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Serotonin Contributes to the Spinal Antinociceptive Effects of Morphine

TERRIANN CRISP, JANET L. STAFINSKY, MARC URAM, VEERAIAH C. PERNI, MICHAEL F. WEAVER AND LINDA J. SPANOS

Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

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CRISP, T., J. L. STAFINSKY, M. URAM, V. C. PERNI, M. F. WEAVER AND L. J. SPANOS. *Serotonin contributes to the spinal antinociceptive effects of morphine*. PHARMACOL BIOCHEM BEHAV **39**(3) 591–595, 1991.—This study was designed to determine if morphine administered intrathecally (IT) interacts with serotonergic or noradrenergic nerve terminals in the spinal cord to produce analgesia on the spinally mediated tail-flick test. Male Sprague-Dawley rats were fitted with IT catheters. One week later, animals were spinally pretreated with receptor antagonists selective for opioid, serotonin or α-adrenoceptors, and the ability of these agents to alter spinal morphine-induced antinociception was assessed. Morphine dose-dependently elevated tail-flick latency in a naltrexone-reversible manner. The serotonin receptor antagonists spiroxatrine (5-HT_{1A}), pindolol (5-HT_{1B}), ritanserin (5-HT_2) and ICS 205-930 (5-HT_3) attenuated the spinal analgesic effects of morphine. In contrast, the α_1 and α_2-adrenoceptor antagonists prazosin and yohimbine, respectively, did not alter morphine-induced elevations in tail-flick latency. These data substantiate earlier reports that spinal morphine-induced antinociception relies on an opioid receptor-mediated component in addition to a local serotonergic component. The finding that the α-adrenoceptor antagonists did not alter the antinociceptive effects of IT morphine suggests that spinal norepinephrine does not contribute to the analgesic effects of the opiate.

Analgesia Antinociception Intrathecal Morphine sulfate Norepinephrine Serotonin Spinal cord

IT is well established that opiates interact with opioid receptors on primary afferent nerve terminals in the spinal cord to inhibit nociceptive transmission (18). In addition to an opioid receptor-mediated component underlying the antinociceptive effects of opiates, a number of studies has demonstrated that neurotransmitters such as serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE) contribute to the full expression of opiate analgesia (1, 3, 15). For instance, when morphine was systemically injected in spinally transected rats, an elevation in tail-flick latency (TFL) ensued which was opioid-mediated (naloxone-reversible) and serotonergically mediated [methysergide-reversible; (11)]. Additionally, morphine administered into the periaqueductal gray region of the rat midbrain produced elevations in TFL that were reversed by intrathecal (IT) injections of methysergide and phentolamine (16). Studies such as these were among the first to identify a monoaminergic component contributing to the systemic and supraspinal antinociceptive action of opiates (15).

Recently, an attempt was made to study the relative involvement of spinal serotonergic and noradrenergic neuronal systems in the analgesic action of opiates administered IT (1,5). β-Endorphin is a potent epsilon opioid receptor agonist and, when injected IT in rats, produces a robust analgesic response on the tail-flick test (17). When 5-HT and α-adrenoceptor antagonists were injected into the rat subarachnoid space prior to IT β-endorphin, the antinociceptive effects of the opioid were inhibited (3). Similarly, morphine-induced spinal analgesia was attenuated by subcutaneously injected methysergide (1) but not by α-adrenoceptor antagonists (5). An investigation was recently made of the spinal antinociceptive properties of the selective μ and δ opioid receptor agonists DAMPGO [(D-Ala^2,N-methyl-Phe^4,Gly^5-ol) enkephalin] and DPDPE [(D-Pen^2,D-Pen^5) enkephalin], respectively. Following IT administration, both of these opioid peptides produced a naloxone-reversible analgesic response which was not mediated by local spinal 5-HT or NE (12). Apparently, some opiates rely on a monoaminergically mediated link in the spinal cord to produce analgesia (1, 3, 11, 15), whereas others do not (12).

