

# Evidence for a functional role of connexin 43 and 45 in human neurogenic detrusor overactivity

Véronique Phé<sup>1</sup>, Delphine Behr-Roussel<sup>2</sup>, Stéphanie Oger-Roussel<sup>2</sup>, Emmanuel Chartier-Kastler<sup>1</sup>, Tarek Ghoneim<sup>3</sup>, Thierry Lebre<sup>3</sup>, Gilles Karsenty<sup>4</sup>, Eva Compérat<sup>1</sup>, Philippe Camparo<sup>3</sup>, Pierre Validire<sup>5</sup>, François Giuliano<sup>2,6</sup>  
(1) La Pitié-Salpêtrière Hospital, Paris, (2) EA 4501, Orsay, (3) Foch Hospital, Suresnes, (4) La Conception Hospital, Marseille, (5) Institut Mutualiste Montsouris, Paris, (6) Raymond Poincaré Hospital, Garches, FRANCE

## BACKGROUND

Altered intercellular communication is suspected to participate in NDO pathophysiology. Modulation of detrusor connexin (Cx) expression has been reported in rats and humans with neurogenic detrusor overactivity (NDO) but their functional role in the pathophysiology of NDO patients is unknown.

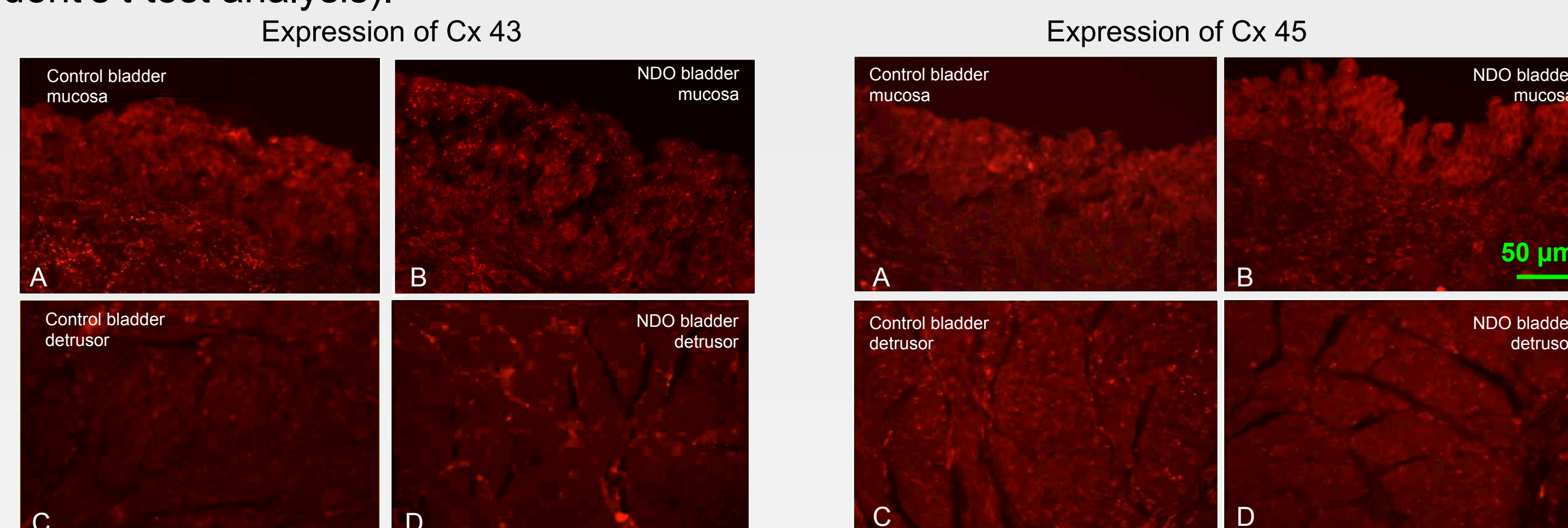
## OBJECTIVES

- 1/To determine the effect of Cx40, 43 and 45 selective inhibition on detrusor contractions from NDO patients.
- 2/To study the expression of Cx40, 43 and 45 in the bladder from NDO patients.

