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Differential effects of substance P on serotonin-modulated spinal nociceptive reflexes

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Abstract. Recent immunohistochemical studies indicate the presence of a bulbospinal substance P (SP) system, as well as a bulbospinal serotonin (5-HT) system, involved in spinal pain transmission. Although electrophysiological studies indicate that SP may modulate the effects of 5-HT on postsynaptic spinal nociceptive neurons, the functional relationship between SP and 5-HT on "pain behavior" remains obscure. To bridge this gap between mechanism and behavior, the purpose of the present study was to determine specific postsynaptic behavioral effects of SP and 5-HT on local spinal nociceptive reflexes in spinally transected animals. Administration of the 5-HT agonists 5-methoxydimethyltryptamine (5-MeODMT) (0, 0.5, 1.5, 2.0 mg/kg) and quipazine (0, 5, 10, 20 mg/kg) 2 days after transection significantly expanded the receptive field (RF) areas of three spinal reflexes, as previously reported. Intrathecal administration of SP alone (0, 0.25, 2.5, 7.5 ng) also resulted in hyperalgesia, indicated by a significant expansion of the RF areas of all three nociceptive reflexes. However, administration of SP, in animals pretreated with 5-HT agonists, decreased the 5-HT-induced expansion of RF size. Therefore, SP had opposite effects on spinal nociceptive reflexes depending on whether or not the animal was pretreated with 5-HT agonists, i.e., hyperalgesia in the absence of 5-HT agonists, and analgesia in the presence of 5-HT agonists. The two effects of SP on local spinal reflexes may be related to the anatomical organization of the two spinal SP systems: 1) SP released from primary afferents facilitates nociceptive reflexes, and 2) SP associated with the descending bulbospinal system interacts with the descending bulbospinal 5-HT system and inhibits nociceptive reflexes. The present results help explain contradictory literature regarding the effect of SP on spinal nociceptive reflexes.

Key words: Serotonin – Substance P – Spinal cord – Colocalization – Receptor binding – Spinal reflexes

The involvement of the descending bulbospinal serotonin (5-HT) system in pain transmission has been well established. However, depending upon experimental procedures, recent physiological and behavioral studies indicate that 5-HT may either facilitate or inhibit pain transmission (Dennis and Melzack 1980; Vasko et al. 1984). In particular, 5-HT agonist administration generally results in behaviorally-defined analgesia in intact animals (Basbaum et al. 1976) while 5-HT agonist administration has the opposite effect on pain transmission in the spinal animal even when the same behavioral endpoint is measured (Zemlan et al. 1983). One possible explanation is that 5-HT may differentially affect sensory input related to local spinal reflexes as opposed to supraspinal nociceptive information.

Recent studies using histochemistry and immunohistofluorescence have identified the presence of a descending bulbospinal substance P (SP) system distinct from the descending 5-HT and 5-HT/SP systems (Bowker et al. 1981, 1983; Johansson et al. 1981; Hokfelt et al. 1984). Under varying experimental conditions, SP has been shown to differentially affect pain transmission. Specifically, Yashpal and Henry (1982) found that intrathecal administration of SP (7.4 nmol) abruptly but transiently (<6 min) reduced reaction time in the tail-flick test. However, this hyperalgesic effect was followed by analgesia (i.e., increased response time) which lasted 10 min. Additional studies have demonstrated that low doses of SP injected intraventricularly produced analgesia (Frederickson et al. 1978) while intrathecal administration of SP, using comparable doses, produced dose-related hyperalgesia (Moochhala and Sawynok 1984). The conflicting data for SP, like 5-HT, may be due to differential effects of SP on sensory input related to the underlying anatomical organization of two spinal SP systems, i.e., SP associated with primary afferents (Jessell 1981) and SP associated with a descending bulbospinal system.

