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An isobolographic analysis of the effects of N-methyl-D-aspartate and NK_1 tachykinin receptor antagonists on inflammatory hyperalgesia in the rat

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1 The interaction between N-methyl-D-aspartate (NMDA) and NK_1 tachykinin receptors was analyzed isobolographically in rats with inflammatory hyperalgesia induced by intraplantar injection of complete Freund's adjuvant-saline emulsion (CFA, 100 μg *Mycobacterium* tuberculosis).

2 Thermal hyperalgesia of the inflamed paw, determined by paw withdrawal response to a heat stimulus, was dose-dependently attenuated by intrathecal administration of an NMDA receptor antagonist, dextrorphan (2.5–40 μg, $ED_{50}=7.2$ μg), and two NK_1 tachykinin receptor antagonists, WIN 51,708 (0.01–200 μg, $ED_{50}=10.4$ μg) or CP-96,345 (5–200 μg, $ED_{50}=82.1$ μg). There was no effect of these agents on the nociceptive threshold of the non-inflamed paw. CP-96,344, an enantiomer of CP-96,345 that is inactive as an NK_1 tachykinin receptor antagonist, slightly attenuated hyperalgesia at a dose of 200 μg.

3 Combinations of dextrorphan and WIN 51,708 were administered at fixed ratios (10%:90%; 41%:59%; 90%:10%). Isobolographic analysis revealed that the ED_{50}s obtained from the three combination ratios were not significantly different from those that were expected from a simple additive effect.

4 Thus, an additive interaction was demonstrated between NMDA and NK_1 tachykinin receptor systems at the spinal level. These results suggest that both NMDA and NK_1 tachykinin receptors are activated in response to peripheral inflammation, but that they may contribute independently to development of hyperalgesia.

Keywords: NMDA receptor antagonist; dextrorphan; tachykinin receptors; NK_1 tachykinin receptor antagonists; CP-96,345; WIN 51,708; isobologram; hyperalgesia

Introduction

Both N-methyl-D-aspartate (NMDA) and NK_1 tachykinin receptors are present in the rat spinal cord (Hershey & Krause, 1990; Henley *et al.*, 1993). There is coexistence of glutamate and substance P, the preferred endogenous ligand for the NK_1 tachykinin receptor, in dorsal root ganglion cell bodies and primary afferent terminals in the superficial dorsal horn (Battaglia & Rustioni, 1988; De Biasi & Rustioni, 1988). Spinal NMDA and NK_1 tachykinin receptors have been shown to be involved in dorsal horn hyperexcitability and behavioural hyperalgesia. The intrathecal (i.t.) administration of substance P or NMDA induces behavioural nociception or hyperalgesia in rats (Hylden & Wilcox, 1983; Aanonsen & Wilcox, 1987; Coderre & Melzack, 1991). Both spinal substance P and NMDA receptors are involved in the central sensitization of the nociceptive flexor reflex (Xu *et al.*, 1992). The administration of the NMDA receptor antagonists that act at agonist recognition or glycine modulatory sites, or bind to receptor coupled ion channels, inhibits dorsal horn nociceptive neuronal activity and selectively attenuates hyperalgesia (Haley *et al.*, 1990; Ren *et al.*, 1992a,b). In a rat model of inflammatory hyperalgesia, there is an increase in preprotachykinin mRNA (Iadarola & Draisci 1988; Minami *et al.*, 1989; Noguchi & Ruda, 1992) and an induction of NK_1 tachykinin receptor mRNA in dorsal horn neurones (Schäfer *et al.*, 1993; McCarson & Krause, 1994). The NK_1 tachykinin receptor antagonists, CP-96,345 (Snider *et al.*, 1991) and RP67580 (Garret *et al.*, 1991), inhibit responses of dorsal horn neurones to noxious stimuli (Radhakrishnan & Henry, 1991; Chapman & Dickenson, 1993; Neugebauer *et al.*, 1995). Intrathecal injection of anti-substance P antibody attenuates carrageenan-induced hyperalgesia (Satoh *et al.*, 1992). CP-96,345 appears to be antinociceptive in inflammatory pain models (Birch *et al.*, 1992; Nagahisa *et al.*, 1992b; Neugebauer *et al.*, 1995), although the specificity of this agent has been questioned (Nagahisa *et al.*, 1992a; Schmidt *et al.*, 1992).

