Pharmacology Biochemistry & Behavior, Vol. 26, pp. 445–451. Copyright © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Monoamine and Opioid Interactions in Spinal Analgesia and Tolerance[1]

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LOOMIS, C. W., K. JHAMANDAS, B. MILNE AND F. CERVENKO. *Monoamine and opioid interactions in spinal analgesia and tolerance.* PHARMACOL BIOCHEM BEHAV 26(2) 445–451, 1987.—Noradrenergic and serotonergic neurons, originating in the brainstem and terminating in the dorsal horn, modulate the spinal processing of nociception. The inhibitory effects of norepinephrine (NE) and serotonin (5-HT) on elements of nociceptive transmission may be direct, or secondary to the release of neuromodulators such as opioid peptides. Two major criteria have been used in pharmacological studies of spinal opioid and monoamine interactions: the ability of opioid antagonists to attenuate the antinociceptive effects evoked by stimulating the release of endogenous NE and 5-HT in the lumbar spinal cord, or by the intrathecal injection of exogenous NE and 5-HT; and the development of cross tolerance between opioids and each of NE and 5-HT. Evidence regarding the spinal interaction between opioids and monoamines in mediating behavioural analgesia is reviewed. Recent results from this laboratory indicate that IT (−)naloxone but not (+)naloxone produces dose-dependent antagonism of IT NE-induced antinociception in the rat. This effect was not due to hyperalgesia. In rats made tolerant to spinal morphine using continuous IT infusion, the antinociceptive effect of continuous IT NE was significantly attenuated. However, no cross tolerance was observed between morphine and 5-HT. Observations from a variety of studies support the hypothesis of a spinal opioid link which contributes, in part, to NE-induced antinociception. However, this interaction remains to be conclusively established.

Spinal analgesia Monoamines Opioids Norepinephrine Serotonin Morphine Naloxone Cross tolerance

THE identification of multiple opioid binding sites in the substantia gelatinosa of the spinal cord [9, 17, 25, 32] raised the possibility that both exogenous and endogenous opioids could interfere with the processing of nociceptive information directly in the spinal cord. Electrophysiological studies using microiontophoretic application of opioids to discrete areas of the spinal cord demonstrated the potent depressant effects of these drugs on dorsal horn neurons activated by noxious stimuli [4, 8, 18]. These effects could be blocked by opioid antagonists. It became apparent that opioids, at appropriate doses, could selectively depress the activity of small diameter afferent fibres without affecting large diameter fibres.

A major question regarding electrophysiological studies of spinal cord neurons was whether these results had relevance to the analgesic actions of opioids. Chronic catheterization of the subarachnoid space in a number of animal species made it possible to apply these drugs to the spinal cord in conscious animals [41]. Using this technique, it has been repeatedly shown that exogenous opioids produce dose-dependent, stereospecific, behavioural analgesia which can be blocked by naloxone [42, 43, 45]. Anatomical studies have also shown that enkephalin-immunoreactive nerve terminals make direct synaptic contact with the soma and dendrites of dorsal horn thalamic projection neurons [29]. It is now clear that opioids produce profound antinociception at the spinal level in addition to their effects at supraspinal sites. Electrophysiological, anatomical and pharmacological data, which have been extensively reviewed elsewhere [36,39], are consistent with the hypothesis that opioids regulate the processing of nociceptive information directly in the spinal cord.

Monoaminergic neurons, originating in medullary and pontine nuclei, and terminating in the dorsal horn of the spinal cord, have also been implicated in the spinal modulation of noxious stimuli (see reviews [3,44]). Noradrenergic innervation of the spinal cord seems to arise exclusively from supraspinal sites. Recent studies with two specific retrograde transport techniques suggest that, in the rat, the majority of these fibres originate from pontine cell groups. These include the locus coeruleus, subcoeruleus, the Kollicker-Fuse nucleus and the medial and lateral parabrachial nuclei [34]. Spinopetal projections from medullary nuclei seem to be composed primarily of epinephrine containing neurons. The nucleus raphe magnus (NRM), located in the midline of the rostral ventral medulla, is the major source of descending 5-HT neurons. Pharmacological and electrophysiological studies have shown that focal electrical or chemical stimulation (e.g., microinjection of morphine or glutamate) of these brainstem nuclei depress the discharge of dorsal horn

