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Hyperalgesia produced by intrathecal substance P and related peptides: desensitization and cross desensitization

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1 The hyperalgesic effect of intrathecally administered substance P (SP), physalaemin, eledoisin and eledoisin-related peptide (ERP) was investigated in the rat tail flick test.

2 Hyperalgesia produced by SP (2.5–15 μg, 1.9–11 nmol) was maximal 10–20 min after injection, lasted 30 min and was dose-related. The effect was mimicked by all of the peptides examined. The rank order of potency was physalaemin > SP > eledoisin > ERP.

3 Desensitization to the hyperalgesic effect of SP was produced by three repeated intrathecal injections. Rats desensitized to SP no longer responded to physalaemin or ERP, indicating cross-desensitization. Phentolamine continued to produce hyperalgesia following such desensitization.

4 The demonstration of a hyperalgesic effect for SP provides further support for a role for SP in nociceptive transmission. The receptor mediating this effect appears to be a SP-P subtype. Cross-desensitization between peptides suggests an action on the same receptor.

Introduction

Evidence from anatomical, biochemical and electrophysiological studies indicates that substance P (SP) may be a transmitter or modulator of nociceptive information at the primary afferent synapse in the spinal cord (reviewed by Jessell, 1982; Henry, 1982). Recent studies in which SP has been administered intrathecally provide further evidence in support of this notion. In behavioural studies, intrathecal SP produces a caudally directed biting and scratching syndrome resembling that produced by cutaneous irritants which suggests that SP is perceived as a noxious stimulus (Piercey *et al.*, 1981; Rackham *et al.*, 1981; Hylden & Wilcox, 1981). In tests for nociceptive activity, intrathecal SP decreases the response latency in both the hot plate (Hayes & Tyers, 1979; Akerman *et al.*, 1982) and tail flick tests (Yasphal *et al.*, 1982; Pillay & Sawynok, 1983) suggesting that SP induces a hyperalgesic state. Hyperalgesia in the hot plate test is transient, lasting up to 10 min. In the tail flick test, the hyperalgesia reported in one study (Yasphal *et al.*, 1982) was transient, but that observed in this laboratory is more sustained lasting up to 30 min (Pillay & Sawynok, 1983). Conversely, the intrathecal administration of agents which interfere with SP function increase nociceptive thresholds. Analogues of SP with antagonistic properties (Akerman *et al.*, 1982; Rodriguez *et al.*, 1983) and capsaicin, which depletes the spinal cord content of SP (Yaksh *et al.*, 1979) produce analgesia (increase in response latency) in the tail flick and hot plate tests. It should however be noted that the effects of these agents on nociceptive threshold may not be specific in that intrathecal administration of SP antagonists produce an alteration in motor function as well (Rodriguez *et al.*, 1983) while intrathecal capsaicin also depletes peptides other than SP (Micevych *et al.*, 1983).

Recently, the existence of two types of SP receptors was proposed on the basis of the relative potencies of a series of tachykinins and their analogues compared to SP in a number of isolated organ systems and supported by desensitization studies (Lee *et al.*, 1982; Watson *et al.*, 1983). These receptors were termed SP-P and SP-E because physalaemin and eledoisin were used as key discriminant compounds. The present study was undertaken to determine whether the prolonged hyperalgesic effect of SP in the tail flick test was due to activation of a SP-P or SP-E type receptor by comparing the effects of SP to those of physalaemin, eledoisin and eledoisin-related peptide (ERP). An additional objective was to determine whether desensitization to the effect of SP could

be demonstrated in a test for nociceptive activity, and if so to determine whether these other tachykinins act on the same receptor by examining cross-desensitization. Desensitization to SP and cross-desensitization between SP and physalaemin and eledoisin have been demonstrated in the guinea-pig ileum, and this is believed to represent one method of determining that these peptides are acting on the same receptor (Lembeck & Fisher, 1967). A preliminary account of these findings has been published previously (Moochhala & Sawynok, 1983).