Previous studies have suggested that there is an interaction between spinal NMDA and NK_1 tachykinin receptor systems during the processing of nociceptive information. Substance P modulates NMDA responses in rat dorsal horn neurones *in vitro* (Rusin *et al.*, 1993). (+)-HA966, a partial agonist at the glycine site of the NMDA receptor, enhances the antinociceptive effect of NK_1 tachykinin receptor antagonists (Seguin & Millan, 1994). A number of studies suggest that the nature of the NMDA-substance P receptor interaction is synergistic (Dougherty & Willis, 1991; Mjellem-Joly *et al.*, 1992). However, this interaction has not been analyzed in a rigorous fashion (see Tallarida, 1992; Caudle & Williams, 1993). Since either NMDA or substance P alone has an effect on nociceptive neurones and behavioural responses, an additive action of the two receptors cannot be ruled out unless a careful analysis of the dose-response relationship of their interaction has been determined. In the present study, an isobolographic analysis was performed to characterize further the interaction between NMDA and NK_1 tachykinin receptors in the rat model of inflammatory hyperalgesia. The effects of two selective non-peptide NK_1 tachykinin receptor antagonists, CP-96,345 and WIN 51,708 (Venepalli *et al.*, 1992) were first compared, and WIN 51,708 was subsequently chosen to be co-administered with dextrorphan, a non-competitive NMDA receptor antagonist acting at the phencyclidine-binding site of the receptor

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(Mendelsohn *et al.*, 1984; Church *et al.*, 1985). The results suggest an additive rather than a synergistic interaction between NMDA and NK$_1$ tachykinin receptors in this model.

Methods

Surgery

Adult male Sprague-Dawley rats (300–400 g) were prepared for intrathecal (i.t.) injections two to seven days before experimentation. The surgery was performed under methohexitone anaesthesia (50 mg kg^{-1} i.p.). The atlanto-occipital membrane was exposed, and a 7.0-cm length of PE-10 tubing was inserted into the subarachnoid space, through a slit made in the membrane and advanced to the level of the lumbar spinal cord (Yaksh & Rudy, 1976). The catheter was filled with saline (approximately 7 μl) and the outer end was plugged with a short length of stainless steel wire. Upon recovery from anaesthesia, animals were examined for gross signs of motor impairment resulting from spinal cord damage. Such animals were excluded from the study. Rats were killed by administration of CO_2 at the conclusion of the experiments.

Induction of inflammation

The animals were housed in cages in which the floor was covered with sawdust and food and water were available *ad libitum*. Complete Freund's adjuvant (CFA, *Mycobacterium* tuberculosis, Sigma) suspended in an oil : saline (1 : 1) emulsion was used as the inflammatory substance. Rats received s.c. injections of 0.2 ml (100 μg *Mycobacterium* tuberculosis) CFA into the plantar surface of one hindpaw. The CFA injection produced an intense inflammation associated with behavioural hyperalgesia, the characteristics of which have been described previously (Iadarola *et al.*, 1988; Hylden *et al.*, 1989). The injected hindpaw became maximally inflamed by 2–3 h after injection. Maximal thermal hyperalgesia occurred at 6–24 h after CFA administration and resolved over one to two weeks. After induction of inflammation, rats demonstrate normal eating and grooming behaviour and normal levels of exploration activity (Iadarola *et al.*, 1988; Hylden *et al.*, 1991), but tend to guard the inflamed paw. This animal model has been approved by the National Institute of Dental Research Animal Care and Use Committee. The ethical guidelines of The International Association for the Study of Pain were adhered to in these experiments (Zimmermann, 1983).