Morphine interacts with at least three different opioid receptors, namely mu (μ), delta (δ) and kappa (κ) sites (13), while DAMPGO and DPDPE are more selective for μ and δ opioid receptors, respectively. Perhaps the preferential selectivity of an opiate for a particular opioid receptor determines the potential involvement of 5-HT or NE in the spinal analgesic effects of the opiate. The purpose of the present study was to determine the extent to which monoaminergic influences may contribute to morphine-induced spinal analgesia. Rats were pretreated with selective 5-HT or NE receptor antagonists IT, and the ability of these agents to alter the spinal effects of morphine was evaluated on the tail-flick analgesiometric assay. The data suggest that an opioid-mediated component and a serotonergically mediated component contribute to the spinal antinociceptive effects of morphine.

METHOD

Intrathecal Cannulation Procedure

Male Sprague-Dawley rats (350 ± 50 g) were anesthetized with ketamine and chronically implanted with IT polyethylene

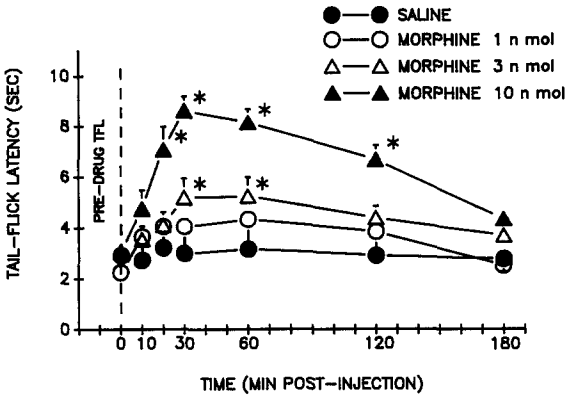

FIG. 1. Dose-response and duration of action for intrathecal morphine. Values represent the TFL means ± S.E.M. of at least 6 rats per treatment group. *$p<0.05$ versus IT saline control values.

(PE-10) catheters (6) to allow for the spinal injection of morphine, naltrexone and the various 5-HT and NE receptor antagonists. The PE-10 cannula was inserted through a slit in the atlanto-occipital membrane and passed distally approximately 8.5 cm to the rostral edge of the lumbar enlargement. Rats were allowed at least seven days to recover from the intrathecal surgery, and only those animals that displayed unimpaired motor function after spinal catheterization were used in the experiments. An interval of at least two weeks was maintained between repeated injections, and rats were not injected more than twice. After the second injection, rats were killed and cannula placement was checked using methylene blue dye.

Analgesiometric Test

The spinally mediated tail-flick test (Model 33 Tail-Flick Analgesia Meter; IITC Life Science Instruments) was used as the nociceptive measure. The rat's tail was blackened beforehand with India ink and placed in a depression over a photocell in the analgesic meter. The time (in tenths of s) required for the rat to remove its tail from the heat source was automatically determined and expressed as tail-flick latency (TFL). Four predrug TFL values were obtained at 10-min intervals and used to calculate individual predrug means. The heat source on the tail-flick meter was set at an intensity that would produce predrug values within a 2–4-s range. A 10-s maximum exposure to the heat source was employed as the cutoff time to preclude tissue damage to the tail during multiple measurements, and animals not responding within the allotted 10 s were assigned a TFL of 10.

Spinal Antinociceptive Properties of IT Morphine

Initial studies were conducted to determine the analgesic efficacy of different doses of morphine administered spinally in rats. Morphine was dissolved in physiological saline and administered in IT doses of 1, 3 and 10 nmol/10 μl saline (0.4, 1 and 3.8 μg, respectively). All IT injections were made using an infusion pump which was preset to deliver 10 μl of drug or saline per min (Braintree Scientific, Inc.). Each drug injection was followed by saline (10 μl) to flush the IT catheter. Drug-induced changes in TFL were recorded 10, 20, 30 and 60 min following IT drug injections. The dose-response and duration studies were carried out for 180 min (see Fig. 1).