## RESULTS

### Connexin expression in the bladder wall

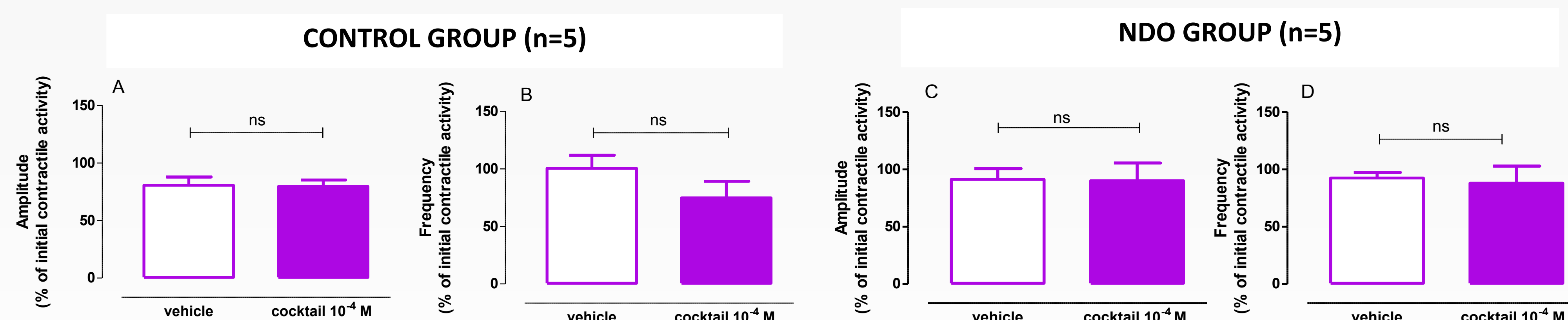
- In control and NDO groups, Cx43 and Cx45 immunofluorescent staining was observed in the mucosa and the detrusor. No difference in Cx43 and Cx45 expression both in mucosa and detrusor was observed between the two groups (Student's *t* test analysis).



- Cx43 labeling was significantly increased in the mucosa compared to the detrusor ( $p < 0.01$ ), either in control group or NDO group. There was no significant difference in Cx45 labeling between mucosa and detrusor, whatever the groups.
- Cx40 staining was very slightly detected in the bladder wall of control and NDO groups.

### Effect of a cocktail of Cx40, 43 and 45 selective inhibitors on spontaneous contractile activity

- A cocktail of Cx40, 43 and 45 selective inhibitors had no significant effect, neither on the amplitude nor the frequency of spontaneous contractions of bladder strips, neither in control nor in NDO groups (ns: non significant, Student's *t* test analysis)



## MATERIALS & METHODS

### Human bladder samples preparation

Human bladder samples were obtained from 17 patients (6 men, 11 women, mean age 46.9±14.1 years) with urodynamically documented NDO (11 SCI, 4 multiple sclerosis, 1 spina bifida, 1 cerebral tumor) refractory to antimuscarinics and/or intradetrusor botulinum toxin-A injections, and undergoing partial or total cystectomy (NDO group), and from 30 patients (27 men, 3 women, mean age 65.2±8.7 years) undergoing cystectomy for bladder cancer with no symptoms of overactive bladder (control group) according to the validated questionnaire USP. The collection and use of bladder samples were carried out in accordance with all relevant laws, regulations and codes of practice in force in France, including obtaining informed written consent from patients.

