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Inescapable Shock-Induced Potentiation of Morphine Analgesia in Rats: Sites of Action

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Inescapable shock (IS) enhances analgesia to systemic morphine (MOR) 24 hr later. IS activates serotonin neurons in the dorsal raphe nucleus (DRN), rendering them hyperexcitable. These studies tested whether IS potentiates the analgesic effect of MOR microinjected in the DRN, as predicted by this hypothesis. To test site specificity, the effect of previous IS was examined on MOR microinjected lateral to the DRN and into 2 other sites that support MOR analgesia, the nucleus raphe magnus (NRM) and spinal cord. Twenty-four hours after IS, potentiated analgesia was observed after 0.5 μg MOR microinjected into, but not lateral to, the DRN. Potentiated analgesia was also observed after NRM (1.0 μg) and spinal cord (3.0 μg) MOR microinjections. These data suggest that IS-induced excitability changes within the DRN synergize with opiates microinjected in other analgesia areas and that this potentiates the responses to opiates 24 hr after IS.

Exposure to inescapable shock (IS) leads to widespread behavioral and physiological consequences that are not observed after an equal amount of escapable shock (Anisman, Zalcman, Shanks, & Zacharko, 1991). These consequences are called *learned helplessness* effects because they depend on the uncontrollability of the stressor (Maier & Seligman, 1976) and include deficits in escape learning (Overmier & Seligman, 1967), decreased aggression (Williams, 1982), decreased social dominance and social interaction (Short & Maier, 1993), increased fear conditioning (Osborne, Mattingly, Redmon, & Osborne, 1975), increased rewarding qualities of morphine (Will, Watkins, & Maier, 1998), and a sensitization to the analgesic effects of opiates (Sutton, Lea, et al., 1997). These phenomena follow a distinct time course, developing approximately 2 hr after inescapable shock and dissipating 48–72 hr later (Jackson, Maier, & Rapaport, 1978).

The diverse effects of IS suggest an underlying neural mechanism that is widespread. Serotonergic projections from the dorsal raphe nucleus (DRN) have been implicated in mechanisms by which IS produces these phenomena, in part because these projections innervate many different nuclei that mediate behaviors altered by IS. It has been suggested that IS leads to more serotonin (5-HT) release than does escapable shock (ES) and that this alters the DRN in such a way as to lead to an increased release of 5-HT by DRN projection neurons in response to stimuli 24 hr after treatment (Maier, Busch, Maswood, Grahn, & Watkins, 1995; Maier, Grahn, Maswood, & Watkins, 1995; Maier, Grahn, & Watkins, 1995; Maier, Kalman, & Grahn, 1994; Maier & Watkins, 1998; Sutton, Grahn, Wiertelak, Watkins, & Maier, 1997).

Substantial evidence supports this hypothesis. Electrolytic lesions within the DRN block the effects of IS (Maier, Grahn, et al., 1993), and experiments using in vivo microdialysis find more extracellular 5-HT within the amygdala, a DRN projection region, 24 hr after IS as compared with ES (Maier, Amat, Matus-Amat, Sparks, & Watkins, 1998). As noted above, learned helplessness is associated with increased anxiety and decreased escape behavior, and the release of 5-HT by DRN terminals in the amygdala and frontal cortex appears to increase anxiety, whereas 5-HT released by DRN terminals in the dorsal periaqueductal gray (dPAG) decreases escape behavior (Graeff, Guimaraes, De Andrade, & Deakin, 1996).

Furthermore, stressful events can activate 5-HT neurons within the DRN (Maswood, Barter, Watkins, & Maier, 1998), and such 5-HT activation during IS is thought to sensitize the DRN to later stimulation. Moreover, pharmacological manipulations that decrease DRN 5-HT activity also block IS effects. IS effects on fear conditioning and escape are blocked by inhibiting DRN 5-HT neurons before shock through activation of inhibitory autoreceptors using the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT; Maier, Grahn, & Watkins, 1995). Identical effects are observed after the administration of benzodiazapine agonists that increase inhibition of DRN neurons by gamma aminobutyric acid (GABA; Maier et al., 1994). It is interesting that a benzodiazepine inverse agonist, which is expected to increase DRN 5-HT activity, produces increased fear conditioning and escape behavior deficits in the absence of shock (Maier, Busch, et al., 1995).

