ELSEVIER

European Journal of Pharmacology 308 (1996) 41–48

ejp

SR 48968 specifically depresses neurokinin A- vs. substance P-induced hyperalgesia in a nociceptive withdrawal reflex

Kiran Yashpal [a,*], Christina W.Y. Hui-Chan [b], James L. Henry [a]

[a] *Departments of Psychiatry and Physiology, McGill University, 3655 Drummond Street, Montreal, Quebec H3G 1Y6, Canada*
[b] *School of Physical and Occupational Therapy, McGill University, Montreal, Quebec H3G 1Y6, Canada*

Received 7 December 1995; revised 28 March 1996; accepted 2 April 1996

Abstract

To determine the role of neurokinin A and tachykinin NK_2 receptors in processing of nociceptive information at the spinal level, the selective NK_2 receptor antagonist, SR 48968 (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-[phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide, was tested for its effects on the hyperalgesia produced in the tail flick reflex by intrathecal administration of neurokinin A and of substance P. SR 48968 was also tested in a model in which noxious peripheral stimulation has been shown to produce hyperalgesia via a substance P mechanism. SR 48968 given intrathecally had a dose-dependent inhibitory effect on both the behaviour and the hyperalgesia induced by neurokinin A but not on either of these effects produced by substance P. In addition, systemic administration of SR 48968 depressed the hyperalgesic effect of intrathecal administration of neurokinin A. First, this evidence indicates a unique role for neurokinin A in the spinal cord as distinct from that of its homologue, substance P, and confirms that neurokinin A acts via the tachykinin NK_2 receptor, rather than non-specifically via the NK_1 receptor. Second, the data indicate that in this model substance P does not express any of its effects non-selectively via activation of NK_2 receptors. Third, SR 48968 appears to have access to the spinal cord upon systemic administration. Fourth, intrathecal administration of the NK_1 receptor antagonist, CP-96,345 [(2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxy-phenyl)-methyl]-1-azabicyclo[2.2.2]-octan-3-amine], had no effect on the responses to intrathecal administration of neurokinin A. Finally, the hyperalgesia produced by sustained noxious thermal stimulation of the tip of the tail was unaffected by intrathecal administration of SR 48968; thus, it remains to find a physiological response in which endogenous neurokinin A and NK_2 receptors at the spinal level are involved in the rat in vivo.

Keywords: Substance P; Neurokinin A; Tachykinin NK_1 receptor; Nociception; Analgesia; Hyperalgesia

1. Introduction

There is plentiful physiological, anatomical, immunocytochemical and molecular information implicating the tachykinin, substance P, and the tachykinin NK_1 receptor, for which substance P is the preferential ligand, in somatosensory mechanisms in the spinal cord. Relatively less is known about the involvement of neurokinin A and the NK_2 receptor, for which neurokinin A is the preferential ligand. As both peptides derive from the preprotachykinin-I gene and from β and γ preprotachykinin mRNA (Nawa et al., 1983; Krause et al., 1987), are colocalized in the same primary afferents (Hua et al., 1985) and are released simultaneously from spinal tissue (Saria et al., 1986), one might expect a function of neurokinin A in somatosensory physiology somewhat similar to that attributed to substance P. However, neurokinin A has been reported to excite a population of non-nociceptive dorsal horn neurones which are not excited by substance P (Salter and Henry, 1991), suggesting an involvement of NK_2 receptors in pathways which do not involve NK_1 receptors. Furthermore, when given intrathecally in the tail flick test, neurokinin A has less hyperalgesic effect than does substance P (Cridland and Henry, 1986), suggesting either that NK_2 receptors are less abundant in nociceptive pathways or that effects of neurokinin A on nociception are expressed via NK_1 receptors. In view of the possibility that neurokinin A expresses at least some of its effects via non-specific activation of NK_1 receptors (Geraghty et al., 1992; Nagahisa et al., 1992; Hosoki et al., 1994; Guo et al., 1993), it was considered important to reassess the type of receptors activated by neurokinin A using the selective

* Corresponding author. Department of Physiology, McGill University, 3655 Drummond Street, Montreal, Quebec H3G 1Y6, Canada. Tel.: (1) (514) 398-6003; fax: (1) (514) 398-7452; e-mail: mc70@musica.mcgill.ca

PII S0014-2999(96)00262-2

non-peptide NK_2 receptor antagonist, SR 48968.

Thus, an attempt was made to determine the role of NK_2 receptors in processing of nociceptive information at the spinal level. The objective was to resolve the apparent contradiction between the expected similarity of effects of neurokinin A and substance P, as argued above, and data indicating a different role of NK_2 vs. NK_1 receptors in nociceptive processing at the spinal level. Thus, the present study was done to test the effect of intrathecal injection of SR 48968 on the hyperalgesia in the tail flick reflex induced by intrathecal administration of neurokinin A and of substance P.

In addition, as only one study (Poncelet et al., 1993), has information regarding access of SR 48968 to the central nervous system (CNS) from the periphery, experiments were done to determine whether i.p. administration of this antagonist alters the response to intrathecal administration of neurokinin A.