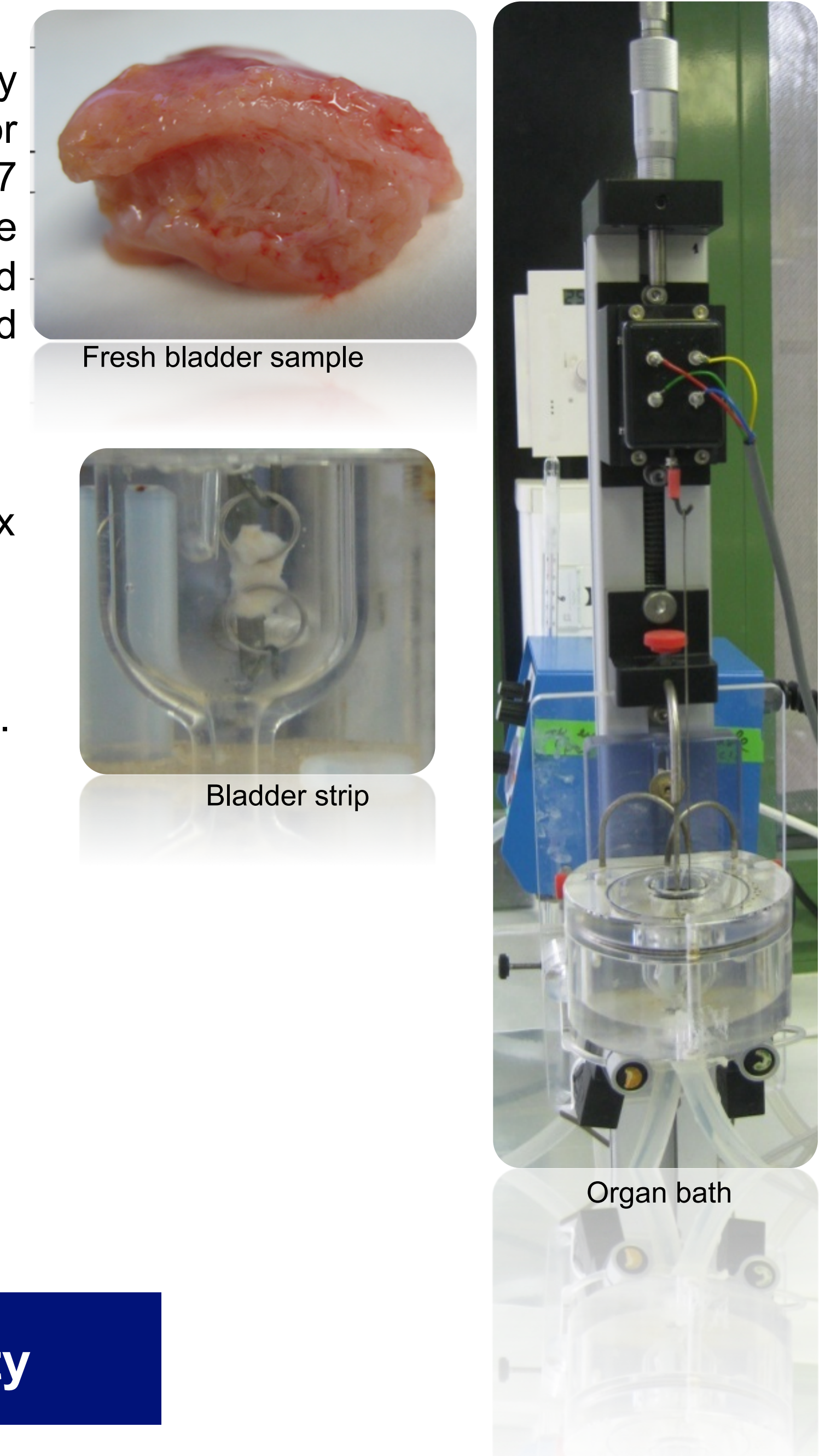
### Immunofluorescent study

The expression of Cx 40, 43 and were studied by immunofluorescence. The fluorescence mean intensity corresponding to Cx labeling was measured.