To determine the involvement of opioid or serotonergic systems in spinal morphine-induced analgesia, IT doses (15 μg/10 μl) of the opiate antagonist naltrexone (40 nmol) or the 5-HT antagonists spiroxatrine (5-HT_{1A}; 40 nmol), pindolol (5-HT_{1B}; 60 nmol), ritanserin (5-HT_2; 31 nmol) or ICS 205-930 (5-HT_3; 53 nmol) were injected as pretreatments 10 min prior to a fixed analgesic dose of morphine (10 nmol). Data from preliminary studies demonstrated that these doses of naltrexone (present study) and the various 5-HT receptor antagonists effectively reversed the analgesic effects of morphine or 5-HT, respectively (2, 3, 12). Tail-flick latency values were recorded 10, 20, 30 and 60 min after morphine injection.

To determine if the analgesic properties of IT morphine relied upon a spinal noradrenergic component, another group of rats received IT pretreatments (15 μg) of the α_1-adrenoceptor antagonist prazosin (36 nmol) or the α_2-adrenoceptor blocker yohimbine (38 nmol) 10 min prior to IT morphine. The effects of the α-adrenoceptor antagonists on spinal morphine-induced elevations in TFL were assessed at 10, 20, 30 and 60 min after morphine injection. A separate group of animals received either prazosin or yohimbine alone, and these agents were tested for an ability to alter TFL by themselves.

Statistical Analyses

The effects of the opioid, 5-HT and NE receptor antagonists on the antinociceptive effects of IT morphine were compared to controls receiving IT saline 10 min prior to morphine injections. The IT pretreatment conditions (antagonists and saline controls) were also injected alone and tested for an ability to alter TFL by themselves. Results are expressed as TFL means ± S.E.M., and all data are derived from experiments having an n of at least 6 rats per treatment condition. Statistical analyses were done using a repeated-measures analysis of variance and a Dunnett's test, as applicable, to calculate significant differences between the means. Statistical significance was accepted at the 5% level ($p<0.05$).

Drugs

Naltrexone and spiroxatrine were obtained from commercial sources (Research Biochemicals, Inc.). Morphine sulfate (National Institute on Drug Abuse), ritanserin (Janssen Life Sciences Products), ICS 205-930 (Sandoz Pharmaceuticals) and phentolamine (CIBA-GEIGY) were graciously donated. Drugs were prepared daily in 0.9% saline (morphine, naltrexone and yohimbine) or dissolved in dimethyl sulfoxide (DMSO) and diluted to volume with saline (spiroxatrine, pindolol, ritanserin, ICS 205-930 and prazosin).

RESULTS

The Analgesic Effects of Morphine Administered Spinally

Morphine administered IT (1, 3 and 10 nmol/10 μl saline) dose-dependently elevated TFL in rats (Fig. 1). For instance, the 1 nmol dose of morphine (N = 6) did not significantly elevate TFL above saline control values, but the 3 nmol dose (N = 15) produced an antinociceptive response which was significantly greater than IT saline TFL values ($p<0.05$) at 30 and 60 min postinjection. The onset of action for the 10-nmol dose of morphine (N = 12) occurred within 20 min postinjection, and these effects were elevated above IT saline control TFL values for 120 min (Fig. 1).

To determine if the analgesic effects of IT morphine were opioid receptor-mediated, rats were pretreated with naltrexone

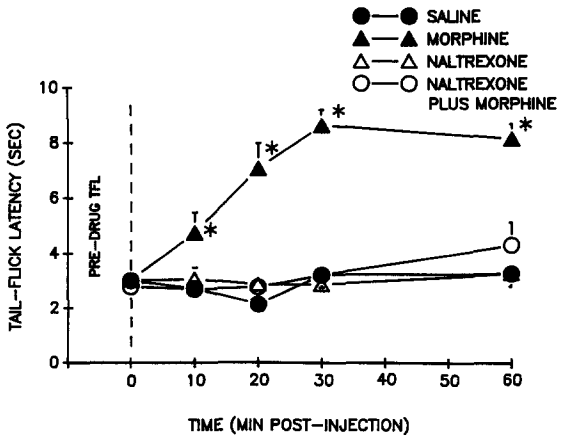

FIG. 2. Naltrexone (40 nmol) injected spinally 10 min prior to IT morphine (10 nmol) reversed the spinal antinociceptive effects of morphine. Each data point represents an n of 6–8 rats. Naltrexone produced no significant changes in TFL when administered alone. *$p<0.05$ compared to IT saline controls.

