

Netupitant, a potent and highly selective NK₁ receptor antagonist in the guinea-pig isolated urinary bladder: comparison with aprepitant

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Objectives

Substance P (SP), a neuropeptide largely distributed in the body of mammals, displays a wide variety of physiological functions and acts mainly through stimulation of NK₁ receptors. SP is located in capsaicin-sensitive primary afferent neurons that innervate the urinary bladder⁽¹⁾. Pharmacological studies using selective antagonists have demonstrated that this peptide plays an important role in the sensory control of bladder contractions. Functional NK₁ and NK₂ receptors have been previously demonstrated in guinea-pig urinary bladder smooth muscle⁽²⁾.

The aim of the study was to compare the effects of netupitant and aprepitant, two potent and selective NK₁ receptor antagonists, on guinea-pig isolated detrusor muscle contracted with the selective NK₁ receptor agonist, substance P methylester (SP-OMe). Specificity of the two antagonists was tested by investigating their effects on contractions induced by carbachol and KCl.

Methods

- Female guinea-pigs were sacrificed by cervical dislocation and the whole urinary bladder excised. The detrusor muscle of the posterior face of the bladder was dissected free from connective tissue and urothelium and cut into 2 equal strips.
- Strips were mounted in 5 mL organ baths containing a Krebs-Henseleit solution and 0.3 μM GR159987 (NK₂ receptor antagonist), 1 μM propranolol and 1 μM indomethacin. A resting tension of 1 g was applied and contractile responses were measured using isometric tension transducers connected to amplifiers and to a data acquisition system. Strips were challenged with 80 mM KCl in order to test tissue viability.
- A first cumulative concentration-response curve (CRC) to SP-OMe was performed between 1 nM and 10 μM. After 30 min, tissues were incubated for 90 min with netupitant at 1, 3, 10 and 30 nM, aprepitant at 3 and 10 nM or their corresponding solvents (0.03% ethanol, 0.003% DMSO, respectively) and a second CRC to SP-OMe performed.
- In another series of experiments, a first cumulative CRC to carbachol (0.1 to 100 μM) or KCl (10 to 100 mM) was performed. Tissues were then incubated for 90 min with netupitant and aprepitant at concentrations of 0.3 and 1 μM or the corresponding solvents before a second CRC to carbachol or KCl (for netupitant only) was constructed.
- Data were analyzed using the Chart® software. Results were expressed as % of the maximal response obtained in the first CRC to the agonist. EC₅₀ values for SP-OMe, carbachol or KCl in the absence and presence of each concentration of netupitant, aprepitant or solvent were calculated by nonlinear regression and statistically compared using the software Graph Pad Prism® v 4.0. Since it was not possible to construct a Schild Plot, the potency of netupitant and aprepitant was calculated using the formula $pK_B = \log [\text{antagonist}] + \log (\text{dose ratio} - 1)$.

Conclusions

These results demonstrate that netupitant and aprepitant are potent antagonists of contractions evoked by the activation of NK₁ receptors in the guinea-pig isolated urinary bladder. The potency of netupitant (mean $pK_B = 9.24$) was very similar to its reported affinity for human recombinant NK₁ receptors ($pK_i = 9.01$)⁽³⁾. The potency of aprepitant (mean $pK_B = 10.03$) was also similar to its affinity for native NK₁ receptors in human brain ($pK_i = 10.11$) confirming that the pharmacology of the guinea-pig NK₁ receptor is very similar to that of the human receptor⁽⁴⁾.

At low concentrations both netupitant and aprepitant are competitive antagonists at guinea-pig NK₁ receptors based on the parallel rightward shifts in the CRC's to SP-OMe observed. However, at concentrations higher than 10 nM, both antagonists clearly reduced E_{max} values. Netupitant and aprepitant did slightly antagonize contractile effects induced by carbachol but only at concentrations 1000 times greater than their pK_B values at NK₁ receptors. We conclude that aprepitant and netupitant are potent and selective antagonists of NK₁ receptors in the guinea-pig urinary bladder.

It was recently hypothesized that NK₁ receptor antagonists, by blocking neurotransmission through afferent fibres could be the drug of choice for treating overactive bladder⁽⁵⁾. Because of its novel mechanism of action coupled with its selectivity over muscarinic receptors, netupitant could produce positive effects on bladder function in overactive bladder patients without reductions in bladder contractility (and other non-bladder effects of muscarinic blockade) frequently observed with antimuscarinic drugs.