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5-HT neurons terminate in efferent targets, as well as on neighboring DRN 5-HT cells (Matos, Urban, & Yocca, 1996), so that 5-HT release leads to increased 5-HT in efferent terminals as well as within the DRN itself. In vivo microdialysis studies have confirmed that more 5-HT is released within the DRN during IS, as compared with ES (Maswood et al., 1998). As noted above, increases in DRN 5-HT activity are thought to be regulated by 5-HT_{1A} autoreceptors on serotonergic cell bodies (De Vry, 1995; Radja et al., 1991), and alterations in this regulation could change the response properties of DRN 5-HT neurons. Activated 5-HT_{1A} receptors inhibit the further release of 5-HT. In this way, increased 5-HT within the DRN normally inhibits the further release of 5-HT into projection areas. Receptors often desensitize as a result of prolonged exposure to a stimulus, and 5-HT_{1A} receptors have been shown to desensitize to a single administration of agonist (Kennett, Marcou, Dourish, & Curzon, 1987). Desensitization of 5-HT_{1A} receptors for a period of time after IS would be predicted to cause increased stress-induced release of 5-HT in terminal fields such as the amygdala. If 5-HT_{1A} receptor desensitization involves processes such as down-regulation of receptor protein, these changes would likely not become apparent until after IS. This hypothesis correlates well with the observed time course of learned helplessness behaviors.

In the absence of IS, 5-HT activity in the DRN is tonically constrained by GABA interneurons (Nishikawa & Scatton, 1983). Pharmacological evidence suggests that the mechanism underlying the IS-induced exaggerated release of DRN 5-HT (Maswood et al., 1998) involves both endogenous opioids and GABA. Administration of the opiate receptor antagonist naltrexone before IS blocks learned helplessness effects (Maier & Minor, 1993; Sutton, Grahn, et al., 1997), implying that IS induces endogenous opioid release. Studies have shown opiate receptors to be inhibitory (Mansour, Fox, Akil, & Watson, 1995), and there is anatomical evidence for expression of opiate receptors on GABA interneurons within the DRN (Nicoll, Alger, & Jahr, 1980). Endogenous opioid binding of opiate receptors on DRN GABAergic interneurons during IS could lead to increased DRN 5-HT activity by inhibiting the tonic GABAergic inhibition known to occur on DRN 5-HT neurons (Harandi et al., 1987). This disinhibition would lead to an increased release of 5-HT within the DRN and in projection regions. Tao and Auerbach (1994) have confirmed that injections of morphine into the DRN increases 5-HT release in terminal fields.

Interactions between DRN 5-HT and opioid systems after IS may explain IS-induced sensitization to the analgesic effects of morphine (Sutton, Grahn, et al., 1997). Rats exposed to IS exhibit analgesia, as measured by both the tailflick and hot plate test, to a dose of morphine that does not elicit analgesia in ES or nonshocked controls (Sutton, Lea, et al., 1997). The time course of IS-induced sensitization of morphine analgesia follows a similar pattern to other learned helplessness effects (Sutton, Lea, et al., 1997). Tail temperature and motor function are not affected, suggesting that the increased latencies in these tests represent true analgesia (Sutton, Lea, et al., 1997). Pharmacological manipulations also suggest this to be a learned helplessness effect. Naltrexone administered into the DRN before IS blocks the morphine sensitization (Sutton, Grahn, et al., 1997) just as it blocks escape deficits, and benzodiazepine inverse agonists and antagonists administered into the DRN produce and block (respectively) the effect in a manner consistent with other learned helplessness phenomena (Sutton, Grahn, et al., 1997). Last, inhibition of DRN 5-HT neurons by 8-OH-DPAT before shock blocks IS-induced morphine sensitization as well (Sutton, Grahn, et al., 1997).

The DRN is a focus in the literature concerning learned helplessness but has also been strongly implicated in bulbospinal analgesia systems (for review, see Wang & Nakai, 1994). Electrical stimulation of the DRN produces analgesia (Oliveras, Besson, Guilbaud, & Liebeskind, 1974; Oliveras, Guilbaud, & Besson, 1979) and potentiates the analgesic effects of systemic morphine (Samanin & Valzelli, 1971). Morphine also produces analgesia when microinjected into the area of the DRN (Tseng & Tang, 1989). Morphine is believed to act through increased 5-HT released by DRN efferent neurons (Tao & Auerbach, 1994). 5-HT projections from the DRN synapse in the nucleus raphe magnus (NRM; Reichling & Basbaum, 1991; Yezierski et al., 1982). 5-HT neurons from the NRM project to spinal cord lamina I, II, and V, where the release of 5-HT, opioids, GABA, and norepinephrine (NE) can separately inhibit second-order nociceptive neurons, causing analgesia (for review, see Fields, Heinricher, & Mason, 1991). Thus, this DRN–NRM–spinal-cord bulbospinal pathway is well documented to exert profound effects on pain.

This relationship between the DRN and analgesia systems is capable of explaining how stress-induced changes in DRN activity could change an organism's responsivity to opiates. Analgesia produced by morphine acting at the DRN results, at least in part, from the release of 5-HT into the NRM and spinal cord (Wang & Nakai, 1994). Indeed 5-HT injected into either the NRM or spinal cord produces analgesia (Llewelyn, Azami, & Roberts, 1983; Yaksh & Wilson, 1979). If the DRN is sensitized after IS, and releases more 5-HT in response to a stimulus such as morphine, then more analgesia should be measured behaviorally. There is no known mechanism by which DRN 5-HT would be affected by morphine injected elsewhere along the bulbospinal analgesic pathway, suggesting that injections of morphine into sites such as the NRM and spinal cord should not produce a potentiated analgesia in IS rats, as the morphine would not affect the sensitized DRN and therefore should not elicit more 5-HT release in these areas. Furthermore, morphine injected into a midbrain site not associated with analgesia should not produce analgesia in IS or control rats, thereby providing a test for site specificity.

