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EFFECTS OF L-ARGININE, AN NO DONOR, ON THE PRIMARY AFFERENT ACTIVITY WITH OR WITHOUT ACROLEIN-TREATED RAT BLADDER

Hypothesis / aims of study
It has been suggested that nitric oxide (NO) affects the afferent pathways innervating the bladder. In addition, acrolein, a metabolite of cyclophosphamide, causes bladder hypersensitivity as a chemical cystitis agent in rats (1). We investigated the direct effects of an NO donor (L-Arginine) on single fiber activities of the primary bladder afferent nerves with or without acrolein-application.

Study design, materials and methods
Female Sprague-Dawley rats were used. Under intraperitoneal urethane anesthesia (1.5 g/kg), for monitoring single unit nerve activity of the primary bladder afferents, fine filaments were dissected from the left L6 dorsal roots and placed across a bipolar electrode. Nerve fibers primarily originating from the bladder were identified by electrical stimulation of the left pelvic nerve and by bladder distension. Nerves of which conduction velocity (CV) is more than 2.5 m/sec were determined as Aδ-fibers and those with less than 2.5 m/sec as C-fibers (2). To facilitate permeability of the bladder urothelium for drugs, protamine sulfate solution (10 mg/ml, 0.3 ml) was instilled intravesically and kept in the bladder for 60 minutes just before the measurement. At the beginning of the experiments, the afferent activity measurements with constant bladder filling were repeated three times and the third measurement served as the control observation. Then, L-Arginine (300 mg/kg) was administrated intravenously. Thereafter, 0.003 % of acrolein or saline was instilled intravesically to obtain another three cycles of investigations (Figure 1). The afferent activity is expressed as a percentage of baseline activity, integrated for the whole filling phase, which is based on pressure and volume. One- and two-way ANOVA followed by Tukey's test was applied for statistical comparisons between groups and between before- and after-L-Arginine-administration.

Results
33 single afferent fibers (Aδ-fibers: n=14, CV: 5.51 ± 1.05 m/sec, C-fibers: n=19, CV: 1.02 ± 0.09 m/sec) were isolated from 26 rats. When bladder was filled with saline-instillation after L-Arginine-administration, bladder compliance significantly increased from the base-line value. In contrast, bladder compliance tended to be decreased by acrolein-instillation with or without L-Arginine-administration. The afferent activities of both Aδ- and C-fibers in response to saline-instillation significantly decreased after L-Arginine-administration (Figures 2, 3). After the vehicle-administration, acrolein-instillation itself significantly increased the activities of the both fibers. Furthermore, these increased responses were inhibited by the pretreatment with L-Arginine (Figure 3).

Interpretation of results
The results of the present study suggest that both mechano-sensitive Aδ- and C-fibers were inhibited by L-Arginine-administration. Moreover, both fibers were activated by intravesical acrolein, and this activation was attenuated by L-Arginine-administration.

Concluding message
The present results clearly demonstrate that L-Arginine, an NO donor, can inhibit both mechano-sensitive Aδ- and C-fibers of the primary bladder afferents in the rat. In addition, L-Arginine can inhibit the activated responses of the both fibers to intravesical acrolein. These findings may give us a new insight into the mechanism of the possible effect of NO system in the treatment of overactive bladder.

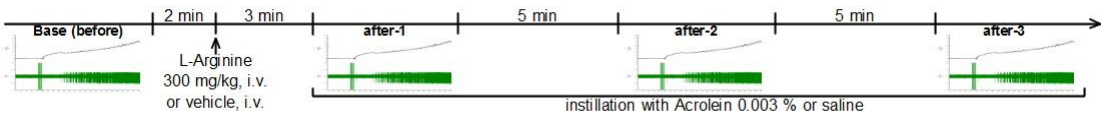

Figure 1. Experimental protocol.

