Opioid nature of learned helplessness and stress-induced analgesia observed without re-exposure to shock

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It has been shown that uncontrollable shocks that produce learned helplessness also produce long-term opioid analgesia if the animal is re-exposed to shock immediately before the test. The present study was conducted in order to investigate if this effect can be observed 24 h after the uncontrollable shock treatment without re-exposure to shock, and if it is opioid mediated. Long-term analgesia was found in the absence of re-exposure to shock, and was prevented by an i.p. injection of naloxone (10 mg/kg) administered 10 min before the test. The learned helplessness effect produced by the same shock treatment was prevented by the administration of 10 and 20 mg/kg of naloxone 10 min before the shuttlebox test, but not by a lower naloxone dose (5 mg/kg). These findings suggest that the shock re-exposure requirement proposed in previous studies is not crucial in determining the long-term analgesia, and that both the long-term analgesia and the learned helplessness effect produced by this shock treatment were opioid mediated.

Keywords: Learned helplessness – Stress-induced analgesia – Opioid analgesia – Uncontrollable shocks – Naloxone – Rat

INTRODUCTION

Exposure to a variety of painful events produces an analgesic reaction that has been called stress-induced analgesia (Amit and Galina, 1986). The existence of different forms of stress-induced analgesia mediated by opioid and nonopioid mechanisms has led to investigations concerning the factors that determine which form will occur (Lewis et al., 1980, 1981; Drugan et al., 1981, 1982). It has been shown that different types of stimuli or even the same stimulus under different conditions may change the nature of stress-induced analgesia. For example, electric footshock, the most frequently employed stimulus in these studies, produces an opioid or nonopioid form of analgesic response depending on its amount, duration, and temporal aspects (Lewis et al., 1980, 1981).

The controllability or uncontrollability of the shock is also important in determining the nature of the resulting analgesic response. Although both controllable and uncontrollable shocks produce an analgesic reaction that dissipates rapidly (short-term analgesia), only uncontrollable shock produces an analgesia that can be reinstated 24 h later by brief re-exposure to shock (long-term analgesia) (Jackson et al., 1979; Maier et al., 1982). Moreover, the short-term analgesia produced by uncontrollable shock is more affected by opiate antagonists than that produced by controllable shock (Hyson et al., 1982) while the long-term reinstated analgesia is reversible by opiate antagonists (Maier et al., 1980) and is cross-tolerant with morphine (Drugan et al., 1981).

Stress-induced analgesia has important implications for the study of animal behavior, especially for investigations of the learned helplessness effect. This effect is shown in relative difficulty in learning an escape and/or avoidance response after exposure to uncontrollable shock but not following equivalent amounts of controllable shock (Maier and Seligman, 1976). It has been postulated that the animal learns that it has no control over the shock and thus does not respond when the shock becomes escapable (Seligman and Maier, 1967). Since the typical procedure which produces learned helplessness is analogous to that which produces long-term opioid analgesia, it has also been argued, however, that learned helplessness occurs because shock is less painful for the animals exposed to the uncontrollable shocks (Jackson et al., 1979).

It is important to note that stress-induced analgesia has
not been observed 24 h after the exposure to uncontrollable shock unless the animals have been re-exposed to shock. It is argued that this procedure reinstates the analgesia that would otherwise be dissipated (Jackson et al., 1979). The typical helplessness procedure also re-exposes the animal to five shocks before the learning test (Jackson et al., 1978; Maier et al., 1973). However, preliminary studies showed that learned helplessness can be demonstrated without re-exposure to shock (unpublished observations).

The purpose of the present work was to assess whether a helplessness procedure which produces learned helplessness without pretest shocks, will also produce analgesia in rats, and if this analgesia and/or the helplessness effects are mediated by opioid mechanisms.

METHODS

Subjects
The subjects were 128 male albino rats (Wistar descendant) bred and raised at the State University of Campinas, weighing 240-300 g at the time of testing.

The subjects were individually housed with water and food freely available and maintained on a 12/12 h light/dark cycle, with daily light onset at 06.00 h. Experiments were carried out during the light phase of the cycle.

Apparatus
Inescapable shocks were delivered in a 21.5 × 21.5 × 20.0 cm plexiglas box located in a sound-attenuating chamber with a constant white noise. The floor consisted of brass grids 0.05 cm in diameter, spaced 1.3 cm apart. Scrambled shocks were delivered through the grid floor by a BRS Foringer AC shock generator. Shock presentation and duration were programmed through electromechanical relay-circuits located in an adjacent room.

The escape learning test was conducted in an automated 50.0 × 15.5 × 20.0 cm plexiglas two-way shuttlebox. The box was divided into two compartments of equal size by a plexiglass wall with a central 7.5 × 6.0 cm hole located 8 cm above the floor providing access to the adjacent compartment. The grid floor, white noise and acoustic isolation were similar to those described for the shock box.