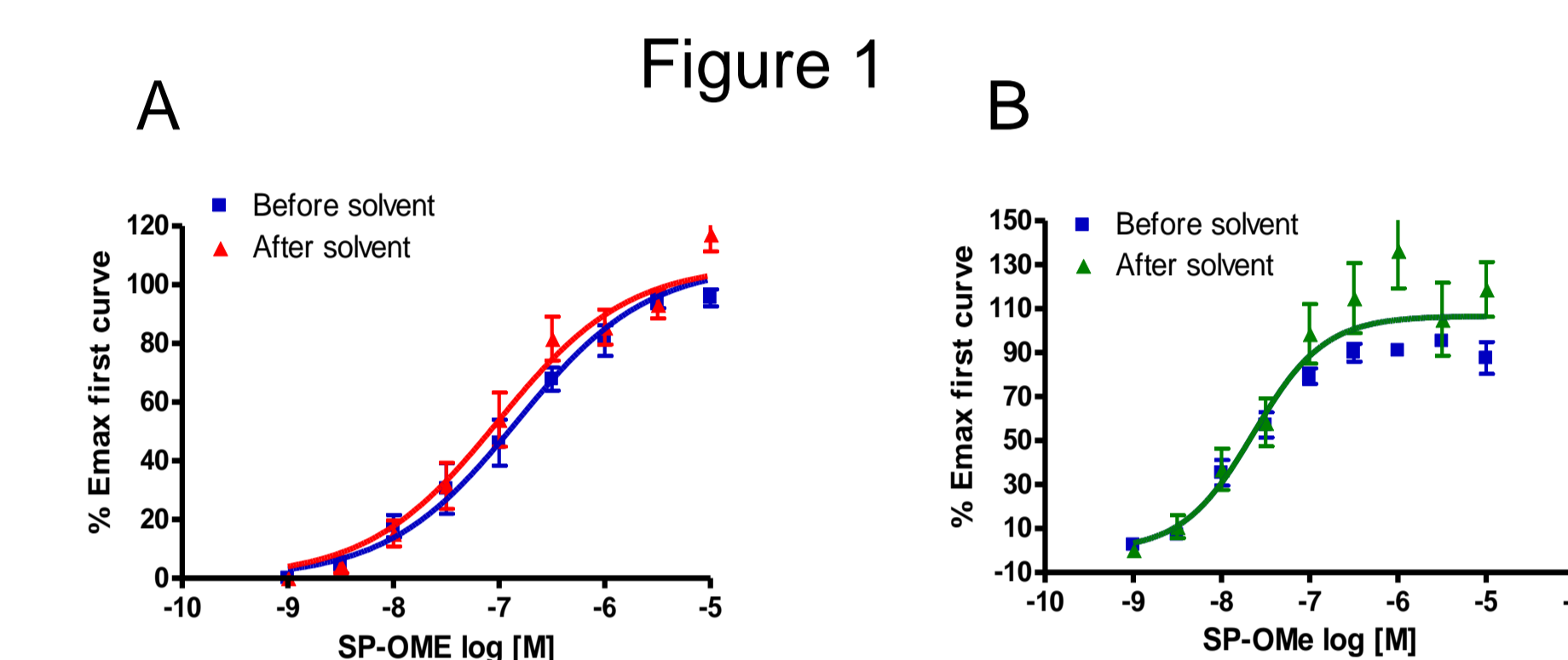
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Results

Effects of increasing concentrations of netupitant or aprepitant on SP-OMe-induced contractions of guinea-pig isolated urinary bladder

