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Effect of tachykinin receptor antagonists in experimental neuropathic pain

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Abstract

The intrathecal effect of 0.1 to 10 μg of RP-67,580 (3*aR*,7*aR*)-7,7-diphenyl-2[1-imino-2(2-methoxyphenyl)-ethyl]perhydroisoindol-4-onehydro chloride, CP-96,345 (2S,3S)-*cis*-(2(diphenylmethyl)-*N*-[(2-methoxyphenyl) methyl]-1-azabicyclo[2.2.2]octan-3-amine), SR-140,333 (*S*)-(1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azonia-bicyclo[2.2.2.]-octane,chloride), all neurokinin $(NK)_1$-receptor antagonists, SR-48,968 (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-[phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide, a tachykinin NK_2 receptor antagonist and SR-142,801 (*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methyl acetamide, a tachykinin NK_3 receptor antagonist, and of their respective inactive enantiomers on thresholds of vocalization due to a mechanical stimulus in mononeuropathic (sciatic nerve ligature) and diabetic rats, was examined. The tachykinin NK_1 and the NK_2 receptor antagonists were antinociceptive in both models, with a higher effect of the former in diabetic rats. The tachykinin NK_3 receptor antagonist was weakly effective in diabetic rats only. This indicates a differential involvement of the tachykinins according to the model of neuropathic pain, suggesting a potential role for tachykinin receptor antagonists in the treatment of neuropathic pain.

Keywords: Tachykinin receptor antagonist; Neuropathic pain; Intrathecal injection

1. Introduction

Patients suffering from neuropathic disorders frequently complain of pain. Classical reference pain treatments such as tricyclic antidepressants are not always sufficiently effective or well tolerated (Onghena and Van Houdenhove, 1993; McQuay et al., 1996) and consequently improved treatments are required. Development of new therapies requires a fuller understanding of the pathophysiological mechanisms of neuropathic pain.

Tachykinins, substance P, neurokinin A and neurokinin B are important in sensory processing in the dorsal horn of the spinal cord (Helke et al., 1990) where they activate tachykinin NK_1, NK_2 and NK_3 receptors, respectively (Helke et al., 1990; Nakanishi, 1991). Behavioural experiments using chemical (Nagahisa et al., 1992; Yamamoto and Yaksh, 1992; Sakurada et al., 1995; Holzer-Petsch and Rordorf-Nikolic, 1995) as well as thermal stimuli (Santucci et al., 1993) clearly demonstrate the pronociceptive role of tachykinin NK_1 and NK_2 receptor agonists. In addition, electrophysiological recordings of neuronal activity indicate the involvement of substance P and neurokinin A in the responses of spinothalamic tract neurons (Dougherty et al., 1994) and provide evidence for a role of these tachykinins in mediating nociceptive inputs in the dorsal horn (Fleetwood-Walker et al., 1990; Munro et al., 1993). Molecular biology has also revealed that chemical activation of nociceptors upregulates the expression of tachykinin NK_1 and NK_3 receptor mRNA in the dorsal root ganglia (McCarson and Krause, 1994). However, the lack of effect of tachykinin NK_1 receptor antagonists in acute pain models suggests that at least substance P may only be involved in conditions of persistent pain (Henry, 1993).

To date, little has been published concerning the role of tachykinin in nociceptive processing in chronic pain syndromes. Donaldson et al. (1992) have reported that monoarthritis increases the expression of preprotachykinin mRNA in the dorsal root ganglia of the rat. Using a model of neuropathic pain, Marchand et al. (1994) have shown

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that tachykinin expression is initially increased and later decreased in dorsal root ganglia neurons. RP-67,580, a non-peptide tachykinin NK_1 receptor antagonist, has been shown to possess antinociceptive properties in a model of chronic painful diabetes, suggesting the involvement of substance P in the hyperalgesia resulting from this metabolic dysfunction (Courteix et al., 1993a). The recent development of some non-peptide tachykinin receptor antagonists and the availability of animal models of chronic pain have provided new research tools to investigate the role of tachykinins in persistent nociception, particularly when this is due to neurogenic changes.

The present experiments were undertaken to compare the spinal antinociceptive properties of tachykinin NK_1 and NK_2 receptor antagonists in two models of neurogenic pain, sciatic nerve ligature (Bennett and Xie, 1988) and streptozocin-induced diabetes (Courteix et al., 1993b), and in normal rats. These two models of neurogenic pain were chosen to determine whether there were differences in tachykinin involvement according to the etiology of the neuropathy (compressive or metabolic), an observation which could help to discriminate between neuropathic pain syndromes. To clarify the role played by neurokinin B at the NK_3 receptor site, the effect of a non-peptide tachykinin NK_3 receptor antagonist was also tested.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, France), initially weighing 200–250 g, were housed four or five per cage under standard laboratory conditions and allowed food and water ad libitum.

As some suffering might result from these experiments, the I.A.S.P. Committee for Research and Ethical Issues Guidelines (Zimmermann, 1983) were followed. Great care was taken, particularly with regard to housing conditions, to avoid or minimize discomfort to the animals.

2.2. Induction of mononeuropathy

The rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and four chronic gut (5-0) ligatures were tied loosely (with about 1 mm spacing) around the common sciatic nerve, according to the method described by Bennett and Xie (1988). The nerve was constricted to a barely discernible degree, so that circulation through the epineural vasculature was not interrupted.

2.3. Induction of diabetes

Animals were intraperitoneally injected with streptozocin (75 mg/kg) (Zanosar®, Upjohn, France) dissolved in distilled water. Diabetes was confirmed 1 week later by measurement of tail vein blood glucose levels with Ames Dextrostix and a reflectance colorimeter (Ames Division, Miles laboratories, France). Blood samples were obtained from the tail by pin prick and only animals with a final blood glucose level > 14 mM were considered diabetic. Weight-matched control rats (normal rats) received only distilled water.