The pain-reactivity test was conducted using a hot plate. It consisted of a 36 × 24 cm aluminium plate placed on top of a thermostatically controlled water bath that maintained a constant surface temperature of 50°C across the plate. A glass cylinder 25 cm in height and 17 cm in inner diameter was fitted on top of the plate to prevent the subjects from leaving it. A manual chronometer was used to record paw lick latencies.

Procedure
The subjects were randomly divided into sixteen groups (n = 8): half of the groups received inescapable shocks (IS groups) and the other half were not exposed to shocks (NS groups). The IS groups were exposed to three experimental sessions at 24 h intervals. In each session, the animal was exposed to 20 1.0 mA shocks of 10.0 s fixed duration, delivered on a variable time 60 s schedule (range 5-120 s). The choice of shock parameters was based on preliminary parametric studies (unpublished) of shock intensity and duration, number of shocks and number of sessions, carried out in order to establish the minimally aversive conditions necessary for the purposes of these experiments. Subjects in the NS group were placed in the experimental chamber without shock.

Twenty-four hours after the last shock session animals were tested either for escape learning or for paw-reactivity. Five groups IS and five NS groups were submitted to the escape learning test. Ten min before this test, four groups were administered an i.p. injection (volume 1 ml/kg) of naloxone (5, 10 or 20 mg/kg) or saline, and one group was not injected. For the test, the rats were placed in the shuttlebox with no shock for 1 min and then received 30 1.0 mA shocks according to the variable time 60 s schedule (range 5-120 s). The shock was interrupted if the rat jumped from one compartment to the other. If 30s had elapsed without a jump response, the shock was turned off, and a 30 s latency was recorded. Each shock started one trial, and the time between the shock onset and termination was recorded as the latency of that trial.

Of the three IS and NS groups tested on pain-reactivity, two received an injection of naloxone (10 mg/kg) or saline, and one group was not injected. Ten minutes later, the rat was placed in the shuttlebox for 1 min with no shock. Then, it was removed from the shuttlebox and immediately placed inside the hot-plate cylinder with the four paws touching the hot surface. This started the chronometer which stopped as soon as the rat licked a hind paw. The test was terminated if the paw-licking had not occurred in 90 s, in which case a 90 s latency was recorded.

Statistical analyses
The escape test results were analysed by a Group X Trial Block ANOVA, and the hot-plate test results were analysed by the Student t-test. Differences with p < 0.05 were considered significant.

RESULTS
Figure 1 shows the mean latencies in the escape learning test (left side) and in the pain-reactivity test (right side) seen in animals not submitted to any injection. It can be seen that the groups exposed to uncontrollable shocks exhibited a higher latency to respond either to thermal stimulation or to escapable shock in the test situation when compared with their no-shock control groups. Despite the
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FIG. 1. Mean escape latencies (left side) and mean paw licking response latencies with corresponding standard error (right side) for groups submitted previously to inescapable shock (IS) or no-shock (NS). Asterisks represent significant differences between NS and IS groups: *p < 0.05, **p < 0.01.

marked group differences during the escape test session, latencies gradually decreased across the trials in both groups. Statistical analysis supported these findings. The analysis of variance indicated yielded reliable effects of shock treatment [F(1,14) = 12.87, p < 0.01] and blocks of trials [F (5,70) = 15.43, p < 0.01] during the escape test; the interaction of groups and blocks was not significant. In the hot-plate assay, latencies of the shocked rats were significantly longer than those of their no-shock controls (p < 0.05).

Figure 2 shows the mean licking response latency for the four groups tested on the hot-plate after injection of saline or naloxone (10 mg/kg). The results of the saline groups replicated those shown in Fig. 1, i.e. shocked animals showed higher latencies than non-shocked ones. However, these differences were not observed in the naloxone groups: shocked rats did not differ from non-shocked animals. Naloxone did not alter paw-lick latencies in animals not exposed to shocks but reversed the increased latencies seen in animals that received inescapable shocks. The statistical analysis confirmed that only the shocked group injected with saline responded more slowly than did other groups (p < 0.05) which did not differ significantly among themselves.

The results presented in Fig. 3 show the mean shuttle-box response latency for the four pairs of groups injected with saline or naloxone (5, 10 or 20 mg/kg) before test. All the groups showed a gradual decrease in latency along trials regardless of the shock or drug condition. However only the IS groups treated with saline or 5 mg/kg of naloxone exhibited higher escape latencies relative to their no-shock controls. The rats that received inescapable shocks and were treated with 10 and 20 mg/kg of naloxone were
not different from their no-shock controls. The statistical analysis indicated significant differences among pairs of no-shock and shocked groups treated with 0 and 5 mg/kg of naloxone \( [F(1,14) = 5.98 \text{ and } 5.65, \text{ respectively, } p < 0.05 \text{ in both cases}] \). Significant changes in response latencies over blocks of trials were found in all groups \( [F(5,70) = 10.86, 15.81, 11.90 \text{ and } 17.30, \text{ for groups treated with } 0, 5, 10 \text{ and } 20 \text{ mg/kg of naloxone respectively, } p < 0.01 \text{ in all cases}] \). Interactions of groups and blocks were not observed.

