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THE CONTRACTILE PROPERTIES OF ISOLATED HUMAN DETRUSOR MUSCLE – EFFECT OF AGE, GENDER AND PATHOLOGY

Hypothesis / aims of study

Hypothesis: The in vitro contractile properties of isolated human detrusor smooth muscle are a function of patient age, gender and lower urinary tract symptoms.

Lower urinary tract symptoms (LUTS) increase in prevalence with age [1] and common urodynamic observations associated with LUTS include detrusor overactivity (DO). Some studies [2], although not all, also suggest that LUTS are more prevalent in women [2]. The purpose of the study was to determine if human detrusor smooth muscle contractility, as assessed by stimulating the muscle with a number of paradigms, was also a function of the age or gender of the patient. Furthermore, were contractile properties different when obtained from patients with stable, overactive or obstructed bladders? Previous studies used relatively small numbers of samples, so that actual differences could be masked in the face of many confounding variables. The study aimed to analyse data from a large number of samples (n>200) to determine if changes to muscle physiology were associated with patient age, gender or bladder pathology to guide therapeutic options in managing the clinical conditions.

Study design, materials and methods

Human biopsy samples were used, from whom patient age, gender and pathology [control - (generally cystectomy samples), neuropathic DO, idiopathic DO, obstructed bladders) were recorded. Strips, after removal of mucosa, were attached to an isometric force-transducer, superfused with Tyrode's solution (24 mM $NaHCO_3$, 5% CO_2, 37^0C) and stimulated by; nerve-mediated (n-m) excitation (0.1 ms pulses at 1-40 Hz, after subtraction of 1μM TTX-resistant responses); addition of agonists (carbachol 0.1-10 μM; □ □ □methylene-ATP (ABMA) 10 μM; or 80-mM KCl solution); or direct muscle electrical stimulation (0.1-20 ms pulses in the presence of the neurotoxin TTX,). N-m contractions were also obtained in the presence of atropine (1 μM) or ABMA (10 μM) to determine the cholinergic or purinergic components. Data are presented as medians [25, 75% interquartiles; sample number] and two or more sets compared by Wilcoxon or Kruskal-Wallace tests, respectively. Association between two variables was assessed by calculation of Spearman's correlation coefficient, r, which assumes no specific relationship between variables. Significance of association was tested by calculation of Student's t-value (n>20). The null hypothesis (no change between sets or variables) was rejected when $p<0.05$.

Results

Table 1 shows the contractile properties of human detrusor smooth muscle from all patients. N-m tension is quoted at one frequency (16Hz) or the estimated value at high frequencies, T_{max}, from the force-frequency relationship. $f_{1/2}$ is the frequency required to achieve $T_{max}/2$. The percentage n-m contraction after atropine or ABMA were added yields the purinergic and cholinergic component respectively. The carbachol contracture magnitude at 10 μM was recorded. The percentage maximum carbachol (10 μM), ABMA (10 μM) and 80 mM KCl/T_{max} ratios were calculated from the magnitude of agonist contractures and T_{max} values in the same preparation. Contractions due to direct muscle (DS) stimulation increased as pulse width increased; the maximum estimated force at high pulse widths, DS T_{max}, and the pulse width achieving DS $T_{max}/2$ (*d*) are shown. The value of *d* is a measure of tissue excitability, smaller values indicating increased excitability.

When comparing results from control and pathology groups only the percentage of atropine-resistance was significantly different, greater in the pathology group. There were no differences between the three pathology groups and so data have been grouped throughout. There were no differences in any variable from male and female patients when the whole data set and individual stable and pathological groups were analysed.

To investigate the effect of patient age, data from control and pathological groups were therefore combined except for atropine-resistance data. There was:

i) a significant negative association between age and n-m contraction magnitude (r = -0.267 and -0.173; 16Hz, T_{max} respectively); $f_{1/2}$ was independent of age (r = 0.090).

ii) no relationship between the maximum carbachol contraction and pEC_{50} and age (r= 0.041, 0.034, respectively).

iii) a significant negative association between age and T_{max}/carb (r = -0.32); there were no age-dependent associations for T_{max}/ABMA and T_{max}/KCl.

iv) no association with age for direct muscle stimulation variables (r = 0.244, 0.147 for DS T_{max} and DS *d*, respectively).

v) within the control and pathology groups no association between age and atropine-resistance percentage (r = 0.077, 0.043; respectively).