2.4. Experimental protocol

The rats underwent the paw pressure test, previously described by Randall and Selitto (1957). Nociceptive thresholds expressed in grams were measured using a Ugo Basile analgesimeter (Apelex, probe tip diameter 1 mm) by applying increasing pressure to the right and the left hind paw for mononeuropathic rats and only to the left hind paw for diabetic and normal rats, until vocalization was elicited. Once two stable vocalization thresholds were obtained, drugs or vehicle was injected. The vocalization thresholds were then determined every 15 min over a 120-min period.

Tests took place 2 weeks (mononeuropathic rats) or 3 weeks (diabetic rats) after induction of the disease. Only rats in which the pain scores were reduced at these times by more than 15% of the value obtained prior to the induction of the disease were included. The rats were randomly allocated to cages, each rat receiving either a drug (antagonists or their enantiomers) or a vehicle of the same volume (10 μl). Injections were given intrathecally in the subarachnoid space between (L_5–L_6) according to the method described by Mestre et al. (1994). All the experiments were performed blind, using the method of equal blocks ($n = 8$ rats for each treatment). Three doses (0.1, 1, 10 μg) of antagonists were chosen, based on literature data and on the affinity of the compounds for the different receptors. The lowest dose of 0.1 μg/rat corresponded to 16 to 24 nmol/rat according to the drug used. The concentrations of the solutions injected ranged from 16 to 24 μM, and were mostly higher than the K_i values of the various antagonists for their respective receptors (from 0.027 to 52 nM (Emonds-Alt et al., 1993; Garret et al., 1993)). Depending on the drug, enantiomers were tested systematically at the same doses or only at the highest dose, 10 μg. These experiments were performed to assess the potential involvement of a non-specific effect especially the anti-calcium channel activity of some NK_1 receptor antagonists, ($n = 8$ for each compound).

2.5. Drugs

The following antagonists and their enantiomers were used (generous gifts from companies).

2.5.1. Tachykinin NK_1 receptor antagonists

RP-67,580(3*aR*,7*aR*)-7,7-diphenyl-2[1-imino-2 (2-methoxyphenyl)-ethyl] perhydroisoindol-4-one hydrochlo-

ride (molecular weight: 438.57) and its enantiomer, RP-68,651 (Rhône-Poulenc Rorer, France), were dissolved in an aqueous solution (1%) of methane sulfonic acid. RP-67,580 has greater affinity for NK_1 subtype receptors in the rat and mouse than in the guinea-pig and man, and possesses anti-calcium channel properties (Rupniak et al., 1993).

CP-96,345 (2*S*,3*S*)-*cis*-(2(diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine) (molecular weight: 412) and its enantiomer, CP-96,344 (Pfizer, France), were dissolved in distilled water. CP-96,345 has a 100 to 500-fold lower affinity for NK_1 receptors in rats than in humans and guinea-pigs and displays anti-calcium channel activity (Schmidt et al., 1992; Delay-Goyet et al., 1992).

SR-140,333 (*S*)-(1-{2-[3-(3,4-dichlorophenyl)-1-(3 isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azonia-bicyclo[2.2.2.]octane, chloride) (molecular weight: 620) and its enantiomer, SR-140,603 (Sanofi, France), were dissolved in the organic solvent, dimethylsulfoxide (DMSO). SR-140,333 is a potent and selective NK_1 receptor antagonist with the same affinity for rodent and human NK_1 receptors, with no other activity identified (Emonds-Alt et al., 1993).

2.5.2. Tachykinin NK_2 receptor antagonist

SR-48,968 (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-[phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide (molecular weight: 552) and its enantiomer, SR-48,965 (Sanofi), were dissolved in distilled water. SR-48,968 is an effective, competitive and highly selective non-peptide antagonist of the NK_2 receptor (Emonds-Alt et al., 1992; Maggi et al., 1993).

2.5.3. Tachykinin NK_3 receptor antagonist

SR-142,801 (*S*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide (molecular weight: 606) and its enantiomer, SR-142,806 (Sanofi), were dissolved in the organic solvent, DMSO. SR-142,801 is markedly species-depen-

