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ROLE OF ISCHEMIA AND OXIDATIVE STRESS IN BLADDER NEURODEGENERATION

Hypothesis / aims of study

A correlation between human bladder overactivity and neurodegeneration has been reported. However, the mechanism of neural injury in the overactive bladder remains unknown. The role of ischemia and oxidative stress in neural functional disability and structural damage has been documented in human polyneuropathy and in the experimental diabetic neuropathy in rat. It is known that nerve growth factor (NGF) regulates reactive oxygen species (ROS) and that excessive ROS is neurotoxic. We hypothesized that "*ischemia-induced bladder overactivity leads to oxidative stress and neural oxidative injury".* Our goal was to search for markers of oxidative stress and oxidative neural injury in the ischemic bladder.

Study design, materials and methods

New Zealand white male rabbits (3-3.5 Kg) were assigned into treatment (n=14) and age-matched control (n=14) groups. In the treatment group, atherosclerosis and bladder ischemia was induced by balloon dilation of the iliac arteries and a short period of 0.5% cholesterol diet. Seven treated and 7 control animals were studied at week 8 and another 7 treated and 7 control animals were studied at week 16 after the induction of ischemia. Bladder ischemia/reperfusion (I/RP) and hypoxia/reoxygenation (H/RO) were documented with a laser Doppler flow probe and an oxygen sensing needle electrode placed directly into the bladder wall. Bladder overactivity was determined with cystometry. Oxidative stress was assessed by enzyme immunoassay (EIA) of a neurotoxic oxidative product isoprostane 8-epi PGF2α. Bladder neural density was examined immunohistochemically using mouse anti-S-100 primary antibody (for Shcwann cells) and mouse anti-neurofilament 70 + 200 primary antibody. The mean number of nerves per high power field was determined using computerized image analysis. Gene expression of NGF and NGF receptor was examined by RT-PCR analysis. The effect of ROS producing agent hydrogen peroxide (H_2O_2) on isoprostane 8-epi PGF2α release and NGF content was examined in a tissue culture model using EIA.

Results

Ischemia increased the frequency and force of spontaneous bladder contractions leading to extreme cycles of I/RP and H/RO (table 1). The magnitudes of I/RP and H/RO during spontaneous contraction in the 8 weeks and 16 weeks ischemic bladders were significantly greater than their age-matched controls.

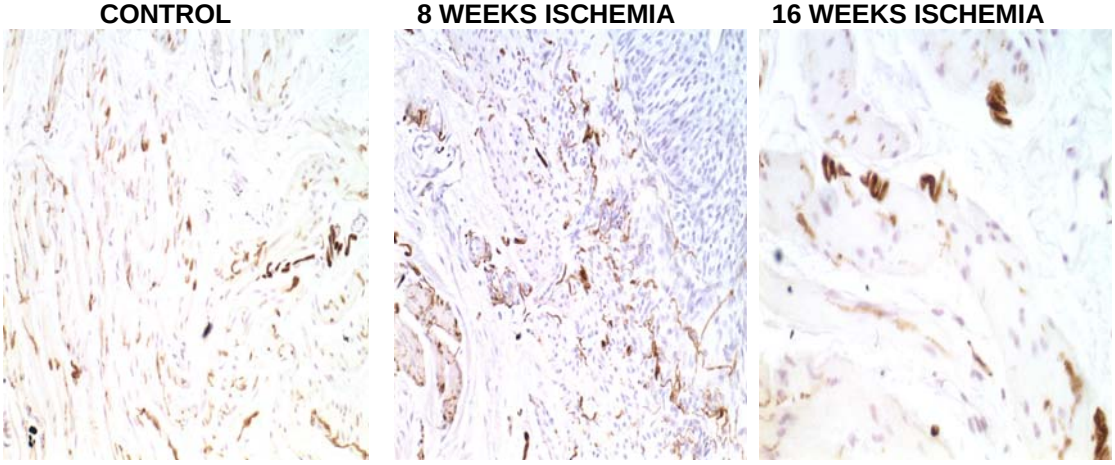
Table 1. Blood flow (ml/min/100 grams tissue) and pO2 (mm Hg) changes with spontaneous contraction in the 8 weeks ischemic and age-matched control bladders * indicates significant differences in the ischemic bladder compared with control.

	CONTROL BLADDER		**ISCHEMIC BLADDER**	
	Blood flow	**pO2**	**Blood flow**	**pO2**
Basal level	**6.8 ± 0.7**	**41.5 ± 6.2**	**3.3 ± 0.4*** (Sustained I/H phase)	**26.7 ± 4.2***
At peak contraction	**4.6 ± 0.5**	**37.3 ± 5.4**	**0.6 ± 0.3*** (Severe I/H phase)	**8.8 ± 3.8***
End of contraction	**8.1 ± 0.6**	**46.4 ± 7.1**	**4.7 ± 0.5*** (RP/RO phase)	**35 ± 5.5***
Contraction Frequency	**3 ± 1.8 / 10 minutes**		**10.0 ± 2.2 / 10 minutes***	

EIA showed progressive increases in the bladder levels of isoprostane 8-epi PGF2α (pg/ml/hour) in the 8 weeks (4.78 ± 0.18) and 16 weeks (7.35 ± 2.4) ischemic bladders in comparison with age-matched controls (1.88 ± 0.8 and 2.13 ± 1.2, respectively). The average number of nerve fibres (mean ± standard error) in the control bladder was 15.7 ± 0.5 per high

power field. The average number of nerve fibres was unchanged in the 8 weeks ischemic bladder (13.2 ± 1.0) but significantly decreased to 8.2 ± 1.7 in the 16 weeks ischemic bladder. The surviving nerves in the 16 weeks ischemic bladder seemed markedly thickened in comparison with the controls (Figure 1).

Figure 1: Neural expression in the control and ischemic bladder. Arrows point to the nerve fibres.



RT-PCR analysis showed an initial increase in NGF gene expression at week 8 and then a marked decrease at week 16 after the induction of bladder ischemia. In contrast to NGF, the NGF receptor expression decreased at week 8 and increased at week 16. EIA showed that tissue treatment with ROS producer H_2O_2 increased isoprostane 8-epi PGF2α release and decreased NGF levels in both ischemic and control tissues.

Interpretation of results

Ischemia exposed the bladder to repeating cycles of I/RP and H/RO and led to neurotoxic oxidative products. Long-term (16 weeks) I/RP and H/RO resulted in marked neurodegeneration. NGF gene appeared to initially increase in the early stage of ischemia (8 weeks), perhaps to protect the bladder from oxidative neural injury. This defensive mechanism seemed to fail in long-term ischemia. The NGF receptor expression decreased in the early stage of ischemia but increased in long-term ischemia, perhaps due to ROS-induced stimulation of its signalling pathway. The tissue culture experiments clearly demonstrated that acute exposure to ROS producer H_2O_2 stimulated neurotoxic oxidative products and downregulated NGF production in the bladder.

Concluding message

Our data suggest that the mechanism of neurodegeneration in the overactive bladder involves oxidative stress and neurotoxic oxidative products.

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