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AGE-RELATED CHANGES IN DETRUSOR PROPERTIES AND GENE EXPRESSION IN THE BLADDER AND DORSAL ROOT GANGLION OF THE RAT AND PREVENTATIVE EFFECT OF LOW CALORIC DIET AGAINST THESE CHANGES

Hypothesis / aims of study

Detrusor overactivity and impaired detrusor contractility are commonly observed among elderly (1). Decreased cholinergic neurotransmission and compensatory increased purinergic transmission in aged human bladder have been reported, which is postulated as a cause of impaired detrusor contractility (2). However, precise pathophysiology of these age-related impairment of bladder function has not yet clarified. No study on the preventative effect of low diet (LCD) against these age-related bladder dysfunctions has been reported. In the present study, we aimed to determine how the contractile properties of rat detrusor change with aging and whether long-term LCD has a prophylactic effect against the age-related changes of the detrusor properties using *in vitro* functional studies. Moreover, to disclose the potential mechanisms involved in the aging-related changes and prophylactic effect of LCD, by using cDNA microarray analysis, we searched the genes upregulated or downregulated with aging and further picked up the genes of which changes with aging are made inconspicuous by LCD.

Study design, materials and methods

Fischer 344 male rats were divided into three groups: young (6 months-old, n=8), old (25-28 months-old) with normal diet (ND: n=8) or LCD (n=8). The LCD group had been fed three days a week since 6 weeks-old. *In vitro* organ bath studies using full-thickness of longitudinal strips taken from the bladder body were carried out to evaluate their contractile responses to high potassium (High K^+: KCl 62 mM), carbachol (CCh: 10^{-3}-10^{-8} M), and also to electrical field stimulation (EFS; 2-20 Hz) in the absence and presence of atropine (10^{-6} M), alpha-beta methyleneATP (M-ATP; 10^{-5} M), and tetrodotoxin (TTX; 10^{-6} M). For the analysis of cDNA microarray, each 4 samples of the bladder and L6 DRG from all 3 groups were examined. The genes expressed more than 2 fold greater or smaller in the old with ND group compared with the young group were searched. Then further selected the genes of which expression differences were less between the old with LCD and young groups and relationship among those detected genes were evaluated by pathway analysis.

Results

There were no significant differences in the contractile responses to High K^+ among the three groups. In contractile responses to CCh, the Emax (122.4±4.5%) in the old with ND group significantly (p=0.024) lower than that (139.6±6.1%) in the young group, while the value (130.3±4.4%) in the old with LCD group was not significantly (p=0.236) different from that in the young group (Figure 1). The contractile responses to EFS were significantly weaker in the old with ND group than the young group, but no significant difference were observed between the old with LCD and young groups. Strips of the old with ND group showed a decreased atropine-sensitive component and an increased M-ATP-sensitive component compared with those of the young and old with LCD groups (Figure 2). The cDNA microarray following by network and heat map analysis revealed some biological functions associated with the upregulated genes of the bladder in old with ND group compared with the young group including ”immune cell trafficking”, “cell-to-cell signalling and interaction”, “cellular movement“ and “inflammatory response” (Figure 3 upper panel). Among those upregulated gene-complex areas in the old +ND group showed less extent or even rather downregulated expression in the old with LCD compared with the young group (figure 3 lower panel). Functions associated with the upregulated genes of the DRG in old with ND group include “cell-to cell signalling and interaction”, “hematological system development and function” and “humoral immune response” (Data not shown). Downregulated genes in old with ND compared with the young group were too few to make network analysis.

Interpretation of results

The results of the present functional study in male rats indicates that the contractility of the detrusor smooth muscle to muscarinic receptor stimulation and EFS is impaired with aging and the impaired contractility is likely to be mainly due to impaired acetylcholine (ACh)-mediated contraction whereas no significant impairment of the response to High K^+ is accompanied, suggesting downregulation of muscarinic receptor function and/or decreased ACh-release from the parasympathetic nerve terminal in the bladder with aging. These findings are in line with those reported in a previous study (3). Interestingly, LCD feeding counteracted such age-related functional impairments of the bladder, suggesting that LCD may prevent these changes. The present microarray analysis of the bladder and DRG innervating the bladder revealed that there are some genes upregulated remarkably in old with ND, and less extent in old with LCD compared to the young group, including those related to “immune cell trafficking“, and “inflammatory response”. These genes might be key molecules for the age-related, and possibly preventative by low calorie diet, bladder dysfunction.