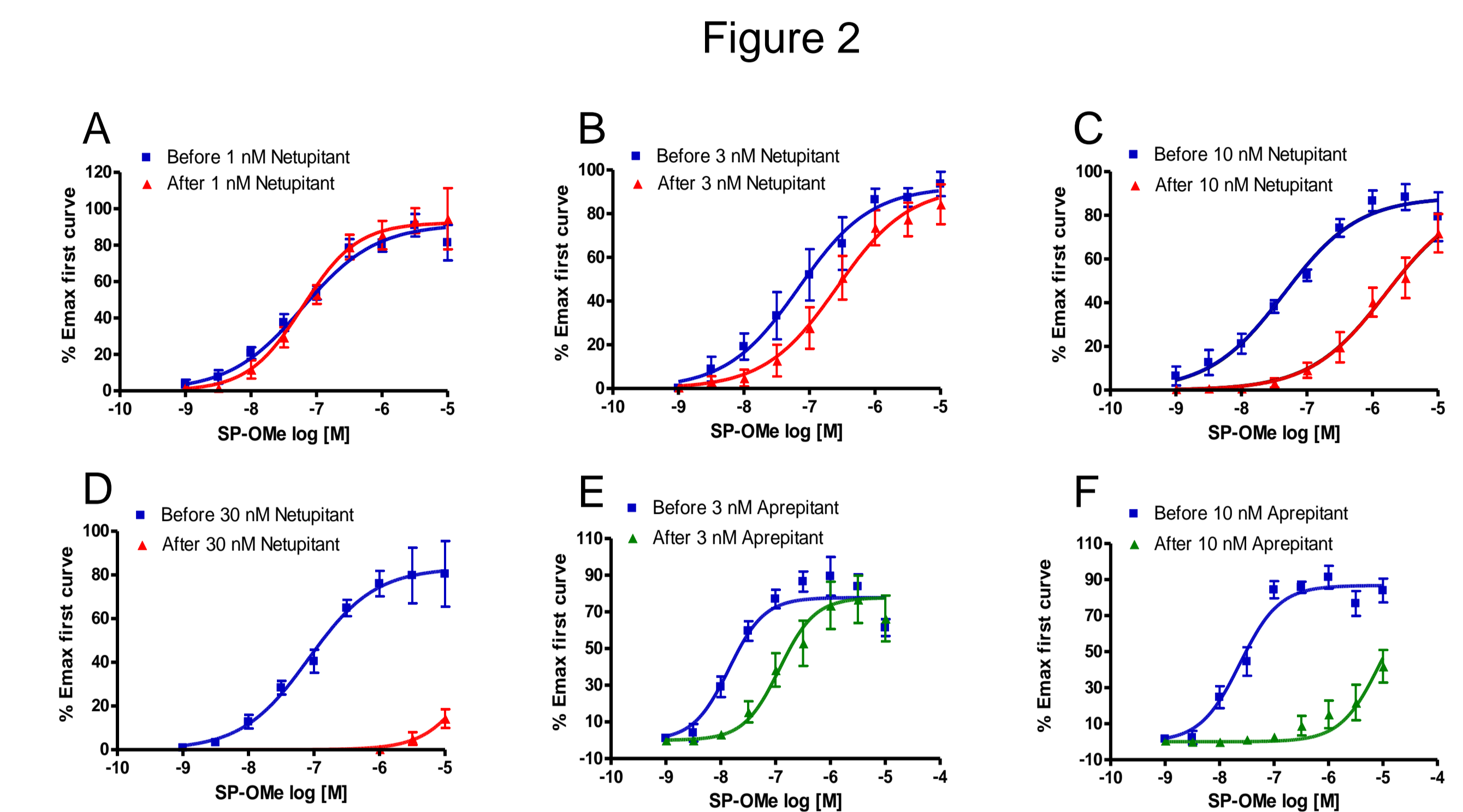
- Two consecutive CRCs to SP-OMe in the same strip of the guinea-pig detrusor muscle were reproducible and not affected by incubation with solvents (Fig. 1A-B).



- Both netupitant and aprepitant inhibited SP-OMe induced contractions in a concentration-dependent manner (Fig. 2).

- Netupitant at 1 nM did not affect the second CRC to SP-OMe (Fig. 2A), however at 3 and 10 nM netupitant produced parallel rightward shifts in the CRC's to SP-OMe (Fig. 2B-C) without affecting the maximal response (E_{max}). At 30 nM, netupitant totally depressed the second CRC (Fig. 2D) making it impossible to use a Schild plot to calculate the pA_2 value. The pK_B value of netupitant was estimated to be 8.95 or 9.54 (mean 9.24) using displacement obtained at 3 and 10 nM, respectively.

