of the response to EFS, but when added to solutions already containing ATP and atropine, it produced little further change to the strips.

Table 1 – Response of smooth muscle strips to 50Hz EFS expressed as a percentage of the maximal response in the Krebs' control.

Krebs'	**Krebs'+ATP**	**Krebs'+atropine**	**Krebs'+ATP+atropine**	**ALL+TTX**
100%	75%	21%	24%	22%
EM	**EM+ATP**	**EM+atropine**	**EM+ATP+atropine**	**ALL+TTX**
40%	24%	23%	27%	22%

Conclusions

Atropine inhibits the cholinergic component of the response to EFS. Overall, both responses in Krebs' and EM were reduced by atropine to the same end point, suggesting that the effects of EM cannot be accounted for by blocking the cholinergic component of the response to EFS alone.

The effect of EM on the non-cholinergic component was unexpected. A non-cholinergic component to EFS contractions has been reported to occur in human detrusor, although it is thought to represent only a small percentage of the total contraction in 'normal' bladders. Some reports have only been able to show a purinergic component to contraction in abnormal bladder (eg obstructed or overactive) (2), where it may be responsible for up to 50% of function. There is good evidence that the transmitter responsible is ATP acting on P_{2x1} receptors. In this experiment, ATP was given continuously to 'normal' bladder to desensitise the P_{2x} receptors. In the normal bladders used in this experiment, this did not significantly effect the responses to EFS in the control, but the addition of ATP to tissues exposed to EM did produce a significant inhibition of muscle contraction. The addition of ATP to tissues exposed to EM and atropine, had no effect, and these responses to EFS were TTX insensitive, suggesting direct muscle activation. EM thus appears to be exposing a purinergic component to produce the inhibitory response, but only when there is a remaining cholinergic response.

References

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