The present experiments were conducted to determine whether IS potentiates the analgesic effects of morphine when injected into DRN, NRM, or spinal cord, and whether the effects of IS are specific to injection sites within the DRN.

Experiment 1

IS effects on systemic morphine reactivity are known to be dependent on an increase in DRN 5-HT activity 24 hr

after IS (Sutton, Grahn, et al., 1997). DRN 5-HT projections are also known to produce analgesia (Wang & Nakai, 1994). If the effects of IS on systemic morphine analgesia are mediated in the DRN, then IS should potentiate analgesia created by injections of morphine within this area. This study was conducted to test this possibility.

Method

Subjects. The subjects were 36 male Sprague-Dawley rats (Holtzman Co., Madison, WI), weighing 325–400 g at the time of surgery. Rats were group-housed in triplets and maintained on a 12-hr light–dark cycle, with all procedures occurring during the light part of the cycle.

Apparatus. Restraint and IS occurred in Plexiglas tubes 17.5 cm in length and 7.0 cm in diameter. The rat's tail extended from the rear of the tube and was attached by adhesive tape to a Plexiglas rod. Computer-controlled 1.0-mA shocks were created by shock sources modeled after the Grason-Stadler Model 700 shock source and were administered to the tail through fixed electrodes.

Procedure. All of the rats were anesthetized with sodium pentobarbital (55 mg/kg ip Nembutal; Abbott Laboratories, Farmer's Branch, TX) supplemented with methoxyflurane (Metofane; Mallinkrodt, Mundelein, IL) and implanted with a guide cannula in the region of the DRN. Guide cannulas were constructed from sterile, 24-gauge stainless steel tubing (Small Parts, Miami Lakes, FL) and were equipped with a stainless steel stylet (Medwire; Small Parts) to maintain patency of the cannula lumen.

Cannulas were implanted stereotaxically according to coordinates based on the atlas of Paxinos and Watson (1986). The cannulas were aimed 1 mm dorsal to the target region of the DRN to prevent damage to that area. DRN coordinates were AP = +1.0 mm, DV = +4.5 mm, and ML = 0, using interaural zero as a reference; the bite bar was set at −3.5 mm.

Rats were allowed 7 days to recover postoperatively before testing. During this time, rats were handled frequently to habituate them to the injection procedures. Rats were randomly assigned to one of four groups: IS + morphine, IS + vehicle, restraint + morphine, or restraint + vehicle. IS treatment consisted of one hundred 5-s tailshocks delivered on a 1-min variable-interval schedule. Control rats were restrained in the identical tubes for a time equivalent to the IS session.

Rats were tested for their threshold responsivity to radiant heat 24 hr after IS or restraint. A modification of the tailflick test (D'Amour & Smith, 1941) was used. Testing occurred in a separate experimental room from that of the IS or restraint treatment. Rats were gently restrained by wrapping them in flannel towels with their tails exposed. After a 1-hr adaptation period, baseline latencies were measured, which were typically 2.5–3.0 s in duration. The tailflick procedure and apparatus have been previously described (Watkins, Wiertelak, & Maier, 1992).

After baseline measurement, each rat was wrapped in a flannel towel with the head exposed and handheld during the injection procedure. The stylet was removed, and rats were microinjected through the guide cannula with 0.5 µg morphine (Mallinkrodt) or an equivalent volume of vehicle (1.0 µl sterile physiological saline). Drug microinjectors extended 1 mm beyond the end of the cannula, so that the injection site was within the DRN. Microinjectors were constructed from 33-gauge stainless steel tubing (Small Parts) and connected to a 50-µl Hamilton syringe by a length of PE-20 tubing. The flow of drug was monitored by means of a small air bubble created in the PE-20 tubing. After injection, the microinjector was left in place for 2 min to allow drug diffusion away from the injector before removal. The stylet was then replaced and rats were rewrapped in the flannel towels, with the tail exposed.

Beginning 15 min after microinjection, three tailflick latencies were recorded every 10 min for 1 hr. For each subject, data at each time point were averaged to obtain a single test-trial latency for analysis.