Methods

Rats (male Sprague-Dawley, 250–300 g) were implanted with chronically indwelling cannulae by the method previously described (Sawynok *et al.*, 1983). Following surgery, animals were housed individually. Experiments were started after a 5–7 day recovery period, and rats were used repeatedly for a number of experiments with at least 2 days between experiments.

Nociceptive thresholds were determined using an automated tail flick unit (Ugo Basile, Italy). The intensity of the light beam was adjusted so that baseline readings were between 6–8 s. Rats were placed in a plastic restraining box which allowed access to the cannula for the duration of the experi-

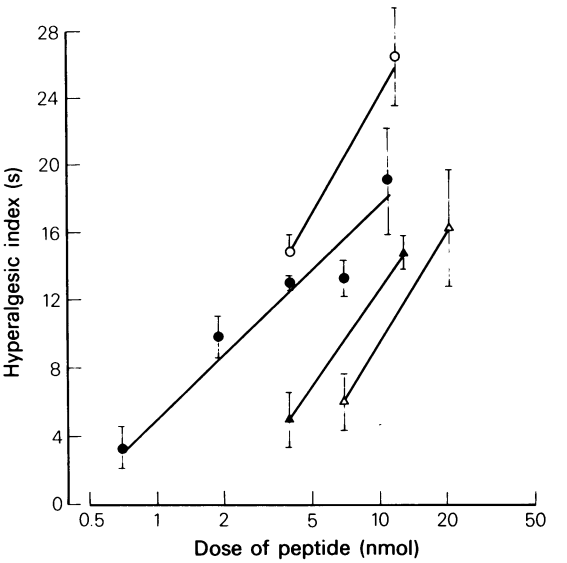

Figure 2 Dose-related hyperalgesia produced by substance P (SP) and related peptides. Data are expressed as a hyperalgesic index which is the sum of differences between recorded latencies and baseline (mean of 2 values) at individual time periods. (●) SP, (○) physalaemin, (▲) eledoisin, (△) eledoisin-related peptide. Values depict mean for 3–9 rats with s.e.mean indicated by vertical lines.

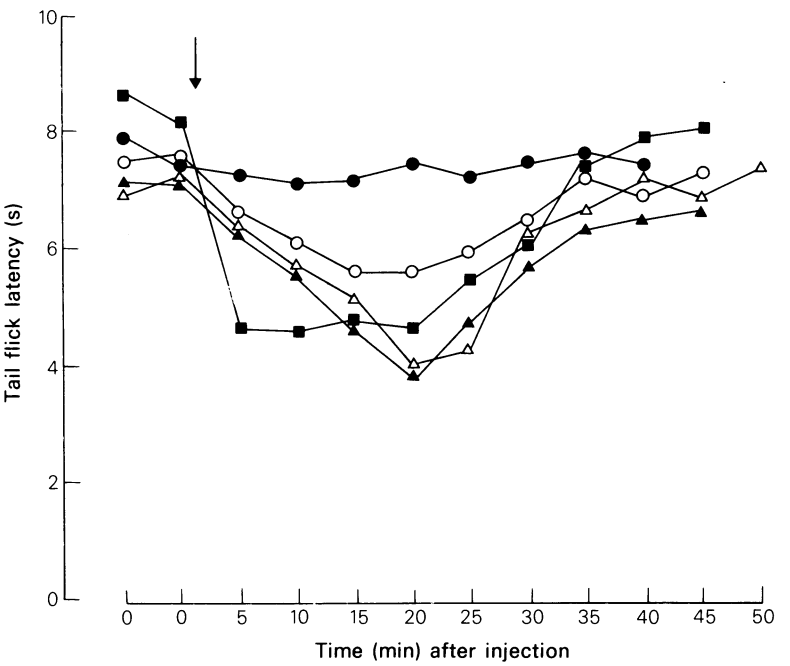

Figure 1 Hyperalgesic effect of substance P (SP) injected intrathecally via a chronic indwelling cannula. Doses of SP: (●) 1 μg;(○) 2.5 μg; (▲) 5 μg; (△) 10 μg; (■) 15 μg. Values represent means from 3–5 rats. Standard errors of the mean were generally 0.5 s or less. The effect of the intrathecal injection of saline is illustrated in Figures 3, 4 and 5.