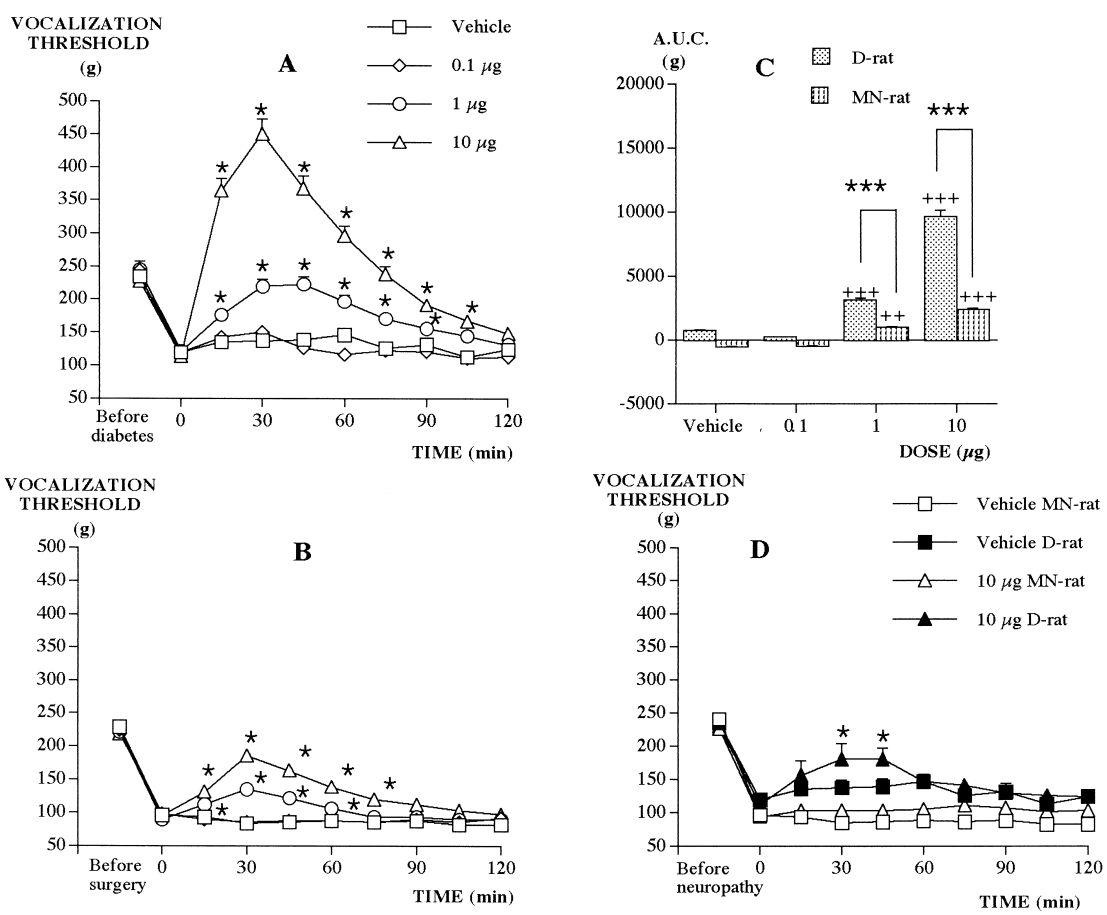

Fig. 1. Time course of the effect of i.t. RP-67,580 on vocalization threshold in diabetic (A) and mononeuropathic (B) rats. Vocalization thresholds determined before (0) and after drug injection are expressed as grams. Bars = + S.E.M. $*\ P < 0.05$ vs. predrug values. (C) Effect of RP-67,580 on pain thresholds in diabetic and mononeuropathic rats. Results expressed as area under the vocalization threshold–time curve (A.U.C.) obtained by joining the mean values of the vocalization thresholds (grams) obtained between 0 and 120 min. Bars = + S.E.M. +: comparisons vs. scores obtained after vehicle. ($+P < 0.05$; $++P < 0.01$; $+++P < 0.001$). *: comparisons vs. scores obtained in diabetic (D-rat) or mononeuropathic (MN-rat) rats. ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$). (D) Time course of the effect of i.t. RP-68,651 (10 μg) in diabetic (filled symbols) and mononeuropathic (open symbols) rats on vocalization thresholds. Results are expressed as grams. Bars = + S.E.M. $*\ P < 0.05$ vs. predrug values.

dent and has low affinity for rat tachykinin NK_3 receptors and a much higher affinity for human and guinea-pig receptors (Emonds-Alt et al., 1995; Patacchini et al., 1995).

2.6. Expression of results and statistical analysis

Results were expressed as means ± S.E.M. of raw data (vocalization thresholds expressed in grams). The mean area under the vocalization threshold–time curve (post-drug–predrug values) was calculated with the trapezoidal rule, using the program Siphar/Win (1-2b, SIMED, Créteil, France). The maximal increase in vocalization threshold was calculated for each animal and drug as follows: maximal post drug value − predrug value/predrug value. Statistical comparisons were made by Two-way analysis of variance (ANOVA) followed by either Dunnett's test for studying the time course of the effect of various treatments or Student's *t*-test for unpaired series. In all cases, the significance level was 0.05.

Student's *t*-test was applied to compare A.U.C.s between diabetic and mononeuropathic rats ($P < 0.05$).

3. Results

Diabetes significantly reduced the vocalization thresholds 3 weeks after streptozocin injection (mean values: 247 ± 15 g before induction vs. 141 ± 10 g after induction). Two weeks after nerve ligation, the vocalization thresholds (mean values: 257 ± 11 g before surgery) were significantly lower (mean values: 107 ± 6 g).

3.1. Studies in normal rats

The tachykinin NK_1, NK_2 and NK_3 receptor antagonists and their enantiomers, all tested at a dose of 10 μg, did not significantly change the vocalization thresholds in normal rats (data not shown).

3.2. Studies in chronic pain models

No behavioural effects were observed after injection of any drug. None of the tachykinin NK_1, NK_2, NK_3 receptor antagonists induced changes in vocalization thresholds