Table 1: Contractile properties of human detrusor. Data show all values and separately those from control or pathology groups. *p<0.05, M=male; F=female

	All patients	Control group	Pathology group
Age, gender, n	66 [43,69]; 138M, 75F	53 [39,68]; 50M, 26F	61 [51,73]; 98M, 49F
n-m, 16Hz mN/mm^2	20.1 [11.0, 29.4; 190]	21.1 [11.0, 27.7; 71]	19.9 [11.3, 30.8; 119]
n-m, T_{max}, mN/mm^2	45.0 [27.0, 66.8; 188]	47.8 [28.2, 69.7; 71]	43.0 [27.1, 66.0, 117]
$f_{1/2}$, Hz	16.8 [12.9, 22.1; 188]	17.8 [13.8, 22.8; 71]	16.5 [12.8, 21.8; 117]
atrop %n-m tens 40Hz	9.4 [0.9, 30.0; 151]	1.7 [0.0, 9.7; 47]	14.7 [1.9, 42.2; 104] *
ABMA %n-m tens 40Hz	88.5 [67.3, 98.8; 134]	94.1 [83.6, 99.3; 46]	86.1 [58.7, 97.0; 88]
Carbach max mN/mm^2	61.9 [34.9, 88.1; 87]	61.7 [45.9, 89.0; 35]	65.5 [31.4, 84.1; 52]
Carbach pEC_{50}	6.13 [5.88, 6.45; 55]	6.24 [5.97, 6.55; 26]	6.04 [5.76, 6.31; 29]
% T_{max}/Carb	89.5 [51.3, 136; 78]	91.9 [58.7, 127; 32]	94.0 [53.7, 141; 46]
% T_{max}/ABMA	219 [126, 377; 45]	296 [162, 401; 18]	225 [130, 359; 27]
% T_{max}/KCl	138 [82.0, 188; 45]	144 [113, 177; 18]	112 [78.8, 191; 27]
DS T_{max} mN/mm^2	26.3 [17.3, 38.5; 45]	32.8 [22.9, 40.4; 19]	22.5 [17.1, 37.2; 26]

DS *d*, ms	1.39 [0.86, 2.38; 45]	1.61 [1.19, 2.57; 19]	1.22 [0.63, 2.04; 26]

Interpretation of results

The data show no association between patient gender or bladder pathology and contractile properties of isolated detrusor muscle, except for confirmation of the previous association between an increased incidence of atropine-resistance and overactivity bladders [3].

With respect to patient age there was a significant decline of the strength of nerve-mediated contractions. However, for direct muscle stimulation variables such as the maximum carbachol contracture and direct muscle stimulated contraction there was no association. This was corroborated by the negative association of the T_{max}/Carb and age. There was no evidence of altered carbachol sensitivity in any group. This may be interpreted that with age there is no decline in the unit contractile properties of muscle but a relative denervation of the tissue. Thus, urodynamic concepts such as impaired contractility need to be re-evaluated in terms of their precise meaning. Of interest also was the fact that the variable DS-*d* was independent of age, implying that detrusor excitability is also unchanging. Atropine-resistance was also age-independent in either the stable or pathology group.

Concluding message

Overall, the unit contractile and excitability properties of human detrusor muscle are independent of gender or pathology, with the exception of greater atropine-resistance pathological samples. Decline of nerve-mediated contractions was associated with increasing age, but direct muscle-stimulated properties were not. Thus, we further hypothesise there is a functional denervation of detrusor with age without change to muscle contractility per se. Therefore, extra-muscular factors need to be sought to explain the occurrence of bladder over- or under-activity and may help to explain why musculotropic agents may have limited effect on reducing functional bladder pathologies.

References

1. Wagg et al., BJU Int 2007; 99: 502-509
2. Milsom etal., Am J Manag Care 2000; 6: 565-573
3. Bayliss et al., J Urol 1999; 162: 1833-1839

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