(40 nmol IT) 10 min prior to spinal morphine injections. The opiate antagonist reversed the antinociceptive response to morphine for 60 min postinjection (Fig. 2). When naltrexone was administered alone IT, TFL values were not significantly different from saline control values for 60 min postinjection.

Figure 3 depicts the inhibitory effects of spiroxatrine (40 nmol; N=7), pindolol (60 nmol; N=6), ritanserin (31 nmol; N=8) and ICS 205-930 (53 nmol; N=7) on morphine-induced spinal analgesia. The duration of action for the blocking effects of the 5-HT receptor antagonists on morphine analgesia varied. For example, the spiroxatrine-induced reversal of morphine analgesia lasted for 60 min after morphine injection, whereas the inhibitory effects of pindolol, ritanserin and ICS 205-930 lasted for 30 min after morphine injection (Fig. 3). Additionally, the effective inhibitory doses of the 5-HT antagonists produced no significant changes in the TFL for 60 min when injected alone.

When animals were pretreated with either prazosin or yohimbine 10 min before IT morphine, it was found that neither of the α-adrenoceptor antagonists altered morphine-induced elevations in TFL (Fig. 4). Additionally, the TFL response to these agents administered by themselves did not differ from the effects of IT saline for 60 min postinjection. These data further substantiate the lack of involvement of spinal noradrenergic neuronal systems in IT morphine-induced analgesia.

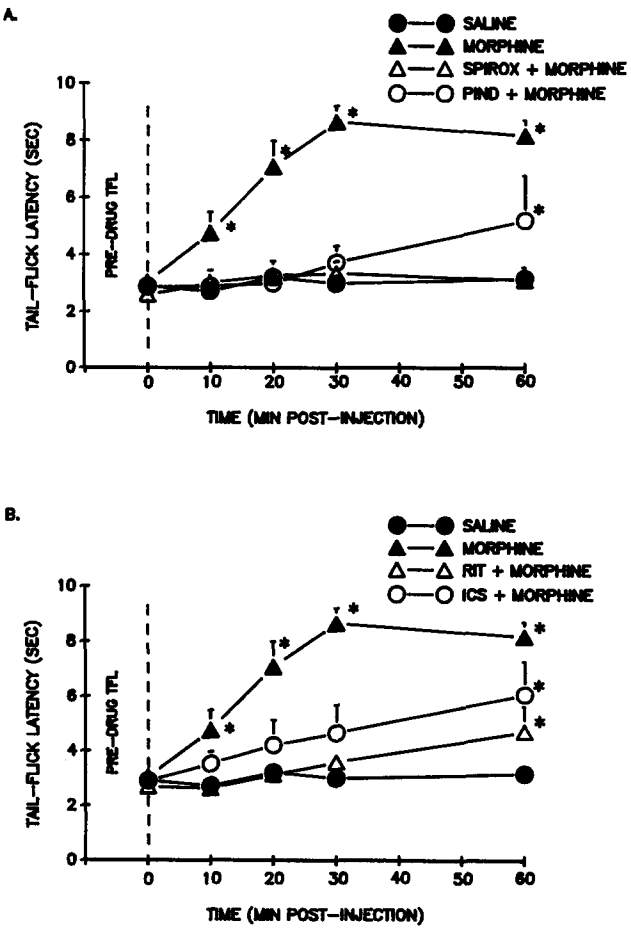

FIG. 3. The analgesic effects of IT morphine (10 nmol) were significantly attenuated by (A) the 5-HT_{1A} receptor antagonist spiroxatrine (40 nmol) and the 5-HT_{1B} blocker pindolol (60 nmol). Morphine-induced spinal antinociception was also decreased by (B) the 5-HT_2 antagonist ritanserin (31 nmol) and the 5-HT_3 receptor antagonist ICS 205-930 (53 nmol). N=6–8 rats per treatment condition. *$p<0.05$ versus morphine 10 nmol IT.

