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The involvement of endogenous opiate systems in learned helplessness and stress-induced analgesia

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Abstract. The participation of endogenous opiate systems in the induction and expression of learned helplessness (LH) and stress-induced analgesia (SIA) was investigated in rats exposed to escapable and inescapable footshock. Following an initial footshock, analgesia was observed only in those animals that could not control their stress exposure and this SIA was prevented by the administration of naloxone. Analgesia was no longer evident in this inescapable group after 48 h. However, exposure to a shuttlebox escape task at this time reinstated the SIA but did not produce SIA in animals previously exposed to escapable footshock. Shuttlebox escape deficits indicative of LH were also exhibited by animals that received an inescapable footshock stress 48 h prior to testing. The analgesia and LH observed in the inescapable group were not affected by the administration of naloxone (3 mg/kg, IP) 10 min before shuttlebox exposure but were prevented when the same dose of naloxone was given before the initial stress. Thus, endogenous opiates clearly participate in the initial induction of LH and SIA and, although the degree of endogenous opiate involvement in the subsequent expression of these behaviors is unclear, it seems evident that their expression can occur in the presence of opiate antagonism and may therefore require the participation of additional transmitter systems.

Key words: Endogenous opiates – Naloxone – Learned helplessness – Escape behavior – Stress-induced analgesia – Footshock

The exposure of rats to uncontrollable footshock has been shown to produce deficits in the subsequent performance of escape behavior (learned helplessness) and to elicit an antinociceptive effect which has been termed stress-induced analgesia (SIA). Since the escape deficits associated with learned helplessness (LH) and the analgesia elicited by a number of environmental stressors can be prevented by the administration of opiate antagonists before exposure to the inescapable stress (Drugan and Maier 1983; Watkins and Mayer 1982), it has been suggested that endogenous opiates participate in the mediation of these behavioral effects. Further, Whitehouse et al. (1983) reported that naltrexone can eliminate escape deficits when administered immediately prior to a shuttlebox escape test conducted 24 h after exposure to inescapable footshock. However, the excessive dose of the opiate antagonist used may be expected to exert non-selective effects (Costa et al. 1973) and was indeed shown to produce escape deficits in non-shocked controls and in animals previously exposed to escapable footshock. Similarly, large doses of naloxone or naltrexone have been shown to prevent the reinstatement of analgesia by shuttlebox exposure 24 h after an initial inescapable shock (Maier et al. 1980). Again, the size of the antagonist doses used prohibits the conclusion that their effects were due to the selective antagonism of endogenous opiate systems.

Several recent reports have described the development of SIA which is not antagonized by naloxone and is, therefore, probably non-opiate in nature (Hayes et al. 1978; Lewis et al. 1980; Watkins and Mayer 1982). Since the SIA observed by Lewis et al. (1980) was either sensitive or non-sensitive to naloxone depending on the duration of the shock exposure, these workers suggested that the temporal characteristics of the stress may be one determinant of the activation of opiate or non-opiate systems. Also, based on the finding that SIA occurred only if induced by an inescapable electrical shock, Jackson et al. (1979) suggested that the controllability of the stressor, rather than its nature, may be critical to the development of an analgesic effect. However, in a subsequent report, Hyson et al. (1982) observed that although SIA could be elicited by exposure to escapable or inescapable shock, the analgesia of inescapably shocked subjects was more sensitive to reversal by naltrexone and only the inescapably shocked subjects became hypersensitive to the analgesic effects of morphine, suggesting that inescapable stress produces a greater activation of an endogenous opiate system. The temporal characteristics and controllability of the stressor are also important determinants in the development of LH. Thus, Maier et al. (1983) have reported that 30 min of intermittent footshock produces an escape deficit and a naloxone-sensitive SIA, whereas 3 min of continuous footshock fails to produce LH and elicits a non-opiate analgesia.

Since previous research appears to indicate clearly that endogenous opiates are involved in the induction of LH and SIA but is unclear with respect to opiate mediation of the subsequent expression of these behavioral changes, and a number of reports indicate the development of a non-opiate SIA, the present study was conducted to replicate several aspects of the previous work utilizing a lower, more selective dose of naloxone in order to clarify the role of endogenous opiate systems in the induction and expression of these behavioral effects of stress.