After testing, rats were anesthetized with 55 mg/kg sodium pentobarbital supplemented with methoxyflurane and injected with Evans blue dye (1 µl) through the guide cannula. Rats were then perfused transcardially with heparinized saline and the skulls (with the cannula intact) were dissected and placed in a 10% (vol/vol) formalin–30% (wt/vol) sucrose solution for at least 3 days. Brains were then removed and prepared for sectioning in a cryostat. Forty-µm sections were mounted on gelatin-treated slides and examined for dye placement before staining with cresyl violet. Slides were then coverslipped and examined for accuracy of cannula placement. Data from rats in which dye was not located within the target tissue were discarded.

Statistical analysis. A three-way, repeated measures (Drug × Stressor × Time) analysis of variance (ANOVA) was conducted to compare drug and shock treatments over time. For post hoc analyses, scores for all postinjection time points were averaged for each group, and Scheffé analyses were conducted.

Results

Only data from rats whose dye placement was within the DRN as specified by Paxinos and Watson (1986) were analyzed. All dye placements were within the following coordinates: AP = +0.7 to +1.5 mm, ML = +0.4 to −0.5 mm, and DV = +3.4 to +4.1 mm. Figure 1 represents baseline tailflick latencies, as well as latencies measured 15–75 min after morphine or vehicle injection into the DRN. A one-way ANOVA did not reveal a difference in baseline tailflick latencies between groups, $F(3, 27) = 0.543$, $p > .05$. Tailflick latencies in rats that received restraint + morphine did not appear to differ from those of restraint +

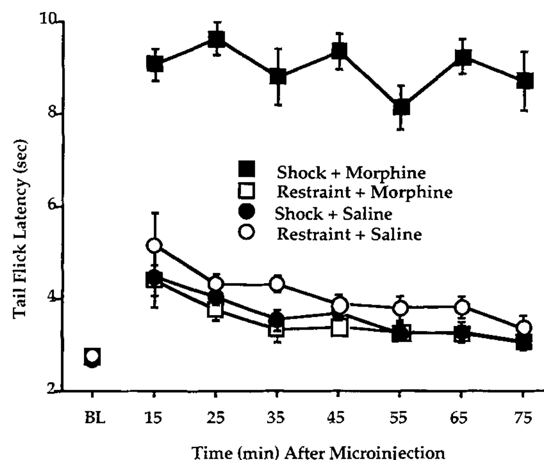

Figure 1. Inescapable shock (IS) potentiates analgesia when morphine (0.5 µg) is microinjected into the dorsal raphe nucleus. Rats were exposed to IS or restraint and microinjected with morphine or an equivalent volume of saline 24 hr later. Rats exposed to IS and tested with morphine displayed significant elevations in tailflick latencies as compared with control groups. BL = baseline.

vehicle rats. However, rats given IS + morphine into the DRN 24 hr later showed much larger increases in tailflick latencies that were still pronounced 75 min after injection.

A repeated measures ANOVA revealed a significant effect of injection, $F(1, 27) = 68.706, p < .0001$, as well as shock treatment, $F(1, 27) = 75.190, p < .0001$. The interaction between drug injection and shock treatment was also significant, $F(1, 27) = 106.188, p < .0001$. The analysis also revealed a significant effect of time, $F(7, 189) = 43.107, p < .0001$, as well as significant interactions between time and injection, $F(7, 189) = 10.682, p < .0001$, and time and shock treatment, $F(7, 189) = 12.152, p < .0001$. A three-way significant interaction was found between time, injection, and shock treatment, $F(7, 189) = 14.986, p < .0001$.

Scheffé analyses revealed that the IS + morphine group showed significantly longer tailflick latencies as compared with the IS + vehicle, restraint + vehicle, and restraint + morphine groups, which did not differ from each other. Thus, IS delivered 24 hr before testing potentiated the analgesia produced by morphine microinjected into the DRN.

Experiment 2

Experiment 1 showed an IS-induced potentiation of analgesia to morphine microinjected into the DRN. Experiment 2 was conducted to determine whether the site of injection was an important factor in Experiment 1, by injecting morphine into neural tissue just lateral to the DRN (off-placement control). Another concern in Experiment 1 was the placement of the cannula through the cerebral aqueduct into the DRN. It can be argued that DRN injections might diffuse along the cannula path into the aqueduct and thus throughout the ventricular system. In addition to the off-placement control just lateral to the DRN, Experiment 2 also examined the analgesia to aqueduct-injected morphine 24 hr after IS (aqueduct control).

Method

The subjects were 23 naive rats, as in Experiment 1. Because vehicle did not produce analgesia in that experiment, vehicle-injected control groups were excluded. Rats were randomly assigned to one of four groups: off-placement control IS, off-placement control restraint (R), aqueduct control IS, and aqueduct control restraint. All of the procedures for Experiment 2 were identical to Experiment 1 except that cannulas were aimed at a site just lateral to the placement area for the DRN (AP = +1.0 mm, DV = +4.5 mm, and ML = +2.2) or into the cerebral aqueduct (AP = −0.2 mm, DV = +5.5 mm). At the time of testing, 0.5 μg morphine (Mallinkrodt) or an equivalent volume (1 μl) of vehicle was injected 1 mm beyond the end of the cannula.