[1]Supported by the Medical Research Council of Canada.

neurons driven by noxious stimulation, and effect significant behavioural analgesia [16, 35, 44]. These effects can be blocked by the depletion of spinal NE and 5-HT, or by appropriate receptor antagonists. The analgesia produced by the stimulation of these nuclei is associated with increased release of NE and 5-HT in the lumbar subarachnoid space [11]. Furthermore, the effects of brainstem stimulation can be mimicked by the iontophoretic administration of NE or 5-HT in anesthetized animals or by intrathecal (IT) injection in conscious animals [12, 26, 27, 37, 38].

Based on pharmacological studies which have been previously reviewed [3,37], it is clear that NE and 5-HT evoke significant antinociception by activating α-adrenoceptors and serotonergic receptors in the dorsal horn of the spinal cord, respectively. The ability of these agonists to produce spinal analgesia via distinct receptors, theoretically makes such agents useful for maintaining spinal analgesia during opioid tolerance and dependence while avoiding the consequences of opioid withdrawal. The objective of this review is to examine some of the current information regarding the interaction between monoamines and opioids in the spinal cord in mediating analgesia.

NALOXONE ANTAGONISM OF SPINAL MONOAMINE-INDUCED ANTINOCICEPTION

A major criterion in determining the spinal interaction between opioids and monoamines is the ability of naloxone to antagonize the antinociceptive effects evoked by stimulating endogenous monoaminergic spinal pathways, or by the intrathecal injection of exogenous monoamines in the lumbar spinal cord. Table 1 summarizes the results obtained with naloxone in previous investigations of endogenous and exogenous monoamine-induced antinociception.

In two early studies, the systemic injection of naloxone reduced or abolished the antinociceptive effect evoked by electrical stimulation of the periaqueductal grey (PAG) in the rat, and the inferior raphe nucleus of the cat [1,23]. It was suggested that stimulation in these sites caused the release of endogenous opioids which contributed to the observed analgesia. In the rat and the cat, the nucleus raphe magnus (NRM) and nucleus reticularis paragigantocellularis (NRPG) of the ventolateral medulla make significant contributions to the spinal dorsal horn via the dorsolateral funiculus (DLF) [2,19]. In the rat, low threshold electrical stimulation (<10 μA) of the NRM or the NRPG significantly elevated tail-flick latency; an effect antagonized by the systemic injection of naloxone [47]. Other brainstem sites requiring higher threshold stimulation to produce behavioural analgesia (e.g., nucleus reticularis gigantocellularis) were not antagonized by naloxone. These data suggest that endogenous opioids, either in descending bulbospinal neurons or in segmental spinal interneurons, and released during stimulation of the NRM or NRPG, were contributing to the observed analgesia. In a related study, low threshold electrical stimulation in the rostral ventromedial medulla (VMM) in the rat, which includes the NRM and NRPG, inhibited the tail-flick response to noxious heat [46]. This effect was attenuated by the IT injection of naloxone into the lumbar spinal cord but not by IT naloxone in the cervical spinal cord or by systemic naloxone. Naloxone alone had no effect on baseline tail-flick latency. These results are in agreement with the previous investigation, implicating the involvement of endogenous opioids in the behavioural analgesia observed with VMM stimulation. In this regard, naloxone has been shown to partially antagonize the inhibition of dorsal horn cell activity evoked by electrical stimulation of the VMM in the rat [28]. Previous studies showing dense localization of opioid receptors and enkephalin-immunoreactive nerve terminals in the dorsal horn provide a possible anatomical substrate for this opioid-mediated analgesia.