Finally, we designed a model in which noxious peripheral stimulation provokes thermal hyperalgesic effects in the tail flick reflex similar to those of substance P and neurokinin A (Cridland and Henry, 1988). As this hyperalgesia is depressed by peptide and non-peptide NK_1 receptor antagonists (Cridland and Henry, 1988; Yashpal et al., 1993), implying endogenous release of a ligand, the possible participation of NK_2 receptors in this response was also investigated.

2. Materials and methods

Three types of experiment were run. In one, the effect of intrathecal administration of SR 48968 (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-[phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide, CP-96,345 [(2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxy-phenyl)-methyl]-1-azabicyclo[2.2.2]-octan-3-amine], an NK_1 receptor antagonist, or of artificial cerebrospinal fluid was tested on the thermal hyperalgesia in the tail flick reflex provoked by intrathecal administration of neurokinin A or of substance P. In a second group of experiments, the effect of SR 48968 given systemically was tested on the thermal hyperalgesia induced by intrathecal administration of neurokinin A. In the third group, the effects of SR 48968 on the thermal hyperalgesia produced by immersion of the tip of the tail for 90 s in water at 55°C were tested. All experiments monitored effects on the tail withdrawal reflex.

2.1. Animal preparation

Male Sprague Dawley rats (250–300 g) were used. In all cases, the guidelines described in The Care and Use of Experimental Animals, of the Canadian Council on Animal Care, Volumes I and II, were strictly followed. In addition, the experimental protocols were reviewed and approved by the McGill University Animal Care Committee.

Each rat was implanted with an indwelling intrathecal catheter (Intramedic PE-10) under chloral hydrate anaesthesia (300 mg/kg i.p.). This catheter was inserted through an incision in the dura at the atlanto-occipital junction and was positioned so that the inner tip lay at the lower lumbar vertebral level. Spinous processes were used as landmarks for this positioning (Yashpal et al., 1985). The outer end of the catheter was fixed with dental cement to a screw embedded in the skull. The rats were allowed to recover for 4–6 days after implantation of the catheter and only those animals which were free of any neurological deficit were used in the experiments. In addition, the viability of the intrathecal catheter was checked before experimentation by injecting 20 μl of lidocaine (a 1% aqueous solution); only those rats showing a temporally reversible sensory deficit and motor paralysis were used in the experiments. The exact location of the inner tip of the catheter was verified routinely during post-mortem examination. Results were included only if the tip of the catheter was confirmed to lie at the lower lumbar or sacral level.

2.2. Tail flick test

The awake rat was placed in a plastic restrainer covered with a black cloth and a projector bulb was focused on the tail 4 cm from the tip, at a site which had been blackened to promote the absorption of heat (Yashpal et al., 1982). A flick of the tail exposed the light beam to a photodetector which in turn stopped a timer giving reaction time measured to 0.01 s (Isabel et al., 1981). Reaction time was measured at 5 min intervals. The intensity of the stimulus was set to give baseline reaction times of 6–10 s. Rats were used only if the reaction time of 3 successive tail flick tests had a standard deviation less than 10% of the mean. This normally occurs with the first three or four trials.

2.3. Intrathecal experiments

Once tail flick latency had stabilized, SR-48968 or CP-96,345 was given intrathecally at a dose of 6.5 or 65 nmol in 10 μl. After three more readings, neurokinin A or substance P was given intrathecally in a single dose of 6.5 nmol (as determined by peptide content) in 10 μl of artificial cerebrospinal fluid (CSF; an aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 1.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$, phosphate- buffered to pH 7.33). This was followed by an additional 10 μl of CSF to flush the catheter (approximate internal volume was 8 μl). The end of the flush was timed to occur 1 min before the next reading; this was done because the maximum effect of intrathecal administration of these peptides under these conditions is at 1 min (Yashpal et al., 1982). CSF replaced the antagonist solution in control rats. It was found earlier (Cridland et al., 1987) that following intrathecal administration, substance P penetrates into the spinal cord over a

rostro-caudal distance of 1 cm around the injection site. Also, diffusion of substance P into the circulation is less than 4% of the amount injected 15 min after intrathecal administration. Five more readings were taken after the second intrathecal administration.

2.4. Systemic administration of SR 48968

In other experiments, the NK_2 receptor antagonist or saline was given systemically. Neurokinin A was administered intrathecally. The experiments began with preadministration of SR 48968 (at a dose of 1 mg/kg) or saline (0.9% NaCl) i.p. in a volume of 0.1 ml/100 g body weight. Three more readings were taken to allow access of the antagonist to the spinal cord and to determine any effect of the antagonist on baseline readings. Then, neurokinin A was given intrathecally in a single dose of 6.5 nmol as above, again timed to end 1 min before the next reading. Following neurokinin A administration, 5 more readings were taken.