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Materials and methods

Experimental subjects were 300–400 g male Sprague-Dawley rats (Harlan) maintained in individual cages on a 12 h light/dark cycle with free access to food and water. The animals were randomly divided into three experimental groups which received escapable, yoked inescapable or no initial shock, respectively. Single animals from each experimental group were tested as a block.

Initial stress was induced in individual plexiglass Skinner boxes (28 cm long, 20 cm wide, 21 cm high). The floor grid was constructed from 0.3 cm stainless steel bars spaced 1.3 cm apart and a lever (5.1 cm wide, 1.8 cm long, 1.0 cm thick) was mounted 7.0 cm above the floor. Scrambled footshock was delivered to unrestrained animals at an intensity of 2 mA for a maximum duration of 30 s with a 30 s interval between trials. Control animals were exposed to the apparatus but received no shock. Escapable animals received 360 s of shock under an escape contingency in which an FR-2 bar press response produced immediate termination of the shock for both the escapable and yoked animals. Thus, yoked inescapable animals received shock of an identical duration and frequency to the escapable subjects but had no control over their stress exposure. However, bar presses by both the escapable and yoked inescapable animals were recorded and a minimum ratio of 2:1 (escapable:yoked) was required for their continuation in the experiment. This requirement appeared to insure that the appropriate shuttlebox escape deficit (LH) would occur during subsequent testing in non-drug-treated animals.

Animals were tested for analgesia immediately and 48 h after initial stress exposure by a modification (Bass and Vander Brook 1952) of the tail flick procedure of D'Amour and Smith (1941). Baseline tail flick latency was determined immediately prior to the initial stress and the intensity of the focused heat stimulus was adjusted to produce a baseline (BL) of 3–4 s. A 12-s cutoff was used in the absence of a response and the degree of analgesia (DA) was expressed as a percentage derived from the ratio of the observed change in response time (T) from baseline to the maximum possible change according to the following formula (Mayer and Hayes 1975): $DA = 100\ (T-BL)/(12-BL)$. The mean and standard error of these ratios were calculated through the use of an arcsine transformation (Sokal and Rohlf 1969) and significant differences between groups were determined by analysis of variance and multiple range tests ($\alpha = 0.05$).

Following analgesic testing 48 h after initial stress exposure, individual animals were placed in a shuttlebox (61 cm long, 20 cm wide, 19.5 cm high) consisting of two compartments divided by an electrified stainless steel barrier which extended 5.7 cm above the floor. Scrambled footshock was applied through a stainless steel floor grid (0.3 cm diameter bars spaced 1.3 cm apart) at an intensity of 1 mA for a maximum of 15 s with a 60 s interval between trials. All animals were given 50 trials of escape testing with a two way (FR-2) shuttle task (i.e. animals were required to jump the barrier and return to their original compartment in order to terminate the shock). Animals were again tested for analgesia immediately after completion of the 50 shuttlebox trials. Escape response latencies for the 50 trials were added and the sums were log transformed. Significant differences ($\alpha = 0.05$) in escape latencies between control, escapable and yoked inescapable groups were determined by an analysis of variance of the transformed sums and subsequent multiple range tests.

The above procedures were conducted with drug naive animals and with animals which received naloxone (3 mg/kg, IP) either 10 min before initial stress exposure or 10 min before escape testing in the shuttlebox.

Results

Lever press responding during initial stress exposure ranged between 131 ± 21 and 161 ± 20 (mean $\pm$ SEM) total bar presses for the escapable groups and was not significantly affected by pretreatment with naloxone. Similar results were obtained with the yoked inescapable groups, whose total bar press responses ranged between 32 ± 9 and 50 ± 16. Thus, the ratio of bar press responding between escapable and yoked inescapable groups was between 3:1 and 4:1, indicating that sufficient differences in the apparent learning of controllability were observed to predict subsequent shuttlebox escape deficits in the yoked inescapable groups. This prediction proved accurate for the drug-naive animals and for those animals that received naloxone 10 min before the shuttlebox test (Fig. 1). Under these conditions, the yoked inescapable groups exhibited significantly ($F = 17.51$; $P < 0.01$) greater escape latencies than either the escapable or non-shocked control groups, whose escape latencies were not significantly different from each other. However, no significant differences among the groups ($F = 1.01$; $P > 0.05$) were observed when naloxone was administered 10 min before the initial stress exposure (Fig. 1). Thus, naloxone prevented the predicted escape deficit in the yoked inescapable animals when administered before exposure to the initial stress but did not prevent this deficit when administered 48 h later, immediately prior to the shuttlebox test.