Studies using chemical stimulation of brainstem nuclei have also provided evidence for the participation of endogenous opioids in spinal analgesia. The focal injection of the excitatory amino acid glutamate into the rat PAG or VMM was reported to significantly increase tail-flick and hot-plate latency [14]. In the tail-flick test, pretreatment with IT naloxone antagonized the effect of glutamate in the VMM but not in the PAG. Intrathecal methysergide or phentolamine antagonized the effect of glutamate in the PAG and in the VMM. None of the IT antagonists reduced the effect of brainstem glutamate in the hot-plate test. These results are consistent with the hypothesis that activation of cells in the VMM inhibit the spinal processing of nociceptive information by a mechanism involving release of endogenous opioids in the spinal cord. Similar results have been reported using microinjections of morphine to activate opiate receptors in the PAG, NRM or the NRPG [15]. Intrathecal naloxone antagonized but did not abolish the elevation in tail-flick latency evoked by morphine in the NRM or the NRPG, but had no effect on morphine-induced antinociception in the PAG. The effect of morphine in the NRM on tail-flick latency was maximally antagonized by IT methysergide; the effect of morphine in the NRPG was maximally antagonized by IT phentolamine. Intrathecal methysergide and phentolamine were equally effective in attenuating the increase in tail-flick produced by morphine in the PAG. The reduction in morphine-induced antinociception produced by the IT

TABLE 1

SUMMARY OF STUDIES EXAMINING THE EFFECT OF NALOXONE ON STIMULATION-PRODUCED ANTINOCICEPTION AND ON SPINAL MONOAMINERGIC ANTINOCICEPTION

Dose and Route of Naloxone	Species	Method and Site of Stimulation	Effect of Naloxone	Ref.
1.0 mg/kg IP	Rat	Electrical—PAG	+	[1]
0.3 mg/kg IM	Cat	Electrical—IRN	+	[23]
5.0 mg/kg IP	Rat	Electrical—NRM	+	[47]
		Electrical—NRPG	+	
15–25 μg IT	Rat	Electrical—VMM	+	[46]
10 μg IT	Rat	Glutamate—PAG	−	[14]
		Glutamate—VMM	+	
10–20 μg IT	Rat	Morphine—PAG	−	[15]
		Morphine—NRM	+	
		Morphine—NRPG	+	
2.0 mg/kg IP	Cat	Serotonin—IT	−	[38]
	Rabbit	Serotonin—IT	−	
2.0 mg/kg IP	Rat	Norepinephrine—IT	−	[26]
2.0 mg/kg IP	Rat	Norepinephrine—IT	−	[27]
1.0 mg/kg IM	Monkey	Clonidine—IT	−	[40]

IRN—Inferior raphe nucleus; NRM—Nucleus raphe magnus; NRPG—Nucleus reticularis paragigantocellularis; PAG—Periaqueductal grey; VMM—Ventromedial medulla.

antagonists was not due to physiological antagonism (e.g., hyperflexia). There were obvious differences in the ability of each antagonist to reverse the effects of morphine in the PAG, NRM and NRPG. These data indicate that the activation of opioid receptors in the NRM and NRPG results in excitation of spinal monoamine and opioid receptors which modulate thermally evoked, spinally mediated reflexes.

Overall, these studies provide evidence that both monoamines and endogenous opioids play a significant role in mediating the behavioural analgesia evoked by stimulation of monoaminergic brainstem nuclei. While it has been shown that electrical stimulation of the NRM and NRPG increases the efflux of endogenous 5-HT and NE from the spinal cord [11], increased release of endogenous opioid peptides from the spinal cord has not yet been shown. The release of endogenous opioids in the spinal cord could involve the activation of a direct spinopetal enkephalinergic system or the activation of intrinsic spinal enkephalinergic interneurons via descending monoaminergic neurons. Anatomical evidence supporting the latter possibility has been reported in the descending 5-HT system of the cat. Using combined ultrastructural localization of enkephalin immunoreactivity and [^{3}H] 5-HT uptake sites, 5-HT containing axons were shown to contact enkephalin immunoreactive cell bodies and small dendrites in the dorsal horn [10]. The radiolabelled 5-HT nerve terminals presumably were derived from the NRM. Comparable studies of the anatomical relationship between the descending noradrenergic and spinal opioid neurons have not been reported. There is also anatomical evidence that some 5-HT neurons projecting from the raphe to the spinal cord contain one or more peptides, including enkephalin, as cotransmitters [6]. The functional significance of this observation is unknown, but if 5-HT and opioid peptides are co-contained in the same raphe-spinal neurons, this may explain the opioid component of raphe stimulation produced analgesia. On the other hand, not all raphe-spinal neurons are serotonergic and these peptides may be located in non-serotonergic fibres [30].

