Amitriptyline Potentiates Morphine Analgesia by a Direct Action on the Central Nervous System

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Trycyclic antidepressants are often effective in the management of neuropathic pains. To elucidate the mechanism of tricyclic-induced analgesia, amitriptyline and other drugs were injected into lightly anesthetized rats either systemically or via lumbar intrathecal cannulas. Analgesia was assessed by measuring the latency of the tail flick reflex. Using this model, intrathecal amitriptyline (30 μg) significantly enhanced the analgesic effect of an intraperitoneal dose of morphine (0.5 mg/kg) that by itself produced no measurable effect. Given systemically, amitriptyline (30 or 100 μg intraperitoneally) was ineffective. Cocaine (30 μg) also potentiated morphine analgesia, but iprindole, a tricyclic antidepressant with a very weak inhibitory effect on monoamine uptake, was ineffective. This enhancement of analgesia by intrathecal amitriptyline was prevented by pretreating the rats with *p*-chlorophenylalanine (300 mg/kg). These results are consistent with the hypothesis that amitriptyline produces analgesia by blocking serotonin uptake and therefore enhancing the action of serotonin at the spinal terminals of an opioid-mediated intrinsic analgesia system.

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Tricyclic antidepressants are effective in the management of certain painful conditions including postherpetic neuralgia [33, 35] and headache [4, 6, 15]. They have also been shown to produce analgesia in animals and to potentiate the analgesic effect of morphine [7, 21, 27]. The mechanism by which tricyclics produce or potentiate analgesia is not known, but the discovery of intrinsic analgesia systems within the brain provides a possible explanation. The intrinsic analgesia system that has been most extensively studied has well-defined connections from the midbrain periaqueductal gray matter to the rostral ventromedial medulla and from there to the dorsal horn of the spinal cord [1, 9]. Immunohistochemical and microinjection studies have implicated endogenous opioid peptides in the operation of this system at midbrain, medullary, and spinal levels [9, 14, 18]. This system can be activated by the systemic administration of narcotic analgesics such as morphine [1, 18], which presumably mimic the action of endogenous opioid peptides. In addition to endogenous opioid links, serotonin-containing neurons form an important part of the medullospinal connections of this intrinsic analgesia system [2].

There is evidence that tricyclics act at serotonergic synapses in the brain. Some tricyclics interfere with serotonin reuptake into nerve terminals [16, 25, 28]. In addition, certain tricyclic antidepressants alter serotonin binding to receptors on neural tissue [11, 29]. Either or both of these actions could be related to tricyclic-induced analgesia. The hypothesis that tricyclics enhance the action of morphine by potentiating the serotonergic synapses of the intrinsic analgesia system is supported by the recent finding that both the opioid antagonist naloxone and the 5-hydroxytryptamine (5-HT; serotonin) antagonist methysergide block the analgesic effect of systemic administration of the tricyclic drug clomipramine [7]. Although this hypothesis is attractive, tricyclic antidepressants have actions in addition to those at serotonergic synapses in the brain. For example, they are known to inhibit catecholamine uptake, to have an anticholinergic action [13], and to alter the blood-brain barrier [23]. Furthermore, the tricyclic drug desipramine significantly increases concentrations of intravenously administered methadone in both plasma and brain [20]. A first step, then, in unraveling the sequence of events leading to tricyclic-induced analgesia requires a technique that eliminates peripheral factors. In the present experiments this problem was addressed by using intrathecal administration of doses of tricyclics too low to act systemically.

The present studies were undertaken to investigate whether the tricyclic antidepressant amitriptyline produces analgesia by a direct action on the central nervous system. Because of the evidence implicating

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medullospinal serotonergic neurons in morphine analgesia, amitriptyline was administered intrathecally at the level of the lumbosacral spinal cord, i.e., in the region of terminals of descending serotonergic neurons. Although amitriptyline did not produce analgesia by itself, it markedly enhanced the effect of systemically administered morphine.

Methods

Female Sprague-Dawley rats weighing 200 to 300 gm were cannulated intrathecally. Cannulas were placed in the lumbar intrathecal space as described by Yaksh and Rudy [38]. Briefly, drug-naive rats were anesthetized with pentobarbital, 70 mg per kilogram of body weight. PE 10 tubing, flushed with a balanced ion solution (BIS; 0.13 M sodium chloride, 0.0025 M potassium chloride, 0.001 M magnesium chloride · 6 H_2O, and 0.001 M calcium chloride), was then inserted into the intrathecal space 8.5 cm beyond the atlantooccipital membrane and glued in place. Sufficient recovery from deep anesthesia was allowed so that reflexes induced by noxious stimuli were observable.