### In vitro experiments on contractile activity of bladder strips

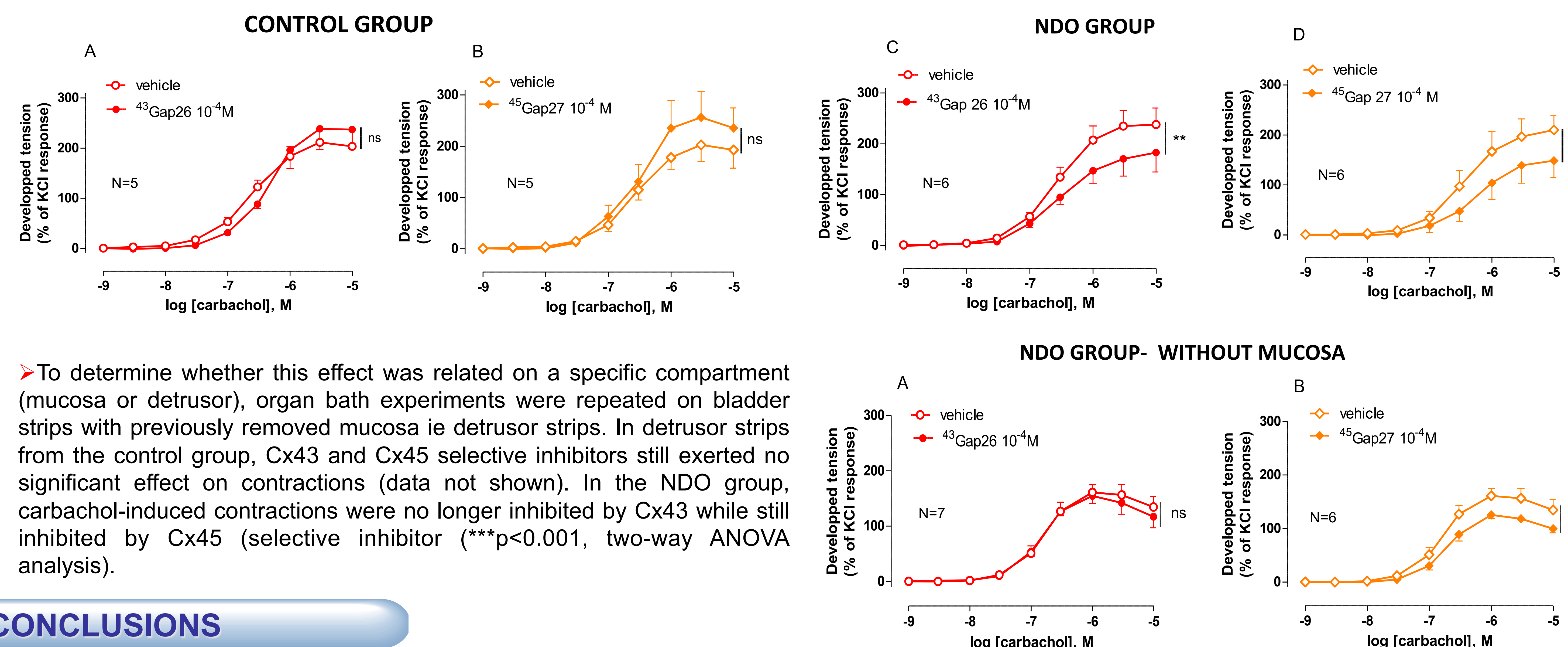
Strips were excised from each donor bladder sample. The serosal layer was removed, and strips of 8×6×4 mm were isolated. Depending on the experiment, mucosa was carefully removed to obtain detrusor strips or kept to obtain bladder strips. Strips were suspended in 5-ml organ chambers filled with Krebs-HEPES buffer (37°C, continuously bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, pH 7.4) and connected to force transducers for isometric tension recording (LCM Systems, Newport, UK). An initial tension of 0.5–1 g was applied. After amplification, the tension changes were computerized with Mac Lab™/8 using Chart™5 software (AD Instruments Ltd, Chalgrove, UK). The tissue preparations were allowed to equilibrate for 90 min while being washed periodically with fresh Krebs-HEPES buffer.

Inhibitory effects of Cx selective inhibitors, the Cx mimetic peptides <sup>43</sup>Gap26, <sup>45</sup>Gap27 and <sup>40</sup>Gap27 (Proteogenix, Oberhausbergen, France) were assessed on spontaneous and carbachol-induced contractile activity of bladder strips.