Results

Only data from rats whose dye placement was lateral to the DRN as specified by Paxinos and Watson (1986) or in the aqueduct (dye diffused within the ventricular system) were analyzed. All dye placements for the aqueduct control experiment were within the following coordinates: AP = +0.7 to +1.4 mm, ML = +1.6 to −2.5 mm, or DV = +3.4 to +4.0 mm. Figure 2 presents baseline tailflick latencies, as well as latencies measured 15–75 min after morphine injection lateral to the DRN or the cerebral aqueduct. Morphine injected lateral to the DRN did not produce analgesia as measured by tailflick latencies in rats that received either restraint or IS 24 hr earlier. A repeated measures ANOVA did not reveal a significant effect of shock treatment, $F(1, 9) = 0.050, p > .05$. The effects of time $F(7, 63) = 1.902, p > .05$, and interactions between time and shock treatment, $F(7, 63) = 0.673, p > .05$, were also not significant.

Morphine injected into the cerebral aqueduct produced a slight but significant elevation in tailflick latencies in rats that received IS 24 hr earlier. Morphine had no apparent analgesic affect in rats receiving restraint 24 hr earlier. A repeated measures ANOVA revealed no effect of shock treatment, $F(1, 10) = 2.529, p > .05$. There was, however, a significant effect of time, $F(7, 70) = 2.349, p < .05$, and a significant interaction between time and shock treatment, $F(7, 70) = 3.114, p < .05$.

A visual inspection of the aqueduct control data suggests that morphine injected into the cerebral aqueduct is slightly potentiated by IS and that the elevation in tailflick latencies is much less than seen when morphine is injected into the DRN. It is therefore unlikely that the results obtained in Experiment 1 can be explained by morphine diffusing up the cannula tract and into the aqueduct. It is more probable that the slight elevation in tailflick latencies seen in Experiment 2 is the result of aqueduct-injected morphine acting on DRN neurons.

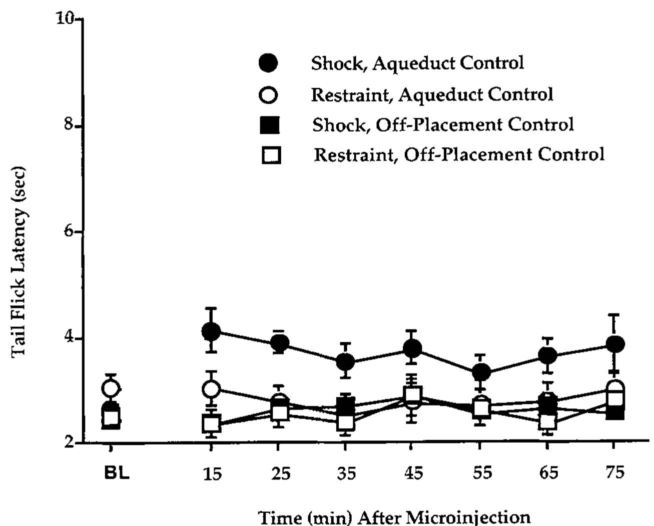

Figure 2. Inescapable shock (IS) does not potentiate analgesia when morphine (0.5 μg) is microinjected into a region just lateral to the dorsal raphe nucleus. IS slightly potentiated analgesia when morphine (0.5 μg) was microinjected into the cerebral aqueduct. This slight elevation in tailflick latencies is much less than that seen in Experiment 1. Rats were exposed to IS or restraint and microinjected with morphine 24 hr later. BL = baseline.

Experiment 3

IS is argued to sensitize 5-HT neurons in the DRN 24 hr later, so that morphine stimulates these sensitized neurons to release excess 5-HT into DRN projection sites. The NRM has been implicated in the modulation of nociception and receives projections from the DRN (Reichling & Basbaum, 1990; Yezierski et al., 1982). Both electrical stimulation of (Oliveras et al., 1979) and morphine microinjection into (Llewelyn, Azami, & Roberts, 1986) the NRM produces analgesia. Systemic injection of morphine has been shown to increase NRM 5-HT activity (Rivot, Pointis, & Besson, 1988, 1989). Furthermore, destruction of NRM 5-HT neurons attenuates analgesia created by systemic morphine (Mohrland & Gebhart, 1980). The NRM sends efferents to the dorsal horn of the spinal cord (Basbaum & Fields, 1979), where pain-evoked neuronal activity is inhibited by NRM stimulation (Llewelyn et al., 1986). This well-established DRN–NRM–spinal-cord pathway is referred to here as the *bulbospinal analgesia pathway.*

The learned helplessness model predicts that the DRN is sensitized but silent 24 hr after IS. In turn, this predicts that behavioral differences would be observed only between IS and restraint groups after stimulation of the DRN by agents including morphine. If this model is correct, then potentiation of analgesia should not occur when morphine is injected into DRN projection sites such as the NRM. On the other hand, factors such as the stress of testing may interact with morphine, leading to exaggerated responses at all levels of the bulbospinal pathway. Experiment 3 was conducted to clarify these issues.