Nociceptive test

Twenty-four hours after CFA injection, rats were tested for behavioural hyperalgesia to a thermal stimulus. The rats were placed in a clear plastic chamber on a glass surface and allowed to acclimatize to their environment for 15–30 min before testing. The heat source consisted of a high intensity projector lamp bulb (Osram 58-8007, 8 V, 50 W) placed beneath the glass floor and projecting through a 5 × 10 mm aperture in the top of a movable case. The heat stimulus was directed onto the plantar surface of each hindpaw. A photoelectric cell aimed at the aperture detected light reflected from the paw and turned off the lamp and the electronic clock when paw movement interrupted the reflected light. The paw withdrawal latency to the nearest 0.1 s was determined. If an animal failed to withdraw its paw by 20 s, the trial was terminated by the investigator. A detailed description of this method has been published elsewhere (Hargreaves *et al.*, 1988). This testing method allows for side-to-side comparisons of drug effects on inflamed and non-inflamed paws within subjects.

Drugs

Dextrorphan (Hoffmann-La Roche, Nutley, NJ, USA), a non-competitive NMDA receptor antagonist; CP-96,345 ((2S,3S)-[*cis*-2-(diphenylmethyl)-N-[2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2.]octan-3-amine]) (Pfizer, Groton, CT. U.S.A.) and WIN 51,708 (17-β-hydroxy-17-α-ethynyl-5-α-androstanol[3,2-*b*]pyrimido[1,2-*a*]benzimidazole) (Sterling Winthrop, Rensselaer, NY, U.S.A.), both selective NK$_1$ tachykinin receptor antagonists, were used. CP-96,344 ((2**R**,3**R**)-[*cis*-2-(diphenylmethyl)-N-[2-methoxyphenyl)-methyl]- 1-azabicyclo[2.2.2.]-octan-3-amine]) (Pfizer, Groton, CT, U.S.A.), an inactive enantiomer of CP-96,345, was used for comparison. Dextrorphan, CP-96,345 and CP-96,344 were dissolved in normal saline. WIN 51,708 was dissolved in dimethyl sulphoxide (DMSO, Sigma) and diluted in saline (final DMSO concentration: 1–10%).

Intrathecal injection

All drug solutions for i.t. injection were prepared fresh on the day of the experiment. The drug to be injected was initially loaded into a PE-10 connecting tubing and separated from the saline solution in the remaining portion of the connecting tubing by an air bubble. The i.t. catheter was connected to the pre-filled connecting tubing. Drugs were injected in a volume of 5–10 μl, followed by a flush of 10 μl of normal saline. The travel of the air bubble in the tubing was carefully observed to ensure that the drug solution entered the intrathecal cannula. The details of this i.t. injection procedure have been described elsewhere (Hylden *et al.*, 1991). Vehicle solutions for the receptor antagonists were administered in the same manner.

Data analysis

Data are presented as means ± s.e.mean. Analysis of variance (ANOVA) was used for overall effects, with the Newman-Keuls test for *post-hoc* analysis for differences between means. $P<0.05$ was considered significant. The drug effect was determined by converting paw withdrawal latencies from thermal stimuli to % reversal of hyperalgesia according to the formula:

$$\%\ \text{reversal of hypersalgesia} = \frac{\text{postdrug} - \text{predrug}}{\text{baseline} - \text{predrug}} \times 100$$

Baseline paw withdrawal latency was measured immediately before CFA injection and averaged from 15–24 paw withdrawal latencies for one experiment; predrug paw withdrawal latency was measured 24 h after injection of CFA when behavioural hyperalgesia was apparent. The return of paw withdrawal latencies to the pre-CFA level was taken as the reversal of hyperalgesia. The ED_{50}, defined as the dose of a drug that produces a 50% reversal of hyperalgesia, was determined by computer-assisted, least-squares analysis of the graded dose-response curves.