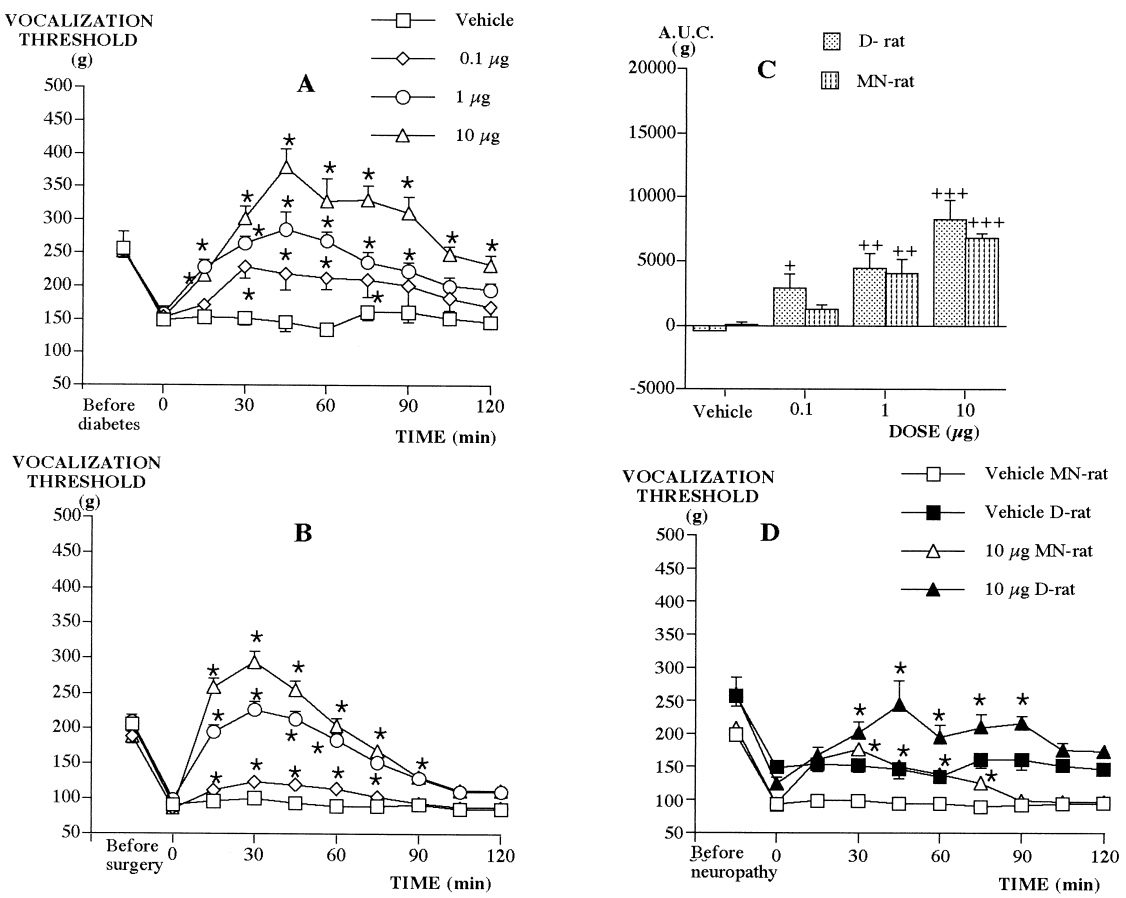

Fig. 2. Time course of the effect of i.t. CP-96,345 on vocalization threshold in diabetic (A) and mononeuropathic (B) rats. Vocalization thresholds determined before (0) and after drug injection are expressed as grams. Bars = + S.E.M. * $P < 0.05$ vs. predrug values. (C) Effect of CP-96,345 on pain thresholds in diabetic and mononeuropathic rats. Results expressed as area under the vocalization threshold–time curve (A.U.C.) obtained by joining the mean values of the vocalization thresholds (grams) obtained between 0 and 120 min. Bars = + S.E.M. +: comparisons vs. scores obtained after vehicle. (+ $P < 0.05$; + + $P < 0.01$; + + + $P < 0.001$). (D) Time course of the effect of i.t. CP-96,344 (10 μg) in diabetic (filled symbols) and mononeuropathic (open symbols) rats on vocalization thresholds. Results are expressed as grams. Bars = + S.E.M. * $P < 0.05$ vs. predrug values.

of the non-ligatured paw in mononeuropathic rats. The following data for these animals therefore refer only to the ligated paw.

3.2.1. Effect of tachykinin NK_1 receptor antagonists

3.2.1.1. RP-67,580 (Fig. 1). At the lowest dose (0.1 μg), RP-67,580 did not affect the vocalization threshold in diabetic and mononeuropathic rats. The doses of 1 and 10 μg induced antinociception in both diabetic and mononeuropathic rats (Fig. 1A and B, respectively). The maximum increase induced by RP-67,580 (10 μg) was $+337 \pm 13$ g at 30 min in diabetic rats and $+90 \pm 8$ g at 30 min in mononeuropathic rats; these effects were significantly different. As shown in Fig. 1C, the global effect of RP-67,580 (at 1 μg and 10 μg) as assessed by A.U.C.s was significantly higher in diabetic than in mononeuropathic rats ($P < 0.001$).

The enantiomer of RP-67,580, RP-68,651, inactive after 0.1 and 1 μg/rat, induced significant changes in diabetic rats (maximal increase: $+69 \pm 16$ g at 30 min) at 10 μg (Fig. 1D). The corresponding A.U.C. (2248 ± 796 g × min) was significantly smaller ($P < 0.05$) than that obtained after 10 μg of the active isomer (9647 ± 2476 g × min) in the same model (data not shown). The enantiomer was inactive in the chronic constriction injury model.

3.2.1.2. CP-96,345 (Fig. 2). CP-96,345 significantly and dose dependently raised the vocalization thresholds in both diabetic and mononeuropathic rats (Fig. 2A and B, respectively). The maximal increase at the dose of 10 μg did not differ significantly between diabetic ($+227 \pm 27$ g) and mononeuropathic rats ($+208 \pm 10$ g). However, at this dose, the antinociceptive effect was longer-lasted in diabetic (greater than 120 min) than in mononeuropathic (90 min) rats. The mean A.U.C. for the change in vocalization threshold with time for the dose of 10 μg was not significantly different between in two models (Fig. 2C).

The enantiomer of CP-96,345, CP-96,344, produced a significant antinociceptive effect in diabetic and in mononeuropathic rats ($+120 \pm 36$ g and $+84 \pm 7$ g, re-