Method

The subjects were 27 naive rats, as in Experiment 1. Experiment 3 was identical to Experiment 1 except that (a) cannulas were aimed at a site 1 mm above the NRM (AP = −2.3 mm, DV = +1.0 mm, ML = 0) and (b) 1 μg morphine (Mallinkrodt) or an equivalent volume (1 μl) of vehicle was injected 1 mm beyond the end of the cannula, into the NRM, at the time of testing.

Results

Only data from rats whose dye placement was within the NRM as specified by Paxinos and Watson (1986) were analyzed. All of the dye placements were within the following coordinates: AP = −1.5 to −2.6 mm, ML = +0.4 to −0.4 mm, and DV = −0.4 to 0 mm. Figure 3 presents baseline tailflick latencies, as well as latencies measured 15–75 min after morphine or vehicle injection into the NRM. A one-way ANOVA did not reveal a difference in baseline tailflick latencies between groups, $F(3, 23) = 1.262$, $p > .05$. Tailflick latencies in the restraint + morphine group did not appear to differ from those of the restraint + vehicle group. Rats given NRM morphine 24 hr after IS, however, showed much larger increases in tailflick latencies that were still pronounced 75 min after injection.

A repeated measures ANOVA revealed a significant effect of injection, $F(1, 23) = 36.228$, $p < .0001$, as well as shock treatment, $F(1, 23) = 30.892$, $p < .0001$. The interaction between drug injection and shock treatment was also significant, $F(1, 23) = 41.889$, $p < .0001$. The analysis also revealed a significant effect of time, $F(7, 161) = 17.973$, $p < .0001$, as well as significant interactions between time and injection, $F(7, 161) = 8.056$, $p < .0001$, and time and shock treatment, $F(7, 161) = 6.371$, $p < .0001$. A significant three-way interaction was found between time, injection, and shock treatment, $F(7, 161) = 8.169$, $p < .0001$.

Scheffé analyses revealed that the IS + morphine group had significantly longer tailflick latencies as compared with the IS + vehicle, restraint + vehicle, and restraint + morphine groups, which did not differ from each other. Thus, IS delivered 24 hr before testing potentiated the analgesia produced by morphine microinjected into the NRM.

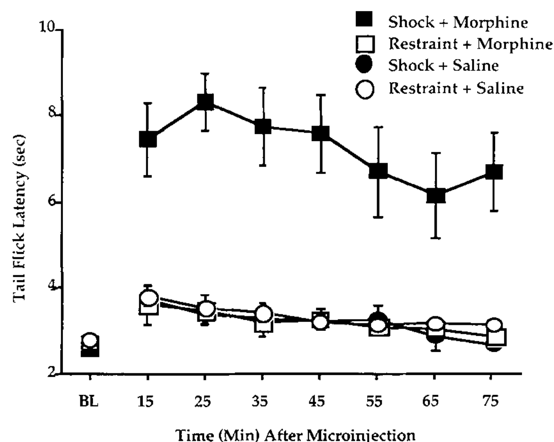

Figure 3. Inescapable shock (IS) potentiates analgesia when morphine (1 μg) is microinjected into the nucleus raphe magnus. Rats exposed to IS or restraint and microinjected with an equivalent of saline 24 hr later did not show significant elevations in tailflick latencies. Likewise, rats exposed to restraint and tested with morphine were not analgesic. In contrast, rats exposed to IS and tested with morphine displayed significant elevation in tailflick latencies 24 hr later. BL = baseline.

Experiment 4

IS potentiated morphine when injected into the NRM, suggesting that morphine plus the stress associated with behavioral testing may have interacted to activate the bulbospinal analgesia pathway. Experiment 4 was conducted to determine whether IS-induced potentiation of analgesia would also occur with morphine injected at the most distal point of the bulbospinal pathway, namely the spinal cord. If stress associated with the testing session creates a low level of activation at all levels of the bulbospinal pathway, and morphine synergizes with this activation, then injections of morphine onto the lumbosacral spinal cord should also be potentiated by IS.