2.5. Tail immersion experiments

In these experiments, each rat was lightly anaesthetized by an i.p. injection of a freshly prepared solution of sodium pentobarbital (20 mg/kg) and chloral hydrate (120 mg/kg). This level of anaesthesia is sufficient to prevent any overt sign of discomfort to the rat during experimentation, yet a stable response is obtained in the tail flick test for about 45 min. Pinching of the tail or hind leg produced only a withdrawal response; there was no vocalization, spontaneous movement or visible sign of distress. In this case, to measure tail flick latency, a portion of the tail close to the base (12–14 cm from the tip) was blackened to facilitate the absorption of heat. This portion was positioned above the radiant heat source used to provoke the tail flick reflex as in the previous series of experiments. After baseline readings were taken, sustained noxious thermal stimulation was applied by immersing the distal 4 cm of the tail for 1.5 min in water at 55°C. This usually produced an initial withdrawal response which subsided rapidly so that the tail remained flaccid in the water throughout the immersion. Just after the third reading following immersion, 10 μl of CSF or 65 nmol of SR 48968 was administered intrathecally and three more readings were taken. In all cases, the catheter was then flushed with 10 μl of CSF. Then, the tail was immersed a second time, as above, and readings were continued.

2.6. Statistical analysis

The mean of the 3 readings immediately prior to the first intrathecal administration was taken as 100% response for each rat. All subsequent reaction times for each rat were expressed as a percentage of this mean baseline reaction time. The combined data were evaluated by analysis of variance (ANOVA) and Tukey's wholly significant difference test was used for post-hoc pairwise comparisons (Myers, 1979). The level of statistical significance was $P < 0.05$.

3. Results

3.1. Effects of intrathecal administration of SR 48968 on neurokinin A-induced hyperalgesia in the tail flick reflex

The results of these experiments are illustrated in Fig. 1. In rats pretreated with CSF ($n = 5$), intrathecal administration of neurokinin A transiently reduced the reaction time to 58.6 ± 4.5% of the baseline value at 1 min after its administration. The reaction time then increased toward the control levels by the second reading and, at 4 min after administration, was 89.3 ± 6.8% of the baseline value.

In the second group of rats ($n = 8$), 6.5 nmol of SR 48968 was administered instead of CSF, 15 min prior to neurokinin A administration. Administration of SR 48968 had no significant effect on baseline reaction time in this or subsequent groups of animals. At the reading 1 min after neurokinin A administration, reaction time had decreased to 59.85 ± 7.59% of the baseline value. The next reading was 68.90 ± 4.23% of this baseline value. The ANOVA indicated that there was no difference at any sample time between the readings in this group vs. those in the group given CSF.

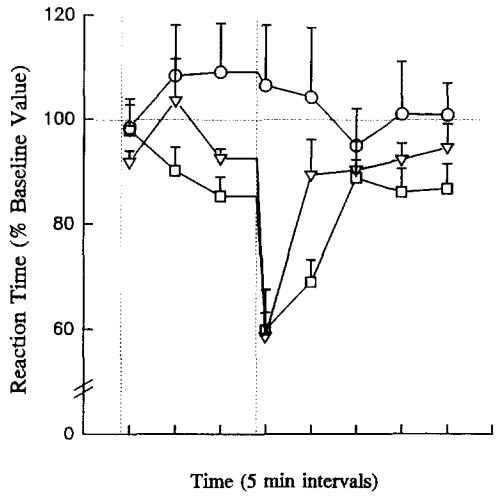

Fig. 1. Effects of intrathecal administration of SR 48968 on thermal hyperalgesia in the tail withdrawal reflex induced by intrathecal administration of neurokinin A (6.5 nmol; given at the time indicated by the vertical dotted line on the right). Withdrawal of the tail was provoked by a noxious radiant heat stimulus applied to the tip of the tail. Rats were pretreated intrathecally with CSF (▽–▽; $n = 5$), with 6.5 nmol SR 48968 (□–□; $n = 8$) or with 65 nmol SR 48968 (○–○; $n = 6$) at the time indicated by the vertical dotted line on the left. Statistical analysis indicated that only the value at 1 min after administration of neurokinin A in the group given 65 nmol SR 48968 was different from the respective value in the CSF-pretreated group. Vertical bars represent ± S.E.M.

The third group ($n = 6$) was given 65 nmol of SR 48968 instead of CSF. Following neurokinin A administration, the reaction times were 106.5 ± 11.6 and 104.3 ± 13.3% of the baseline values at 1 and 4 min after administration, respectively. At 1 min after administration, the value was significantly different ($P < 0.01$) from that of the CSF-treated group; there was no difference at any other time.

3.2. Effects of intrathecal administration of SR 48968 on substance P-induced hyperalgesia in the tail flick reflex

The results of these experiments are illustrated in Fig. 2. In rats pretreated with CSF ($n = 8$), intrathecal administration of substance P transiently reduced the reaction time to 43.7 ± 4.3% of the baseline value at 1 min after administration. The reaction time then increased toward control levels by the second reading. Substance P also produced behavioural effects which suggested that the animal had perceived a noxious stimulus. These latter effects usually consisted of movement, defecation and restlessness in the restrainer, lasting about 1 min and an allodynia such that there was a squeak and withdrawal of the tail from a light mechanical stimulus; this effect lasted about 2 min.