If monoaminergic neurons do make synaptic connections with enkephalin containing spinal interneurons, IT naloxone would be expected to antagonize the effects evoked by the IT injection of NE or 5-HT in the lumbar spinal cord. In an early study, naloxone (2 mg/kg IP) injected 10 minutes before IT 5-HT, had no effect on baseline response latencies or on 5-HT-induced spinal analgesia (measured as either the maximum percent effect (MPE) or as the area under the MPE vs. time curve) [38]. This dose of naloxone was previously shown to antagonize the antinociceptive effects of IT morphine [42]. Systemic naloxone was reported to have no effect on the maximum increase in tail-flick and hot-plate latency evoked by IT NE in the rat [26]. In this study, naloxone (2 mg/kg IP) was injected 10 minutes after IT NE. Using a similar protocol, systemic naloxone did not antagonize the increase in tail-flick and hot-plate latency recorded 30 minutes after the IT injection of NE [27]. Naloxone (1 mg/kg IM), injected after IT clonidine or IT ST-91 (a polar analogue of clonidine), did not antagonize the elevation in shock titration threshold in the monkey [40].

The failure of naloxone to antagonize the hot-plate effect of IT NE or 5-HT is in keeping with the results observed using focal brainstem stimulation. The failure of naloxone to antagonize the effect of IT 5-HT or NE on tail-flick latency must be interpreted with caution. In these studies, there was only a 4 second range between baseline and the cutoff time in the tail-flick test. Furthermore, the dose of IT NE which was used yielded a response latency just below the cutoff. As shown in experiments using stimulation produced analgesia, IT naloxone only partially antagonizes the effects of NRM and NRPG activation. If the effects of IT 5-HT or NE on thermally evoked spinal reflexes are only mediated, in part, by the release of endogenous opioids, then naloxone antagonism may not have been detected under these conditions. It is also noteworthy that pretreatment with naloxone is required to reverse some of the analgesic effects of supraspinal morphine and may be required for the reversal of IT monoamine analgesia [20].

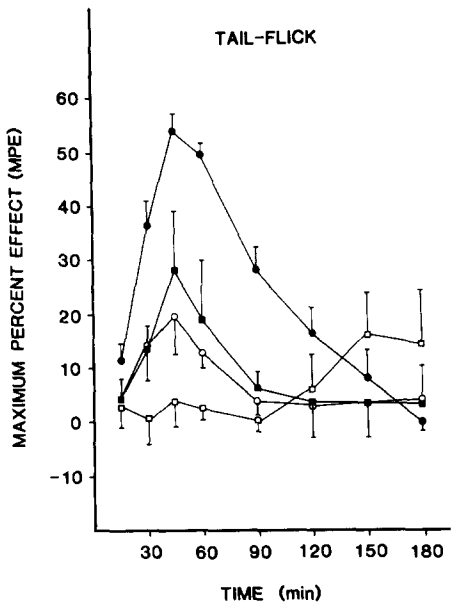

FIG. 1. The effect of intrathecal naloxone on the antinociceptive activity of intrathecal norepinephrine (NE) in the tail-flick test. Naloxone (2, 4 or 10 μg) was infused through an intrathecal catheter terminating in the lumbar subarachnoid space immediately before the intrathecal injection of norepinephrine (15 μg). Data are expressed as the mean±S.E.M. of 5–10 rats. Saline Vehicle + NE 15 μg (●), (−)Naloxone 2 μg + NE 15 μg (○), (−)Naloxone 4 μg + NE 15 μg (■), (−)Naloxone 10 μg + NE 15 μg (□).

