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CONVERGENCE OF BLADDER AND SKIN PRIMARY SENSORY NEURON EXPRESSING TRPM8

Hypothesis / aims of study

It is known that some stimuli on skin such as cool temperature or manual stimulation of certain areas within sacral and lumbar dermatomes affects bladder contraction. To investigate the skin and bladder interaction we investigated the convergence of bladder and skin sensation in primary sensory neuron level.

Study design, materials and methods

Double labelling with retrograde neuron tracers was performed on male Sprague-Dawley rats. Cholera toxin subunit B recombinant (CTB) Alexa Fluor 594 conjugate and Fast Blue(FB) were used as retrograde neuron tracers. CTB was injected circumferentially and subserosally into the urinary bladder. On the following day, FB was injected intracutaneously from upper scrotum to the base of the tail meaning L6-S1 level in the male rat. Paired L6-S1 DRGs were dissected and sections were observed with fluorescence microscope to detect CTB- and FB-positive neurons. The total cell number, cell number of CTB- and Fast Blue-positive neurons were calculated for each ganglion of representative 5 sections per one animal.

Localization of the TRPM8 mRNA in the DRG neurons was determined by in situ hybridization. The sections obtained from preceding procedure were used.

Electrophysiological recordings were performed to detect electrical skin-to-bladder interaction. Animals were anaesthetized with ethyl-carbamate (1.2 g/kg i.p.). Laminectomy was performed on the vertebral arch of the L4 and L5 vertebrae and cauda equina was transected between L4 and L5 lumbar vertebrae level. A branch of the pelvic nerve arising from the urinary bladder was dissected in bundle form and used. The nerve activity was integrated and plotted as voltage histogram. The stimulation point was on upper scrotum to the base of the tail meaning L6-S1 level of dermatome and precordia as control skin area. Electrical activity was recorded at control temperature, cold stimuli, and post-test duration and calculated the ratio to the baseline activity.

Results

Double-labeled neurons were observed and cell numbers of the double-labelled cells were 1.4 ± 0.2, 1.0 ± 0.3 and 2.4 ± 0.2 per animal at the L6, S1 and L6-S1 levels, respectively, on the same sections, implying that approximately 0.057, 0.11 and 0.071% of the L6, S1 and L6-S1 neurons were labeled with both tracers, respectively.

It was found that cross-sectional areas of the double-positive neurons ranged from 292 to 1622 (μm)2, corresponding to cell somata diameters between 93 and 517 μm. The majority of the neurons fell within the area range between 400 and 800 (μm)2.

In situ hybridization experiments revealed that approximately 8.0 ± 3.0% of the double-labeled L6-S1 neurons expressed TRPM8 transcripts.

In electrophysiological analysis, we recorded cold-induced reactions in 8 out of 24 rats. In detail, when control warm water (approximately 28ºC) was applied to the stimulus skin area, the background bladder nerve activities could not substantially increase, indicating that the temperature stimuli and accompanying mechanical stimuli themselves evoked no action potentials. Next, when cold stimuli were applied to the same area, the whole nerve activities significantly increased, and these reactions calmed down immediately after the stimuli were stopped. The relative nerve activities against basal ones at the control temperature, cold and post stimuli were 98.9 ± 0.6%, 115.0 ± 2.6% and 102.2 ± 1.4%, respectively (n = 8), and the differences between the cold stimuli and the warm or post stimuli were statistically significant ($p < 0.01$). In control experiments, no reaction was observed against the same stimuli applied onto the precordia in the same animal. The relative nerve activities against basal ones at the control temperature, cold, and post stimuli to the precordia were 98.9 ± 1.3%, 97.5 ± 1.3% and 99.4 ± 1.2%, respectively.

Interpretation of results

Double-labeled neurons indicate that the DRD cells had dichotomizing or dually projecting axons, innervating the skin and bladder, simultaneously.

Distribution of dichotomizing neurons indicate that the greater part of the population was comprised of small-to-medium-diameter cells.

In situ hybridization showed that the sub-population of the dichotomizing neurons expressed the TRPM8 channels respond to cold stimuli. As TRPM8 and TRPA1, another cold-sensitive cation channel, are located in the distinct populations of DRG neurons, these results also suggest that the cold activation of the TRPM8 channels in skin afferents leads to urinary urgency via backfiring of other terminals in the bladder.

Electrophisiological analysis indicated that some sub-population of the nerve bundles generated action potentials in response to the cold stimuli to the skin that corresponded to the L6-S1 dermatomes, suggesting that some dichotomizing DRG neurons innervate both organs simultaneously.

Concluding message

We detected dichotomizing DRG neurons supplying the bladder and the skin simultaneously with concomitant retrograde labeling technique of both urinary bladder and skin afferents, and with electrophysiological technique. A fraction of these double-labeled neurons express TRPM8 channels were detected with in situ hybridization technique. These referred-pain-like axon reflex relationship between skin and bladder may provide a neuronal substrate for trigger voiding in patients with spinal cord injury or urinary urgency evoked by cold sensation or drop of temperature.

Specify source of funding or grant	**none**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Center of Experimental Animal Sciences at Nagoya City University**