Method

The subjects were 32 naive rats, as in Experiment 1. Rats were anesthetized with sodium pentobarbital (55 mg/kg ip Nembutal;

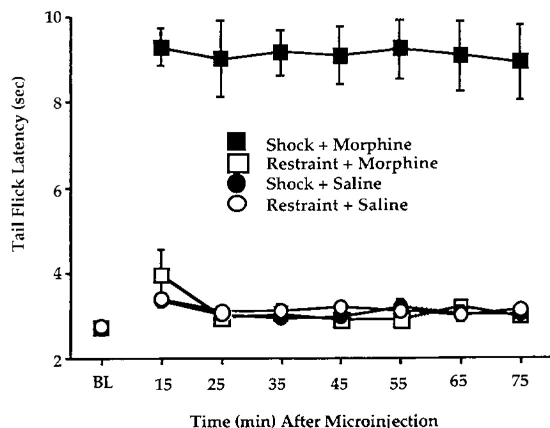

Figure 4. Inescapable shock (IS) potentiates analgesia when morphine (3 μg) is administered intrathecally. Rats exposed to IS or restraint and injected with an equivalent of (10 μl) saline 24 hr later did not show significant elevations in tailflick latencies. Likewise, rats exposed to restraint and tested with morphine were not analgesic. Rats exposed to IS 24 hr previously and tested with morphine displayed significant elevations in tailflick latencies. BL = baseline.

Abbott Laboratories) and implanted with intrathecal catheters. Supplements of methoxyflurane (Metofane; Mallinkrodt) were administered as needed during surgery. Catheterization allowed for drug injection into the cerebrospinal fluid surrounding the lumbosacral spinal cord and has been described in detail previously (Watkins & Mayer, 1982).

Rats were treated with IS or restraint in the same manner as in Experiment 1. Twenty-four hours later, rats received baseline tailflick testing and were injected through the intrathecal catheter with 3 μg morphine (Mallinkrodt) or an equivalent volume (1 μl) of vehicle. Injection was followed by a 10-μl sterile saline flush over 30 s. All other procedures were the same as in Experiment 1.

Results

Figure 4 represents baseline tailflick latencies, as well as latencies measured 15–75 min after intrathecal morphine or vehicle. A one-way ANOVA did not reveal a difference in baseline tailflick latencies between groups, $F(3, 24) = 0.862$, $p > .05$. Tailflick latencies in the restraint + morphine group did not appear to differ from those of the restraint + vehicle group. Rats given intrathecal morphine 24 hr after IS, however, showed much larger increases in tailflick latencies that were still pronounced 75 min after injection.

A repeated measures ANOVA revealed a significant effect of injection, $F(1, 24) = 70.668$, $p < .0001$, as well as shock treatment, $F(1, 24) = 73.034$, $p < .0001$. The interaction between drug injection and shock treatment was also significant, $F(1, 24) = 74.128$, $p < .0001$. The analysis also revealed a significant effect of time, $F(7, 168) = 41.350$, $p < .0001$, as well as significant interactions between time and injection, $F(7, 168) = 24.831$, $p < .0001$, and time and shock treatment, $F(7, 168) = 25.407$, $p < .0001$. A significant three-way interaction was found between time, injection, and shock treatment, $F(7, 168) = 25.165$, $p < .0001$.

Scheffé analyses revealed that the IS + morphine group had significantly longer tailflick latencies as compared with the IS + vehicle, restraint + vehicle, and restraint + morphine groups, which did not differ from each other. Thus, IS delivered 24 hr before testing potentiated the analgesia produced by intrathecal morphine.

Discussion

IS produced dramatic changes in the analgesic response to morphine 24 hr later. This IS-induced effect was observed using the tailflick test after morphine was microinjected into the area of the DRN, but not into brain tissue lateral to the DRN. The IS-induced effect after morphine was microinjected into the cerebral aqueduct was much less than that seen following DRN microinjection. These results suggest that the DRN is sensitized in response to IS and that DRN morphine may elicit more 5-HT release along the bulbospinal analgesia pathway. Notably, IS also potentiated the analgesic effects of morphine when it was microinjected either into the NRM or over the lumbosacral spinal cord, areas that have been implicated as mediating analgesia but not learned helplessness mechanisms.

The DRN is critical to the development and expression of IS effects (Maier, Busch, et al., 1995; Maier, Grahn, et al., 1995; Maier, Grahn, & Watkins, 1995; Maier et al., 1994; Maier & Watkins, 1998; Sutton, Grahn, et al., 1997) and has been hypothesized to be sensitized by prior IS. Morphine is believed to act through DRN μ and κ opiate receptors (Mansour et al., 1995) to activate the bulbospinal pathway resulting in analgesia, and this activation is thought to be greater after IS. In this model, morphine injected into the DRN should lead to potentiated analgesia 24 hr after IS, but morphine injected elsewhere along the analgesia pathway should not interact with the DRN and thus should not produce potentiated analgesia. The results obtained in these experiments conflict with this hypothesis.

Several potential implications can be noted. One is that the DRN hypothesis is simply incorrect. Another is that it is possible that the hypothesized mechanism for the induction of learned helplessness is not confined to the DRN and that other nuclei are affected in the same way. Morphine binds to μ and κ opiate receptors in the NRM (Mansour et al., 1995) and could produce analgesia by disinhibiting GABA in the same manner as proposed for the DRN, resulting in the activation of NRM 5-HT projections to the spinal cord (Rivot et al., 1988, 1989). If the NRM 5-HT system is sensitized, morphine injected there should produce potentiated analgesia.