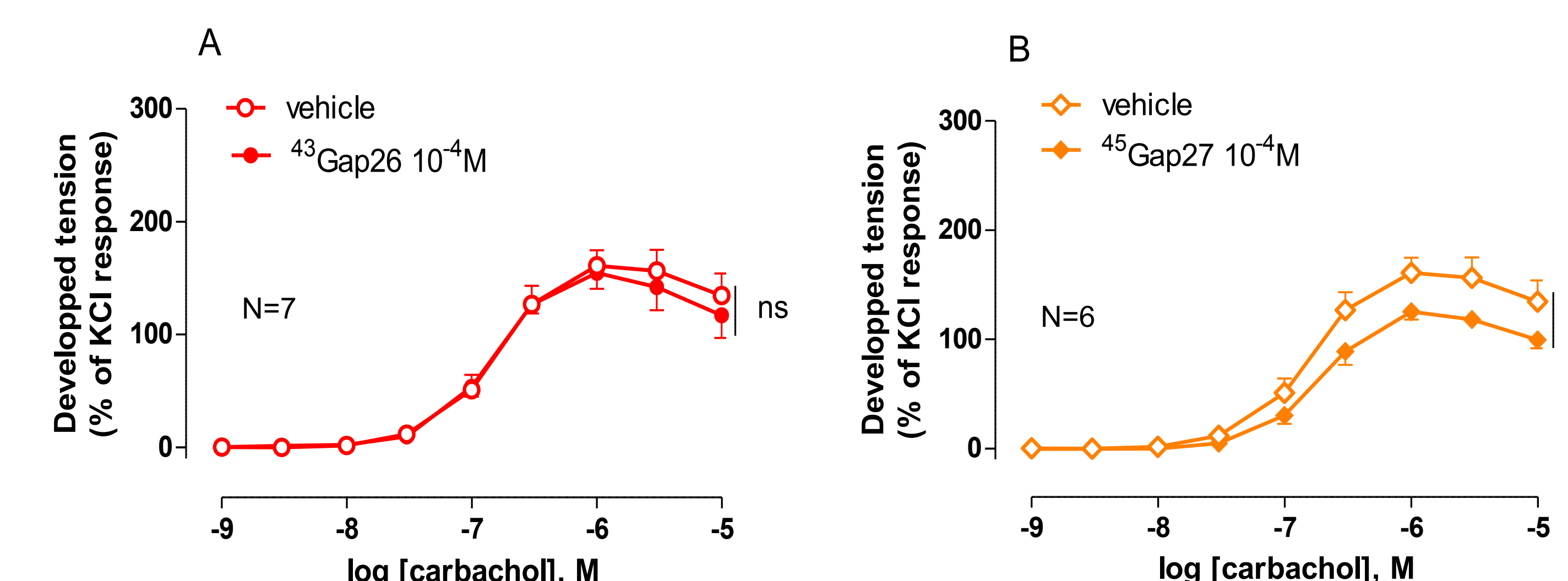
### Effect of Cx43 and Cx45 selective inhibitors on carbachol-induced contractile activity

- In the control group, neither Cx43 nor Cx45 selective inhibitors exerted an effect on bladder strips carbachol-induced contractions. By contrast, in the NDO group, carbachol-induced contractions were significantly inhibited by 23% by the Cx43 and by 29% by the Cx45 selective inhibitors (\*\* $p < 0.01$ , two-way ANOVA analysis).



- To determine whether this effect was related on a specific compartment (mucosa or detrusor), organ bath experiments were repeated on bladder strips with previously removed mucosa ie detrusor strips. In detrusor strips from the control group, Cx43 and Cx45 selective inhibitors still exerted no significant effect on contractions (data not shown). In the NDO group, carbachol-induced contractions were no longer inhibited by Cx43 while still inhibited by Cx45 (selective inhibitor (\*\* $p < 0.001$ , two-way ANOVA analysis).

### NDO GROUP- WITHOUT MUCOSA



## CONCLUSIONS

- This the first evidence in humans with NDO for a functional role of Cx43 and 45 in detrusor activity coordination. The modulation of intercellular communication through Cx43 and 45 could represent a new avenue for pharmacological research for NDO.