In rats pretreated 15 min previously with 6.5 nmol of SR 48968 ($n = 6$), the reaction times following substance P administration were 37.9 ± 4.7 and 82.6 ± 9.3% of the baseline values at 1 and 4 min after administration, respectively. The post-administration values were not significantly different ($P > 0.05$) from those of the CSF-pretreated group at any time. The behavioural response to substance P in this group did not differ from that in the group given CSF.

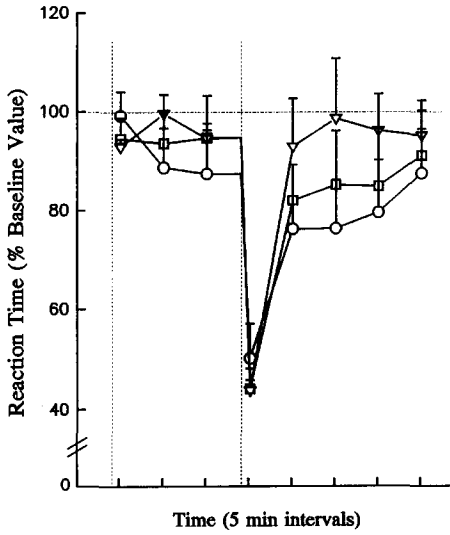

Fig. 2. Effects of intrathecal administration of SR 48968 on thermal hyperalgesia in the tail withdrawal reflex induced by intrathecal administration of substance P (6.5 nmol; given at the time indicated by the vertical dotted line on the right). Rats were pretreated intrathecally with CSF (□–□; $n = 8$), with 6.5 nmol of SR 48968 (▽–▽; $n = 6$) or with 65 nmol of SR 48968 (○–○; $n = 6$) at the time of the dotted line on the left. The experiments were otherwise run in the same way as described in Fig. 1. Statistical analysis indicated that values for all groups were identical at all readings.

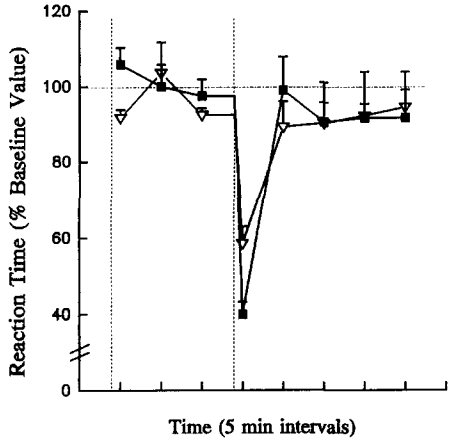

Fig. 3. Effects of intrathecal administration of CP-96,345 on thermal hyperalgesia in the tail withdrawal reflex induced by intrathecal administration of neurokinin A (6.5 nmol; given at the time indicated by the vertical dotted line on the right). Rats were pretreated intrathecally with CSF (▽–▽; $n = 5$) or with 65 nmol C-96,345 (■–■; $n = 6$) at the time indicated by the vertical dotted line on the left. The experiments were otherwise run in the same way as in Fig. 1. Statistical analysis indicated that the values for all groups were identical at all readings.

In rats pretreated 15 min previously with 65 nmol of SR 48968 ($n = 6$), the reaction times following substance P administration were 50.1 ± 6.9 and 76.2 ± 6.7% of the baseline values at 1 and 4 min after administration, respectively. The post-administration values were not significantly different ($P > 0.05$) from those of the CSF-pretreated group at any time. Again, the behavioural response to substance P in this group did not differ from that in the group given CSF.

3.3. Effects of intrathecal administration of CP-96,345 on neurokinin A-induced hyperalgesia in the tail flick reflex

Fig. 3 shows the results from the group of rats pretreated with 65 nmol of CP-96,345 ($n = 6$) to determine whether some of the effects of neurokinin A might be mediated via activation of NK_1 receptors. Administration of CP-96,345 had no effect on baseline reaction time. The reaction times 1 and 4 min following neurokinin A administration were 39.97 ± 3.23 and 99.00 ± 8.83% of the baseline values, respectively. None of the values after neurokinin A administration differed significantly ($P > 0.05$) from those of a group pretreated with CSF rather than CP-96,345 ($n = 5$).

3.4. Effects of systemic administration of SR 48968 on neurokinin A-induced hyperalgesia in the tail flick reflex

To determine whether SR 48968 passes from the periphery into the spinal cord, the antagonist was given

systemically (1 mg/kg i.p.) 20 min prior to intrathecal administration of neurokinin A (6.5 nmol; $n = 5$). The results, illustrated in Fig. 4, show that in this case neurokinin A had no effect on reaction time. The readings at 1 and 4 min after administration were 98.76 ± 8.42 and 102.31 ± 10.34 of the baseline value, respectively. When comparison was vs. a control group given saline instead of SR 48968 ($n = 5$), the two groups differed significantly only at the reading 1 min after neurokinin A administration.