In addition to specific SP effects on pain transmission, recent electrophysiological studies suggest that the SP and 5-HT systems may interact at the level of the spinal cord. Specifically, stimulation of the ventral medulla (VM) inhibited a majority of spinal units responsive to nociceptive input. The ventral medullary (VM) inhibition could be attenuated by either SP antagonists, or 5-HT antagonists (Vaughn et al. 1985), indicating that certain spinal units may be controlled by both descending systems.

The existence of unique descending bulbospinal transmitter systems, and multiple SP systems in the spinal cord attests to the complexity of spinal pain transmission. The purpose of this present study was to identify specific behavioral effects and possible interactive behavioral effects between SP and 5-HT on postsynaptic spinal nociceptive neurons. Therefore, the effects of intrathecal injections of SP and 5-HT agonists were examined on three spinal nocicep-

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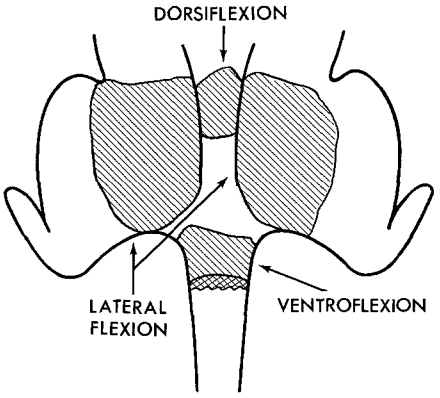

Fig. 1. Spinal nociceptive reflexes: vertebral column ventroflexion, receptive field (RF) area located on tail base and immediately adjacent portion of the dorsal body surface; dorsiflexion, RF located on the dorsal body surface along the midline at the level of the iliac crest; and lateral flexion/tail deviation, RF located on the entire lumbar dorsum off the midline (Zemlan et al. 1983)

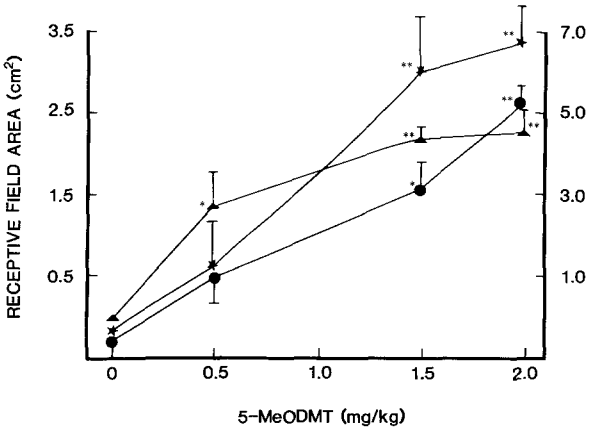

Fig. 2. Dose-response related expansion of three nociceptive reflex RF areas after administration of the 5-HT agonist 5-MeODMT, 2 days after spinal transection. RF area scale for the ventro- and dorsiflexion reflexes are to the left, with the lateral flexion reflex scale to the right. Values are mean RF area ±SEM; $N=7$; * $P<0.05$; ** $P<0.01$ when compared with predrug baseline values. ▲ Ventroflexion; ● Dorsiflexion; * Lateral flexion

tive reflexes freed from supraspinal control by complete spinal transection.

Methods

Reflex studies. Spinal cord transections (male Sprague-Dawley rats, 250–350 g) were performed according to a procedure described by Zemlan et al. (1978) but modified for intrathecal drug administration. Prior to spinal transection at T_{10}, intramedic polyethylene (PE_{10}) tubing (3.5 cm in length) was inserted through the subarachnoid space, terminating over the lumbar enlargement of the spinal cord. Substance P was administered intrathecally while all other drugs were administered intraperitoneally (IP). Previous research (Zemlan et al. 1983) demonstrated that expansion of the nociceptive reflex RF areas was a function of both 5-HT agonist dose and time following transection. Since each reflex is circumscribed by a finite surface area, it was necessary to administer drugs 2 days following spinal transection when baseline recovery of reflexes was near threshold levels. This procedure allowed maximal expansion of the RF areas following drug administration, and minimized confounding results due to ceiling effects.