ment. If restraint caused obvious signs of distress such as squirming or agitation, the rat was not used on this occasion. Three sets of duplicate baseline values were obtained for each rat. The first reading was usually higher than those which followed and is not presented in the figures. Drugs were injected in 15 μl saline and flushed in with 10 μl saline. Following injection, duplicate tail flick determinations were made at 5 min intervals for 45 min in hyperalgesia experiments. In desensitization experiments, additional intrathecal injections were made 30 and 60 min after the initial injection and reaction latencies followed for up to 120 min after the initial injection. Means of duplicate determinations were used for analysis.

Substance P, physalaemin and eledoisin-related peptide were purchased from Sigma and eledoisin from Peninsula Laboratories, while phentolamine HCl was a gift from Ciba-Geigy, Canada. Solutions of peptides were prepared in saline, stored on ice during the experiment, frozen between experiments and used within 3 days of preparation.

Results

The intrathecal injection of SP (2.5–15 μg, 1.9–11 nmol) produced hyperalgesia (a reduction in reaction latency) in the tail flick test when baseline latencies were between 6–8 s (Figure 1). The maxi-

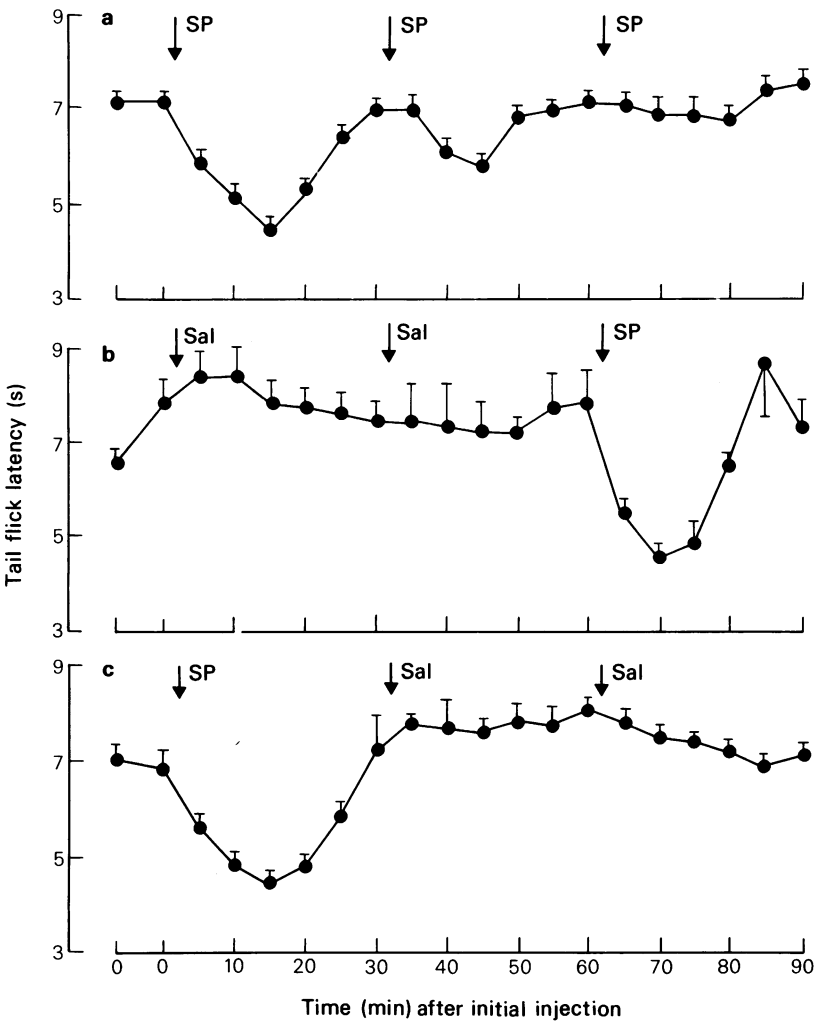

Figure 3 Desensitization to the hyperalgesic effect of substance P (SP). SP (15 μg, 11 nmol) or saline (Sal)were injected at the points indicated and tail flick latencies determined at 5 min intervals. Values in this and subsequent figures depict mean with s.e.mean indicated by vertical lines. In (a) $n = 11$ and in (b) and (c) $n = 3$.