3.5. Effects of SR 48968 on hyperalgesia induced by noxious cutaneous stimulation in the tail flick reflex

The results of these experiments are illustrated in Fig. 5. In the group given CSF ($n = 6$), the reaction time to noxious stimulation of the tail was transiently decreased; the first reading after this first immersion was $60.94 \pm 2.77\%$ of the baseline value ($P < 0.01$). The reaction time showed a recovery toward the baseline value over the next 3 min. After the second immersion, 15 min after the first, there was a hyperalgesia similar to that in response to the first immersion; the reading 1 min after this second immersion was $66.66 \pm 2.20\%$ of the baseline value.

In the second group of rats in this series, following a first immersion of the tail in water at 55°C for 90 s, the rats were treated with 65 nmol of SR 48968 intrathecally instead of CSF ($n = 8$). The first reading after the first immersion was $55.48 \pm 2.09\%$ of the baseline value ($P < 0.01$). The reaction time showed a recovery toward the baseline value over the next 3–6 min. At the second immersion, timed as for the first group in this series, there was a decrease in reaction time to $64.77 \pm 4.52\%$ of the baseline value taken before the first immersion. In this case, there was no difference at any sample time between this group and the group given CSF, including the baseline sample times before immersion and the test sample time at 1 min after the immersion.

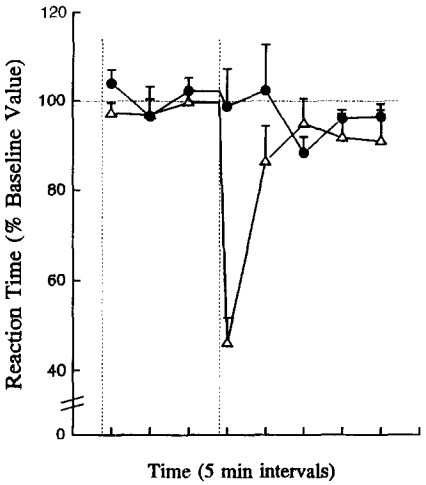

Fig. 4. Effects of systemic administration of SR 48968 on thermal hyperalgesia in the tail withdrawal reflex induced by intrathecal administration of neurokinin A (6.5 nmol; given at the time indicated by the vertical dotted line on the right). Rats were pretreated with i.p. administration of saline (△–△; $n = 5$) or of 1 mg/kg SR 48968 (●–●; $n = 5$) at the time indicated by the vertical dotted line on the left. The experiments were otherwise run in the same way as described in Fig. 1. Statistical analysis indicated that only the value at 1 min after administration of neurokinin A in the group given 1 mg/kg SR 48968 was different from the respective value in the saline-pretreated group.

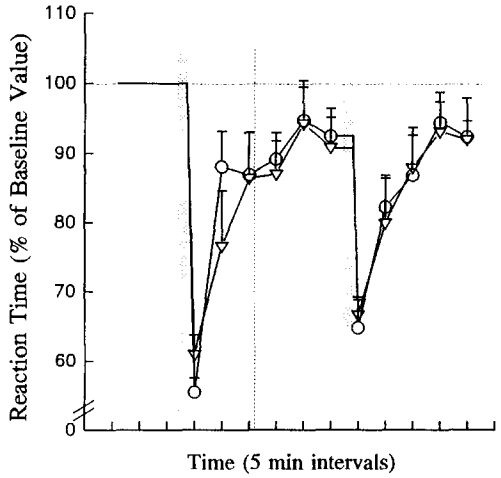

Fig. 5. Effects of intrathecal administration of SR 48968 on thermal hyperalgesia in tail withdrawal reflex induced by immersion of the tip of the tail in water at 55°C for 1.5 min. In these experiments, the tail withdrawal was provoked by a noxious radiant heat stimulus to the base of the tail rather than to the tip. Immersion was done two times, during the periods indicated by the two vertical hatched bars. Rats were treated intrathecally with CSF (▽–▽; $n = 6$) or with 65 nmol of SR 48968 (○–○; $n = 8$) at the time shown by the dotted line between the hatched bars.

4. Discussion

Recently, a number of non-peptide tachykinin receptor antagonists have been developed, providing useful tools for a better understanding of the functions of various tachykinins, specifically substance P and neurokinin A, which were of interest in the present study. Autoradiographic techniques showed NK_1 and NK_2 receptors, for which substance P and neurokinin A are the respective natural ligands (Henry, 1987), to be predominantly located in laminae I and II of the spinal dorsal horn (Yashpal et al., 1990), where nociception is processed (Sugiura et al., 1986; Light and Perl, 1979). However, the density of NK_2 receptors in these areas has been shown to be relatively low compared to that of NK_1 receptors (Yashpal et al., 1990). While the role of substance P in various biological functions, especially in nociception, is becoming well established, the precise role of neurokinin A remains elusive. For instance, it has been shown that intrathecal injection of neurokinin A induces hyperalgesia in the tail flick and flexor reflex tests in the rat (Cridland and Henry, 1986; Xu

and Wiesenfeld-Hallin, 1992), but less potently than substance P (Cridland and Henry, 1986). Neurokinin A similarly administered elicits biting and scratching in the mouse (Sakurada et al., 1992), but the specific link of this response to nociception remains to be established. Electrophysiological studies have also indicated that neurokinin A excites dorsal horn nociceptive neurones and potentiates their response to noxious stimulation (Fleetwood-Walker et al., 1993; Salter and Henry, 1991), but does not share the preferential excitatory effect of substance P on nociceptive dorsal horn neurones (Salter and Henry, 1991). In vitro, dorsal horn neurones are excited by substance P and neurokinin A, and capsaicin-evoked depolarization of dorsal horn neurones is depressed by a peptide NK_2 receptor antagonist (Nagy et al., 1993), but again the specific link to nociception remains to be established.

