Conclusions

The present data suggests that the actions of NS-8 are associated with an efflux of potassium channel on human detrusor smooth muscles, and that NS-8 may be an useful agent for atropine-resistant bladder instability.

References

1. J. Urol., 155, 1454-1458, 1995.

2. Biochem. Biophys. Res. Commun., 213 : 404-409, 1995.

3. J. Pharmcol. Exp. Ther., 283 : 1193-1200, 1997.

83

R. McCoy, G. McMurray, M.J. Drake and A.F. Brading

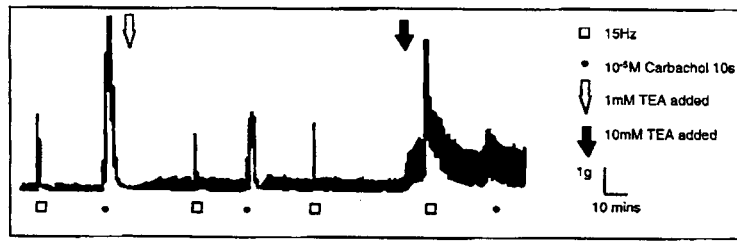
University Department of Pharmacology, Oxford, UK

EFFECT OF TETRAETHYLAMMONIUM CHLORIDE ON ISOLATED HUMAN DETRUSOR SMOOTH MUSCLE STRIP RESPONSES TO CARBACHOL AND ELECTRICAL STIMULATION.

Aim of the study : The blockade of potassium channels in smooth muscle should increase the response of events mediated by action potentials, such as neurotransmitter release and excitation-contraction coupling. The aim of this study was to investigate the role of potassium channels in the contractile responses evoked by carbachol and intrinsic nerve stimulation in isolated human detrusor smooth muscle strips, with the use of, the nonspecific potassium channel blocker, tetraethylammonium chloride (TEA).

Methods : Whole normal human detrusor (N=4) was obtained from cadaveric organ donors and transported to the laboratory in cold Krebs solution (ca 4°C). The dome region of the detrusor was selected for study. The mucosa was carefully removed and smooth muscle strips (1x1x5mm) dissected with the aid of a binocular dissection microscope. The strips were mounted in 0.2ml superfusion organ baths at an initial tension of one gram. The organ baths were constantly perfused with carboxygenated Krebs solution (37±0.1°C, pH 7.4) and the strips were allowed to equilibrate for ninety minutes. Repeatable control responses to both 10^{-5}M Carbachol and 15Hz stimulation were obtained and the mean result determined. Subsequently, responses to carbachol and 15Hz stimulation were determined in the presence of 1mM and 10mM TEA added in a cumulative manner. Responses are expressed in g/mg of tissue and as a percentage of the control tissue response to contractile agents. A paired t-test was used to establish statistical significance of the results, a value of $p<0.05$ proving significance.

Results : An example of an experimental trace is shown below for reference.



A concentration dependent increase, both in frequency and magnitude, of spontaneous contractile activity was observed in all strips studied upon addition of TEA. In addition, an increase in intrinsic tone of a magnitude of 0.15g/mg was observed in only one strip upon addition of 1mM TEA, whereas an increase in tone of 0.29±0.06g/mg was recorded in 44.8% of total strips upon addition of 10mM TEA.

As shown in the table below, isolated human detrusor smooth muscle strip responses to carbachol was significantly reduced during TEA-sensitive potassium channel blockade. In contrast, the responses to intrinsic nerve stimulation were potentiated substantially under the same conditions (10mM TEA present).

	Response to 10^{-5} M Carbachol (n=29, N=4)		Response to 15Hz stimulation (n=26, N=4)	
	g/mg	% of control tissue response	g/mg	% of control tissue response
Control	2.81±0.31	100±0	1.14±0.16	100±0
1mM TEA	2.27±0.41 p<0.05	69.7±6.26 p<0.05	1.13±0.18 NS	109±11.45 NS
10mM TEA	1.93±0.40 p<0.05	56.0±7.12 p<0.05	2.06±0.37 p<0.05	221.7±28.4 p<0.05

Conclusion: The increase in spontaneous contractile activity of normal human detrusor smooth muscle upon addition of TEA suggests that spontaneous activity is an action potential mediated process. Blockade of TEA-sensitive potassium channels profoundly potentiates the effect of intrinsic nerve stimulation in the normal human detrusor, this is consistent with a large increase in transmitter release effected by blockade of potassium channels. However, in contrast, the reduction of the response to carbachol in the presence of TEA may be due to muscarinic receptor antagonism.

84

M. Yoshida, A. Inadome, M. Yono, H. Seshita, Y. Miyamoto, S. Ueda

Department of Urology, Kumamoto University School of Medicine, Kumamoto, Japan

AGE-RELATED CHANGES IN ACETYLCHOLINE AND ADENOSINE TRIPHOSPHATE RELEASES FROM HUMAN BLADDER SMOOTH MUSCLES

Aims of study

With regard to excitatory innervation of urinary bladder, it is generally accepted that, in addition to a cholinergic neurotransmission, an atropine-insensitive, non-adrenergic, non-cholinergic neurotransmission exists in most mammalian bladder. Adenosine triphosphate (ATP) is believed to be the neurotransmitter responsible for non-cholinergic portion of bladder contraction (1, 2). Thus, the neurogenic contraction of bladder is mainly mediated by two neurotransmitters, acetylcholine (ACh) and ATP. It is well known that the aging process affects bladder function. However, little information is as yet available on the age-related changes in neurotransmitters release from bladder smooth muscles. We reported the measurement of ACh release induced by electrical field stimulation (EFS) in rabbit bladder smooth muscles, using microdialysis method (3). In this report, we evaluated the age-related changes in the ACh and ATP releases induced by EFS in human bladder smooth muscles.

Methods

Smooth muscle strips were obtained from detrusor in 25 patients (42 - 82 years) with bladder carcinoma. The strip was suspended in a 20 ml muscle bath filled with Krebs-Henseleit solution, was connected to an isometric force displacement transducer, and an isometric tension development was recorded. The contractions induc by EFS (duration; 0.2 msec, frequency; 5 - 50 Hz and 2 sec train) were constructed. The microdialysis probe (O-P-100-10, Eikom, Kyoto, Japan) was inserted into the strip. Ringer solution was perfused into the probe at a rate of 2 μl/min. The dialysate during EFS (0.2 msec pulse duration, 20 Hz, 2 sec train and 2 min interval for 10 min) was collected. A volume of 10 μl of the each sample was injected into ACh and ATP assay systems, and the amount of ACh and ATP released in the dialysate was measured by HPLC.

Results

Contractile responses induced by 80 mM KCl and the frequency-response curves to EFS did not significantly change with age. In this assay system, the detection limits of ATP and ACh were 0.1 and 0.02 pmol/injection,