Isobolographic analysis was performed according to Tallarida (1992). The potency of individual drugs was first determined. A theoretical simple additive line for a combination of two drugs was then generated. Two agents were mixed in fixed ratio based on weight and the mixture was administered in various doses. The potency and 95% confidence limit of the mixture was compared with the theoretically additive value. An overlap of the 95% confidence limits suggests a simple additive effect of the two agents in the mixture.

Results

Behavioural hyperalgesia

There was no significant difference in paw withdrawal latencies between left and right hindpaws before the injection of CFA. Twenty-four hours after the induction of inflammation, the injected paw was maximally inflamed and the paw withdrawal latency was reduced to 4.2 ± 0.1 s ($n=170$) as compared to 9.6 ± 0.1 s ($n=170$) for the pre-CFA value and 10.2 ± 0.2 s ($n=170$) for the non-injected paw ($P<0.01$). In a control ex-

periment ($n=6$), the magnitude of hyperalgesia did not change over the two-hour test period when needed in the following experiments to examine the effects of the intrathecal receptor antagonists. This animal model has been described in detail previously (Iadarola *et al.*, 1988; Hylden *et al.*, 1991).

Effects of the individual receptor antagonist

The effects of two NK_1 tachykinin receptor antagonists, CP-96,345 and WIN 51,708 were compared (Figure 1). Both agents produced attenuation of hyperalgesia that lasted for 30–60 min. WIN 51,708 produced a maximal 80±11% reversal of hyperalgesia at a dose of 100 μg (227 nmol). The effects of WIN 51,708 were dose-dependent ($F_{4,20}=2.993$, $P<0.05$). The i.t. injection of 200 μg (480 nmol) CP-96,345 resulted in 59±6% reversal of hyperalgesia. The effects of CP-96,345 also were dose-dependent ($F_{5,29}=3.711$, $P<0.05$). Further increases in the dose of CP-96,345 to 400 μg produced agitation during the injection and apparent motor dysfunction, making it difficult to judge the effects of the drug. WIN 51,708 appeared to be 8 fold more potent than CP-96,345 in reducing hyperalgesia (Table 1).

The **2R,3R** enantiomer of CP-96,345, CP-96,344 (Snider *et al.*, 1991), did not have a comparable effect as CP-96,345 on inflammatory hyperalgesia (Figure 1). The i.t. administration of 0.4–40 μg (0.8–80 nmol) of CP-96,344 was inactive and at 200 μg (400 nmol) only 31±12% reversal of hyperalgesia was produced. Further higher dose of CP-96,344 (400 μg) produced motor dysfunction in rats. Rats turned to lie on their back and the paw withdrawal test could not be conducted reliably.

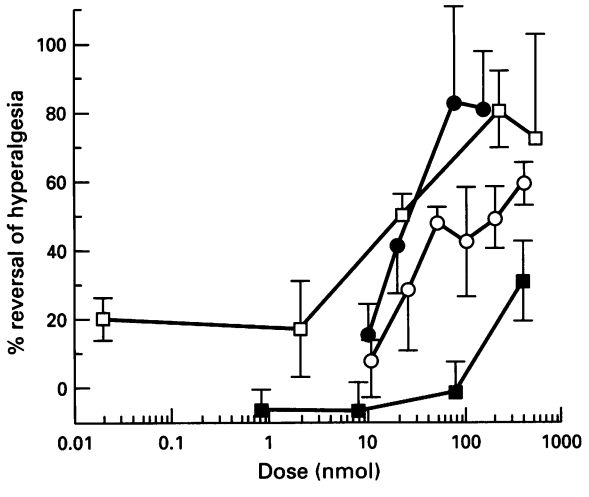

Figure 1 Anti-hyperalgesic effects of the intrathecal N-methyl-D-aspartate (NMDA) receptor antagonist dextrorphan (●) and NK_1 tachykinin receptor antagonists WIN 51,708 (□) and CP-96,345 (○). The effects of an inactive enantiomer of CP-96,345, CP-96,344 (■) are also shown. The effects of the drugs are expressed as % reversal of hyperalgesia. The doses in nmol are plotted on the abscissa scale using a log scale. Each point represents the mean of 5–8 animals. The vertical lines represent 1 s.e.mean.