The degree of analgesia was determined, while the animals were lightly anesthetized, by measuring the latency of the tail flick reflex (TFR) [5]. The stimulus was a beam of light focused on one of five demarcated sections of the rat's tail. The intensity was adjusted to establish a stable and uniform baseline latency (between 4 and 6 seconds). TFR latencies were timed at 5-minute intervals, and stimulation of any one section of the tail was not repeated for 25 minutes.

Morphine (0.5 mg/kg intraperitoneally) or saline vehicle was injected after four consecutive baseline (4- to 6-second) TFR latencies were obtained (see Fig 1A). An additional four measurements of the TFR latency (at 5-minute intervals) followed to evaluate the response to morphine alone. Either amitriptyline (30 μg), cocaine (30 μg), iprindole (30 μg), or vehicle was then administered intrathecally in a 3 μl volume. The stimulating heat was shut off if the tail flick had not occurred by 10 seconds (cutoff). All drugs were coded and unknown to the observer. Because of possible mechanical artifacts of injection, measurements made immediately postinjection were not included for data analysis. After 15 minutes TFR latencies were measured four times. Naloxone (10 mg/kg), when administered, was injected intraperitoneally following the last TFR measurement. The analgesic effect was then evaluated as before.

Some rats were pretreated with *p*-chlorophenylalanine (PCPA). PCPA was suspended in saline and administered intraperitoneally (300 mg/kg) for three consecutive days prior to the day of experiment. Control animals were treated similarly but with saline alone.

Changes in the TFR latency due to drug treatment were expressed as the maximum percent effect (MPE). MPE was calculated using the formula:

$$\text{MPE}\ (\%) = \frac{\text{postdrug latency} - \text{baseline}}{\text{cutoff latency} - \text{baseline}}$$

where cutoff is 10 seconds and baseline is the mean of 4 premorphine TFR latencies. Statistical significance was determined with the Mann-Whitney U test [32].

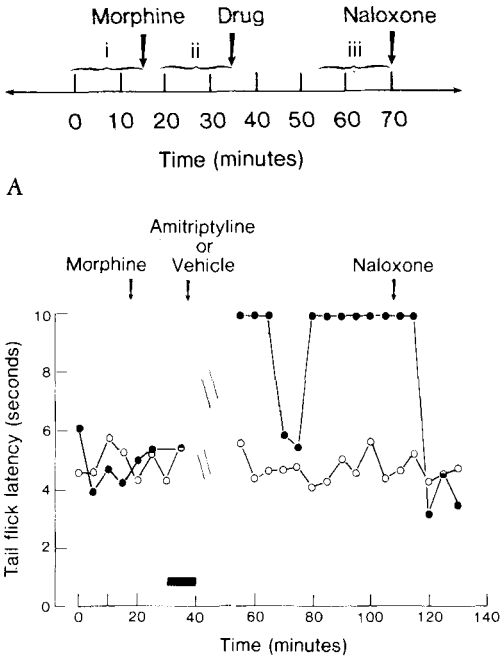

Fig 1. (A) The experimental protocol: i = *time of baseline tail flick latency (TFR) measurements;* ii = *latency measurements following intraperitoneal injection of morphine (0.5 mg/kg);* iii = *measurements of TFR latencies following intrathecal administration of amitriptyline (30 μg), cocaine (30 μg), iprindole (30 μg), or vehicle (BIS). (B) Representative example of an experiment. TFR latency is plotted for two rats studied simultaneously. Both animals received morphine, 0.5 mg/kg intraperitoneally, at the time indicated. Subsequently, either vehicle* (open circles) *or amitriptyline, 30* μg (filled circles) *was injected intrathecally. After 15 minutes, measurements were resumed. Amitriptyline produces a rapid (≤ 15 minute) effect, which persists until naloxone (10 mg/kg intraperitoneally) is administered.*

Results

Pentobarbital-anesthetized rats have a normal TFR latency. Normal TFR latency is also observed in methohexital-anesthetized rats [19]. Once established, a stable baseline latency was observed over the course of the experiment.

