Analgesia following startle-eliciting stimuli

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This study investigated the possibility that startle-eliciting stimuli could produce analgesia, as measured by paw-lick latencies on a hot-plate analgesia test. Rats were treated in a 5 X 2 design: drug (saline, naloxone) X treatment (startle, no startle). First, rats were administered the naloxone (6 mg/kg) or the saline. One animal following injection, the startle groups were presented with nine bursts at 60 sec intervals, and the no-startle groups received 10 min of exposure to the chamber, with no noise bursts. The hot-plate test was then administered. Two hours later, the rats were returned to the startle chamber for 60 sec. The paw-lick latencies of the startle and no-startle groups were significantly longer than those of the no-startle group, suggesting that startle produces stress-induced analgesia. The naloxone-startle group had a shorter latency than the saline-startle group, suggesting that the stress-induced analgesia is partly opioid-based. There were no significant differences between the naloxone-startle and saline-startle rats in startle amplitude or freezing. When returned to the startle chamber 24 h later, however, the naloxone-startle rats froze more than did the saline-startle rats.

Exposure to a variety of different stressors results in decreased responsiveness to pain sensitivity for a period that usually outlasts the duration of the stimulus (Hays, Bennett, & Mayer, 1978). Stress-induced analgesia was once thought to be mediated by endogenous opioid peptides (Aki, Penar, & Barsky, 1976); however, more recent evidence (Weinstein & Mayer, 1982) concludes that there were many forms of stress-induced analgesia, with clear evidence for opioid/hormonal, opioid/hormonal, and nonopioid types. Many different kinds of stimuli seem to produce an analgesia mediated by endogenous opioids; in some of these stimuli are either painful or associated with pain. This experiment examines the possibility that a startle-eliciting stimulus, usually thought to be nonpainful (e.g., Warren, 1982), might also provide an analgesia mediated by endogenous opioids. This might be expected because previous studies (Borsa, Cranney, & Leaton, 1983; Cranney, 1987) suggest that the startle stimulus is capable of supporting conditioned freezing in the experimental context. This implies that the startle stimulus is aversive and may elicit a nociceptive response, and thus produce an analgesia somewhat similar to that induced by low levels of shock.

The freezing response is one of the rat's species-specific defense reactions (Bolles, 1979), and it can be conditioned to the apparatus stimulus that has been associated with an aversive stimulus, such as shock (e.g., Fanselow, 1980). Fanselow (1981, 1984; Fanselow & Bolles, 1979) reported that naloxone, an opioid antagonist, enhanced the reversion to the aversive stimulus; he argued that naloxone enhanced the perceived intensity of the shock by untagonizing endogenous opioid analgesic systems. He also argued that these effects were specific to nociceptive stimuli and, in particular, that these effects did not occur with an acoustic startle stimulus. Borsa et al. (1985), however, reported that the freezing that normally develops during the first few days of startle habituation training was affected by manipulations that affected conditioning, such as latent inhibition and extinction. They concluded that the increased startle responsiveness and freezing behavior that they observed resulted from the formation of an association between the initially aversive startle stimulus and the contextual stimuli of the startle apparatus. In terms of opioid manipulations, Cranney (1987) reports that a higher startle stimulus intensity and naloxone pretreatment increased context-conditioned freezing. Thus appears that the acoustic startle stimulus may have nociceptive or other averse properties that can support a conditioned response, particularly freezing to contextual stimuli, and that the underlying mechanisms may be opioid in nature. If the startle reflex does somehow engage the nociceptive opioid system, then it is possible that it also elicits an opioid-based stress-induced analgesia.

The current experiment tested the possibility that the startle stimulus could produce stress-induced analgesia, as indexed by paw-lick latency. In addition, the opioid antagonist, naloxone, was administered to half of the animals. If endogenous opioids were involved in mediating stress-induced analgesia, then there should be reduced analgesia in the naloxone animals. Startle and freezing responses were also monitored, as the basis of previous work, it was expected that there would be no significant differences in startle and freezing responses between the groups in Session 1 (Borsa et al., 1985; Cranney, 1987), but that the naloxone-treated group would show a higher level of context-conditioned freezing in Session 2.

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METHOD

Subjects
The rats were 16 experimentally naive male albino Wistar rats obtained from the University of New South Wales Animal Breeding and Holding Units and weighing between 370 and 475 g. The rats were housed in large, white plastic boxes (60 x 40 x 20 cm) with 8 animals per box. Water and food were available ad lib. The rats were handled at least 20 sec per day for 4 days before the experiment began. They were handled and tested during the middle of the light portion of a 12:12-h light-dark cycle.

Apparatus
The apparatus was similar to that described by Lister and Rosenberger (1973). Briefly, it consisted of a 20 x 20 x 22 cm opaque chamber enclosed in a sound-attenuated, sound-eliminated box with an observation window in the front wall. Vertical displacement of the chamber moved a piece of plastic-covered film material attached to a rigid structure, in such a way that movement produced a visual image in front of the rat.

The startle stimulus was a 50-msec burst of white noise with a 5-msec rise/fall time, and with a peak intensity of 100 db SPL (re: 0.0002 dyne/cm²). It was delivered through a Teac Piezo Horn (Type 40-175c), attached at an angle to the frame of the startle apparatus. Continuous white noise (75 db SPL re: 0.0002 dyne/cm²) was used as a background auditory stimulus. All animals were superimposed on the background of white noise. Stimulus and background sounds were measured with a field and Kaiser sound level meter (Type 2225), with a microphone placed in a standard, central position within the chamber.