Significant ($P < 0.01$) analgesia was observed only in the yoked inescapable group immediately following initial stress exposure and the analgesic effect was prevented by the administration of naloxone 10 min prior to the foot-

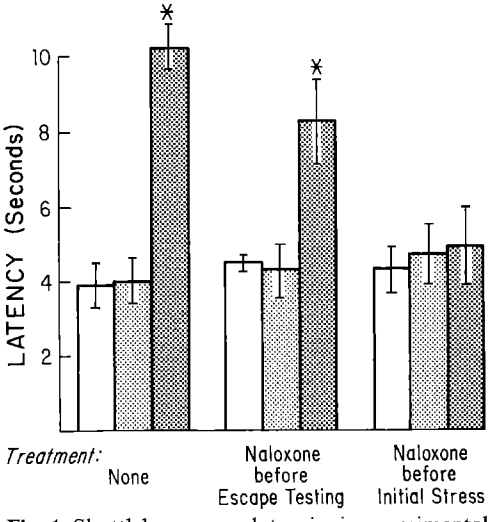

Fig. 1. Shuttlebox escape latencies in experimental groups receiving no treatment, naloxone (3 mg/kg, IP) 10 min before the shuttlebox test or naloxone (3 mg/kg, IP) 10 min prior to the initial footshock stress. The interval between initial stress exposure and shuttlebox testing was 48 h. Data presented as mean $\pm$ SEM; $n = 6$–8 animals per group. *$F = 17.51$; $P < 0.01$ vs control and escapable groups. □Control, ▨Escapable, ▩Yoked-inescapable

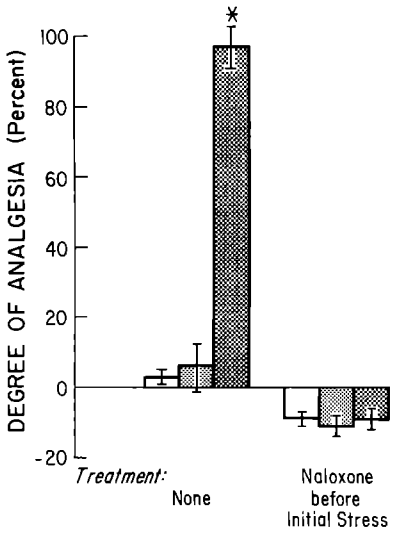

Fig. 2. Analgesia exhibited immediately after initial stress exposure in experimental groups receiving either no treatment or naloxone (3 mg/kg, IP) 10 min prior to the initial footshock stress. Data presented as mean ± SEM; $n = 7$ animals per group; degree of analgesia = 100 (T − BL)/(12 − BL). $*F = 19.23$; $P < 0.01$ vs non-shocked control groups. □ Control, ▨ Escapable, ▩ Yoked-inescapable

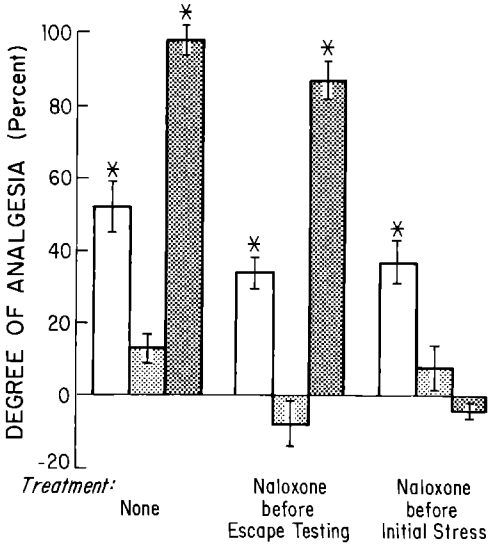

Fig. 3. Analgesia observed immediately after the shuttlebox test in experimental groups receiving no treatment, naloxone (3 mg/kg, IP) 10 min prior to the shuttlebox test or naloxone (3 mg/kg, IP) 10 min prior to the initial footshock stress. The interval between initial stress exposure and shuttlebox testing was 48 h. Data presented as mean ± SEM; $n = 7$ animals per group; degree of analgesia = 100 (T − BL)/(12 − BL). $*F = 34.39$; $P < 0.01$ vs escapable groups. □ Control, ▨ Escapable, ▩ Yoked-inescapable

shock stress (Fig. 2). Thus in animals receiving identical amounts of electrical shock in our initial stress paradigm, analgesia (SIA) occurred only in those which could not control their stress exposure and this SIA was sensitive to naloxone.