Table 1 ED_{50} values of N-methyl-D-aspartate (NMDA) and NK_1 tachykinin receptor antagonists for reversal of hyperalgesia in inflamed paws

	ED_{50}		
Drugs	μg	nmol	n
Dextrorphan	7.2 (3.08–23.3)	28.4 (12.2–92.2)	23
WIN 51,708	10.4 (8.3–12.9)	23.4 (19.0–29.3)	25
CP-96,345	82.1 (39.8–411.4)	197.0 (95.5–986.6)	35

Values shown are means with 95% confidence limits in parentheses.

The NMDA receptor antagonist dextrorphan produced a maximal 82±28% reversal of hyperalgesia at a dose of 20 μg (79 nmol) (Figure 1). A further increase of the dose to 40 μg did not attain greater effect. The effects of dextrorphan were dose-dependent ($F_{3,19}=3.888$, $P<0.05$). Dextrorphan appeared to have the same potency as WIN 51,708 but to be more potent than CP-96,345 (Table 1).

Neither the NK_1 tachykinin nor the NMDA receptor antagonists had effects on the paw withdrawal latency of the non-inflamed paw (not shown). The injection of drug vehicles did not affect the hyperalgesia. The CFA-induced oedema did not appear to be affected by the i.t. injection of a single dose of WIN 51,708 or CP-96,345.

Combined administration of receptor antagonists

It was apparent from the above single drug experiments that CP-96,345 was much less potent and efficacious than WIN 51,708 in attenuating hyperalgesia in this animal model. Thus, WIN 51,708 was selected for the following combination experiment. A combination of dextrorphan and WIN 51,708 was injected i.t. to identify the nature of the interaction between the two agents. Since the peak effects of WIN 51,708 and dextrorphan appeared at approximately 10 and 30 min after i.t. injection, respectively, the injection of WIN 51,708 was preceded by dextrorphan for 20 min to ensure that the peak effects of the two drugs coincided. The two drugs were combined at fixed ratios according to weight. Since the pharmacological interaction of two agents may depend on the ratio of the combination (Adams *et al.*, 1993), two other ratios of the combinations of dextrorphan and WIN 51,708 were also tested. The dose-response effect was established for each ratio (Figure 2) and ED_{50} s calculated (Table 2).

The data for the combinations of WIN 51,708 and dextrorphan are presented in Table 2 and illustrated by an isobologram in Figure 3. For the combination ratio at 41% : 59% (dextrorphan: WIN 51,708), the observed and predicted ED_{50} values clearly overlap. Although the observed ED_{50} s for the drug mixtures at ratios of 10% : 90% and 90% : 10% (dextrorphan: WIN 51,708) were off the theoretical additive line, their 95% confidence limits overlapped with those of the predicted ED_{50} values (Figure 3 and Table 2).