To determine if pretreatment with IT naloxone could antagonize the antinociceptive effect of IT NE, rats were injected with naloxone (2, 4 or 10 μg IT) immediately before the IT injection of NE (15 μg). The time-course of tail-flick latency was then determined from 0 to 180 min. Dose-dependent antagonism was observed with (−)naloxone (Fig. 1), but not (+)naloxone (data not shown). Naltrexone (2.5 μg) was also found to significantly attenuate the effect of IT NE on tail-flick latency (data not shown). The apparent antagonism of NE was not due to hyperalgesia since baseline latencies remained unchanged following IT naloxone or naltrexone alone. Similar results have been observed with naloxone and NE using the tail pinch test (Dr. Y. Kuraishi, personal communication). These data suggest that NE-induced spinal analgesia is mediated, in part, by endogenous opioid peptides which interact with spinal elements involved in the processing of noxious information. While it is possible that IT NE may activate α-adrenoceptors on spinal opioid interneurons to increase

TABLE 2

SUMMARY OF CROSS-TOLERANCE STUDIES BETWEEN MORPHINE AND α-ADRENERGIC AGONISTS WITH RESPECT TO ANALGESIA

Adrenergic Drugs	Species	Analgesia Tests	Cross-Tolerance to Morphine	Ref.
Systemic				
Clonidine (SC)	Rat	Electrical Threshold Vocalization	+	[24]
Clonidine (SC)	Mouse	Tail-Flick Test	−	[31]
Oxymetazoline (IP)	Mouse	Acetic Acid	+	[5]
Clonidine (IP)		Writhing Test	+	
Norepinephrine (IP)			−	
Spinal				
ST-91 (IT)	Rat	Hot-Plate Test Tail-Flick Test	−	[33]
ST-91 (IT)	Monkey	Shock Titration Threshold	−	[40]
Norepinephrine (IT)	Rat	Tail-Flick Test	+	[22]
Clonidine (IT)	Human	Clinical Pain	+	[7]

+Indicates cross-tolerance.
−Indicates no cross-tolerance.

the release of opioid peptides, there is evidence that NE can also influence the disposition of opioid peptides and presumably enhance their antinociceptive effect. Monoamines, including NE and 5-HT, have been reported to inhibit the *in vitro* degradation of Met-enkephalin by the soluble fraction of human brain cortex and by rat striatum with IC_{50} values ranging from 60–200 μM and 260–400 μM for NE and 5-HT, respectively [13]. The effect of NE and 5-HT on the spinal degradation of Met-enkephalin has not been reported. As the role of specific peptidase enzymes in the metabolism of opioid peptides becomes known, and as specific inhibitors of these peptidases are identified, it will be possible to do further *in vitro* studies and to investigate the influence of monoamines on endogenous opioid disposition *in vivo* and its corresponding effect on spinal analgesia. If the biogenic amines have a regulatory role in the metabolism and disposition of opioid peptides, this may partially explain their apparent spinal interaction with opioids in mediating analgesia.

