

THE EFFECT OF OXYBUTYNIN AND EDROPHONIUM ON THE AFFERENT EXCITABILITY INDUCED BY BLADDER IRRITATION IN RAT

Hypothesis / aims of study

It is widely accepted that anticholinergic drugs act on detrusor smooth muscle and improve overactive bladder symptoms by inhibiting detrusor overactivity. However, it is also known that many patients who complain of urgency do not show detrusor overactivity on filling cystometry. In addition, anticholinergic drugs at clinical doses have little effect on voiding contractions. Therefore, attention has focused on new concept that anticholinergic drugs may directly act on afferent pathway in regard to the therapeutic effect on overactive bladder symptoms. On the other hand, recent studies have shown that c-Fos expression in L6 spinal cord is able to use as a marker for afferent neuronal activation induced by bladder irritation in rat. The present study was designed to determine whether oxybutynin chloride (oxybutynin) inhibits afferent activation resulting from bladder irritation, and whether a reduction of afferent activation alters storage or voiding function of the bladder. Moreover, edrophonium chloride (edrophonium), a potent cholinesterase inhibitor, was used to study whether endogenous acetylcholine stimulates the afferent pathway. Thus, we investigated the number of c-Fos positive cells in L6 spinal cord and cystometric parameters between control and drug administrated rats.

Study design, materials and methods

Thirty female Sprague-Dawley rats at the age of 12 weeks were separated into 3 groups. Using osmotic pump (Alzet 2004), oxybutynin group was administrated oxybutynin (0.36mg/kg/day), edrophonium group was administrated edrophonium (0.2mg/kg/day) and control group was given vehicle (distilled water). Continuous cystometry with saline was performed at 4 weeks after pump implantation until each rat micturated 30 times without any anesthesia and restrain. Cystometric capacity, micturition interval and micturition pressure were measured. After cystometry, each rat received cardiac perfusion with 4% paraformaldehyde. Spinal cord was removed and embedded in OCT compound for c-Fos immunostaining. Using anti c-Fos antibody (Ab2 Calbiochem), 20 μ m thick sections of spinal cord (L6) were immunostained. All values are expressed as the mean \pm SE. Statistical analysis of data was performed by one-way ANOVA with the Dunnett's post-test and a probability of $p < 0.05$ was considered significant.

Results

There was no difference between control group and drug administrated groups in regard to micturition pressure (Table). Chronic oxybutynin treatment significantly increased cystometric capacity and lengthened micturition interval (Table). On the other hand, chronic edrophonium treatment decreased cystometric capacity and shortened micturition interval, although the differences did not achieve a statistical significance ($p=0.07$) (Table). Microscopic evaluation of rat L6 c-Fos stain revealed the obvious difference among 3 groups. Continuous oxybutynin administration resulted in about 50% decrease in the number of c-Fos positive cells (20 ± 1.6 cells / section), whereas edrophonium administration resulted in about 50% increase in the number of c-Fos positive cells (100 ± 2.9 cells / section) compared to control animals (46 ± 3 cells / section) (Figure).

Interpretation of results

Oxybutynin acts on the afferent pathway rather than the muscarinic receptor on detrusor smooth muscle because oxybutynin inhibited spinal c-Fos expression without affecting voiding contraction and this reduction of the afferent input results in the increased bladder capacity and extended micturition interval. Furthermore, major action site of acetylcholine is also the afferent pathway during storage phase because the inhibition of acetylcholine resolution with edrophonium increased the afferent pathway activation without affecting voiding contraction. Although the differences did not reach a statistical significance for normal bladder, this increase of the afferent input results in the decreased bladder capacity and shortened micturition interval.

Concluding message

In this study, we demonstrated that oxybutynin acts on the afferent pathway and inhibits the stimulation inputted from bladder. This reduction of the afferent stimulation alters not voiding but storage function. In addition, it is suggested that acetylcholine has an important role in afferent pathway excitability during storage phase.

Table

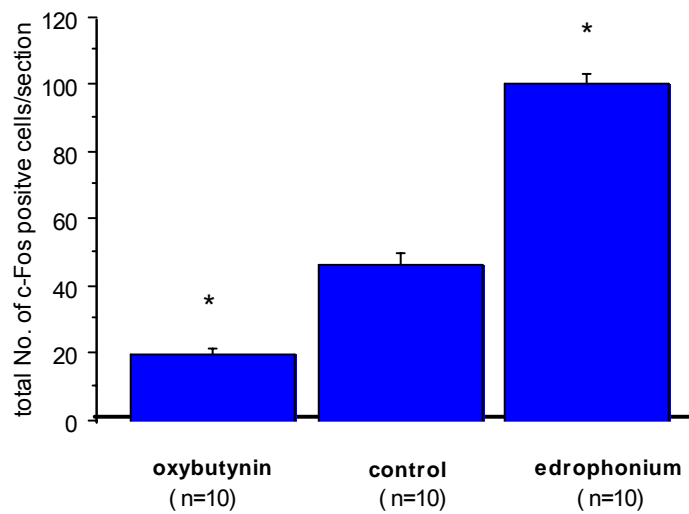
Cystometrical parameters and rat body weight in control, oxybutynin and edrophonium groups

	Control	Oxybutynin	Edrophonium
Rat weight (g)	239 ± 9	253 ± 5	249 ± 7
Micturition pressure (cmH ₂ O)	41 ± 2	50 ± 3	49 ± 3
Cystometric capacity (ml)	0.73 ± 0.01	1.00 ± 0.09*	0.52 ± 0.50
Micturition interval (minute)	4.37 ± 0.99	5.93 ± 0.51*	3.15 ± 0.30

* indicates p=0.01 versus control group.

Figure

The effect of oxybutynin and edrophonium on c-Fos expression induced by bladder irritation.



Error bars indicate SE. * indicates < 0.0001 versus control group.