The results of the present study indicate that the NK_2 receptor antagonist, SR 48968, has a dose-dependent inhibitory effect on expression of the hyperalgesic response to intrathecal injection of neurokinin A in a nociceptive spinal reflex test. SR 48968 did not alter this type of response induced by substance P in this test. Thus, the present results are consistent with the proposed action of SR 48968 as a specific antagonist at NK_2 receptors (Emonds-Alt et al., 1992; Advenier et al., 1992; Burcher et al., 1993), including NK_2 receptors in the CNS (Tremblay et al., 1992; Poncelet et al., 1993).

Our data also indicate that SR 48968 has access to the spinal cord after peripheral administration. Thus, these data support the results of an earlier study indicating that the antagonist has access to the brain after peripheral administration, because turning behavior produced by intrastriatal administration of a neurokinin A analog was blocked by both i.p. and p.o. administration of SR 48968 (Poncelet et al., 1993).

The suggestion that neurokinin A may express its effects via NK_1 rather than NK_2 receptors (Geraghty et al., 1992; Nagahisa et al., 1992; Hosoki et al., 1994; Guo et al., 1993) raises the question of whether SR 48968 might show some effect on NK_1 receptors. In the present experiments, as SR 48968 blocked the hyperalgesia induced by neurokinin A but had no effect on the hyperalgesia induced by substance P, it is suggested that SR 49868 acted selectively on NK_2 receptors. This suggestion is consistent with a recent report that intrathecal injection of SR 48968 blocks the effects of a selective peptide agonist for the NK_2 receptor (Picard et al., 1993). The present data also agree with our earlier evidence that neurokinin A is less efficacious than substance P in nociceptive pathways in the rat (Cridland and Henry, 1986) and that in the cat neurokinin A has effects on non-nociceptive neurones which are insensitive to substance P (Salter and Henry, 1991). Thus, the evidence provided here indicates a unique action of neurokinin A in the spinal cord as distinct from its homologue, substance P, and confirms that neurokinin A acts via the NK_2 receptor, rather than non-specifically via the NK_1 receptor. However, it must be added that our recent electrophysiological studies in the cat indicate that neurokinin A expresses its effects via NK_1, not NK_2 receptors (Radhakrishnan and Henry, 1994); thus, there may be a species difference in the expression of the effects of neurokinin A.

In addition, the observation that the neurokinin A-induced hyperalgesia was not affected by the non-peptide NK_1 receptor antagonist, CP-96,345 (the inactive isomer, CP-96,344, was not used here as it has been shown that this dose of the antagonist has no effect on substance P-induced hyperalgesia in the tail flick test (Yashpal et al., 1993)), indicates that neurokinin A did not express any of its effects via NK_1 receptors. These data are consistent with the earlier report that intrathecal administration of the racemic mixture of CP-96,345 did not block the facilitatory effects of neurokinin A on the flexion reflex (Xu et al., 1992).

As the substance P-induced hyperalgesia was not blocked by SR 48968 in the present experiments, but has been reported to be blocked by CP-96,345 (Yashpal et al., 1993), the conclusion can be drawn that substance P does not appear to act even partially via NK_2 receptors, at least in this test and at the doses used. These data agree with results of our recent studies showing that NK_1 receptor antagonists totally block the effects of substance P on the tail flick reflex (Yashpal et al., 1993) and completely block substance P-induced excitation of spinal nociceptive neurones (Radhakrishnan and Henry, 1991). Thus, it is suggested that the effects of substance P on spinal nociceptive mechanisms are expressed via an action on NK_1 receptors but not on NK_2 receptors.

The NK_2 receptor antagonist did not block the hyperalgesic response induced by noxious thermal stimulation. We reported earlier that an NK_1 receptor antagonist only partially decreases the hyperalgesic response to this noxious stimulation (Yashpal et al., 1993). The fact that intrathecal administration of SR 48968 does not even lessen the original facilitation of the tail flick response suggests the lack of a role of NK_2 receptors, and thus neurokinin A, in this test. This is surprising considering the fact that endogenous neurokinin A is released in the spinal dorsal horn by prolonged thermal stimulation (Duggan et al., 1990) and that both tachykinins can induce hyperalgesia in the tail flick test, although neurokinin A is less potent (Cridland and Henry, 1986). Our data therefore do not provide any evidence indicating a role for this endogenously released neurokinin A. Thus, it remains to find a physiological response, mediated at the spinal level, in which endogenous neurokinin A and subsequently NK_2 receptors, are involved in vivo.