DISCUSSION

The purpose of the present study was to investigate the role of spinal 5-HT and NE in morphine-induced analgesia. The results substantiated earlier reports that morphine injected into the subarachnoid space of the rat spinal cord produces a dose-dependent elevation in TFL (1, 5, 18). Pretreatment with IT naltrexone reversed the spinal effects of morphine, suggesting that the opiate interacts with local spinal opioid receptors to produce analgesia following IT administration.

The demonstration that the 5-HT receptor antagonists also attenuated IT morphine-induced analgesia implies that morphine may interact with local spinal 5-HT nerve terminals to produce elevations in TFL (1, 5, 11). Recent studies have shown that μ_1 opioid receptors play an important role in the mediation of supraspinal analgesia, whereas μ_2 sites have been implicated in spinal analgesia (9,10). Morphine interacts with spinal μ_2 receptors as well as δ and κ sites (13), and it is possible that κ opioid receptors in the spinal cord mediate the 5-HT component that contributes to IT morphine-induced analgesia. For instance, a κ opioid agonist known as U-50,488H has been shown to elevate TFL and hot plate latency in mice, and this effect was antagonized by the 5-HT synthesis inhibitor p-chlorophenylalanine (14). In another recent study, the spinal effects of U-50,488H in mice were reversed by the 5-HT antagonists pindolol and methysergide, again suggesting that κ-mediated analgesia is dependent upon serotonergic mechanisms (4). Further evidence in favor of this concept was provided by the demonstration that μ and δ receptor opioids (DAMPGO and DPDPE, respectively) produced an antinociceptive response which was unaltered by 5-HT receptor antagonists (12). Taken together, these data suggest that the κ opioid receptor may mediate the 5-HT component that contributes to morphine-induced spinal analgesia.

It is not yet clear how morphine interacts with spinal 5-HT neuronal systems to produce analgesia, but it has been suggested that opiates interact with opioid receptors to enhance the release

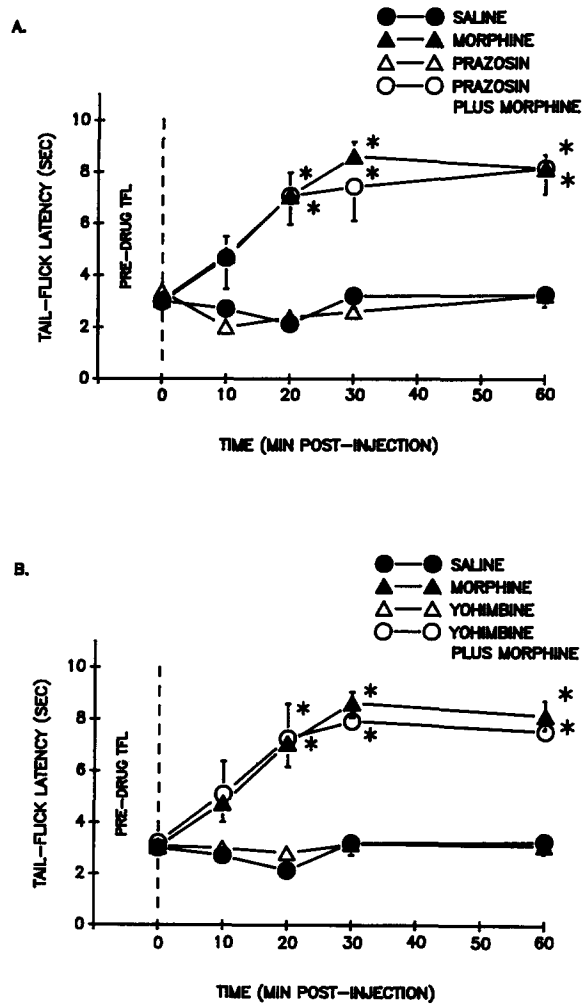

FIG. 4. Morphine-induced elevations in TFL were unaltered by (A) the α_1-adrenoceptor antagonist prazosin (36 nmol) or (B) the α_2-adrenoceptor blocker yohimbine (38 nmol). *$p<0.05$ versus spinal saline control TFL values. N = 6 rats per treatment condition.