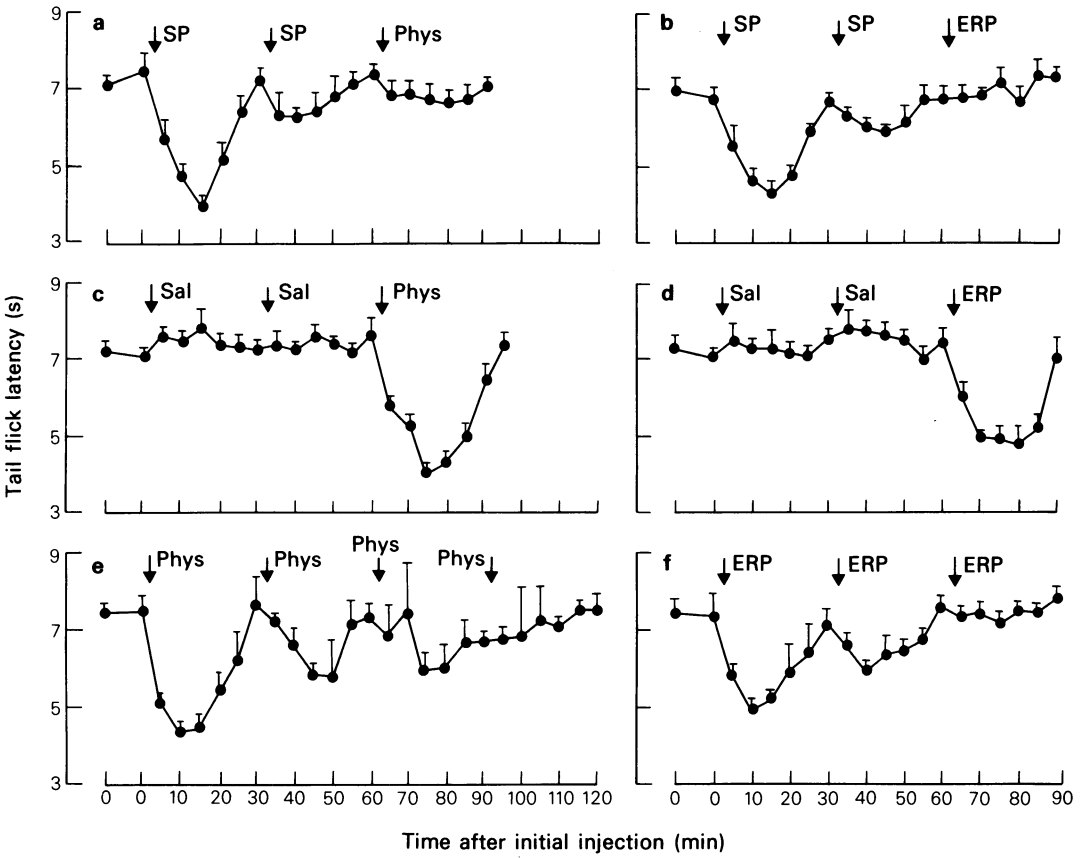

Figure 4 Cross-desensitization between hyperalgesic effect of substance P (SP), physalaemin (Phys) and eledoisin-related peptide (ERP). In (a) and (b), rats were made insensitive to SP by repeated injection of 15 μg doses, then injected with physalaemin (15 μg, 12 nmol, a) or ERP (15 μg, 21 nmol, b). Panels (c) and (d) indicate that the loss of responsiveness is not due to multiple injections and repeated testing; (e) and (f) indicate that the repeated injection of physalaemin and ERP can also induce desensitization to hyperalgesia. $n = 3-4$ in all panels.

mal reduction in latency observed was 40–50% between 10 and 20 min after injection. Recovery occurred within 30–35 min. The dose-related nature of this hyperalgesia is more clearly illustrated in Figure 2. The tachykinins physalaemin, eledoisin and ERP all produced hyperalgesia which was similar to that produced by SP with respect to time course (see Figure 4), but physalaemin was slightly more potent than SP while eledoisin and ERP were less potent than SP (Figure 2).

The repeated intrathecal administration of SP produced a rapid desensitization to hyperalgesia, such that by the third injection, this effect was no longer observed (Figure 3a). The disappearance of the response was not due to repeated determinations at short time intervals because prior