The Influence of Age on Isometric and Isotonic Rat Detrusor Contractions

Ping Kong Lieu, Alfa Sa’adu, E. Olu Orugun, and James G. Malone-Lee

Centre for Geriatric Medicine, University College London Medical School.

**Background.** Reductions in detrusor shortening velocity and detrusor contractility have been observed in association with aging. The reasons for these changes are unclear.

**Methods.** We examined the isometric and isotonic responses of detrusor, taken from the bladder body, to adenosine 5'-triphosphate (ATP), noradrenaline (NA), serotonin, and acetylcholine (Ach) in vitro, using bladders from young (30 days) and old (365 days) Sprague-Dawley rats, to determine whether there were any significant age-related differences.

**Results.** Isometric contractions with ATP at doses of 10^-3 to 10^-2 molar concentrations produced greater contractile forces in old rats when compared to young rats (p = .0136 at 10^-3 mole of ATP). Isotonic contractions at similar concentrations also produced significant differences between the young and the old rats, the latter being faster (p = .0225). Isometric contraction with noradrenaline produced significant differences between young and old rats, the latter being stronger. This became apparent at 10^-4 mole of noradrenaline (p = .0043). Isometric contractions with serotonin also produced significantly greater contractions in the old rats when compared to young rats. The differences became apparent at 10^-4 mole of serotonin (p = .045). There were no age-related differences in isotonic and isometric contractile responses to acetylcholine in the doses used in our experimental setup.

**Conclusions.** Age-related differences in isometric function were detected in response to ATP, NA, and serotonin. Differences in isotonic function were only found in response to ATP and Ach.

**Methods**

Two groups of female Sprague-Dawley rats were used: young (30 days) and old (365 days). The usual mean survival time of these female rats, nonexercised, is 476 days (11). They were reared in an animal house under sterile conditions. The mean weight for the young rats was 260 grams and the mean weight for the old rats was 310 grams.

The rats were sacrificed in a carbon dioxide chamber and their urinary bladders removed. Longitudinal strips of the detrusor muscle from the posterior wall were cut and suspended from the bladder body, and these vary from species to species (7,8). Sympathetic nerve release neuropeptide Y (NPY) and noradrenaline (NA) (9). Noradrenaline, by acting via alpha adrenergic receptors, is known to produce inositol triphosphate as a secondary messenger which releases calcium from sarcoplasmic reticulum. Inositol triphosphate is produced from phosphoinositol biphosphate, which is resident in cell membrane and activated by phospholipase C. Phospholipase C is activated by a noradrenaline/receptor complex, possibly through the G protein (10). The differences in behavior of these agonists may therefore give indirect evidence of the mechanisms responsible for differences observed between the age groups.
which processed the data using the Acqknowledge 2.1 software package. For isometric experiments the strips were tied directly to the force displacement transducers. In isometric experiments, a spring designed to generate a force of 1 gram per centimeter extension was tied to the upper end of each muscle strip. The springs were then tied to the force displacement transducers. A resting tension of 1 gram was applied to all the specimens, and a 30-minute period for equilibration followed before the start of the experiments.

The velocity of shortening during isotonic contractions was calculated by differentiating the change of detrusor length in mm, with respect to time in seconds. Isometric contractions were quantified by measuring the force generated in millinewtons (mN) (g × 9.81).

The effects of adenosine 5'-triphosphate (ATP), noradrenaline, serotonin, and acetylcholine (all obtained from Sigma Chemical, St. Louis) on both isotonic and isometric bladder contractility were studied.

Statistical comparisons were made using the Mann-Whitney test. The W statistic, 95% confidence interval (CI) of the difference between means and the p-value of the difference were quoted. The 95% level of confidence was used to reject the null hypothesis.

RESULTS

Isometric contractile responses of bladder body muscle strips to ATP in old rats were greater than in young rats (mean young rats .644 mN, mean old rats 1.12 mN; 95% CI of the difference .1569 to .9418 mN; p = .0136 at 10^-2 molar concentration of ATP).

Isometric contractions with noradrenaline were greater in the detrusor strips from older rats when compared to younger rats (mean young rats .005 mN, mean old rats 3.57 mN; W = 57; 95% CI of the difference 1.60 to 5.8 mN, p = .0043 at 10^-4 molar concentrations of noradrenaline (Figure 2)).