of 5-HT from local spinal serotonergic nerve terminals (11). Subsequently, 5-HT may contribute to the full expression of morphine analgesia via an activation of spinal 5-HT receptor sites. Although 5-HT produces an elevation in TFL in rats following IT administration (1, 5, 19), investigations of the effects of opiates on [^{3}H]5-HT release from rat spinal cord synaptosomes have not confirmed a presynaptic 5-HT-releasing action of morphine on spinal 5-HT nerve terminals (7). Morphine may, however, activate spinal 5-HT nerves indirectly via interactions with other neurotransmitter systems that impinge upon 5-HT nerve terminals in the spinal cord (7,11). Thus, although a local spinal 5-HT component is apparently involved in mediating the antinociceptive effects of IT morphine, the mechanisms underlying this interaction have not been identified.

Since only one inhibitory dose of the individual 5-HT receptor antagonists (15 μg) was tested against IT morphine in the present study, no inference can be made regarding the 5-HT receptor subtype(s) responsible for mediating the 5-HT component contributing to the spinal actions of the opiate. Studies designed to assess individual ID_{50} values for the effects of the different 5-HT receptor antagonists on morphine-induced spinal analgesia are currently underway in this laboratory. Recent radioligand binding studies have demonstrated that "receptor-selective" 5-HT antagonists (e.g., spiroxatrine and ritanserin) also interact equipotently with μ, δ and κ opioid receptor subtype sites at high concentrations (8). In light of these findings, it is possible that the inhibitory actions of the 5-HT receptor antagonists on morphine analgesia in the present study were due to a naloxone-like blockade of spinal opioid receptors. However, the recent demonstration that the spinal antinociceptive effects of DAMPGO and DPDPE were unaltered by spiroxatrine, pindolol, ritanserin or ICS 205-930 (12) makes it unlikely that the 5-HT receptor antagonists are interacting with opiate sites to inhibit morphine-induced spinal analgesia.

When the α-adrenoceptor antagonists prazosin or yohimbine were injected IT as a pretreatment to morphine, the spinal analgesic effects of the opiate were unchanged. It was proposed earlier that the κ opioid site might be responsible for mediating the 5-HT link associated with the spinal analgesic action of morphine, and it is interesting that the antinociceptive effects of U-50,488H were also unchanged by prazosin and α_2-adrenoceptor antagonist idazoxan (4). In the present study, prazosin and yohimbine (at doses that effectively blocked the spinal antinociceptive effects of NE) failed to reverse morphine-induced analgesia. Taken together, these results provide evidence against a contribution of spinal NE to the analgesic effects of IT morphine (1, 5, 11).

In summary, the present data suggest that morphine produces its spinal antinociceptive effects by interacting with spinal opioid receptors. The finding that 5-HT receptor antagonists, but not α-adrenoceptor antagonists, reverse the analgesic effects of morphine suggests that a local spinal serotonergic link contributes to morphine-induced antinociception. Moreover, since the analgesic effects of κ agonists have been found to be strongly dependent upon the serotonergic system (4,14), it is possible that spinal κ opioid receptors mediate the 5-HT component involved in morphine-induced analgesia. Further studies are required to clarify the relationship between spinal opiate analgesia and the involvement of endogenous monoaminergic neurotransmitters.