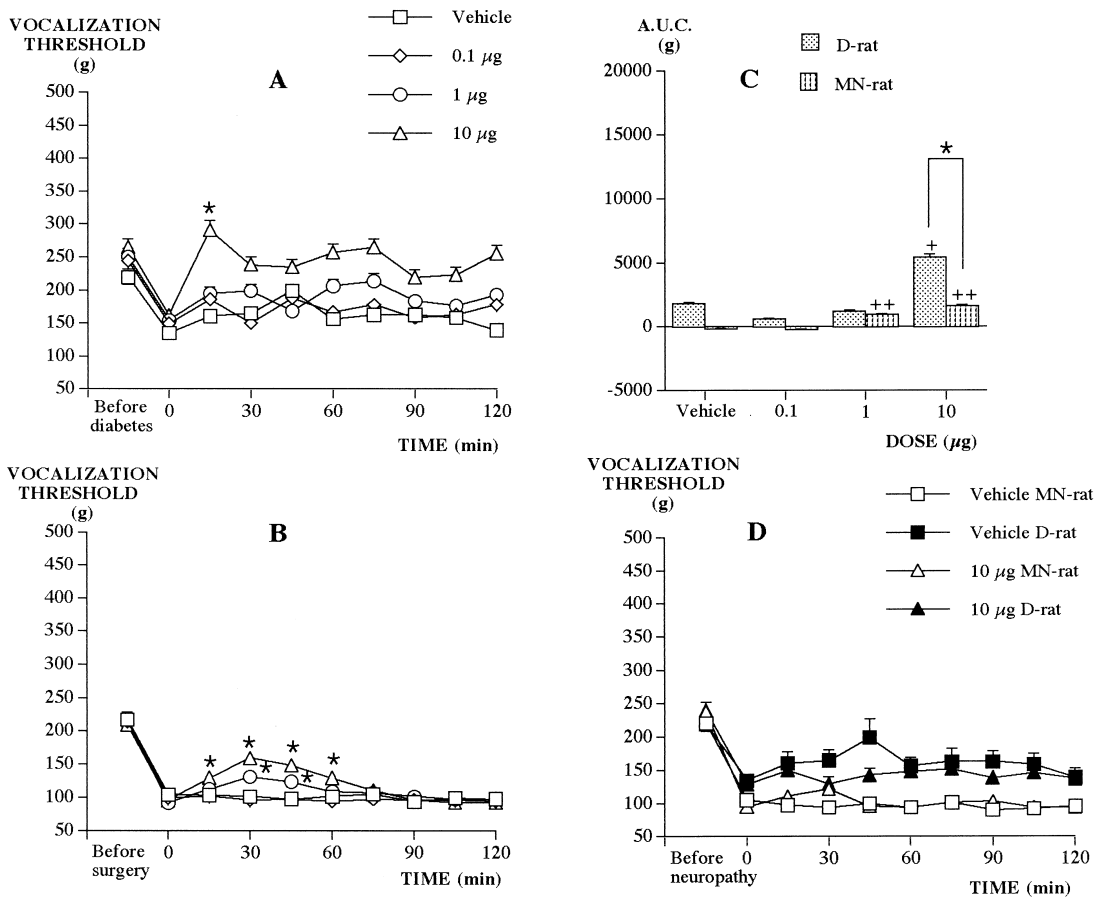

Fig. 3. Time course of the effect of i.t. SR-140,333 on vocalization threshold in diabetic (A) and mononeuropathic (B) rats. Vocalization thresholds determined before (0) and after drug injection are expressed as grams. Bars = +S.E.M. * $P < 0.05$ vs. predrug values. (C) Effect of SR-140,333 on pain thresholds in diabetic and mononeuropathic rats. Results expressed as area under the vocalization threshold–time curve (A.U.C.) obtained by joining the mean values of the vocalization thresholds (grams) obtained between 0 and 120 min. Bars = +S.E.M. +: comparisons vs. scores obtained after vehicle. (+ $P < 0.05$; ++ $P < 0.01$). *: comparisons vs. scores obtained in diabetic (D-rat) or mononeuropathic (MN-rat) rats (* $P < 0.05$). (D) Time course of the effect of i.t. SR-140,603 (10 μg) in diabetic (filled symbols) and mononeuropathic (open symbols) rats on vocalization thresholds. Results are expressed as grams. Bars = +S.E.M.

spectively) at the highest dose of 10 μg (Fig. 2D). No effect was observed at doses of 0.1 and 1 μg (data not shown). Comparing the A.U.C.s, these effects were significantly ($P < 0.05$) lower than those induced by the active enantiomer at the same dose for both diabetic and mononeuropathic rats (data not shown).

3.2.1.3. SR-140,333 (Fig. 3). In diabetic rats, only the dose of 10 μg of SR-140,333 produced a significant elevation in the nociceptive threshold, with a maximal increase of $+128 \pm 35$ g (Fig. 3A). In mononeuropathic rats, significant antinociception was obtained for the doses of 1 and 10 μg (Fig. 3B) but the maximal increase for the highest dose was significantly less ($+64 \pm 4$ g), than that obtained in diabetic rats ($P < 0.05$). Expressed as A.U.C.s (Fig. 3C), for the 10-μg dose, significantly higher scores were obtained with diabetic than with mononeuropathic rats.

The enantiomer of SR-140,333, SR-140,603 (10 μg/rat) was inactive in both models (Fig. 3D).

3.2.2. Effect of a tachykinin NK_2 receptor antagonist (Fig. 4)

SR-48,968 induced a dose-dependent increase of vocalization thresholds with significant changes at the doses of 1 and 10 μg in both models (Fig. 4A,B). The 0.1-μg dose had no effect. It was significantly more effective in diabetic rats. The maximum increase for the dose of 10 μg was significantly greater in diabetic ($+265 \pm 48$ g) than in mononeuropathic rats ($+92 \pm 5$ g). The comparison of A.U.C.s (Fig. 4C) confirmed the significantly lower effect of all the doses of SR-48,968 in mononeuropathic than in diabetic rats.

Its enantiomer, SR-48,965 was inactive at 0.1 and 1 μg in the two models but induced a small, short-lasted, increase in vocalization threshold in diabetic ($+58 \pm 6$ g at 45 min) and mononeuropathic rats ($+35 \pm 4$ g at 30 min) after 10 μg (Fig. 4D). The A.U.C. was significantly lower than that obtained with 10 μg of the active enantiomer (data not shown).