Three spinal withdrawal reflexes were employed to quantify the organism's behavioral response to nociceptive stimulation (graded forceps applied to skin fold): vertebral column ventroflexion, dorsiflexion and lateral flexion (Fig. 1). The nociceptive sensitivity of the reflex was quantified as size of the receptive field area of the reflex in cm^2 ($1\ cm^2 = 8.1$ mg), which is directly proportional to the somatosensory threshold for eliciting the reflex in mm Hg of pressure (Zemlan et al. 1983).

Both raw and transformed data were analyzed by analysis of variance (ANOVA) with repeated measures (Jenrich et al. 1981). Post hoc analysis included *t* tests or Duncan's New Multiple Range test (Kirk 1968).

Drugs. Drugs included the 5-HT receptor agonists 5-hydroxytryptamine creatinine sulfate (5-HT), 5-methoxydimethyltryptamine (5-MeODMT) (Sigma Chemical Co., St. Louis, MO), and quipazine maleate (Miles Laboratories, Elkhart, IN); substance P (Bachem, Torrence, CA); and clonidine HCl (Boehringer-Ingelheim Ltd., Elmsford, NY), an alpha adrenergic receptor stimulant.

Results

Independent effects of 5-HT agonists. Initial studies replicated and expanded earlier work by Zemlan et al. (1983). Following administration of centrally acting 5-HT agonists, a dose-response related increase in reflex RF area was observed. The effect of cumulative doses of 5-MeODMT (0, 0.5, 1.5 and 2 mg/kg) on the withdrawal reflexes 2 days after spinal transection is illustrated in Fig. 2. Prior to drug administration, the reflexes could be reliably elicited from only a small portion of the RF area for each reflex. However, following administration of 2 mg/kg 5-MeODMT, for example, the RF area for the ventroflexion, dorsiflexion and lateral flexion reflexes significantly expanded by 700% to 1500% (P values <0.01). Similarly, administration of the centrally acting 5-HT agonist quipazine (0, 5, 10, and 20 mg/kg) also resulted in a significant expansion of the RF areas of all nociceptive withdrawal reflexes. The maximal per cent expansions (600–1300%) of the RF areas for each reflex following quipazine administration (data not illustrated) were similar to the values obtained for 5-MeODMT.

To determine if the effects above were specific to 5-HT agonists and to determine if expansion of RF areas did not result from ischemic effects of 5-HT agonists, the alpha-2 agonist clonidine HCl was administered. Although clonidine produces hypotension in intact animals, its administration results in hypertension in spinal animals (Kobinger and Pickler 1975). There was no significant expansion observed on the RF area of any of the three nociceptive reflexes after administration of 100 or 300 μg/kg clonidine (P value >0.10).

In order to determine if the effect of 5-MeODMT or quipazine on RF area was mediated by peripheral 5-HT receptors, the effect of IP injection of the peripherally acting 5-HT agonist, 5-HT creatinine sulfate on reflex RF areas

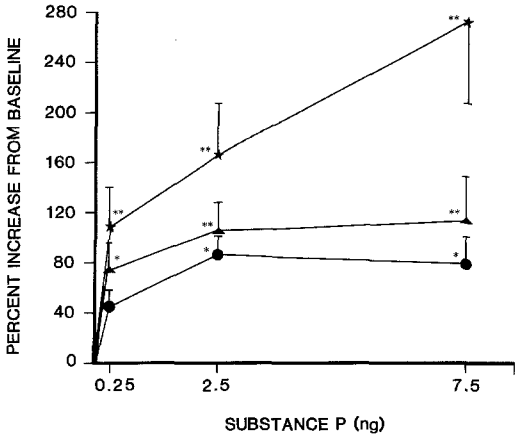

Fig. 3. Effect of cumulative doses (0.25, 2.5 and 7.5 ng) of substance P on the RF area of three nociceptive flexor reflexes in the spinal animal, 2 days after spinal transection. Values are mean RF area expressed as per cent of predrug baseline ±SEM; $N=7$; * $P<0.05$; ** $P<0.01$ when compared with predrug baseline values. ▲ Ventroflexion; ● Dorsiflexion; ∗ Lateral flexion