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REFERENCES

1. Crisp, T.; Smith, D. J. A local serotonergic component involved in the spinal antinociceptive action of morphine. Neuropharmacology 28:1047–1053; 1989.
2. Crisp, T.; Stafinsky, J. L.; Donepudi, H. B.; Perni, S. C.; Spanos, L. J. Spinal serotonin-induced elevations in hot plate latency are reversed by the 5-HT_2 antagonist ritanserin. Pain 5(Suppl.):S162; 1990.
3. Crisp, T.; Stafinsky, J. L.; Hess, J. E.; Uram, M. Spinal β-endorphin analgesia involves an interaction with local monoaminergic systems. Eur. J. Pharmacol. 160:211–217; 1989.
4. Ho, B. Y.; Takemori, A. E. Serotonergic involvement in the antinociceptive action of and the development of tolerance to the kap-

pa-opioid receptor agonist, U-50,488H. J. Pharmacol. Exp. Ther. 250:508–514; 1989.
5. Kellstein, D. E.; Malseed, R. T.; Goldstein, F. J. Opioid-monoamine interactions in spinal antinociception: Evidence for serotonin but not norepinephrine reciprocity. Pain 34:85–92; 1988.
6. LoPachin, R. M.; Rudy, T. A.; Yaksh, T. L. An improved method for chronic catheterization of the rat spinal subarachnoid space. Physiol. Behav. 27:559–561; 1981.
7. Monroe, P. J.; Michaux, K.; Smith, D. J. Evaluation of the direct actions of drugs with a serotonergic link in spinal analgesia on the release of [^{3}H]serotonin from spinal cord synaptosomes. Neuropharmacology 25:261–265; 1986.
8. Monroe, P. J.; Perschke, S. E.; Crisp, T.; Smith, D. J. Evaluation of the interactions of serotonergic ligands with mu, kappa and delta opiate binding sites. Soc. Neurosci. Abstr. 16:851; 1990.
9. Pasternak, G. W. Multiple opiate receptors: Biochemical and pharmacological evidence for multiplicity. Biochem. Pharmacol. 35: 361–364; 1986.
10. Paul, D.; Bodnar, R. J.; Gistrak, M. A.; Pasternak, G. W. Different μ receptor subtypes mediate spinal and supraspinal analgesia in mice. Eur. J. Pharmacol. 168:307–314; 1989.
11. Pekoe, G. M.; Smith, D. J. The involvement of opiate and monoaminergic neuronal systems in the analgesic actions of ketamine. Pain 12:57–73; 1982.
12. Spanos, L. J.; Stafinsky, J. L.; Crisp, T. A comparative analysis of monoaminergic involvement in the spinal antinociceptive action of DAMPGO and DPDPE. Pain 39:329–335; 1989.
13. Takemori, A. E.; Portoghese, P. S. Evidence for the interaction of morphine with kappa and delta opioid receptors to induce analgesia in β-funaltrexamine-treated mice. J. Pharmacol. Exp. Ther. 243:91–94; 1987.
14. Von Voigtlander, P. F.; Lewis, R. A.; Neff, G. L. Kappa opioid analgesia is dependent on serotonergic mechanisms. J. Pharmacol. Exp. Ther. 231:270–274; 1984.
15. Wigdor, S.; Wilcox, G. L. Central and systemic morphine-induced antinociception in mice: Contribution of descending serotonergic and noradrenergic pathways. J. Pharmacol. Exp. Ther. 242:90–95; 1987.
16. Yaksh, T. L. Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effects of morphine in the periaqueductal gray. Brain Res. 160:180–185; 1979.
17. Yaksh, T. L.; Henry, J. L. Antinociceptive effects of intrathecally administered human β-endorphin in the rat and cat. Can. J. Physiol. Pharmacol. 56:754–759; 1978.
18. Yaksh, T. L.; Rudy, T. A. Studies on the direct spinal action of narcotics in the production of analgesia in the rat. J. Pharmacol. Exp. Ther. 202:411–428; 1977.
19. Yaksh, T. L.; Wilson, P. R. Spinal serotonin terminal system mediates antinociception. J. Pharmacol. Exp. Ther. 208:446–453; 1979.