Discussion

The attenuation of inflammatory hyperalgesia by NK_1 tachykinin receptor antagonists suggests the involvement of NK_1 tachykinin receptors in this model. Similar to NMDA receptor antagonists acting at different sites of the NMDA receptor (Ren *et al.*, 1992b), only the hyperalgesic response of the inflamed paw, but not normal nociceptive response of the non-inflamed paw, was inhibited by NK_1 tachykinin receptor antagonists. Birch *et al.* (1992) and Yamamoto *et al.* (1993a) also showed that CP-96,345 blocked carrageenan-induced mechanical and thermal hyperalgesia but had no effect on the nociceptive thresholds of the non-inflamed paw. In electrophysiological experiments, NK_1 tachykinin receptor antagonists selectively blocked dorsal horn nociceptive neuronal responses to sustained, but not short C-fibre intensity stimuli (De Koninck & Henry, 1991; Chapman & Dickenson, 1993). Thus, NK_1 tachykinin receptors may be activated in response to prolonged nociceptive input. Several studies have shown that NK_1 tachykinin receptor antagonists are selective in reducing the second, but not the first, phase of the formalin response (Yamamoto & Yaksh, 1991; Birch *et al.*, 1992; Yamamoto *et al.*, 1993b; Yashpal *et al.*, 1993). CP-96,345 also inhibited acetic acid-induced writhing, a response related to chemical-induced visceral nociception (Nagahisa *et al.*, 1992b). On the other hand, NK_1 tachykinin receptor antagonists did not have an effect on the nociceptive threshold of normal rats (Garces *et al.*, 1992; Xu *et al.*, 1992).

It was found in the present study that WIN 51,708 was

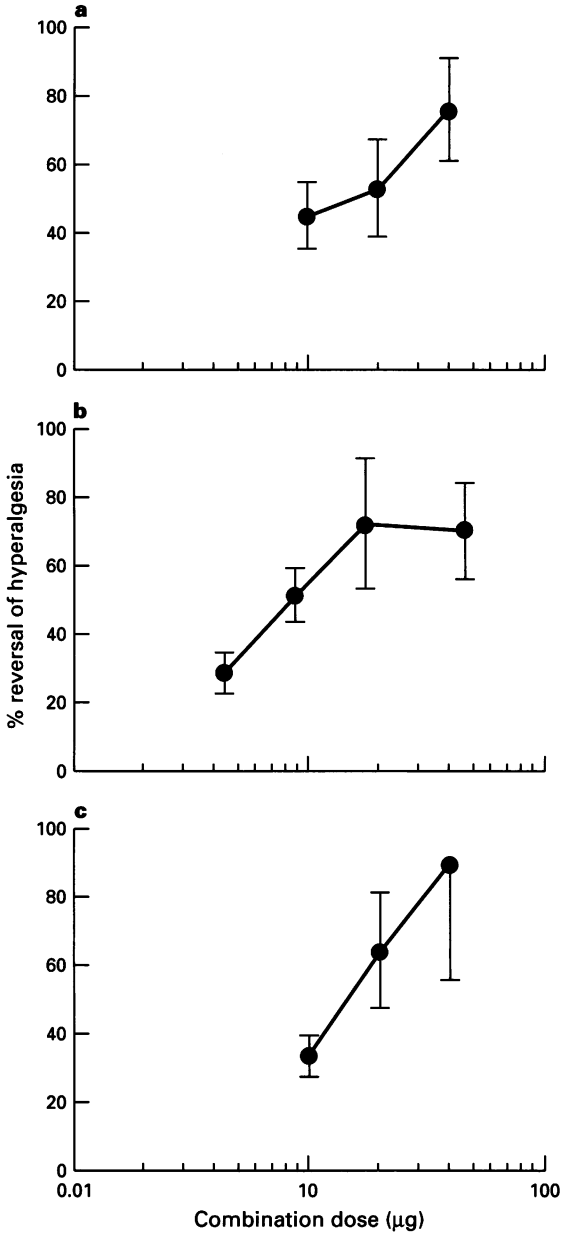

Figure 2 Attenuation of CFA-induced hyperalgesia by the combination of N-methyl-D-aspartate (NMDA) receptor antagonist, dextrorphan, and an NK_1 tachykinin receptor antagonist, WIN 51,708. Each curve was generated by intrathecal administration of a fixed ratio combination of the two drugs expressed in terms of total weight of the two drugs combined. The % compositions of dextrorphan and WIN 51,708 were: (a) 10%:90%; (b) 41%:59% and (c) 90%:10%. Each point represents the mean of 5–8 animals. The vertical lines represent 1 s.e.mean.