injection with saline did not alter the response to SP (Figure 3b). When desensitization to SP was induced, the subsequent injection of physalaemin and ERP no longer elicited the characteristic hyperalgesic response indicating that cross-desensitization occurred between these agents and SP (Figure 4a and 4b). The repeated administration of physalaemin and ERP also produced desensitization to hyperalgesia (Figure 4e and 4f). In order to determine whether cross-desensitization was a receptor-mediated phenomenon or simply a progressive loss of responsiveness of the system, phentolamine was injected after desensitization was induced. This agent was previously shown to produce hyperalgesia in the tail flick test, an action believed to be due to blockade of α-adrenoceptors involved in the tonic gating of nociceptive transmission (Proudfit & Hammond, 1981) and is therefore independent of SP receptors. In Figure 5, it is apparent that phentolamine still produce marked hyperalgesia following repeated injections of SP

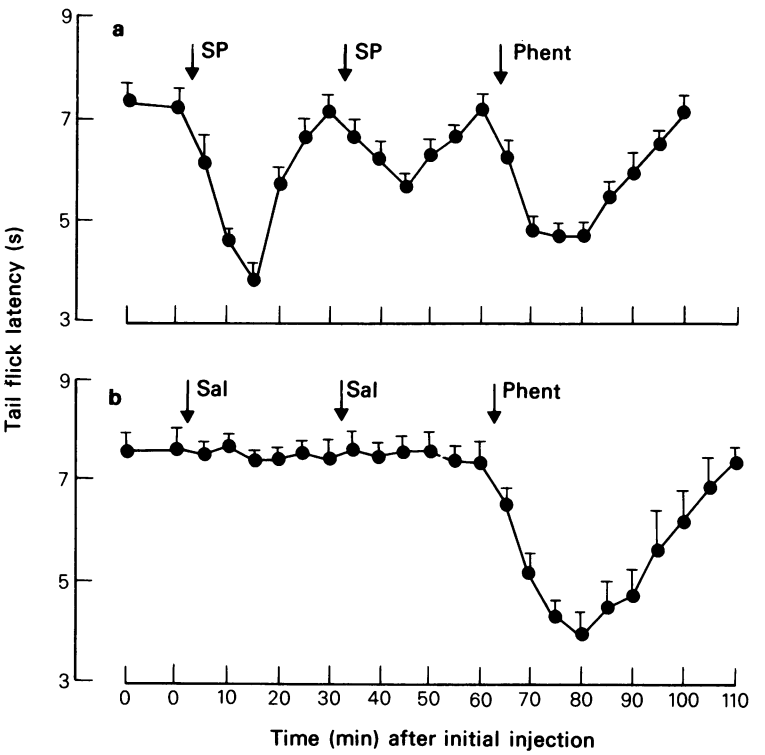

Figure 5 Lack of cross-desensitization between substance P (SP) and phentolamine. In (a) rats were made insensitive to SP by repeated 15 μg injections, then were injected with 50 μg phentolamine (Phent); (b) depicts the response to phentolamine after repeated saline injections. $n = 3$ in both panels.