CROSS-TOLERANCE BETWEEN MONOAMINES AND OPIOIDS

Cross-tolerance studies have also been used to investigate the interaction between opioids and monoamines in mediating behavioural analgesia. The results of some of investigations using both chronic systemic and IT administration are summarized in Table 2. In naive rats, the SC administration of clonidine enhanced the antinociceptive effect of SC morphine in the electrical vocalization threshold test [24]. However, when increasing doses of SC clonidine were given to rats for 7 days, there was a significant reduction in the antinociceptive effect of acute SC clonidine or morphine in the same test. In naive mice, SC clonidine potentiated the SC morphine-induced elevation in tail-flick latency [31]. In the same study, tolerance to morphine was observed in mice implanted with morphine pellets for 4 days but there was no cross-tolerance to clonidine. The morphine pellets were not removed prior to the test. A modified abdominal constriction test was used to investigate the antinociceptive effects of α-agonists and their interactions with opioids [5]. In mice pretreated with IP clonidine or oxymetazoline, marked cross tolerance to IP morphine was observed. Tolerance to morphine occurred in morphine pretreated mice but there was little cross tolerance to clonidine or oxymetazoline. Considering the diversity of drugs, doses, species, analgesia tests, routes and duration of administration that were used in these studies, it is not surprising that conflicting results were observed. Nevertheless, it is clear that there are major differences in the interaction between morphine and α-adrenoceptor agonists after acute versus chronic administration.

Since all drugs were given by systemic administration in the studies cited above, the spinal and supraspinal contributions to the observed analgesia and cross-tolerance cannot be distinguished. To investigate the extent of cross-tolerance between morphine and ST-91 in the rat, drugs were injected in the lumbar spinal cord via chronically implanted IT catheters [33]. The effect of ST-91 on tail-flick latency was determined in naive and morphine-tolerant animals. Tolerance to morphine was induced with daily IT injections of morphine. In this study, no significant difference in the effect of ST-91 was observed between the two groups. However, in animals recovering from morphine tolerance, daily injections of IT ST-91 delayed recovery as compared with IT saline-treatment. In monkeys given daily IT morphine injections to induce tolerance, the IT injection of clonidine significantly elevated the shock titration threshold [39]. In this same study, the IT coadministration of inactive doses of ST-91 and morphine resulted in near maximal analgesia. The development of tolerance to the analgesic

effect of this combination was also delayed compared with tolerance to IT morphine alone. It was suggested that the behavioural analgesia observed with the IT injection of exogenous NE is unlikely to result from the activation of spinal opioid interneurons.

Continuous IT infusion via ALZET® mini-osmotic pumps has also been used to induce spinal tolerance to opioids and to test for cross tolerance to monoamines [21,22]. We have developed a model using sequential spinal infusions of antinociceptive agents to examine the effect of monoamines on nociception after induction of opioid tolerance and dependence in the rat. The time course of analgesia and tolerance during continuous IT morphine infusion is similar to that reported with daily IT morphine injection using the tail-flick test [45]. However, we have shown that the slow infusion rate of mini-osmotic pumps (0.5–1.0 μl/hr) limits the rostral and caudal migration of dye from the tip of the lumbar IT catheter even after 6 days of infusion [21]. Furthermore, animals tolerant to the antinociceptive effect of spinal morphine following continuous IT infusion do not show cross tolerance to systemic morphine [21]. These results suggest that continuous IT infusion is particularly useful for studying the spinal effects of chronic drug administration since the contribution of supraspinal effects are minimized.

Using this double-pump technique, we have shown that the elevation in tail-flick latency produced by NE is significantly attenuated in morphine-tolerant rats as compared with saline-treated rats [22]. The tolerance to NE, and the apparent cross-tolerance to morphine was not due to oxidation of NE in the mini-osmotic pump. In contrast, the analgesic action of IT morphine was not significantly reduced in NE-tolerant rats as compared to saline-treated animals [21]. In this regard, it was recently reported that the increase in paw pressure threshold produced by 2 or 10 μg IT morphine was not significantly affected by 90% depletion of spinal cord NE [24a]. These results suggest that the local infusion of morphine in the lumbar spinal cord does not produce behavioural analgesia by activation of local adrenergic systems. On the other hand, NE locally infused into the lumbar spinal cord of the rat may activate endogenous opioid containing neurons to produce behavioural analgesia. Experiments using continuous IT infusion are currently in progress to determine if the antinociceptive effects of α1- or α2-selective adrenergic agonists are reduced in morphine-tolerant rats.

