IODO-RESINIFERATOXIN A NEW ULTRA-POTENT VR1 ANTAGONIST: PHARMACOLOGICAL PROFILE

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Introduction. Iodo-Resiniferatoxin (6,7-Deepoxy-6,7-didehydro-5-deoxy-21-dephenyl-21-(phenylmethyl)-daphnetoxin, 20-(4-hydroxy-5-iodo-3-methoxybenzeneacetate, I-RTX) is a derivative of the ultra-potent vanilloid receptor-1 (VR1) agonist resiniferatoxin. The iodine substitution has been shown to confer antagonist properties to the new compound that has been studied in the rat VR1 either native or expressed in heterologous systems or in the mouse in an *in vivo* nociceptive test (Wahl *et al.*, 2001. *Mol. Pharmacol.* **59**: 9-15). We have investigated the ability of I-RTX to block activation of VR1 in a range of animal models, including: the guinea pig, rat, and mouse.

Methods. *In vitro* experiments, guinea pigs and rats were killed by cervical dislocation and the urinary bladders were removed and suspended under a resting tension of 1g in Krebs solution (containing phosphoramidon and captopril, aerated at 95 % O_2 and 5% CO_2 at 37°C). The tissues were allowed to equilibrate for 60 mins and washed every 5 min. Cumulative concentration response curves were performed with capsaicin (0.1 nM – 100 µM) and SP (0.1 nM – 1 µM) either in the presence of the selective VR1 antagonists, I-RTX (0.1 – 10 nM) and capsazepine (0.1 – 10 µM) or their respective vehicles.

In vivo plasma extravasation experiments, mice were either pretreated with I-RTX (0.1 – 0.5 µmol/kg i.v.), capsazepine (0.5 – 10 µmol/kg, i.v.) or respective vehicles for 20 or 15 min prior to the injection of Evans Blue (i.v.), respectively. Five min after the injection of Evans Blue mice were transcardially perfused and the urinary bladders removed. The amount of Evans Blue was measured spectrophotometrically at 620 nM.

In vivo writhing test, mice were either pretreated with I-RTX (0.1 – 1 µmol/kg i.p.), capsazepine (1 – 10 µmol/kg, i.p.) or respective vehicles for 20 or 60 min, respectively, prior to the injected of acetic acid. The total number of writhing episodes were counted over the first 20 min.

Results. I-RTX produced a rightward shift of the contractile response to capsaicin in isolated guinea pig and rat urinary bladder with pK_B that were 10.68 $\pm$ 0.24 (n = 12) and 9.63 $\pm$ 0.30 (n = 12), respectively. In comparison, capsazepine showed a pK_B value of 6.56 $\pm$ 0.20 (n = 8) and 6.22 $\pm$ 0.40 (n = 10) in guinea pig and rat urinary bladder (Table 1), respectively. I-RTX, however, did not affect the contractile response produced by substance P (SP).

In *in vivo* studies, I-RTX (0.5 µmol/kg-1, i.p.) blocked capsaicin (1 mg/kg-1, i.v.)-induced plasma extravasation in the mouse urinary bladder (Table 1). Finally, in a nociceptive test in mice I-RTX (0.5 µmol/kg-1, i.p.) blocked citric acid-induced writhing, whereas capsazepine at the same dose was without effect (Table 1).

Conclusions. In both *in vitro* and *in vivo* experiments performed, so far, I-RTX has been proved to be up to ~3 logs more potent than capsazepine in inhibiting VR1 activated responses: this could have important implications for the design of future analgesic and anti-inflammatory drugs with VR1 blocking properties.

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Table 1

Affinities of capsazepine and I-RTX for the VR1 in *in vitro* and *in vivo* studies

	Capsazepine	I-RTX
***In Vitro:* Urinary Bladder**	**pK_B**	**pK_B**
Guinea Pig	6.56 ± 0.20	10.68 ± 0.24
Rat	6.22 ± 0.40	9.63 ± 0.30
***In Vivo:* Mouse**	**ED_{50} (µmol/kg)**	**ED_{50} (µmol/kg)**
Plasma Extravasation	9.03 ± 1.02	0.41 ± 0.08
Writhing (Acetic acid)	7.90 ± 1.13	0.42 ± 0.05