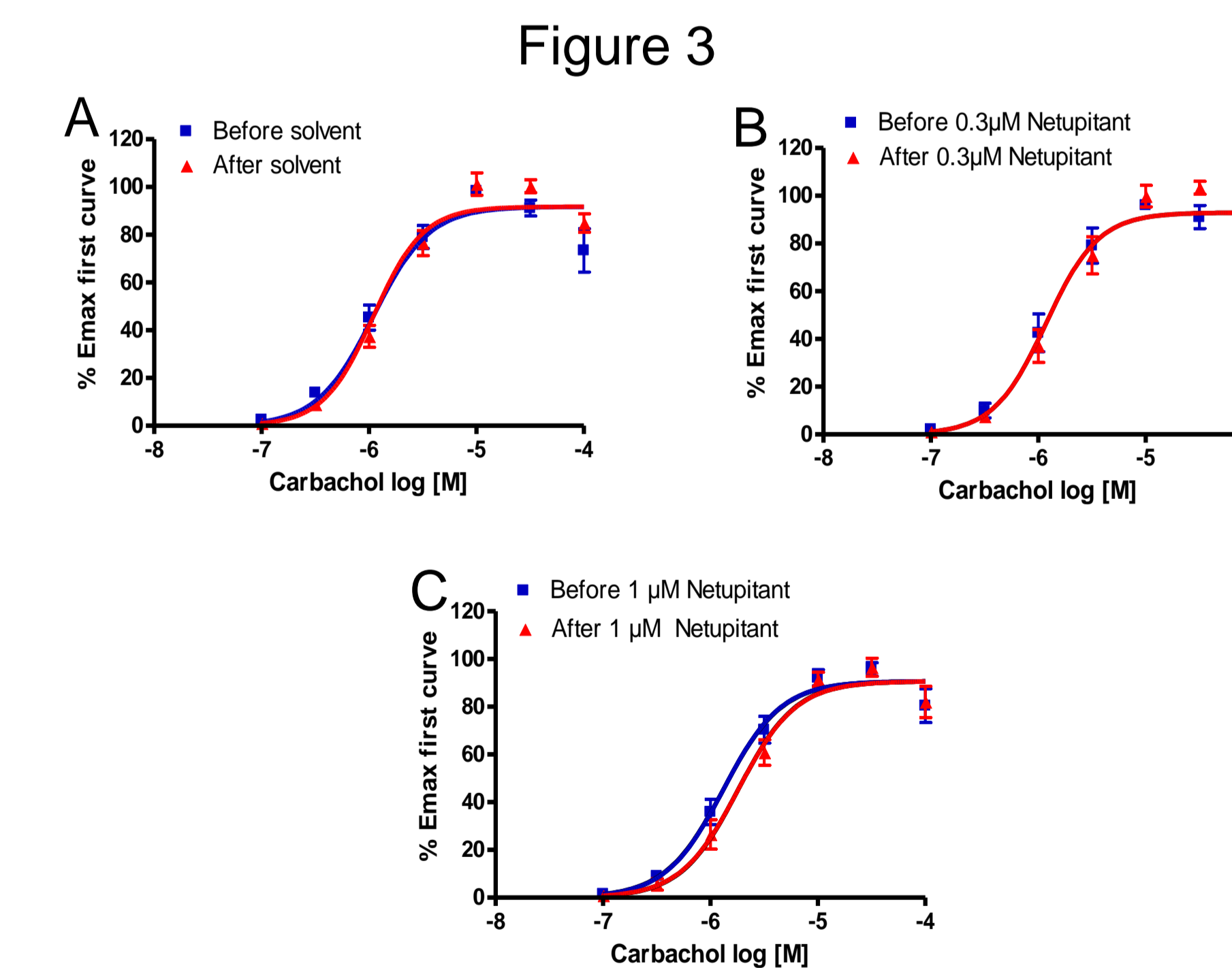
- At 3 and 10 nM, aprepitant also produced rightward shifts in the CRC's to SP-OMe (Fig. 2E-F). A mean pK_B value of 10.03 was calculated based on estimated pK_B values of 9.37 or 10.7 at 3 and 10 nM of aprepitant, respectively.



Effects of increasing concentrations of netupitant or aprepitant on carbachol- or KCl-induced contractions of guinea-pig isolated urinary bladder

- Netupitant at 0.3 μM did not change the second CRC to carbachol (Fig. 3B).
- However, a slight but significant displacement of the CRC to carbachol was observed with 1 μM netupitant (Fig. 3C). The pK_B value was 5.58.

- Netupitant at 1 μM also displaced to the right the CRC to KCl with a calculated pK_B value of 5.38 (data not shown).



- Aprepitant at 0.3 μM did not antagonize the second CRC to carbachol since E_{max} and pEC_{50} values in the absence (6.09 ± 0.04) and presence (6.04 ± 0.04) of aprepitant were not significantly different (Fig. 4B).

- However, at 1 μM, aprepitant slightly but significantly displaced the CRC to carbachol, with an estimated pK_B value of 5.67 (Fig. 4C).

