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SPECIAL REPORT

The nonpeptide neurotensin antagonist, SR 48692, used as a tool to reveal putative neurotensin receptor subtypes

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The nonpeptide neurotensin (NT) antagonist, SR 48692, was recently shown to inhibit NT binding to the cloned rat and human NT receptor and to antagonize NT effects in a variety of *in vitro* and *in vivo* assays. Here, we show that, in contrast to its antagonistic action on NT-induced hypomotility in the rat, SR 48692 failed to antagonize NT-induced hypothermia and analgesia in the mouse and rat. We suggest that these effects might be mediated through a subtype of SR 48692-insensitive NT receptor.

Keywords: Nonpeptide neurotensin antagonist; SR 48692; neurotensin receptor subtypes; hypothermia; analgesia

Introduction Expression cloning studies have identified one type of neurotensin (NT) receptor expressed in the central nervous system and peripheral tissues of mammals (Tanaka *et al.*, 1990; Vita *et al.*, 1993). Based on structure-activity studies with peptide and pseudopeptide agonist analogues of NT, it has been suggested recently that NT exerts its hypothermic and analgesic effects in the rat and the mouse through a central receptor subtype distinct from the cloned NT receptor (Al Rhodan *et al.*, 1991; Labbé-Jullié *et al.*, 1994). Distinction of receptor subtypes on pharmacological grounds can be established with selective antagonists. We recently described the biochemical and pharmacological properties of SR 48692, a potent nonpeptide antagonist of the cloned NT receptor (Gully *et al.*, 1993; Vita *et al.*, 1993). In the present study, we show that SR 48692 antagonizes the hypolocomotor effect induced by i.c.v. injection of NT in the rat. In contrast, the compound fails to inhibit the hypothermic and analgesic responses to i.c.v. injected NT in the mouse and in the rat.

Methods *Studies in mice* Tests were performed on male Swiss albino mice (CD1-Charles River, St-Aubin les Elbeuf, France). NT was dissolved in saline and SR 48692 in dimethyl sulphoxide (DMSO) and diluted in distilled water (final DMSO concentration, 5%). Corresponding vehicles were administered to controls. I.c.v. injections were performed according to Haley & McCormick (1957). SR 48692 was administered either i.p. 30 min before or i.c.v. simultaneously with the i.c.v. injection of NT (100 ng). Hypothermia and analgesia were evaluated 30 min after NT injection. Colonic temperature was measured with a thermistor probe (Ellab RM6, Copenhagen). Analgesic activity was evaluated by the tail flick test of D'Amour & Smith (1941) adapted for mice. Statistical analysis was conducted using two-way ANOVA.

Studies in rats Male OFA rats (130–160 g) from Iffa Credo (l'Arbresle, France) were used for NT-induced hypothermia, analgesia and hypomotility. I.c.v. injections (2 µl) of NT dissolved in sterile saline were made free-hand in the right lateral ventricle of conscious, non-restrained rats. SR 48692 suspended in 0.01% of Tween 80 in distilled water was administered orally (p.o.) at 5 ml kg^{-1}. Corresponding vehicles were administered to controls.

For hypothermia studies, the rectal temperature of all animals was measured with a thermocouple probe (Bailey Instrument) and animal groups with matched mean rectal temperature were constituted. Sixty minutes later, various doses of NT were injected i.c.v. The rectal temperature was measured twice just before and 30 min after NT injection. A dose of 400 ng NT was chosen for studies with SR 48692 which was administered p.o. at the indicated doses 60 min before NT injection.

For NT-induced antinociception against phenyl-*p*-benzoquinone-induced writhings (PBQ test), the animals were fasted 12 h before the test and isolated throughout the experiment. PBQ (20 mg kg^{-1}, 12.5 ml kg^{-1}) was given i.p. immediately after i.c.v. injections of increasing doses of NT. A dose of 100 ng NT was selected for antagonism studies with SR 48692 which was given p.o. at the indicated doses, 60 min before NT injection. Abdominal writhings were recorded visually between 5 and 30 min post PBQ.

