

## NETUPITANT, A POTENT AND HIGHLY SELECTIVE NK<sub>1</sub> RECEPTOR ANTAGONIST IN THE GUINEA-PIG ISOLATED URINARY BLADDER: COMPARISON WITH APREPITANT

### Hypothesis / aims of study

Functional NK<sub>1</sub> receptors have previously been demonstrated in guinea-pig urinary bladder smooth muscle (1). The aim of the present study was to compare the effects of netupitant and aprepitant, two selective NK<sub>1</sub> receptor antagonists, on guinea-pig isolated detrusor muscle contracted with the selective NK<sub>1</sub> 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.

### Study design, materials and methods

Female guinea-pigs were sacrificed by cervical dislocation and the whole urinary bladder excised. The detrusor muscle 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  $\mu$ M GR159987 (NK<sub>2</sub> receptor antagonist), 1  $\mu$ M propranolol and 1  $\mu$ M 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  $\mu$ M. 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  $\mu$ M) or KCl (10 to 100 mM) was performed. Tissues were then incubated for 90 min with netupitant at concentrations of 0.3 and 1  $\mu$ M, aprepitant at the concentrations of 0.3 and 1  $\mu$ M or corresponding solvents before a second CRC to carbachol or KCl (for netupitant only) was constructed. Results were expressed as % of the maximal response obtained in the first CRC to the agonist.

### Results

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

Netupitant at 1 nM did not affect the second CRC to SP-OMe, however at 3 and 10 nM netupitant produced parallel rightward shifts in the CRC's to SP-OMe without affecting the maximal response (E<sub>max</sub>). At 30 nM, netupitant totally depressed the second CRC (Figure D) making it impossible to use a Schild plot to calculate the pA<sub>2</sub> value. The pK<sub>B</sub> 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. A mean pK<sub>B</sub> value of 10.03 was calculated based on estimated pK<sub>B</sub> values of 9.37 or 10.7 at 3 and 10 nM of aprepitant, respectively.

A slight but significant displacement of the CRC to carbachol was observed with 1  $\mu$ M netupitant. The pK<sub>B</sub> value was 5.58. Netupitant at 1  $\mu$ M also displaced to the right the CRC to KCl with a calculated pK<sub>B</sub> value of 5.38.

Aprepitant at 0.3  $\mu$ M did not antagonize the second CRC to carbachol since E<sub>max</sub> and pEC<sub>50</sub> values in the absence (6.09±0.04) and presence (6.04±0.04) of aprepitant were not significantly different. However, at 1  $\mu$ M, aprepitant slightly but significantly displaced the CRC to carbachol, with an estimated pK<sub>B</sub> value of 5.67.

Incubation of the strips with the solvents for netupitant and aprepitant did not affect CRC's to SP-OMe, carbachol or KCl.

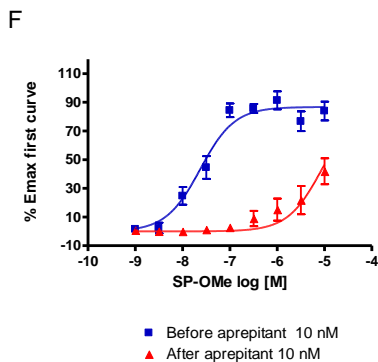
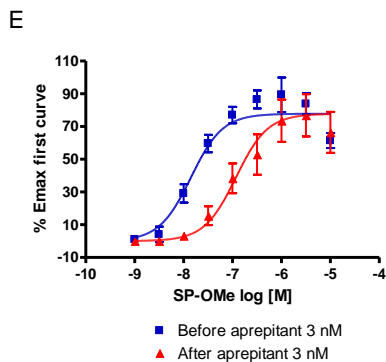
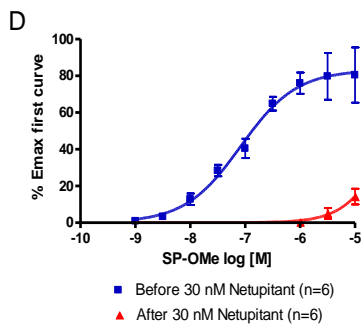
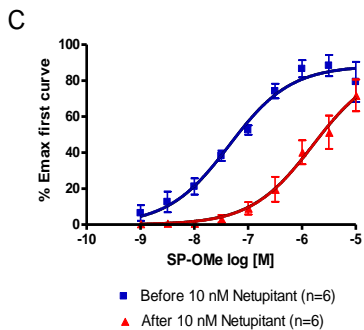
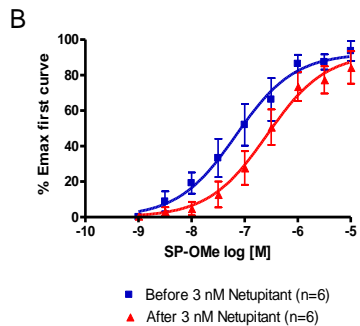
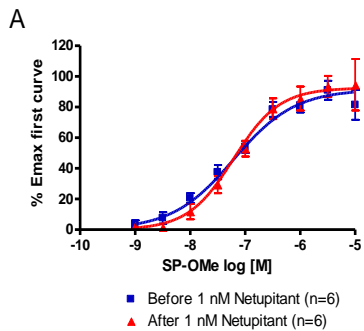
### Interpretation of results

These results demonstrate that netupitant and aprepitant are potent antagonists of contractions evoked by the activation of NK<sub>1</sub> receptors in the guinea-pig isolated urinary bladder. The potency of netupitant (mean pK<sub>B</sub> = 9.24) was very similar to its reported affinity for human NK<sub>1</sub> receptors in transfected cells (pK<sub>i</sub>=9.01) (2). The potency of aprepitant (mean pK<sub>B</sub>= 10.03) was also similar to its affinity for native NK<sub>1</sub> receptors in human brain (pK<sub>i</sub>=10.11) confirming that the pharmacology of the guinea-pig NK<sub>1</sub> receptor is very similar to that of the human receptor (3). At low concentrations both netupitant and aprepitant are competitive antagonists at guinea-pig NK<sub>1</sub> receptors based on the parallel rightward shifts in the CRC's to SP-OMe observed. However, at concentrations > 10 nM both antagonists clearly reduced E<sub>max</sub> values, suggesting a non-competitive antagonism. Netupitant and aprepitant did slightly antagonize contractile effects induced by carbachol but only at concentrations 1000 times greater than their pK<sub>B</sub> values at NK<sub>1</sub> receptors. We conclude that aprepitant and netupitant are potent and selective antagonists of NK<sub>1</sub> receptors in the guinea-pig urinary bladder.

### Concluding message

It was recently hypothesized that NK<sub>1</sub> receptor antagonists, by blocking neurotransmission through afferent fibres could be the drug of choice for treating overactive bladder. Netupitant is a new potent and selective NK<sub>1</sub> receptor antagonist undergoing clinical studies in overactive bladder patients. 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 treatment.

First and second CRCs to SP-OMe in guinea-pig isolated urinary bladders incubated with netupitant at A) 1 nM; B) 3 nM; C) 10 nM; D) 30 nM or aprepitant at E) 3 nM; F) 10 nM.



## References

1. Eur. J. Pharmacol. 222: 167-70, 1992
2. Bioorg. Med. Chem. Lett. 16: 1362-1365, 2005
3. Br. J. Pharmacol. 148: 39-45, 2006

**Specify source of funding or grant**

HELSIN SA

**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**

Comité d'éthique Midi Pyrénées pour l'expérimentation animale