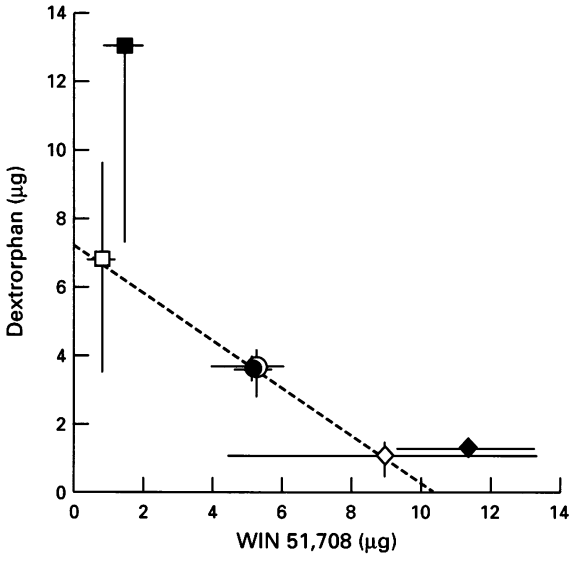

Figure 3 Isobologram for ED_{50} of dextrorphan and WIN 51,708 combinations in attenuating CFA-induced hyperalgesia. The dashed line represents the theoretical additive interaction levels. The intercepts of the dashed line on the ordinates and abscissae are the observed ED_{50} values for dextrorphan and WIN 51,708 alone, respectively. Each data point represents an ED_{50} value for a given combination (dextrorphan:WIN 51,708): 10%:90% (◆, ◇), 41%:59% (●, ○), and 90%:10% (■, □) and WIN 51,708 (abscissa scale) components. The 95% confidence limits for dextrorphan and WIN 51,708 are also resolved into the dextrorphan (ordinate scale) and WIN 51,708 (abscissa scale) components and shown by vertical and horizontal bars, respectively. The predicted and observed ED_{50} values are shown by open and solid symbols, respectively.

about 8 fold more potent than CP-96,345 in attenuating inflammatory hyperalgesia in rats. This is consistent with results from radioligand binding studies. WIN 51,708 and related compounds have been shown to be more potent in tissues from rats than from man or guinea pigs (Appell *et al.*, 1992). On the other hand, CP-96,345 is more potent in human or guinea-pig tissues than in rats or mice (Snider *et al.*, 1991; Beresford *et al.*, 1991). When CP-96,345 was given 2 h after carrageenan injection, it failed to attenuate hyperalgesia (Yamamoto *et al.*, 1993a). This may be explained by the low potency of this compound in rats. Two hours after the induction of inflammation, behavioural hyperalgesia begins to reach its peak magnitude, and dorsal horn hyperexcitability continues to develop (Iadarola *et al.*, 1988; Hylden *et al.*, 1989). Apparently, CP-96,345 was not able to reverse this intense phase of inflammation and hyperalgesia. In the present study, 24 h after the injection of CFA, the maximal reversal of hyperalgesia by CP-96,345 was only 59%. Recent studies suggest that in addition to blockade of NK_1 tachykinin receptors, part of the effects of some NK_1 tachykinin receptor antagonists may be attributable to their non-specific action on neurotransmission (Wang *et al.*, 1994) or their interaction with Ca^{2+} channels (Schmidt *et al.*, 1992; Guard *et al.*, 1993; Rupniak *et al.*, 1993). It is not clear at this time if WIN 51,708 also possesses such non-specific activity. The results from the injection of CP-96,344, an enantiomer of CP-96,345, suggest that the blockade

Table 2 Observed and predicted additive ED_{50} values of the mixture of dextrorphan and WIN 51,708 for reversal of hyperalgesia in inflamed paws