While NE-induced spinal analgesia seems to involve an opioid component, this same component is apparently lacking in 5-HT-induced spinal analgesia. No cross-tolerance was observed between morphine and 5-HT following continuous IT infusion using the tail-flick test. Figure 2 shows the time-course of analgesia during the continuous IT infusion of morphine for 4 days followed by 5-HT, and saline for 4 days followed by 5-HT. For morphine infusion, the tail-flick latency was significantly greater than control at all time points with maximum antinociception on day 2. Tolerance, as indicated by a decline in response latency during IT infusion, was apparent by day 3. The continuous IT infusion of 5-HT to saline- and morphine-pretreated rats produced a significant and prolonged increase in response latency. Area under the curve (AUC) determinations of the analgesia vs. time during 5-HT infusion were not significantly different in the two groups. These data suggest that 5-HT-induced spinal analgesia is not mediated by the release of spinal opioid peptides but rather, is a direct effect on elements which process nociceptive information in the spinal

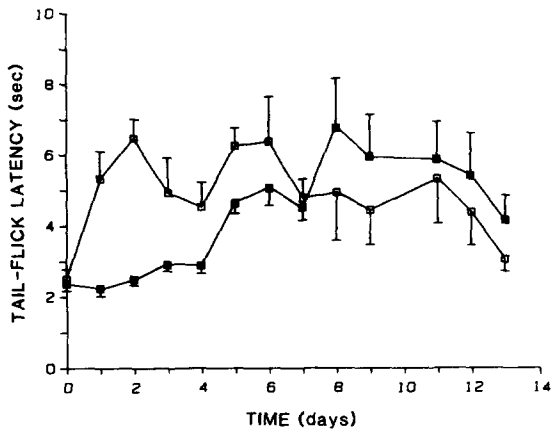

FIG. 2. The effect of continuous intrathecal infusion of: (1) Morphine (□, 10 μg/hr) for 4 days followed by serotonin (□, 53 μg/hr) for 7 days on tail-flick latency, and (2) Saline (■, 1 μl/hr) for 4 days followed by serotonin (■, 53 μg/hr) for 7 days on tail-flick latency. Data are expressed as the mean±S.E.M. of 5 rats. Area under the curve determinations of the tail-flick latency versus time curve during serotonin infusion were 40.1±14.0 and 41.6±11.8 (mean±S.D.) for groups 1 and 2, respectively.

cord. Thus, while there is some anatomical evidence supporting an opioid link in 5-HT induced spinal analgesia, the limited pharmacological evidence available does not support this hypothesis. In contrast, pharmacological studies suggests that there is an opioid link in NE-induced spinal analgesia but the corresponding anatomical substrate has not been demonstrated.

The experiments described above have involved the continuous IT infusion of morphine and their interaction with exogenously administered monoamines. In future studies, it will be important to determine if cross tolerance with the monoamines is observed following the continuous IT infusion of δ and κ receptor selective agonists. In addition, it would be useful to know whether, after continuous IT opioid infusion, there is an altered response to the antinociceptive action of endogenous monoamines released by stimulation of descending bulbospinal neurons.

The presence of an opioid link and its contribution to the antinociceptive effects of spinal monoamines has not been conclusively established. Observations from a variety of studies support the hypothesis of an opioid link but, as indicated in this review, inconsistent and conflicting results have been reported. Pharmacological studies have focussed primarily on the ability of systemic and IT naloxone to antagonize the behavioural analgesia evoked by endogenous and exogenous spinal monoamines, and the extent of cross-tolerance between each of the monoamines and morphine. In view of diversity of peptides and neurotransmitters which are located in the dorsal horn of the spinal cord, both as cotransmitters and in separate neurons, and the fact that when released, these may affect different target cells or modify the same dorsal horn neurons differently, inhibition of nociceptive transmision in the spinal cord is probably very complex. Clearly, the concerted efforts of anatomists, electrophysiologists and pharmacologists will be required to elucidate the nature of monoamine and peptide spinal interactions, including those with the endogenous opioids.

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