Concluding message

Contractile property to muscarinic stimulation and nerve-mediated, especially cholinergic nerve-mediated contractility are impaired with aging in male rats. These impairments of bladder contractility are likely to be preventable by long-term low calorie diet feeding. The genes expressed in the balder and DRG in harmony with these functional changes of the bladder might be key molecules as a target for the treatment and/or prevention of the age-related bladder dysfunction.

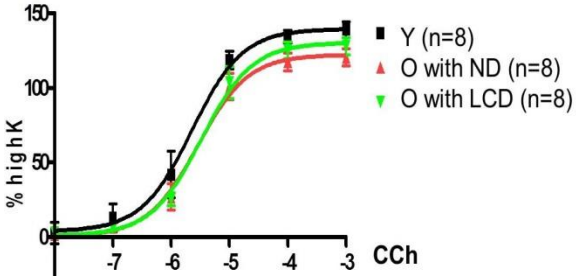

Figure 1 Concentration-response curves for carbachol of the bladder strips in the young (Y), old with normal diet (O with ND) and old with low calorie diet (O with LCD) groups

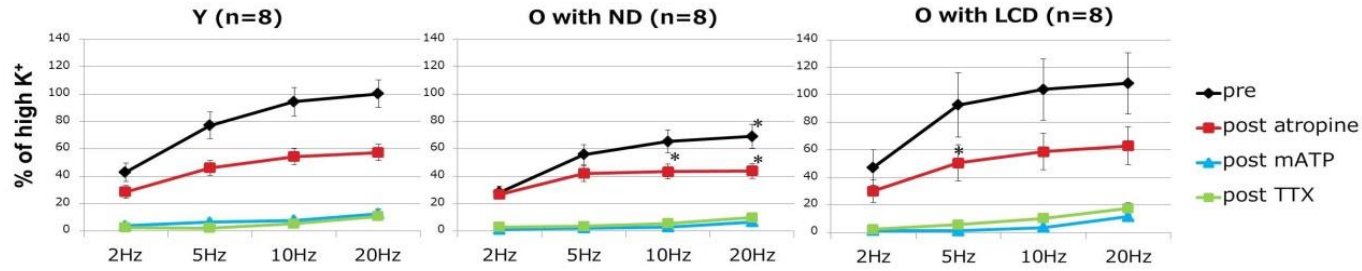

Figure 2. Frequency-response curves of the EFS-induced bladder contractions in the young (Y), old with normal diet (O with ND) and old with low calorie diet (O with LCD) groups
*P<0.05 significant difference from young at same frequency of EFS (Mann-Whitney U test)

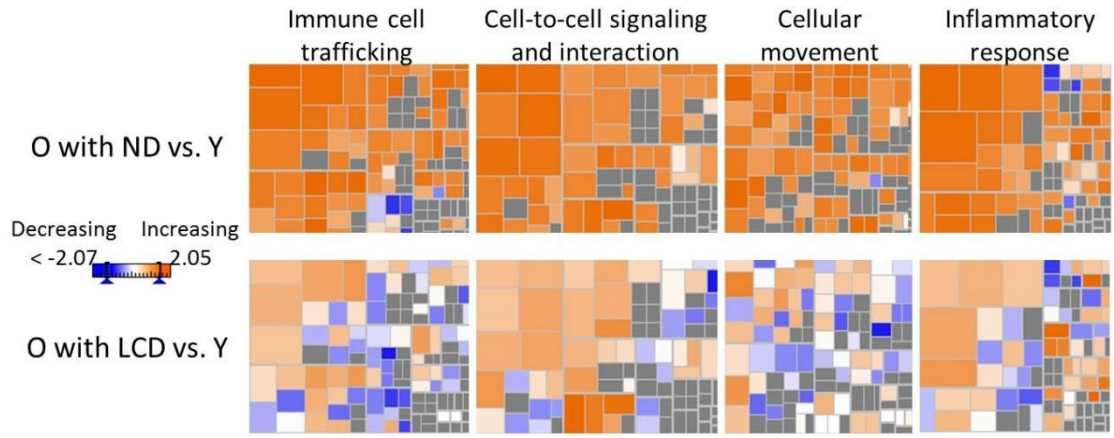

Figure 3. Gene expression levels related to specific functions demonstrated by heat map analysis in the old with normal diet (O with ND) group (upper panel) and in the old with low calorie diet (O with LCD) group (lower panel) compared with the young group (Y). Note some areas upregulated in the O with ND group showing less extent or rather downregulated in the O with LCD group compared with the Y group.

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