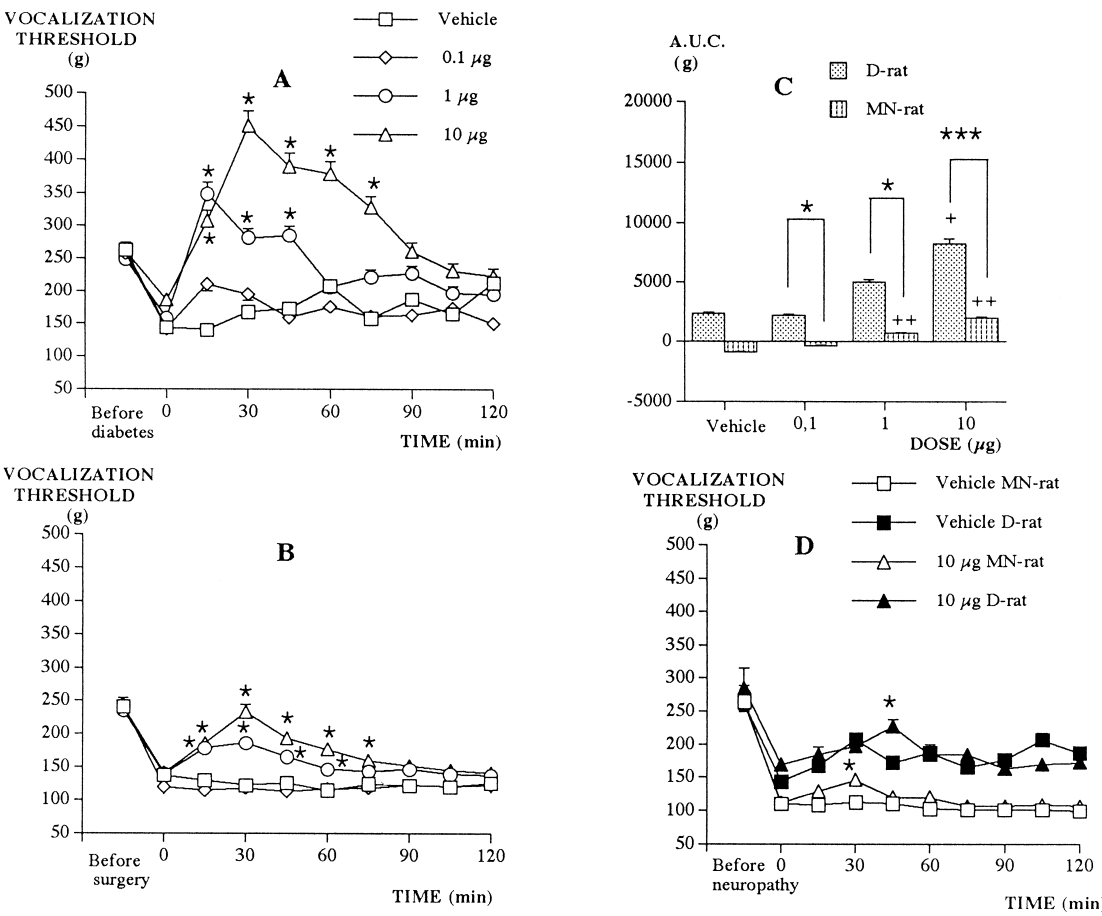

Fig. 4. Time course of the effect of i.t. SR-48,968 on vocalization threshold in diabetic (A) and mononeuropathic (B) rats. Vocalization thresholds determined before (0) and after drug injection are expressed as grams. Bars = +S.E.M. $*$ $P < 0.05$ vs. predrug values. (C) Effect of SR-48,968 on pain thresholds in diabetic and mononeuropathic rats. Results expressed as area under the vocalization threshold–time curve (A.U.C.) obtained by joining the mean values of the vocalization thresholds (grams) obtained between 0 and 120 min. Bars = +S.E.M. +: comparisons vs. scores obtained after vehicle ($+P < 0.05$; $++P < 0.01$). $*$: comparisons vs. scores obtained in diabetic (D-rat) or mononeuropathic (MN-rat) rats. ($*P < 0.05$; $***P < 0.001$). (D) Time course of the effect of i.t. SR-48,965 (10 μg) in diabetic (filled symbols) and mononeuropathic (open symbols) rats on vocalization thresholds. Results are expressed as grams. Bars = +S.E.M. $*$ $P < 0.05$ vs. predrug values.