(hyperalgesic index of 10.8 ± 1.9 compared to 14.6 ± 1.1 after saline, $P < 0.2$, Student's t test).

In addition to hyperalgesia, intrathecal injection of peptides produced a characteristic biting, scratching and grooming behaviour. This effect was transient, lasting only 1–2 min and was over before the first tail flick determination 5 min after injection. All doses of peptides used except for the lowest SP dose (< 1 nmol) produced this response. Although no attempt was made to quantitate these behavioural effects, it was noted that the response to the highest dose of eledoisin (21 nmol) was particularly strong, being more pronounced than that observed with similar doses of SP and physalaemin. Following the repeated injection of peptides, desensitization to behavioural effects occurred in parallel to desensitization in the tail flick response. For SP and ERP, this effect was no longer apparent after the third injection, while for physalaemin, it disappeared after the fourth injection. All doses of peptides were observed to produce vasodilatation as seen by a marked reddening of the ear. This effect was still present following repeated injections of the peptides.

Discussion

The present study indicates that the intrathecal injection of SP can produce hyperalgesia in the tail flick test and provides behavioural evidence in support of SP subserving a mediating or modulatory role in the transmission of noxious information in the spinal cord. The maximum reduction in tail flick latency was 40–50% and generally required 10 min to develop. Hyperalgesia was sustained for up to 30 min following injection. The magnitude and duration of this hyperalgesia confirm earlier observations in this laboratory (Pillay & Sawynok, 1983), but differ from that reported by Yasphal *et al.* (1982) where the maximal effect of intrathecally injected SP was a 70% reduction in latency one minute after injection. This hyperalgesic effect was transient, lasting only 5–6 min. It is not clear why these differences occur, but different experimental conditions (e.g. initial tail flick latencies of 20 s and different methods of handling the animals in the Yasphal *et al.* study) may be contributing factors. Hyperalgesia in the tail flick test is not observed when shorter baseline latencies

(2–3 s) are used (Sawynok *et al.*, 1983). In another study, intrathecal SP has been reported to produce analgesia in the tail flick test (Doi & Jurna, 1981). The doses of SP used, initial tail flick latencies and time course of analgesia are similar to those used in the present study. Analgesia following intrathecal SP is blocked by naloxone (Doi & Jurna, 1981) suggesting it is due to the release of endogenous opioids. These conflicting observations on the effects of SP following intrathecal administration are reminiscent of those reported following intracerebral or intraperitoneal administration (see Frederickson *et al.*, 1978; Oehme *et al.*, 1980 and references therein). It is not clear why different laboratories obtain these different responses, but it is not surprising that intrathecal SP may have multiple effects on nociceptive thresholds because SP is present in primary afferent terminals (Hökfelt *et al.*, 1975), in axons containing 5-hydroxytryptamine which descend from the brainstem (Hökfelt *et al.*, 1978) and in dorsal horn neurones (Hunt *et al.*, 1981). In producing hyperalgesia, SP may activate mechanisms at the primary afferent synapse, while analgesia may result from activation of mechanisms associated with descending pathways. Bulbospinal 5-hydroxytryptaminergic pathways depress nociceptive transmission in the spinal cord (Basbaum & Fields, 1978), but the role of SP which may be co-released with 5-hydroxytryptamine from these pathways is not yet established.