In preliminary studies, acute administration of amitriptyline alone, either systemically (4 mg/kg) or intrathecally (30 μg), produced no consistent changes in TFR latency. However, following systemical administration of morphine (0.5 mg/kg intraperitoneally), intrathecal administration of amitriptyline (30 μg) resulted in a dramatic increase in TFR latency (Figs 1, 2). No enhancement of systemic morphine occurred following intrathecal injection of vehicle (BIS). The mean MPE after amitriptyline (30 μg intrathecally) and morphine was 78% (N = 11) compared to 4% for vehicle and morphine (N = 10; difference significant at $p \leq 0.001$ by the Mann-Whitney U test). This effect had a time course of 30 to 120 minutes, though as Figure 1

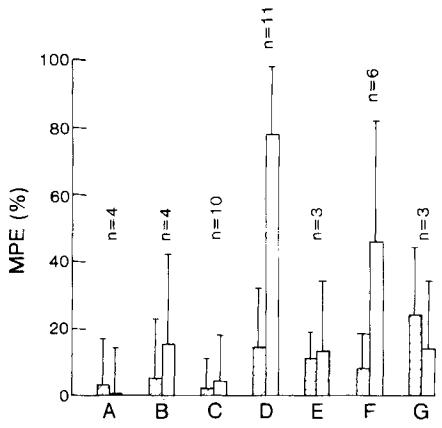

Fig 2. Mean tail flick response (TFR) latencies, expressed as the maximum percent effect (MPE), *for rats following administration of various drugs. All rats received morphine (0.5 mg/kg) or saline intraperitoneally as their first drug, followed by an active substance or balanced ion solution (BIS) vehicle intrathecally as their second drug. Group A (N = 4): saline/BIS; Group B (N = 4): saline/amitriptyline (30 μg); Group C (N = 10): morphine/BIS; Group D (N = 11): morphine/amitriptyline; Group E (N = 3): rats were preinjected with p-chlorophenylalanine (300 mg/kg) for 3 days prior to administration of morphine/amitriptyline; Group F (N = 6): morphine/cocaine (30 μg); Group G (N = 3): morphine/iprindole (30 μg). Hatched bars represent mean ± SEM of 4 TFR latencies following intraperitoneal administration of morphine or BIS (first drug); open bars represent mean ± SEM of 4 TFR latencies following intrathecal drug administration (second drug). The dose of morphine given (0.5 mg/kg) was insufficient to produce a significant change in tail flick latency. Compared to BIS (Group C), significant increases in latency were observed only when amitriptyline* ($p \leq 0.001$) *or cocaine* ($p \leq 0.01$) *was given intrathecally following intraperitoneal morphine injection.*

illustrates it was immediately reversed by naloxone (10 mg/kg intraperitoneally; N = 6). When the same paradigm was used but amitriptyline was injected intraperitoneally instead of intrathecally (30 μg and 100 μg in 4 rats each), there was no change in tail flick latency and therefore no enhancement of the effect of systemically administered morphine. Thus, amitriptyline is much more potent when injected directly onto the lumbosacral spinal cord.

The morphine-potentiating effect of amitriptyline could be prevented by prior administration of PCPA (see Fig 2). PCPA alone or followed by morphine intraperitoneally or BIS intrathecally had no effect on TFR latencies.

In an effort to elucidate the mechanism of enhancement of morphine analgesia, two further drugs were studied: cocaine, which is not a tricyclic antidepressant and presumably has no direct effect on postsynaptic 5-HT receptor binding [22] but does block 5-HT reuptake [28]; and iprindole, an atypical tricyclic antidepressant which only weakly inhibits 5-HT reuptake [8, 26] but does enhance binding to the postsynaptic receptor [11]. Cocaine significantly ($p \leq 0.01$) enhanced morphine analgesia, but iprindole did not (see Fig 2).