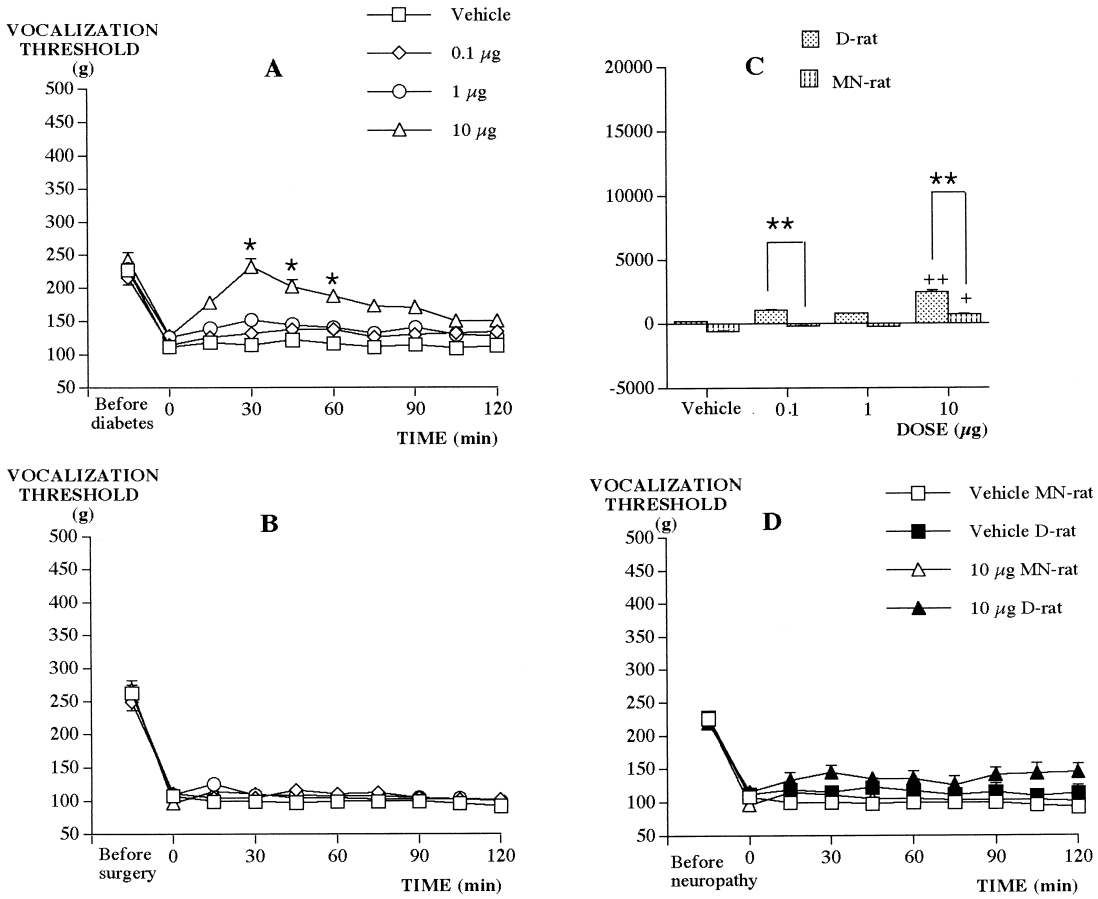

Fig. 5. Time course of the effect of i.t. SR-142,801 on vocalization threshold in diabetic (A) and mononeuropathic (B) rats. Vocalization thresholds determined before (0) and after drug injection are expressed as grams. Bars = +S.E.M. $* P < 0.05$ vs. predrug values. (C) Effect of SR-142,801 on pain thresholds in diabetic and mononeuropathic rats. Results expressed as area under the vocalization threshold–time curve (A.U.C.) obtained by joining the mean values of the vocalization thresholds (grams) obtained between 0 and 120 min. Bars = +S.E.M. +: comparisons vs. scores obtained after vehicle. ($+P < 0.05$; $++P < 0.01$). *: comparisons vs. scores obtained in diabetic (D-rat) or mononeuropathic (MN-rat) rats. ($**P < 0.01$). (D) Time course of the effect of i.t. SR-142,806 (10 μg) in diabetic (filled symbols) and mononeuropathic (open symbols) rats on vocalization thresholds. Results are expressed as grams. Bars = +S.E.M.

3.2.3. Effect of a tachykinin NK_3 receptor antagonist (Fig. 5)

In mononeuropathic rats, SR-142,801 was inactive at the three doses tested (Fig. 5B) but it induced a significant antinociceptive effect in diabetic rats at the highest dose of 10 μg, beginning at the 30th min (maximal increase: $+105 \pm 27$ g at 30 min) and lasting up to 60 min (Fig. 5A). The A.U.C.s (Fig. 5C) confirmed differences in the effect of SR-142,801 between diabetic and mononeuropathic rats at 0.1 and 10 μg.

The enantiomer of SR-142,801, SR-142,806 (10 μg) was ineffective in both models (Fig. 5D).

4. Discussion

The results obtained show an antinociceptive effect of the tachykinin NK_1 receptor antagonist, RP-67,580, in mononeuropathic rats and confirm its activity in diabetic rats, as previously reported by Courteix et al. (1993b). Two other tachykinin NK_1 receptor antagonists, CP-96,345 and SR-140,333, were also antinociceptive in diabetic and mononeuropathic rats (Snider et al., 1991; McLean et al., 1991; Rouissi et al., 1991; Emonds-Alt et al., 1993). All these compounds were shown to be ineffective in healthy rats. These findings confirm several previous reports showing a lack of effect of tachykinin NK_1 receptor antagonists under acute pain conditions in normal rats (Fleetwood-Walker et al., 1990, 1993; Laird et al., 1993; Thompson et al., 1993; Fleetwood-Walker, 1995). This supports the recently widely accepted view that the role of substance P differs from that of nociceptive neurotransmitters (Henry, 1993). During the development of neuropathic hyperalgesia, dorsal root ganglia and dorsal horn substance P levels decrease (Munglani et al., 1995), and increases in NK_1 receptor number have been observed (Aanonsen et al., 1992). No data are available for diabetic rats, concerning substance P and preprotachykinin mRNA in dorsal root ganglia. Some electrophysiological studies have, however, shown that the K^+-evoked release of substance P from

spinal cord slices from diabetic rats is higher than that from samples from healthy rats (Kamei et al., 1990). These authors also suggested that supersensitivity to substance P develops in the spinal cord of diabetic rats and that the basal level of substance P is decreased in the spinal cord of diabetic rats (Kamei et al., 1990).

RP-67,580 and CP-96,345 induced greater increases in the pain threshold than did SR-140,333 in both mononeuropathic and diabetic rats. However, their enantiomers, RP-68,651 and CP-96,344, induced a significant effect in diabetic only or in both diabetic and mononeuropathic rats, respectively. The enantiomer of SR-140,333 was ineffective. RP-67,580 and CP-96,345 are known to block calcium (Schmidt et al., 1992; Rupniak et al., 1993) and sodium (Nagahisa et al., 1992) channels, and calcium channel blockers have been shown to increase nociceptive thresholds in inflammatory or neuropathic pain models (Miranda et al., 1992; Rupniak et al., 1993). This may account partially for the effect of active compounds, RP-67,580 and CP-96,345, for the antinociception observed with the enantiomer, RP-68,651, in diabetic rats and CP-96,344 in both models. Nagahisa et al. (1992) have also shown that, for carrageenan-induced hyperalgesia in the rat, the putative 'inactive' enantiomer, CP-96,344, appears to be as active as CP-96,345.