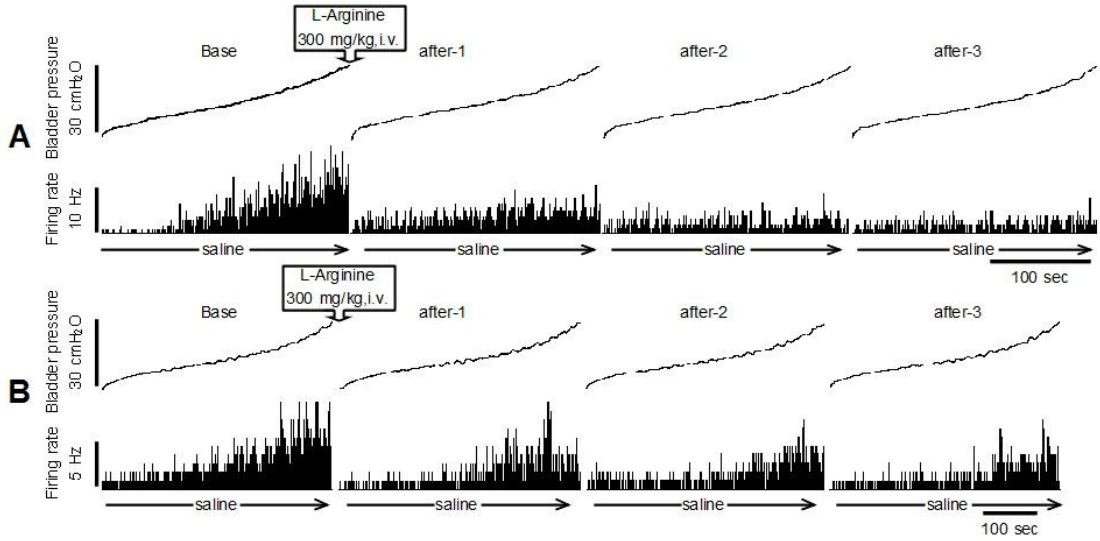

Figure 2. Intravesical pressure (upper tracing) and firing rate (lower tracing) of Aδ-fiber (A) and C-fiber (B) during bladder filling with saline before (Base) and after (-1, -2, -3) L-Arginine-administration.

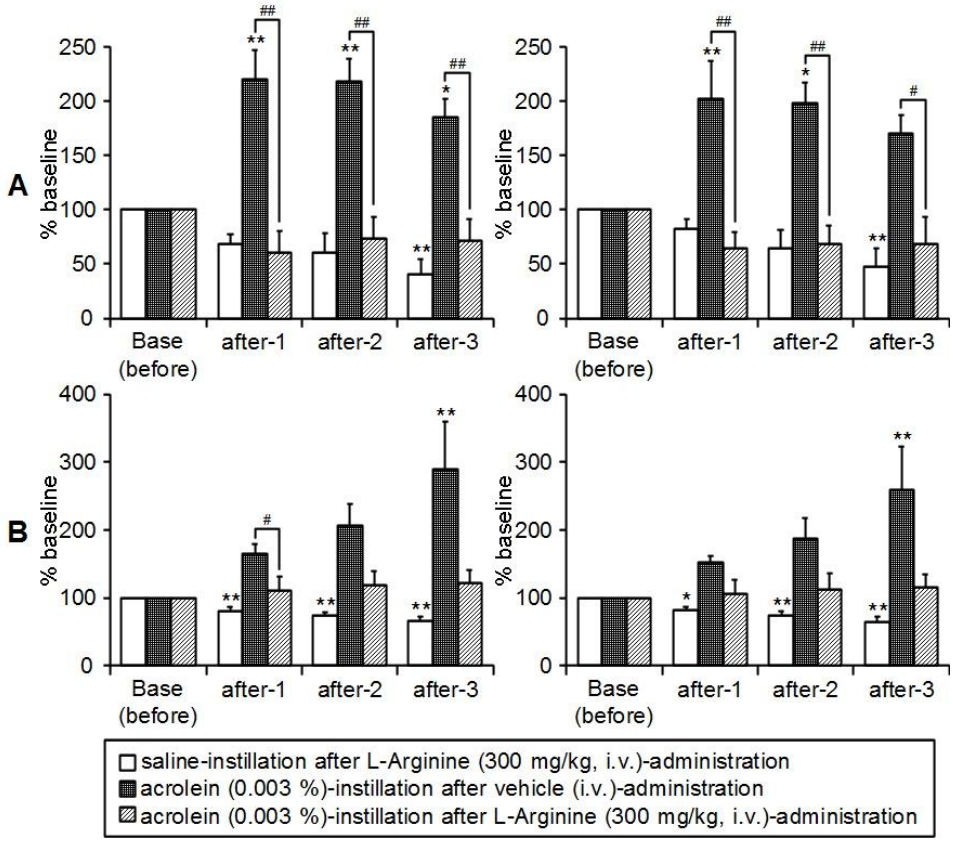

Figure 3. Responses of the Aδ-fibers (A) and C-fibers (B) integrated during the whole filling phase.
Left: based on pressure, Right: based on volume.
**P*<0.05, ***P*<0.01: significant difference from Base (two-way ANOVA followed by Tukey's test).
$^{\#}P$<0.05, $^{\#\#}P$<0.01: significant difference between three groups (one-way ANOVA followed by Tukey's test).

References
1. J Urol 1999; 162: 2211
2. J Neurophysiol 1994; 72: 2420

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	**Animal Ethics Committee, University of Antwerp Faculty of Medicine**