For hypomotility experiments, animals were injected i.c.v. with vehicle or various doses of NT. A dose of 100 ng NT was selected for antagonism studies with SR 48692 which was given p.o. at the indicated doses 60 min before NT injection. Immediately after NT injection, the rats were placed in the test cages, motility being automatically recorded (digital image analysis system, Videotrack 512, View Point) during a 30 min period.

Statistical analysis was performed using ANOVA for motility studies and the Kruskall Wallis test for the other studies.

Drugs SR 48692, {2-[1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl) pyrazol-3-yl) carbonylamino] tricyclo (3.3.1.1.^{3,7}) decan-2-carboxylic acid}, was synthesized at Sanofi Recherche (Montpellier, France). NT was from Neosystem (Strasbourg, France) or Sigma (St Louis, MO, U.S.A.). Phenyl-*p*-benzoquinone (PBQ) from Sigma was solubilized with 5% ethanol in distilled water.

Results *Studies in mice* SR 48692 (0.1–2.5 mg kg^{-1}, i.p.) did not significantly affect the hypothermic and analgesic responses to i.c.v. injections of 100 ng NT (Table 1). Note also that SR 48692 alone has no intrinsic activity on body temperature and tail flick latency (Table 1). When mice were injected i.c.v. with a mixture of NT (100 ng) and SR 48692 (1 µg), their body temperature (32.7 ± 0.5°C) and tail flick latency (7.5 ± 0.5 s) as measured 30 min later were similar to

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Table 1 Effect of SR 48692 on the hypothermic and analgesic resposes to neurotensin (NT) in mice

Treatment	*Colonic temperature* (°C)	*Tail flick latency* (s)
Vehicle	37.0 ± 0.2	4.8 ± 0.4
NT 100 ng	33.0 ± 0.1*	7.8 ± 0.5*
SR 48692 0.1 mg kg^{-1}	37.0 ± 0.1	4.8 ± 0.6
SR 48692 0.3 mg kg^{-1}	36.7 ± 0.4	5.6 ± 0.5
SR 48692 2.5 mg kg^{-1}	37.2 ± 0.1	5.6 ± 0.6
SR 48692 0.1 mg kg^{-1} + NT 100 ng	33.5 ± 0.5*	7.8 ± 0.6*
SR 48692 0.3 mg kg^{-1} + NT 100 ng	33.2 ± 0.3*	7.2 ± 0.4*
SR 48692 2.5 mg kg^{-1} + NT 100 ng	32.7 ± 0.2*	8.6 ± 0.4*

SR 48692 was administered i.p. 30 min before i.c.v. injection of NT. Colonic temperature and tail flick latency were measured 30 min after NT injection. Values represent the mean ± s.e.mean from 6–15 mice per group. Two-way ANOVA indicates a lack of interaction between SR 48692 and NT, and a lack of intrinsic activity of SR 48692 in both tests.
*$P<0.001$ as compared to vehicle-injected animals.

those measured in animals injected with NT alone (32.9 ± 0.3°C; 7.7 ± 0.4 s) (means ± s.e.mean from 6 mice per group). It was also noted that SR 48692 (1 μg, i.c.v.) displayed no intrinsic effect on either parameter.

Studies in rats As shown in Figure 1a left, NT (400 ng, i.c.v.) induced a significant hypothermia in rats. SR 48692 (0.5, 1, 2, 4 mg kg^{-1}) did not significantly modify the hypothermia induced by 400 ng NT (Figure 1a, right). In the dose-range studied, SR 48692 by itself did not affect body temperature.

NT (100 ng, i.c.v.) significantly reduced the number of writhings induced by PBQ in rats (Figure 1b, left). SR 48692 at 0.5, 1, 2 and 4 mg kg^{-1} did not significantly affect the analgesia induced by 100 ng NT (Figure 1b, right).