**DISCUSSION**

In agreement with previous studies (Maier and Seligman, 1976; Amit and Galina, 1987), the results obtained in this series of experiments demonstrate that exposure to uncontrollable shocks produces both stress-induced analgesia and helplessness. The present study has produced a new finding however, namely that the occurrence of stress-induced analgesia 24 h after exposure to uncontrollable shocks does not depend on pre-test re-exposure to shock. While Jackson et al. (1979) established re-exposure to shocks immediately before the analgesia test as a requirement for the occurrence of stress-induced analgesia 24 h after the exposure to uncontrollable shocks, the present study suggests that re-exposure is not a necessary condition.

It is possible that these different results could be explained by some procedural differences. Previous studies have indicated that both stress-induced analgesia and helplessness depend on many experimental variables, e.g. the amount, duration or temporal aspects of shock, the region of the body shocked, and the strain of rats (Maier and Seligman, 1976; Lewis et al., 1980; Drugan et al., 1982; Urca et al., 1985a, b). While Jackson et al. (1979) restrained their rats for exposure to 80, 1 mA unscrambled inescapable shocks delivered to electrodes taped to the tail, rats in the present study were not restrained and received 60, one mA scrambled inescapable shocks delivered by the grid floor to their feet. It is possible that some of these procedural differences could have led to the different findings. Furthermore, similarities among stimuli presented in training and test sessions in the present studies could be important since Pavlovian conditioning might have occurred: if conditioned stimuli which were associated with uncontrollable shocks had been also present during the test session, the responses elicited by the uncontrollable shocks would have been elicited by these stimuli. In the present work, the animal was placed in the shuttlebox before the hot-plate test, and this shuttlebox was quite similar to the environment in which the animals had been shocked: both had the same background white noise and illumination, were made of Plexiglas walls, had a gridfloor, etc. So, if the shock induced the release of analgesia-producing substances, and if Pavlovian conditioning occurred during the shock session, then the conditioned stimuli present in the shuttlebox could have produced the release of these substances immediately after the animal was placed there. In this case the analgesic reaction observed is conditioned, controlled only by environmental cues rather than by shock per se. Jackson et al. (1979) also confined their rats in the shuttlebox with no shock before the hot-plate test. However, as these animals were preshocked in the tail in a very different apparatus (i.e. a restraining device), probably fewer conditioned aversive stimuli were present in the shuttlebox than in the present studies and, consequently, the conditioned analgesia was not observed. This Pavlovian interpretation of the differences observed between the two studies is hypothetical and thus needs to be investigated. It will be neces-
sary to compare the procedure in the present work with a procedure that does not expose the animal to the shuttlebox so as to minimize the possibility that the environmental cues elicit a conditioned response in the hot-plate test. Without this test, it cannot be asserted that the stress-induced analgesia observed here is conditioned analgesia, insofar as it is not impossible to argue that the stress-reduced analgesia observed by Jackson et al. (1979) and Maier et al. (1982) was unconditioned.

Another clear result shown by the present experiments was that both analgesia and the retarded escape acquisition produced by uncontrollable shocks were reversed by an opiate antagonist. This is an apparent disagreement with Hemingway and Reigle (1987), who reported that the analgesia and learned helplessness produced by uncontrollable shocks were not affected by the administration of naloxone before shuttlebox exposure. However, the dose of naloxone administered may account for these different results: Hemingway and Reigle administered only a 3 mg/kg dose of naloxone, a lower dose than our low effective dose of naloxone (10 mg/kg). Moreover, it has been demonstrated that whether stress-induced analgesia is mediated by opioid or nonopiod mechanisms depends on many characteristics of stressful events and/or environmental stimuli associated with these events. For example, the analgesia shown by rats chronically exposed to signal-shock pairings (Bersh et al., 1986) was mediated by opioid systems, whereas the analgesia observed 1 min after a series of intermittent uncontrollable shocks or after 3 min of a continuous shock was nonopioid (Lewis et al., 1980; Maier et al., 1982).

The present results suggest that both the stress-induced analgesia and the helplessness are opioid-mediated. The fact that the same condition that produces or prevents retarded acquisition of escape responses might also produce or prevent opioid analgesia may support an interpretation that the learned helplessness effect simply reflects decrements in pain sensitivity, rather than learning that the aversive outcome cannot be controlled. However, some reports are not consistent with this interpretation. For example, Urca et al. (1985b) found that a procedure that induced marked deficits in learning an escape task produced enhancement, rather than attenuation, of analgesia after an opioid antagonist treatment. So, it is possible that independent neurochemical mechanisms activated by uncontrollable shocks could be responsible for the opioid analgesia as well as for the helplessness effect. Only further investigation will be able to elucidate this point.

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REFERENCES

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