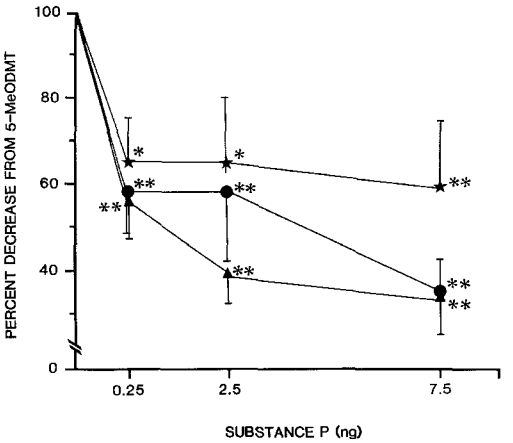

Fig. 4. Decrease in RF areas for three nociceptive withdrawal reflexes following administration of cumulative doses of substance P (0.25, 2.5 and 7.5 ng) in spinal animals pretreated with 5-MeODMT. Values are mean RF areas expressed as per cent decrease from 5-MeODMT dose ±SEM; $N=28$; * $P<0.05$; ** $P<0.01$ when compared to 5-MeODMT values. ▲ Ventroflexion; ● Dorsiflexion; ∗ Lateral flexion

was examined. At doses equipotent to the centrally acting 5-HT agonists (i.e., 0.1, 0.6 or 3.6 mg/kg 5-HT), no effect of 5-HT on any of the spinal reflex RF areas was observed.

Independent effects of substance P. Since the possibility of synergistic effects (i.e., ceiling effects) between SP and 5-MeODMT on expansion of reflex RF areas existed, initial dose-finding studies for SP were conducted. The purpose of these studies was to determine the smallest possible dose resulting in a significant per cent expansion in RF area while minimizing expansion of the absolute RF area (cm^2) of each reflex. Intrathecal administration of SP (0, 0.25, 2.5 and 7.5 ng) resulted in significant increases in RF areas for all three flexor reflexes ($F=7.51$, $df=2$, $P<0.001$). The effect of cumulative doses of SP expressed as per cent increase from baseline is presented in Fig. 3. For the ventro- and dorsiflexion reflexes, the effect of SP was extremely potent, with the lowest dose of SP (0.25 ng) producing the largest per cent increase (40–75%) in their respective RF areas. For the lateral flexion reflex only, administration of increasing concentrations of SP resulted in significantly increasing expansion (P values <0.05) of RF areas at each increasing dose of SP.

Interaction of 5-HT agonists and SP. Pretreatment with the 5-HT agonist MeODMT (1.5 mg/kg) resulted in an expansion of the three reflex RF areas (Fig. 4) similar to that indicated in Fig. 1 ($P<0.01$). Initial studies indicated that the effect of 5-MeODMT on RF area is constant for 2 h after administration so that subsequent repeated administration of SP at 20-min intervals occurred within this 2-h period when the 5-MeODMT effect is constant. Administration of increasing doses of SP significantly decreased the 5-MeODMT-induced expansion of the RF areas of the three nociceptive withdrawal reflexes (Fig. 4). For each of the reflexes, the greatest reduction in RF area (approximately 30–40%) occurred following administration of the initial dose of SP (0.25 ng). This potent effect of SP on RF areas is similar to the potency of SP observed in animals not pretreated with 5-MeODMT (Fig. 3).