Fixed-ratio by weight (Dextrorphan: WIN 51,708)	*ED_{50} (μg[a])* Observed	Predicted	n
10%:90%	12.6 (10.2–14.7)	9.9 (4.9–14.8)	16
41%:59%	8.6 (7.8–9.4)	8.8 (6.5–10.0)	24
90%:10%	14.5 (8.1–19.7)	7.4 (3.9–10.7)	16

Values shown are means with 95% confidence limits in parentheses.
[a]Drug doses are expressed as the total weight of the drug combination.

of calcium channels may not play a significant role in the attenuation of behavioural hyperalgesia by CP-96,345 (also see Radhakrishnan & Henry, 1995; Neugebauer *et al.*, 1995), since CP-96,344 also blocks calcium channels but is inactive at NK_1 tachykinin receptors (Snider *et al.*, 1991).

Dextrorphan is a non-competitive NMDA receptor antagonist (Mendelsohn *et al.*, 1984; Church *et al.*, 1985) which binds to ion channels associated with the NMDA receptor, prevents ion fluxes, and blocks the activation of the NMDA receptor. It was shown recently that dextrorphan alleviates neuropathic hyperalgesia in rats (Mao *et al.*, 1993; Tal & Bennett, 1993). The release of excitatory amino acids during spinal cord ischaemia is inhibited by dextrorphan (Rokkas *et al.*, 1994). Dextromethorphan, the parent compound of dextrorphan, can selectively reduce temporal summation of second pain in humans (Price *et al.*, 1994). Like other NMDA receptor antagonists (Ren *et al.*, 1992b), dextrorphan selectively attenuated thermal hyperalgesia in unilaterally inflamed rats in the present study.

The results of isobolographic analysis suggest that the non-competitive NMDA receptor antagonist, dextrorphan, and the NK_1 tachykinin receptor antagonist, WIN 51,708, act in an additive manner to attenuate inflammatory hyperalgesia. In all three ratios tested, the potency of the combinations was not significantly different from that of the theoretical simple additive action. The implication of these results is that the NMDA and NK_1 tachykinin receptor systems may be co-activated in response to peripheral inflammation, but that they contribute independently to the development of hyperalgesia. Aanonsen & Wilcox (1986) have shown that NMDA and substance P induce nociceptive-like behaviour in mice through distinct receptors. The combination of NMDA and substance P does not produce a supra-additive interaction (Aanonsen & Wilcox, 1986). This is consistent with the finding that capsaicin, an agent that depletes substance P, does not affect glutamate release in the rat spinal cord (Singer *et al.*, 1982). However, the present results are in contrast to those of Mjellem-Joly *et al.* (1991, 1992), which suggest a supra-additive interaction between NMDA and NK_1 tachykinin receptors in evoking nociceptive behaviour in the mouse. It should be remembered that dextrorphan only binds to the phencyclidine site of the NMDA receptor. Since there are multiple binding sites on the NMDA receptor, it remains to be determined if the NMDA receptor antagonists acting at the agonist recognition or glycine modulatory site of the NMDA receptor could reveal a synergistic interaction between NMDA and NK_1 tachykinin receptor systems.

Substance P often enhances NMDA-induced responses of spinal dorsal horn neurones (Dougherty & Willis, 1991; Rusin *et al.*, 1993). However, the nature of the interaction has not been analysed in detail. Although a single combination of the two agonists or antagonists of NMDA and NK_1 tachykinin receptors can produce an apparent greater effect than that produced by individual agents (Xu *et al.*, 1992; Song & Zhao, 1994), it does not necessarily mean the two agents act supra-additively, especially when each agent can produce its own separate effect (see also Ossipov *et al.*, 1990; Adams *et al.*, 1993). To demonstrate a supra- or sub-additive drug interaction, an analysis of the dose-response curves is required (Tallarida, 1992; Caudle & Williams, 1993). The conclusion of the present study does not contradict the concept that corelease of substance P and glutamate from primary sensory neurones may potentiate behavioural hyperalgesia. It simply points out that although the nociceptive response is enhanced, the two receptor systems may act independently.

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