Analgesia was not evident in the yoked inescapable group 48 h after initial stress exposure (data not shown). However, immediately after completion of the shuttlebox escape test, significant ($P < 0.01$) analgesia was observed in the drug-naive control group that had not been exposed to the initial stress (Fig. 3). This analgesic effect was significantly ($P < 0.05$) reduced but not totally prevented by treatment with naloxone either 48 h or 10 min before the shuttlebox escape test. Analgesia was not produced in the escapable groups but was again elicited in the drug-naive, yoked inescapable group (Fig. 3). The analgesic effect in this group was not antagonized by the administration of naloxone 10 min before the shuttlebox escape test but did not occur if animals were treated with naloxone before exposure to the initial stress.

Discussion

In agreement with earlier reports (Maier and Seligman 1976; Watkins and Mayer 1982; Maier et al. 1983), the present results again demonstrate that exposure to an initial stressful event can elicit analgesia and subsequent deficits in escape behavior and that the degree of control that an animal can exert over the stressor is critical to the development of these behavioral effects (Seligman and Maier 1967; Jackson et al. 1979). Present findings further indicate that the effects of initial stress on escape behavior persist for at least 48 h and that the apparent learning of controllability can be predicted by the bar press data obtained during initial stress exposure, since the escapable animals were found to respond with a 3–4 times greater frequency than their yoked inescapable counterparts.

Escape deficits following exposure to an uncontrollable stress have been considered indicative of the development of a learned helplessness (LH) phenomenon, while the loss of pain sensation has been termed stress-induced analgesia (SIA) and evidence has been provided which suggests that endogenous opioid peptides are involved in the mediation of these effects (Drugan and Maier 1983; Maier et al. 1983; McCubbin et al. 1984). Present data support the proposal that endogenous opiates are involved in the induction of LH and SIA, since the escape deficit and analgesia produced by the initial footshock stress were prevented by administration of the opiate antagonist naloxone 10 min prior to stress exposure and, in this respect, naloxone appeared to mimic controllability of the stressor. Also of interest is the slight but nonsignificant hyperalgesia observed in the control and escapable groups after the administration of naloxone (Fig. 2), indicating that an endogenous opiate system exerts a tonic influence on pain sensitivity in these groups and that this influence is further activated by uncontrollable footshock stress. However, since hyperalgesia is difficult to demonstrate with initial baseline latencies of 3–4 s, further studies using longer baseline latencies will be required to evaluate the significance of this apparent tonic opiate influence on pain sensitivity.

In apparent disagreement with previous findings (Maier et al. 1980; Whitehouse et al. 1983), however, the same dose of naloxone (3 mg/kg, IP) failed to prevent the expression of LH and SIA when administered prior to the shuttlebox escape test 48 h after initial stress exposure. Whitehouse et al. (1983) report that SC injections of 1 mg/kg naltrexone reduced and 10 mg/kg naltrexone eliminated escape deficits when given 15 min before the shuttlebox escape test and Maier et al. (1980) demonstrated that a 50 mg/kg dose of naloxone or 14 mg/kg naltrexone prevent the reinstatement of analgesia when given 24 h after exposure to inescapable shock. Strain differences and the possibility of a stronger opiate component when testing is conducted 24 h after initial stress may account for these discrepancies. However,

the inability of naloxone to prevent the deficit in escape responding in the present study cannot be attributed to a direct depressant effect of the drug on escape performance, since response deficits did not occur in the naloxone-treated shuttlebox control or escapable groups. Also, our data demonstrate a slight but non-significant decrease in escape latency in the inescapable group when naloxone is administered prior to the shuttlebox test (Fig. 1), indicating the possibility of some opiate involvement in the expression of this effect. Thus, it is possible that the administration of a higher dose of naloxone prior to the shuttlebox test would have prevented both the escape deficit and analgesia evoked in the control or inescapable groups in the present study.