In diabetic rats, if the maximal increases obtained with the active compounds, RP-67,580 and CP-96,345 ($+337 \pm 13$ g and $+227 \pm 27$ g, respectively), are corrected for the response observed for the respective enantiomers, RP-68,651 and CP-96,344 ($+69 \pm 16$ g and $+120 \pm 36$ g, respectively), the maximal increases are markedly reduced to approximately $+268$ g ($+237\%$) for RP-67,580 and $+107$ g ($+67\%$) for CP-96,345.

Under the same experimental conditions, SR-140,333 raises the pain threshold ($+128 \pm 35$ g) corresponding to a total suppression of diabetes-induced hyperalgesia. Since SR-140,333 is devoid of the calcium channel blocking effect of the NK_1 receptor (Emonds-Alt et al., 1993) and since its 'inactive' enantiomer is ineffective, the antinociceptive effect observed is specific and corresponds to a block of the NK_1 receptor action and consequently indicates that substance P is involved in diabetes-induced pain. However, SR-140,333 seems weakly effective whereas it has been shown to be highly effective against noxious thermal stimulation in healthy rats (Jung et al., 1994). These discrepancies may be due to the nature of the nociceptive stimulation (mechanical vs. thermal) and to the response studied (reflex in the tail flick test vs. the highly integrated response of vocalization in the paw pressure test).

Finally, the efficacy of these compounds (after correction for the effects of the enantiomers), assessed both as the maximal increase in vocalization threshold and as A.U.C.s, is different and may be ranked as: RP-67,580 > SR-140,333 > CP-96,345. The reduced activity of CP-96,345 could be related to its reduced ability to inhibit ^{3}H-substance P binding to rat NK_1 receptors ($K_i = 52$ nM) (Emonds-Alt et al., 1993).

In mononeuropathic rats, the enantiomers, RP-68,651 and SR-140,603, failed to exert any specific effect, whereas CP-96,344 enhanced pain thresholds. This suggests that some of the effect of CP-96,345 is non-specific. The corrected maximal increase in the vocalization threshold was about 124 g rather than 208 ± 10 g if the enantiomer effect is not taken into account. However, in this model this compound was more effective than the other thachykinin receptor antagonists investigated. Nevertheless, in contrast to CP-96,345, 0.1 and 1 μg of CP-96,344 were ineffective in the two models, confirming the specific effect mediated by CP-96,345.

Whatever the differences found between these various drugs, the global effects (A.U.C.s) of RP-67,580, SR-140,333 were systematically and significantly higher in diabetic than in mononeuropathic animals. The greater activity of these tachykinin NK_1 receptor antagonists in diabetic rats could indicate a greater role for substance P in this metabolic pain model. This would suggest that the use of different animal models of neuropathic pain could help to identify differences in the pathophysiology of neuropathic pain syndromes according to their etiology. Further study may lead to different therapeutic approaches.

Investigation of the effect of SR-48,968, which binds selectively to NK_2 receptors (Emonds-Alt et al., 1992; Maggi et al., 1993; Yashpal et al., 1996) and acts as an antagonist, yields further interesting results. In normal rats this drug has no antinociceptive effect. This result is consistent with that reported by Santucci et al. (1993). Using the same drug produced no alteration in the response to nociceptive mechanical stimulation and, in keeping with the reduced efficacy of this non-peptide tachykinin NK_2 receptor antagonist, did not suppress mechanically evoked responses when applied to dorsal horn neurons of normal rats (Fleetwood-Walker et al., 1990). Conversely SR-48,968 induced a dose-dependent antinociceptive effect in neuropathic pain models. The enantiomer, SR-48,965, was slightly effective in both diabetic and mononeuropathic rats at the highest dose. Thus, taking into account the effect of SR-48,965, the maximal increase in vocalization threshold due to the active drug, SR-48,968 (10 μg), was limited to approximately 207 g in diabetic rats and 57 g in mononeuropathic rats. This result suggests a greater efficacy in diabetic than in mononeuropathic rats. Similarly, the A.U.C.s were significantly greater for diabetic than for mononeuropathic rats. These results suggest the involvement of neurokinin A in neuropathic pain models with a more marked involvement of this neuromediator in diabetes-induced hyperalgesia.

The pathophysiological role of NK_3 receptors and neurokinin B can be investigated by studying the effect of a recently described selective and potent antagonist of the NK_3 receptor, SR-142,801 (Emonds-Alt et al., 1995). However, the weak ability of this compound to inhibit the

binding of [^{125}I] iodohistidyl-[MePhe^7] neurokinin B to tachykinin NK_3 receptors from rats ($K_i = 15 \pm 3$ nM) compared with those from humans ($K_i = 0.21 \pm 0.03$ nM) could limit its usefulness in studies with this species. In the present work, SR-142,801 was only effective in diabetic rats; the highest dose reversed hyperalgesia without increasing vocalization thresholds in comparison with predrug values. This result contrasts with reports that stimulation of NK_3 receptors with a specific agonist such as senktide, induces antinociception in healthy rats (Helke et al., 1990; Altier and Stewart, 1997), suggesting a possible involvement of neurokinin B as a pronociceptive mediator, only under conditions of metabolic neuropathic pain.

To conclude, we have now shown that the use of tachykinin antagonists reveals a neuropathic pain model-related pharmacological heterogeneity, expressed by their different analgesic activities in the diabetic and mononeuropathic rats. Thus, the present results offer evidence that substance P ergic and neurokinin A ergic systems, which are not involved after an acute noxious stimulus, are stimulated during neuropathic pain, whether of metabolic or of compressive etiology. Consequently, tachykinin NK_1 and NK_2 receptor antagonists may be useful in the therapy of neuropathic pain syndromes, and especially in diabetic neuropathic pain. The neurokinin B ergic system seems to be involved only in diabetes-induced hyperalgesia. The greater sensitivity of the diabetic model demonstrated in the present behavioural study, must be confirmed by further neurochemical investigations to confirm whether the different pharmacological effects of tachykinin antagonists observed could be linked to pathophysiological differences between these two models of pain.

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