I.c.v. administration of NT induced a dose-dependent and significant reduction of spontaneous locomotor activity in the rat (Figure 1c, left). SR 48692 (2 mg kg^{-1}) significantly ($P<0.05$) antagonized the hypomotility induced by 100 ng NT (Figure 1c, right).

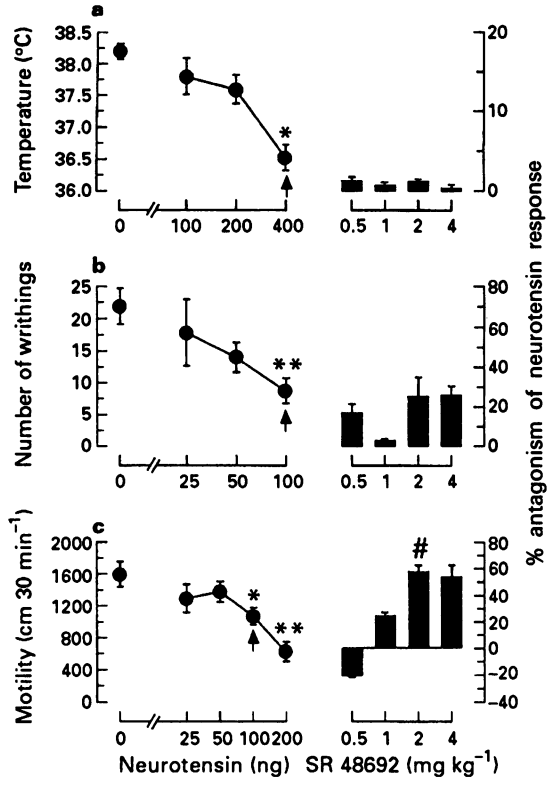

Figure 1 Effect of SR 48692 on neurotensin (NT)-induced hypothermia (a), analgesia (b) and hypomotility (c) in rats. Left panels present the NT dose-response relationships, right panels present the effects of SR 48692 against NT i.c.v. injected at the dose indicated by arrows in the corresponding left panels. In all studies, SR 48692 was administered p.o. 60 min prior to NT injection. Rectal temperature was measured 30 min after NT or vehicle injection. Antinociception (writhing test) was evaluated between 5 and 30 min post phenyl-*p*-benzoquinone (PBQ) which was given i.p. immediately after NT or vehicle injection. Motility was monitored during a 30 min period immediately following NT or vehicle injection. Values represent the mean ± s.e.mean from 10–30 rats per group. *$P<0.05$; **$P<0.01$ as compared to controls. #$P<0.05$ as compared to NT alone.

Discussion Previous studies have shown that SR 48692 inhibited the binding of NT to and antagonized NT responses mediated through the cloned rat and human NT receptors (Gully *et al.*, 1993; Vita *et al.*, 1993). The present data show that SR 48692 fails to antagonize the hypothermic and analgesic responses elicited by NT in the mouse and the rat. A lack of central bioavailability of i.p. or p.o. administered SR 48692 can be ruled out since both routes of administration of the antagonist led to a marked inhibition of the turning behaviour induced by intrastriatal injection of NT in the mouse (Gully *et al.*, 1993). Furthermore, SR 48692 given p.o. to rats significantly antagonizes the hypokinetic effect elicited by NT as reported here. Finally, we show that direct injection of SR 48692 in the central nervous sytem also fails to antagonize the hypothermic and analgesic effects of NT in the mouse. We therefore propose as a possible explanation for the present findings that NT-induced hypothermia and analgesia may be initiated through a receptor pharmacologically distinct from the cloned NT receptor. This conclusion is in full agreement with that recently proposed on the basis of structure-activity studies with NT agonists (Al-Rhodan *et al.*, 1991; Labbé-Jullié *et al.*, 1994). Further studies will be necessary to characterize the structure, biochemical properties and tissue distribution of the SR 48692-insensitive NT receptor.

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