To test for spontaneous startle, the background of white noise was maintained during all trials except for trials in which the startle stimulus was presented. A loudspeaker positioned in a sound-attenuated box (50 x 40 x 30 cm) was used to deliver non-food reinforcers (crushed maguey seed) to the rats. The housing room was maintained at 54° C.

Procedure
The animals were fasted overnight, and then randomly assigned in one of four cells formed by 2 x 2 factorial design: drug (saline, nicotine) X treatment (sitarrine, no sitarrine). The rats were administered either saline or nicotine (4 mg/kg) 10 min before the startle apparatus. After 60 sec, half of the animals in each drug group were exposed to the background auditory stimulus, while the other half were exposed to the non-food reinforcers. The other half of the animals were left in the chamber for 10 min, no startle stimulus was presented. Immediately following the end of the trial, all rats were given the liquid diet. This consisted of placing the rats in the cylinder and measuring the latency to the first paw lick.

RESULTS

Table 1 presents the mean paw lick latencies for each of the groups. Planned t-tests indicated that the paw lick latencies of the sitarrine groups were significantly greater than those of the no-sitarrine groups (t(14) = 2.64, p < 0.05). This finding suggests that sitarrine produces analgesia. The nicotine-sitarrine group had a shorter latency than the saline-sitarrine group (t(6) = 2.78, p < 0.05). This finding suggests that the analgesia is at least in part nicotine-based.

For Session 1 data, a two-way analysis of variance of the first and last trial responses of the two groups revealed no significant main or interaction effects (see Table 1). The mean percentage freezing over the 10-sec periods prior to the 10-sec stimulus was also calculated. Across the 10 trials in Session 1, the no-sitarrine groups did not freeze at all, and there was no difference between the saline-sitarrine and nicotine-sitarrine groups freezing levels. During the 60 sec prior to the first stimulus in Session 2, the nicotine-sitarrine group showed more freezing than did the saline-sitarrine group (t(6) = 1.93, p = 0.05; see Table 1).

DISCUSSION

These findings support the notion that the presentation of repeated, moderately high-intensity startle-eliciting stimuli elicits analgesia, and this analgesia may be opiate-based, as indicated by the attenuation of analgesia by morphine administration. On the basis of the freezing data, it could be argued that the sitarrine groups had

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>t-test Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sal-No-Sitarrine</td>
<td>1.4</td>
<td>0.5</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Nic-No-Sitarrine</td>
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<td>0.4</td>
<td>2.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Sal-Sitarrine</td>
<td>1.0</td>
<td>0.6</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Nic-Sitarrine</td>
<td>0.8</td>
<td>0.4</td>
<td>2.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Note: Sal = saline, Nic = nicotine. Freezing for Session 1 is the mean of the 10-sec periods prior to each trial stimulus. Freezing for Session 2 is the mean of the 60-sec observation period 2 hr after Session 1.
nociceptive properties that contributed to the analgesia, although it should be noted that the intensity used (120 dB) is well below the level at which tissue damage occurs. It may not be necessary, however, to invoke a nociceptive mechanism, because, although shock is commonly employed experimentally to produce analgesia, other stimuli which would not activate nociceptors, such as restraint (e.g., Fanselow & Sigmundi, 1986), also produce analgesia. It could be argued that stimuli that are not necessarily painful, but that may be associated with situations of adaptive relevance (e.g., sudden loud noises may signal the approach of a predator) will evoke an opioid-based analgesia and defensive behavior, such as freezing.

The finding of a stimulus-induced analgesia has implications for the use of the startle stimulus to assess other phenomena. In particular, investigators who use the startle stimulus to assess different forms of analgesia (e.g., Leitner, 1985; Watten & Butt, 1982) should be aware that the startle stimulus itself may produce stress-induced analgesia and concomitant biochemical changes.

The finding that the startle groups showed freezing to the experimental context 2 h after the initial session suggests the development of context-conditioned freezing, and corroborates previous reports of this phenomenon (Borst et al., 1985; Crasneck, 1987). That there was more context-conditioned freezing in the naloxone–startle group corroborates a similar finding by Crasneck (1987), and suggests an opiate basis to context condition- ing. It should be noted that, in Session 1, the average freezing during the 10 prestartle periods was similar for the two groups. This reflects the similar development of freezing in the two groups both started the session with no freezing, but after three to four startle stimulus presentations, they started to freeze, and by the end of the session, most animals were freezing during the whole of the prestartle sample. In contrast, during the 60-sec observation period of Session 2, there was a difference in the freezing levels of the two groups, with the naloxone–startle group freezing more, at about the same average level for Session 1. These data suggest that naloxone does not increase the within-session "prestartle" freezing response, which contrasts with Fanselow's (1981; Fanselow & Bolles, 1979) report that naloxone enhances the postshock freezing response. Rather, in this study, naloxone enhanced the longer term contextual-conditioned freezing in Session 2. This finding suggests that naloxone has a specific enhancing effect on the context-startle association process.

The results of this experiment suggest that startle can elicit analgesia, and that this analgesia is opioid-based. In addition, the previously reported finding of naloxone-enhanced context-conditioned freezing with startle stimuli was replicated. Future work could investigate the extent to which context conditioning and stress-induced analgesia interact and are mediated by opioid mechanisms; in particular, it may be that the startle-induced analgesia is conditionable to contextual stimuli.

REFERENCES


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