It is important to point out that despite the fact that the facilitatory effect of neurokinin A was depressed by SR 48968, the antagonist had no effect on the baseline reaction time in any group. Therefore, NK_2 receptors, and presumably endogenous neurokinin A, do not participate in

the fast nociceptive response to the brief noxious thermal stimulus which provokes the tail flick reflex. Our data therefore fail to support the results of recent in vivo (Fleetwood-Walker et al., 1993) and in vitro (Thompson et al., 1993) electrophysiological studies indicating a role of NK_2 receptors in nociceptive responses to brief noxious stimuli.

Conclusions which can be drawn from this study are that neurokinin A acts via NK_2 receptors in the rat spinal cord, that SR 48969 has access to the spinal cord from the circulation, that the role of endogenous neurokinin A and thereby NK_2 receptors in nociception in vivo remains to be determined and, finally, that the roles of NK_1 and NK_2 receptors in nociception differ.

Acknowledgements

This work was funded by the Canadian Medical Research Council, the Fonds de Recherche en Santé du Québec and by Sanofi Recherche, Montpellier, France.

References

Advenier, C., N. Rouissi, Q.T. Nguyen, X. Emonds-Alt, J.-C. Breliere, G. Neliat, E. Naline and D. Regoli, 1992, Neurokinin A (NK_2) receptor revisited with SR 48968, a potent non-peptide antagonist, Biochem. Biophys. Res. Commun. 184, 1418.

Burcher, E., T. Badgery-Parker, X.-P. Zeng and S. Lavielle, 1993, Characterisation of a novel, selective radioligand, [^{125}I][Lys^5, Tyr(I_2)^7,MeLeu^9,Nle^{10}]neurokinin A-(4-10), for the tachykinin NK_2 receptor in rat fundus, Eur. J. Pharmacol. 233, 201.

Cridland, R.A., K. Yashpal, V.V. Romita, S. Gauthier and J.L. Henry, 1987, Distribution of label after intrathecal administration of ^{125}I-substance P in the rat, Peptides 8, 213.

Cridland, R.A. and J.L. Henry, 1986, Comparison of the effects of substance P, neurokinin A, physalaemin and eledoisin in facilitating a nociceptive reflex in the rat, Brain Res. 381, 93.

Cridland, R.A. and J.L. Henry, 1988, Facilitation of the tail-flick reflex by noxious cutaneous stimulation in the rat: antagonism by a substance P analogue, Brain Res. 462, 15.

Duggan, A.W., P.J. Hope, B. Jarrott, H.-G. Schaible and S.M. Fleetwood-Walker, 1990, Release, spread and persistence of immunoreactive neurokinin A in the dorsal horn of the cat following noxious cutaneous stimulation. Studies with antibody microprobes, Neuroscience 35, 195.

Emonds-Alt, X., P. Vilain, P. Goulaouic, V. Proietto, D. Van Broeck, C. Advenier, E. Naline, G. Neliat, G. Le Fur and J.C. Brelière, 1992, A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor, Life Sci. 50, PL101.

Fleetwood-Walker, S.M., R.M.C. Parker, F.E. Munro, M.R. Young, P.J. Hope and R. Mitchell, 1993, Evidence for a role of tachykinin NK_2 receptors in mediating brief nociceptive inputs to rat dorsal horn (laminae III–V) neurons, Eur. J. Pharmacol. 242, 173.

Geraghty, D.P., C.J. Mussap and E. Burcher, 1992, Radioiodinated substance P, neurokinin A, and eledoisin bind predominantly to NK1 receptors in guinea pig lung, Mol. Pharmacol. 41, 147.

Guo, J.-Z., K. Yoshioka, M. Yanagisawa, R. Hosoki, R.M. Hagan and M. Otsuka, 1993, Depression of primary afferent-evoked responses by GR71251 in the isolated spinal cord of the neonatal rat, Br. J. Pharmacol. 110, 1142.

Henry, J.L., 1987, Discussions of nomenclature for tachykinins and tachykinin receptors, in: Substance P and Neurokinins, eds. J.L. Henry, R. Couture, A.C. Cuello, G. Pelletier, R. Quirion and D. Regoli (Springer-Verlag, New York) p. xvii.

Hosoki, R., M. Yanagisawa, J.-Z. Guo, K. Yoshioka, T. Maehara and M. Otsuka, 1994, Effects of RP 67580, a tachykinin NK_1 receptor antagonist, on a primary afferent-evoked response of ventral roots in the neonatal rat spinal cord, Br. J. Pharmacol. 113, 1141.

Hua, X.-Y., N.E. Theodorsson, E. Brodin, J.M. Lundberg and T. Hökfelt, 1985, Multiple tachykinins (neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive sensory neurons in the guinea-pig, Regul. Pept. 13, 1.

Isabel, G., D.M. Wright and J.L. Henry, 1981, Design for an inexpensive unit for measuring tail flick latencies, J. Pharmacol. Methods 5, 241.

Krause, J.E., J.M. Chirgwin, M.S. Carter, Z.S. Xu and A.D. Hershey, 1987, Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A, Proc. Natl. Acad. Sci. USA 84, 881.

Light, A.R. and E.R. Perl, 1979, Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers, J. Comp. Neurol. 186, 133.