A possible alternative explanation for the observed decrease in reaction latency produced by SP is hyperreflexia due to depolarization of motoneurones. SP is known to depolarize motoneurones in the amphibian and mammalian spinal cord by a direct action (Konishi & Otsuka, 1974a, b). However, dissociations between depolarizing effects and decreases in tail flick latency occur, such that this action cannot be the primary mechanism for the apparent hyperalgesia. Thus, depolarization by SP is mimicked by a number of substances such as L-glutamate, γ-aminobutyric acid, glycine, acetylcholine and noradrenaline (Otsuka & Yanagisawa, 1980), but intrathecal noradrenaline increases tail flick latency (Reddy & Yaksh, 1980). In addition, eledoisin is more potent than physalaemin and SP in depolarizing motoneurones (Konishi & Otsuka, 1974a), while in this study, physalaemin is more potent than SP and eledoisin in decreasing tail flick latency.

The rank order of potency of peptides in producing hyperalgesia is physalaemin > SP > eledoisin > ERP. The receptor mediating this response thus resembles the SP-P type receptor previously described for various peripheral tissues sensitive to SP (Watson *et al.*, 1983). SP is approximately 4 times as potent as ERP in producing this response in agreement with the potency difference observed by Yasphal *et al.* (1982) for the transient hyperalgesia. Conversely, the biting scratching syndrome produced by the intrathecal injection of SP (Piercey *et al.*, 1981; Rackham *et al.*, 1981; Hylden & Wilcox, 1981) may be mediated by a SP-E type receptor. Eledoisin and physalaemin were reported to be 17 and 2.5 times more potent respectively than SP at eliciting the biting scratching syndrome following intracerebral injection (Share & Rackham, 1981), an effect believed to be due to activation of spinally located receptors (Piercey *et al.*, 1981). No attempt was made to quantitate this syndrome in the present study, but the highest dose of eledoisin clearly produced the strongest effect. It appears that the biting scratching syndrome and the hyperalgesia produced by SP may be mediated by different SP receptor subtypes.

One assumption inherent in the characterization of SP receptors by using the relative potency of peptides is that such potency differences reflect different receptor affinities. Although differences in access to the receptor site in the grey matter of the spinal cord from the surrounding cerebrospinal fluid could be a contributing factor, other studies have indicated that there is no simple relationship between hydrophobicity of SP analogues and observed potency differences (Sandberg *et al.*, 1980) and have concluded that such differences were most likely to be due to variable receptor affinities. An additional source of pharmakokinetic variation could be in the rate of metabolic degradation. However, it should be noted that in the present study, the duration of the effect produced by each of the peptides was very similar.

In this study, cross-desensitization between SP and physalaemin and ERP was used to determine whether the same receptors mediated their effects (Lembeck & Fisher, 1967) as an alternative to using a specific receptor antagonist. SP antagonists are available, but their specificity for receptor subtypes has not been established and they produce analgesia in the tail flick test (Yasphal, 1982) such that interpretation of results using combinations of SP and antagonists may be complicated. Repeated intrathecal administration of SP, physalaemin and ERP produced complete desensitization to hyperalgesia by the third or fourth application. In addition, in rats desensitized to SP, physalaemin and ERP no longer produced hyperalgesia indicating a complete cross-desensitization and implying an action at the same receptor. Rats desensitized to SP still responded to the intrathecal injection of phentolamine, an α-adrenoceptor antagonist previously shown to produce hyperalgesia in the tail flick test (Proudfit & Hammond, 1981), indicating that desensitization was a receptor-mediated phenomenon rather than a non-specific loss of responsiveness following repeated injections and testing. The SP-P type receptor

in the spinal cord mediating hyperalgesia thus is similar to that in the guinea-pig ileum both with respect to the rank order of potency and in its ability to exhibit desensitization, providing evidence that SP receptor subtypes in the central nervous system resemble those in peripheral tissues (see Watson *et al.*, 1983).

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