Discussion

The present experiments replicated and expanded earlier findings of Zemlan et al. (1983). Although scaling of the data was different from Zemlan's study, administration of the 5-HT agonists, 5-MeODMT and quipazine, resulted in significant and comparable expansion (i.e., per cent increase) of the RF areas of the three nociceptive withdrawal reflexes. Expansion of RF areas reflects an increased sensitivity to nociceptive somatosensory stimulation in the spinal animal following 5-HT agonist administration. The effects of the 5-HT agonists appeared mediated by central mechanisms rather than by ischemic or peripheral effects, since administration of the alpha-2 agonist clonidine HCl or the peripherally-acting 5-HT creatinine sulfate did not affect RF size.

The observed expansion of the reflex RF area following administration of the centrally-acting 5-HT agonists in spinal animals is opposite to the effects seen in intact animals, in which administration of 5-HT agonists results in behaviorally-defined analgesia (Basbaum et al. 1976; Yaksh and Wilson 1979). Thus, the present experiments support the hypothesis that 5-HT may differentially affect local spinal sensory input as opposed to supraspinal sensory input. The former system may subserve local spinal nociceptive reflexes and appears facilitated by the bulbospinal 5-HT system, whereas the latter system may relay nociceptive input supraspinally and be inhibited by the descending 5-HT system.

The present studies also demonstrate that intrathecal administration of SP results in expansion of the RF area of three nociceptive reflexes in the spinal animal. This work is consistent with earlier studies employing intact animals which indicated that SP released from primary afferents in response to capsaicin results in behaviorally-defined hyperalgesia in rats (Jhamandas et al. 1984; Moochhala and Sawynok 1984). Therefore, the present study suggests that increased sensitivity of nociceptive reflexes following intrathecal administration of SP and hyperalgesia resulting from SP released from primary afferents may involve similar mechanism(s).

The present studies are also the first to examine the interactive effects of 5-HT and SP on spinal nociceptive reflexes, independent of descending control. In animals pretreated with 5-MeODMT, intrathecal administration of SP reversed the 5-HT-induced expansion of reflex RF areas. These results are consistent with results obtained in the intact animal (Yaksh et al. 1979) in which the effects of depletion of primary afferent SP by capsaicin were compared to destruction of descending raphe-spinal SP neurons by 5,6-dihydroxytryptamine (5,6-DHT) injections. Although both treatments produced a 40–50% reduction in SP in the spinal cord, the former treatment resulted in analgesia whereas the latter treatment resulted in hyperalgesia using hot plate and tail-flick latency as measures. The authors concluded that the primary afferent and raphe-spinal SP systems exert opposite effects on pain. Consistent with these data, the present studies support the hypothesis that the primary afferent SP system has a facilitatory effect on pain transmission. However, in the present study administration of SP, only in the presence of a 5-HT agonist, resulted in analgesia. Thus, it appears that a functional interaction between the bulbospinal SP and 5-HT systems, not the influence of the descending SP system alone, inhibits spinal pain transmission. Further, since no 5-HT receptors are localized on capsaicin-sensitive primary afferents (Singer and Sperk 1980) it is unlikely that the interactive effects between SP and 5-HT occur at these primary afferents. Thus, the present studies suggest a physiological function for the SP and 5-HT neurons of the raphe magnus – namely, inhibition of spinal pain transmission involving postsynaptic receptors in the spinal cord innervated by the descending bulbospinal SP and 5-HT systems.

In summary, the present studies support the hypothesis that the descending 5-HT system facilitates local sensory input while inhibiting supraspinal sensory input. Further, administration of SP alone facilitates spinal pain transmission probably involving mechanisms related to primary afferents. However, in animals pretreated with 5-HT agonists, SP demonstrates an analgesic effect inhibiting the 5-MeODMT-induced facilitation of pain transmission. This second mechanism of action may be related to the descending SP and 5-HT systems interactively affecting postsynaptic spinal neurons.

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