Myers, J.L., 1979, Fundamentals of Experimental Design (Allyn and Bacon, Boston).

Nagahisa, A., Y. Kanai, O. Suga, K. Taniguchi, M. Tsuchiya, J.A. Lowe, III and H.-J. Hess, 1992, Antiinflammatory and analgesic activity of a non-peptide substance P receptor antagonist, Eur. J. Pharmacol. 217, 191.

Nagy, I., C.A. Maggi, A. Dray, C.J. Woolf and L. Urban, 1993, The role of neurokinin and *N*-methyl-D-aspartate receptors in synaptic transmission from capsaicin-sensitive primary afferents in the rat spinal cord in vitro, Neuroscience 52, 1029.

Nawa, H., T. Hirose, H. Takashima, S. Imayama and S. Nakanishi, 1983, Nucleotide sequences of cloned cDNAs for two types of bovine brain substance P precursors, Nature (London) 306, 32.

Picard, P., S. Boucher, D. Regoli, B.D. Gitter, J.J. Howbert and R. Couture, 1993, Use of non-peptide tachykinin receptor antagonists to substantiate the involvement of NK_1 and NK_2 receptors in a spinal nociceptive reflex in the rat, Eur. J. Pharmacol. 232, 255.

Poncelet, M., C. Gueudet, X. Emonds-Alt, J.-C. Brelière, G. Le Fur and P. Soubrié, 1993, Turning behavior induced in mice by a neurokinin A receptor agonist: Stereoselective blockade by SR 48968, a non-peptide receptor antagonist, Neurosci. Lett. 149, 40.

Radhakrishnan, V. and J.L. Henry, 1991, Novel substance P antagonist, CP-96,345, blocks responses of spinal dorsal horn neurons to noxious cutaneous stimulation and to substance P, Neurosci. Lett. 132, 39.

Radhakrishnan, V. and J.L. Henry, 1994, Electrophysiological evidence that neurokinin A acts via NK-1 receptors in the cat spinal dorsal horn, Peptides and Their Antagonists in Tissue Injury, a Satellite Symposium of the XII IUPHAR Pharmacology Congress (Abstract).

Sakurada, T., Y. Manome, K. Katsumata, H. Uchiumi, K. Tan-No, S. Sakurada and K. Kisara, 1992, Naloxone-reversible effect of spantide on the spinally mediated behavioural response induced by neurokinin-2 and -3 receptor agonists, Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 69.

Salter, M.W. and J.L. Henry, 1991, Responses of functionally identified neurones in the dorsal horn of the cat spinal cord to substance P, neurokinin A and physalaemin, Neuroscience 43, 601.

Saria, A., R. Gamse, J. Petermann, J.A. Fischer, N.E. Theodorsson and J.M. Lundberg, 1986, Simultaneous release of several tachykinins and calcitonin gene-related peptide from rat spinal cord slices, Neurosci. Lett. 63, 310.

Sugiura, Y., C.L. Lee and E.R. Perl, 1986, Central projections of identified, unmyelinated (C) afferent fibers innervating mammalian skin, Science 234, 358.

Thompson, S.W.N., L. Urban and A. Dray, 1993, Contribution of NK_1 and NK_2 receptor activation to high threshold afferent fibre evoked ventral root responses in the rat spinal cord in vitro, Brain Res. 625, 100.

Tremblay, L., M.-L. Kemel, M. Desban, C. Gauchy and J. Glowinski, 1992, Distinct presynaptic control of dopamine release in striosomal- and matrix-enriched areas of the rat striatum by selective agonists of NK1, NK2, and NK3 tachykinin receptors, Proc. Natl. Acad. Sci. USA 89, 11214.

Xu, X.-J., C.-J. Dalsgaard and Z. Wiesenfeld-Hallin, 1992, Intrathecal CP-96,345 blocks reflex facilitation induced in rats by substance P and C-fiber-conditioning stimulation, Eur. J. Pharmacol. 216, 337.

Xu, X.-J. and Z. Wiesenfeld-Hallin, 1992, Intrathecal neurokinin A facilitates the spinal nociceptive flexor reflex evoked by thermal and mechanical stimuli and synergistically interacts with substance P, Acta Physiol. Scand. 144, 163.

Yashpal, K., D.M. Wright and J.L. Henry, 1982, Substance P reduces tail-flick latency: implications for chronic pain syndromes, Pain 14, 155.

Yashpal, K., S.G. Gauthier and J.L. Henry, 1985, Substance P given intrathecally at the spinal T9 level increases adrenal output of adrenaline and noradrenaline in the rat, Neuroscience 15, 529.

Yashpal, K., T.-V. Dam and R. Quirion, 1990, Quantitative autoradiographic distribution of multiple neurokinin binding sites in rat spinal cord, Brain Res. 506, 259.

Yashpal, K., V. Radhakrishnan, T.J. Coderre and J.L. Henry, 1993, CP-96,345, but not its stereoisomer, CP-96,344, blocks the nociceptive responses to intrathecally administered substance P and to noxious thermal and chemical stimuli in the rat, Neuroscience 52, 1039.