Increases in DRN activity can be correlated with increases in NRM activity (Auerbach, Fornal, & Jacobs, 1985), so that it is likely that both DRN and NRM 5-HT activity are increased during the IS session. One might speculate that this increased NRM 5-HT activity could sensitize the NRM by the same mechanism as proposed for the DRN. However, the NRM contains a different population of 5-HT receptor subtypes than the DRN, so that an injection of 5-HT in the

NRM generally excites neurons and produces analgesia (Llewelyn et al., 1983), whereas 5-HT injection into the DRN produces a complete inhibition of firing (Aghajanian, Haigler, & Bloom, 1972). In addition to a different 5-HT subtype population, there are differences in 5-HT_{1A} somatodendritic autoreceptor sensitivity in different raphe nuclei. In caudal raphe nuclei such as the NRM, 5-HT_{1A} somatodendritic autoreceptors are substantially less responsive to agonists than are rostral raphe nuclei such as the DRN (Baumgarten & Gothert, 1997). Because of these differences in receptor subtype and sensitivities, it is unlikely that the NRM is sensitized by the mechanism proposed for the DRN.

The internal circuitry of the spinal cord differs even more greatly from that of the DRN (see Basbaum & Fields, 1984, for review), and the role of spinal 5-HT_{1A} receptors in pain modulation is unclear (Sawynok, 1997), making predictions difficult. The data presented here cannot counter the possibility that IS-induced changes at the level of the spinal cord sensitize spinal analgesic systems to morphine injected 24 hr later, although such changes would not be expected to explain the potentiation of morphine injected supraspinally.

For the DRN hypothesis to be viable, there must be interaction between the DRN and morphine injected at other sites along the bulbuspinal analgesic pathway. The DRN has been shown to be sensitive to different types of stress (Fornal, Litto, Morilak, & Jacobs, 1987; Krukoff & Khalili, 1997; Pezzone, Lee, Hoffman, Pezzone, & Rabin, 1993), and it is possible that the testing procedure is itself a stressor. Thus, the DRN might respond to the testing session by releasing 5-HT into its projection regions. This might result in a mild bias toward analgesia that is not detected by the tailflick procedures followed in the present studies. Indeed, when the tailflick test is modified to yield longer baseline latencies, a trend toward analgesia can be observed in rats exposed to IS 24 hr earlier (Hammack, Maier, & Watkins, unpublished observation 1998).

If the above is true, then it could explain how prior IS exaggerates morphine analgesia elicited by microinjection into sites outside of the DRN, such as the NRM or spinal cord. That is, 5-HT released by the sensitized DRN may synergize with morphine microinjected into a DRN-projection region.

Indeed, synergy has been found in many nuclei implicated in pain modulation. Rossi and colleagues found that injections of 1 μg morphine into the PAG, a region that contains the DRN, or rostral ventral medulla (RVM), a region that contains the NRM, do not result in analgesia, but simultaneous injections into these nuclei synergize to produce profound analgesia (Rossi, Pasternak, & Bodnar, 1993). PAG morphine has also been found to synergize with morphine in the amygdala (Pavlovic & Bodnar, 1998) and locus coeruleus (Bodnar, Paul, & Pasternak, 1991), but the most effective sites for morphine synergy were the PAG and RVM (Rossi et al., 1993).

Morphine analgesia produced by intrathecal microinjection onto the spinal cord was also potentiated by IS 24 hr earlier. These results can potentially be explained by the same model proposed for the NRM, in which the stress of testing in rats previously exposed to IS synergizes with spinal injections of morphine. Synergy at the level of the spinal cord has been previously observed. Opiates administered into the supraspinal sites synergize with opiates injected spinally (Roerig & Fujimoto, 1989). The potentiation of intrathecal morphine by IS suggests that activation of the bulbospinal analgesic systems by stress might also synergize with spinal morphine to produce analgesia. As previously mentioned, the separate release of 5-HT, opioids, GABA, or NE onto the spinal cord all have been implicated in the analgesia produced by bulbospinal activation. Intrathecal morphine has been shown to interact with intrathecal 5-HT and intrathecal NE agonists producing analgesia that is additive or greater (Arts, Holmes, & Fujimoto, 1991; Stone, MacMillan, Kitto, Limbird, & Wilcox, 1997), so that the spinal release of these neurotransmitters in response to stress might interact with intrathecal morphine to produce potentiated analgesia in IS rats.

Whatever the interpretation, the data are clear in indicating that IS potentiates the analgesia produced by morphine acting at each of the loci in the DRN–NRM–spinal-cord bulbospinal pathway. The IS–DRN hypothesis as presently stated can only explain these findings if the analgesia testing procedure is sufficient to activate DRN 5-HT neurons, a possibility that remains for further investigation.

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