GATING OF THE MICTURITION REFLEX BY ACTIVATION OF BLADDER C-AFFERENTS - AN EXPERIMENTAL STUDY IN THE CAT

Hypothesis / aims of study
Bladder A and C afferents are known to influence bladder function through convergence of excitation onto spinal parasympathetic preganglionic neurones. Their respective pathways to the common target neurone are largely different, however. While A afferents, which drive the ordinary micturition reflex, mediate their effect via a spino-ponto-spinal pathway, C afferents utilize a spinal segmental pathway. From this arrangement it is obvious that C afferents can facilitate and enhance the amplitude of the micturition reflex. It is less clear if they would have any effect on the gating of the micturition reflex, i.e. the micturition reflex threshold. The latter is generally believed to be set at the pontine micturition centre. To address this problem we have now determined the micturition threshold with or without natural stimulation of bladder or urethral C afferents connected to cold receptors. By menthol exposure these receptors can be selectively activated at normal body temperature. For comparison the receptors were also activated by natural cold stimuli.

Study design, materials and methods
Adult female cats were anaesthetized with α-chloralose (55 mg/kg). Using an extra-peritoneal approach, a thin catheter was inserted into the bladder through a slit in proximal urethra and used for fluid infusions and detrusor pressure recordings. Another catheter, introduced in the distal direction, was used for urethral perfusion. Micturition threshold was assessed by repeated control cystometries with body warm saline at 2 - 5 ml/min followed by similar infusions with menthol (0.006 - 0.06 mM) added to the fluid or by cold saline at 4°C. Each session consisted of 4 successive control and test infusions, the first of which was used for wash out and accordingly omitted from the analysis. In some trials the cystometry was instead combined with a slow urethral perfusion with body-warm or cold saline with or without menthol. Threshold volume and pressure were the main variables but peak pressure of isovolumetric micturition contractions was also assessed. Mean values from each trial session were used to calculate the group mean ± c.i. (95% confidence interval), differences were evaluated by paired t-tests. Experimental procedures were approved by the local Animal Research Ethical Committee.

Results
A total of 13 trial sessions with bladder exposure to menthol were performed in five animals. Menthol consistently reduced the threshold volume of the micturition reflex from a group mean of 19.2 ± 6.9 ml to 6.7 ± 1.8 ml (p<0.01). With the used menthol concentration, the effect was fully reversible with control values obtained after a single wash-out cystometry. There was no change in bladder compliance, implying that the bladder pressure at threshold was somewhat lower with menthol, 0.7 ± 0.3 kPa compared to 1 ± 0.2 kPa at control (p<0.01). A similar but less pronounced decrease in threshold volume was obtained after cold infusions, control 22.2 ± 6.7 ml, cold 14.4 ± 5.2 ml (p<0.01; 11 trial sessions in 3 animals). In this case the threshold pressure was unchanged, 0.6 ± 0.2 kPa in both cases. The peak micturition pressure was unchanged after menthol, 6.1 ± 2.0 kPa compared to 5.6 ± 2.8 kPa, while it was slightly increased after cold infusions 4.9 ± 0.7 kPa compared to 4.2 ± 0.4 kPa (p<0.05). Comparable results were obtained when cystometries were combined with a slow urethral perfusion of body warm or cold saline or warm menthol solution (5 cold and 3 menthol sessions in two animals). In all sessions the micturition threshold volume was decreased while the threshold pressure and peak pressure were unchanged.

Interpretation of results
Menthol or cold exposure of the bladder (or urethra) consistently reduced the threshold volume of the micturition reflex. This change in reflex gating occurred without any detectable decrease in bladder compliance, if anything the threshold pressure was also reduced. Since bladder A afferents are insensitive to menthol and have reduced sensitivity when cooled, the observed change can only be explained by a concomitant activation of bladder cold receptors.
of C fibre type. There are two possible explanations for this gating effect by selectively activated bladder C afferents. The threshold setting in the pontine micturition centre may be modulated by ascending activity from bladder C afferents. Alternatively, the gating mechanism is located at the spinal segmental level. In the latter case, permissive descending ponto-spinal signals would, by impinging on spinal interneurones, shift convergent A and C spinal pathways from a storage mode of operation to emptying mode.

**Concluding message**
Identification of the correct site for gating of the micturition reflex may help to clarify the mechanism underlying phasic detrusor overactivity as well as explain the diverse effect of spinal cord injury on bladder function.