Discussion

These results are consistent with previous studies indicating that tricyclic antidepressants have an analgesic action [4, 6, 15, 33, 35]. Furthermore, they provide the first evidence that this analgesic effect is due to a direct action on the central nervous system. The cellular mechanism (or mechanisms) by which amitriptyline produces analgesia is uncertain. However, for the following reasons, the data presented here are consistent with the hypothesis that amitriptyline potentiates the action of an intrinsic pain-modulating network with both opioid and serotonergic links. First, with our experimental paradigm using the TFR to assess analgesia, morphine is required for amitriptyline analgesia. The pain-modulating system is activated by morphine, which presumably acts by mimicking the action of endogenous opioids at central synapses. The involvement of serotonin in morphine analgesia is indicated by the observations that morphine analgesia is reduced by either serotonin depletion [34] or intrathecal administration of serotonin antagonists [37] and that morphine increases serotonin metabolites in the spinal cord [36]. Second, the morphine-potentiated analgesia produced by amitriptyline is reversed by the opiate antagonist naloxone. Furthermore, using a different methodology to assess pain, naloxone reverses analgesia produced by the tricyclic agent clomipramine in unanesthetized rats that have not received narcotic analgesics [7]. In this latter study, perhaps the stress and discomfort were sufficient to activate the intrinsic analgesia system without morphine administration. Third, PCPA, which markedly depletes 5-HT [17], antagonizes the amitriptyline enhancement of morphine analgesia. Finally, the serotonin antagonist methysergide blocks the analgesic effect of the tricyclic clomipramine. Thus, tricyclic drugs appear to produce analgesia by potentiating a serotonergic link of the endogenous opioid-mediated analgesia system.

Although we did not systematically study the site of action of amitriptyline, it is likely to be at the level of the spinal cord. Yaksh and Rudy [38], by injecting a 5 μl volume of dye, showed that the maximum diffusion was 1.5 cm rostral to the catheter tip at 10 minutes, while 20 μl extended as far as 5 cm rostral. Furthermore, by using a tritiated form of the highly lipid soluble compound naloxone, they showed that most of the drug remains within 2 cm of the injection site. In view of the rapid onset of the analgesic effect of spinally administered amitriptyline, it would be necessary to assume particularly rapid diffusion of the drug in order to postulate a supraspinal site of action. Direct studies are needed to clarify this point.

Two receptors relevant to tricyclic action have been defined in the brain. One, a high-affinity binding site for tricyclics, is eliminated by raphe lesions and presumably is located on the terminals of serotonergic neurons [30]. This presynaptic receptor on the terminals of 5-HT neurons is apparently the binding site for drugs that block 5-HT reuptake [16, 25, 28, 31]. The other receptor is a high-affinity 5-HT binding site that is linked to adenylate cyclase [12]. This 5-HT binding site survives 5,6-dihydroxytryptamine lesions [29] but is destroyed by local kainic acid lesions, which are relatively selective for cell bodies [10]. This latter receptor is thus presumably located on neurons that are postsynaptic to serotonergic terminals. Imipramine, desipramine, and amitriptyline bind with high affinity to the presynaptic receptor, while iprindole, which only weakly inhibits 5-HT uptake, does not [24].

Until recently, the antidepressant action of tricyclics was thought to be the result of reuptake blockade and subsequent increase in synaptic serotonin [3]. Yet, this hypothesis is inconsistent with several observations. Some tricyclics used clinically for depression, such as iprindole, do not block monoamine reuptake [8, 26]. Other drugs that do inhibit monoamine uptake, such as cocaine, which binds to the presynaptic receptor with moderate affinity [16, 24], have little clinical antidepressant action [22]. Tricyclic antidepressant drugs (including the atypical iprindole) may increase the affinity of the postsynaptic serotonin binding site [11] and either reduce the number of binding sites [29] or inactivate the 5-HT-sensitive adenylate cyclase or do both [11]. Changes in the postsynaptic 5-HT binding site have been postulated to be involved in the mechanism of the antidepressant action of the tricyclic drugs [11].

To determine which tricyclic action may be mediating the analgesic effect of amitriptyline (which affects both presynaptic and postsynaptic receptors), cocaine and iprindole were administered intrathecally. Cocaine caused a significant and rapid increase of TFR latency, while iprindole had no immediate effect. These observations plus the rapid onset of analgesia suggest that the cellular mechanism of tricyclic-induced analgesia is blockade of monoamine reuptake.

Amitriptyline and cocaine have actions on both 5-HT and catecholamine uptake systems [13]. Furthermore, there is evidence that both 5-HT and norepinephrine may be involved in pain suppression [37]. However, the fact that PCPA pretreatment blocked amitriptyline analgesia does suggest a major contribution by serotonergic systems. Further studies are necessary to determine the relative contributions of these two biogenic amines to the analgesic effect of amitriptyline.

These results confirm previous studies indicating that tricyclics can potentiate the analgesic action of opiates and indicate that combining small doses of narcotic analgesics with serotonin reuptake inhibitors could be a useful therapeutic strategy. Our results further suggest that the relevant anatomical site for amitriptyline's analgesic action is the spinal terminals of descending monoaminergic neurons which form a link in the intrinsic opioid-mediated analgesia system.

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