The chosen 3 mg/kg dose of naloxone is equivalent in potency to 1 mg/kg naltrexone (Blumberg and Dayton 1973) and has been repeatedly shown to be sufficient for effective endogenous opiate antagonism (Watkins and Mayer 1982; Chatterjee and Gebhart 1984; Tricklebank et al. 1984). Further, this dose of naloxone was clearly capable of preventing LH and analgesia when administered before the initial inescapable stress (Figs. 1, 2 and 3). A 10 mg/kg dose of naltrexone appears to be somewhat excessive and may be expected to exert a non-selective action (Costa et al. 1973), which could explain the effects of this dose on escape behavior in non-shocked control and escapable animals observed by Whitehouse et al. (1983). Thus, although endogenous opiate systems activated during the initial stress appear to be responsible for the induction of LH and SIA and may make some contribution to their expression 24 or 48 h later, the full expression of these behavioral effects seems to depend on the involvement of additional non-opiate transmitter systems or pathways. This conclusion is further strengthened by the report that morphine administration before shuttlebox testing does not mimic the escape deficit produced by inescapable shock (Mah et al. 1980).

The finding that analgesia was no longer evident in the yoked inescapable animals after 48 h also indicates that the inescapable shock did not produce a persistent activation of endogenous opiate systems. However, exposure to the shuttlebox escape test at this time elicited analgesia in both the inescapable and non-shocked control groups (Fig. 3). Jackson et al. (1979) have also observed the reinstatement of analgesia in rats 24 h after exposure to inescapable shock. However, these investigators used mild shock procedures that did not produce analgesia in animals that had received escapable or no shock 24 h earlier. Further, it has subsequently been shown that this reinstated analgesia exhibits cross tolerance with morphine (Drugan et al. 1981). Thus, the reinstated analgesia reported by Jackson et al. (1979) is apparently different from that elicited by shuttlebox escape testing in the present study. It appears that the shuttlebox escape procedure can produce either non-opiate or a combination of opiate and non-opiate SIA depending on the exposure to previous stress or perhaps the duration of the shuttlebox stress, since prolonged escape latencies were observed in the yoked inescapable group. However, the comparable average escape latencies observed in the control and escapable groups (Fig. 1) tends to indicate that shuttlebox stress duration is not a major determining factor for the induction of opiate or non-opiate analgesia. Since the non-shocked control group had not previously experienced an initial stress or escape responding, the moderate and partially naloxone sensitive analgesia exhibited by these animals after the shuttlebox escape test may indicate an initial perception that the shock received was uncontrollable until the appropriate response was learned.

The administration of naloxone and exposure to escapable or inescapable stress clearly have long-term effects on the expression of pain sensitivity. Controllability of the initial stressor prevents the occurrence of analgesia following the shuttlebox escape test and the administration of naloxone prior to an initial inescapable stress also prevents the expression of analgesia following shuttlebox exposure 48 h later. Further, the administration of naloxone 48 h prior to shuttlebox testing prevents the full expression of analgesia in control animals not exposed to an initial stress (Fig. 3). Thus, the initial induction of SIA appears to require the activation of endogenous opiate systems by uncontrollable stress. However, once induced, SIA may be subsequently expressed in the absence of endogenous opiate mediation following exposure to an escapable stress.

It is evident from the results of the present study and others that endogenous opiate systems contribute to the induction of LH and SIA. Further evaluation of these data indicates that additional non-opiate transmitter systems are most probably also involved in the development and expression of these behavioral changes. Highly complex interactions among these systems are clearly possible. For example, Drugan et al. (1984) have recently reported that a benzodiazepine antianxiety drug, which presumably acts by facilitating neurotransmission mediated by gamma-aminobutyric acid (GABA), prevents the development of LH and SIA when administered prior to an initial inescapable stress. Interestingly, the benzodiazepine had little effect when given only prior to the shuttlebox test. Thus, endogenous opiates released during induction may elicit a level of anxiety or fear which can be antagonized by benzodiazepines. Other investigators have emphasized the involvement of norepinephrine and other monoamines in LH and SIA (Anisman et al. 1980; Weiss et al. 1981; Coderre and Rollman 1984). LH has been associated with a depletion of brain norepinephrine and is considered to be an animal model of depression, since it can be prevented by antidepressant drugs (Petty and Sherman 1980). Brain monoamines are apparently also involved in the production of analgesia by opiates (Mayer and Price 1976; LoPachin and Reigle 1978) and stress (Watkins et al. 1984). Thus, relationships between pain and depression will be further clarified by future studies designed to evaluate the interactions among transmitter systems involved in the induction and expression of LH and SIA.

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