Isometric contractions of detrusor strips with serotonin showed significant differences between the young and old rats (mean young rats 1.04 mN, mean old rats 2.27 mN; W = 26; 95% CI of the difference .019 to 2.52; p = .0453 at 10^-4 molar concentration of serotonin) (Figure 3). However, within the range of noradrenaline and serotonin concentrations from 10^-4 to 10^4 molar used, no isotonic responses were elicited in both old and young rats in our experimental setup.

Although higher values for isotonic and isometric contractile responses of bladder muscle strips to acetylcholine were observed in older rats when compared to young rats, these did not reach statistical significance in the concentrations used (10^-4 to 10^-2 molar concentrations of acetylcholine).

DISCUSSION

In our study, isometric contractile responses were observed with all the autonomic agonists tested. Significant age-related isometric contractile differences were observed with adenosine triphosphate (ATP), noradrenaline (NA), and serotonin despite the initial length of the detrusor strips being the same. At the initial load of one gram, isotonic contractile responses were only observed with ATP and acetylcholine (Ach). We demonstrated age-related changes in isotonic contractions only to ATP.

The age-related isometric differences observed with ATP, NA, and serotonin, when compared to Ach, probably reflect...
Figure 3. Isometric contraction with serotonin. Comparison of the in vitro dose response curves to serotonin of detrusor muscle strips of young (n = 6) and old (n = 6) rats. Bars represent standard deviations.

Differences in activation of contraction (6). ATP and serotonin effect their responses by depolarizing muscle membrane which results in increases in intracellular calcium (7). Noradrenaline mediates its action via inositol triphosphate which is formed by the noradrenaline/alpha-1 adrenoceptor receptor complex stimulating the hydrolysis of the membrane lipid-phosphatidylinositol (4,5)-diphosphate. This reaction is carried out by a phospholipase C (10). It is therefore possible to speculate that the age-related changes in isometric responses reflect, indirectly, changes in the detrusor muscle membranes of young and old rats. This is supported by the fact that acetylcholine, which has little effect on muscle membrane but mediates its responses via a receptor gated channel (6), was able to produce similar forces of contraction in young as in old rats. This observation is also indirect evidence that the contractile mechanisms including intracellular calcium, actin x myosin interactions, and intracellular calmodulin x calcium interactions are probably the same in the age groups studied.

Isotonic contractile responses were only elicited with acetylcholine and ATP. This would suggest that ATP and acetylcholine were the only agonists capable of generating enough force to overcome the initial calibrated tension load of 1 gram force in our experimental setup. We chose a 1 gram load because of the inertia experienced with smaller loads. However, the 1 gram load may have been too high. The differences in isotonic contraction between the age groups observed with ATP, reflect the observed changes in isometric contractions between the same age groups. Our study provides indirect evidence of changes at the detrusor membrane level between young and old rats. Conclusive results would require direct physiological studies on the detrusor membrane. If the findings are substantiated, it may help in explaining some of the urodynamic changes observed during voiding in later life. Research into the understanding of the differences between age groups would need to be concentrated on the detrusor membrane.

In conclusion, we have demonstrated significant age-related differences in isometric contractile responses with respect to ATP, noradrenaline, and serotonin in the detrusor strips from rat bladder body. Age-related isotonic contractile responses were demonstrated only to ATP. We suggest that the differences observed reflect changes in mechanisms of activation of contraction, and this may be at the level of the detrusor membrane.

Acknowledgments

This study was supported by the Sandoz Gerontological Research Foundation.

We are grateful to Mr. Dave Sheppard for his kind assistance with the breeding of the animals. We thank Dr. Irfan Wahedna for his comments on the manuscript.

Dr. Lieu is now with the Department of Geriatric Medicine, Tan Tock Seng Hospital, Singapore 308433.

Address correspondence to Dr. James G. Malone-Lee, Centre for Geriatric Medicine, University College London Medical School, St. Pancras Hospital, St. Pancras Way, London NW1 OPE. E-mail: james.malone-lee@ucl.ac.uk

References


Received May 25, 1994
Accepted May 17, 1996