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EFFECT OF AGE ON HYDROGEN PEROXIDE-MEDIATED CONTRACTION DAMAGE IN THE RAT BLADDER

Aims of Study

Urinary bladder dysfunction in the elderly is common. There is also in-vitro evidence that bladder function decreases with age in rat and rabbit studies (1,2). In addition, recent studies have introduced the concept that cyclical ischemia / reperfusion induced by benign prostatic hyperplasia (partial outlet obstruction in animals) results in the generation of reactive oxygen species (ROS) and that ROS participates in the progressive deterioration of bladder function (3). These findings indicate that ROS may play a key role in the detrusor contractile dysfunction of aged men. However, the relation between age and the sensitivity of the bladder to ROS is unclear. The present study was designed to determine whether the sensitivity of bladder to ROS changes with age. Using hydrogen peroxide (H_2O_2) to simulate ROS damage, we investigated the contractile responses and the level of peroxidation of bladder smooth muscle isolated from young and aged rats.

Methods

Three month-old (young) and twelve month-old (aged) male Sprague-Dawley rats were used (24 rats each group). Each rat was anesthetized and the bladder was removed. Two longitudinal (10 × 3 mm.) strips were cut from the each bladder body. H_2O_2 was added to Tyrode's to make the following final concentrations of H_2O_2: 0.0625%, 0.125%, 0.25%, 0.5% and 1% concentration. Normal Tyrode's solution (0% H_2O_2) was used as control. The strips were mounted in individual 15 ml baths containing oxygenated Tyrode's solution at 37°C. The contractile responses to EFS (80 V, 32 Hz, 1msec), carbachol (20μM) and KCl (120mM) were determined. Then normal Tyrode's solution was exchanged with one of the H_2O_2 – Tyrode's solutions. After 1 hour exposure of H_2O_2 the tissues were washed three times with fresh normal Tyrode's and the responses to EFS, carbachol and KCl were measured again. At the end of the experiment the strips were weighed and frozen at –70°C for malondialdehyde (MDA) analysis. Each frozen bladder strip was thawed and homogenized. Homogenates were spun and supernatants were incubated in KCl Tris buffer and stimulated with ferrous sulfate in glass tubes. In addition, non-stimulated samples were also evaluated for basal MDA levels. Tubes were centrifuged and supernatants were incubated with perchloric /thiobarbituric acid solution. To each tube, 1-butanol was added and each tube was spun. The upper layer was measured fluorometrically at 532 nm. Quantity of total protein was performed using Micro BCA Protein Assay and results are expressed as pmol MDA/mg protein.

Results

The magnitude of the contractile responses of the young and aged rat bladder strips to all forms of stimulation were not significantly different (Table 1). One hour H_2O_2 exposure resulted in a dose-dependent decrease in the maximal contraction of both young and aged bladder strips to all forms of stimulation (Table 1). However, the aged bladders were significantly more sensitive to H_2O_2 damage than the young bladders (Table 1). H_2O_2 resulted in a dose-dependent increase in basal MDA concentration of both young and aged bladder strips (Table 2). MDA generation of bladder strips from aged rats was significantly greater than those from young rats at all H_2O_2 concentrations (Table 2). This indicates that although the basal level of MDA is similar for young and aged rats, the capacity to generate oxidative products is significantly greater in the aged rats.

Table 1. Effects of age on bladder contraction (g tension / 100mg tissue) after H_2O_2 exposure. Each value represents the mean ± SE of 8 individual preparations (rats). ***** $p < 0.05$ vs. young

	EFS			Carbachol			KCl		
H_2O_2	0%	0.125%	0.5%	0%	0.125%	0.5%	0%	0.125%	0.5%
Aged	27±0.6	13±0.7*	3.8±0.5*	20±1.2	6.7±0.3*	0.9±0.1*	14±0.9	3.1±0.3*	0.9±0.1*
Young	28±0.5	18±1.6	10±1.0	21±0.8	10±0.5	3.1±0.4	16±0.7	5.6±0.3	2.6±0.3

Table 2. Effects of age on basal and generated MDA (pmol / mg protein) after H_2O_2 exposure. Each value represents the mean ± SE of 8 individual preparations (rats). ***** $p < 0.05$ vs. young

	Basal MDA			Generated MDA		
H_2O_2	0%	0.125%	0.5%	0%	0.125%	0.5%
Aged	404±120	772±153	1222±203	81±33*	79±20*	135±49*
Young	558±60	681±242	1020±117	33±3.8	52±16	51±7.7

Conclusions

This data supports the conclusion that the aged bladder is more sensitive to oxidative damage than the young bladder. Thus, it is conceivable that antioxidant protection decreases with age and that antioxidant therapy would be beneficial in the treatment of aging and obstructive bladder dysfunction.

References

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3. J Urol, 166, 341-346, 2001.