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NALOXONE HYPERALGESIA AND STRESS-INDUCED ANALGESIA IN RATS

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SUMMARY

Since past studies concerning the effects of naloxone on nociception have yielded inconclusive findings, the variables of pain test, baseline sensitivity, and stress condition were examined. Within a pure-bred strain of rats, consistent individual differences did not occur. All three measures of pain responsiveness demonstrated hyperalgesic effects of naloxone, but they differed in their capacity to reflect the effects of analgesia produced by continuous or intermittent electrical shock. By some measures, naloxone reversed the stress-induced analgesia due to intermittent shock; it did not influence the analgesia produced by continuous stress. The data support a model of pain inhibition involving both opioid and non-opioid systems and suggest that the hyperalgesic effects of naloxone can sometimes give rise to erroneous conclusions concerning apparent naloxone-reversability of putative analgesic procedures.

Narcotic antagonists such as naloxone, which occupy the receptor sites for opioid binding, block the analgesic properties of procedures which release the endogenous polypeptides, endorphins (1). If endorphins are tonically active pain inhibitors in the central nervous system, administration of naloxone would produce hyperalgesia, an intensification of the behavioral responses to noxious stimulation.

Past reports concerning the effects of naloxone on pain responses have been inconclusive. For instance, several human studies have indicated that naloxone did not affect ratings of cold pressor pain (2) or threshold and tolerance measures for electric shock (3), while one study (4) did demonstrate a significant increase in reported pain intensity following a dental extraction. In the animal literature, there are studies in which naloxone had no effect on tail-flick (5), shock escape (6), or writhing after formic acid injection (7) and there are others in which squeak (8), writhing (9), or jump (10) latency was reduced. These differences, of course, may not be entirely inconsistent, since the experiments differed in nociceptive measure, species, dosage, and other variables. Within one study (11), naloxone had no effect on paw-lick but reduced latency in a jump test. The present study deals, in part, with the issue of pain measurement: does it matter how we assess nociception in the rat?

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A second issue is that of individual differences. Organisms appear to differ considerable in endorphin levels, characteristics of binding sites, and other variables which influence the response to pain (12). Several investigators, employing both humans (13) and lower animals (14), reported pain-insensitive subjects (with a possible high level of endorphin) showed hyperalgesia after naloxone, but pain-sensitive ones (possible low endorphin level) did not. This study examines whether individual differences in baseline sensitivity of rats predicts their response to naloxone.

The third issue deals with the neurochemical effects of different analgesic procedures. Certain treatments produce analgesia which is reversed by naloxone while other yield increases in pain tolerance which are not reversed (e.g. 15). Consequently, there appear to be multiple pathways mediating antinociception which involve both opioid and non-opioid systems. Different methods of assessing pain may be sensitive to changes produced in one or the other system; others may respond to both.

Amir and Amit (16), for instance, found that immobilization stress produced analgesia when measured by jump latency from a hotplate, but no alteration in paw-lick latency. The former effect was naloxone-reversible, suggesting to them that the stressor influenced an endorphinergic system which mediates a long-latency affective response but not a short-latency sensory one.

Lewis, Cannon, and Liebeskind (17) described two forms of stress induced analgesia which appear to operate through distinct neurochemical systems. Prolonged intermittent foot-shock (every 5 seconds over 30 minutes) produced an increase in tail-flick latency which was reversed by naloxone; 3 minutes of continuous shock led to a similar analgesic effect which was not reversed. They suggested that the former is opioid-mediated whereas the latter involves non-opioid mechanisms.

This report examines the factors of nociceptive measure, individual differences, and stress condition as critical variables in naloxone studies of analgesia.

METHODS

The subjects were 40 male Sprague-Dawley rats, weighing between 200 and 300 g, whose pain tolerance was tested in three ways:

1. Tail-flick test. The rat was wrapped in a towel, held at a 45° angle to a thermostatically-controlled (Braun Thermomix) water bath set at 53°C in a 12 l Pyrex beaker. The latency between submersion of the tail and its removal from the water by the animal was registered, with a maximum time of 10 seconds.

2. Paw-lick test. The animal was placed on a electrically-heated, thermostatically-controlled (Technilab Instruments, model 11) metal plate set at 53°C in a plexiglass chamber (25 cm by 27 cm, 30 cm high). Latency was determined between entry into the chamber and the onset of licking behavior directed to the understand of a front or hindpaw.

3. Jump test. Using the hotplate apparatus, the time was noted between entry and the animal's jump to avoid contact of the paws with the heated floor. The paw-lick and jump tests were performed concurrently. Both latencies were noted, no matter which response occurred first. The hotplate test was terminated immediately after the second response or the passage of 70 seconds.

Continuous and intermittent constant current shocks (60 Hz, 2 ma) were presented through the grid floor of a Lehigh Valley operant chamber equipped with a solid-state scrambler. The first group received 3 minutes of continuous foot shock following 12 minutes of no shock; the second group was exposed to 1 second of shock every 5 seconds for 15 minutes.

The experimental procedure, always performed during the light phase of the light-dark cycle, began with two baseline trials, separated by five days. After another five days, rats were given a subcutaneous injection of 10 mg/ml/kg of naloxone HCl or 1 ml/kg of normal saline 15 minutes before an evaluation of possible hyperalgesic effects of the drug on all three nociceptive measures. The experimental and control groups were blocked so that they had equal representation of high and low tolerant rats based upon their average baseline paw-lick latencies (median split at 11 seconds). At least five days later, the rats were grouped into blocks of four, based upon baseline paw-lick performance, and then randomly assigned to one of four drug/stress conditions: brief continuous shock or prolonged intermittent shock following injections of 10 mg/ml/kg of naloxone HCl or 1 ml/kg or normal saline. The injections were given immediately prior to the stress period. Following the stressor, all rats received the tail-flick, paw-lick, and jump tests.

RESULTS

Pearson product-moment correlations between the three response latencies were determined both within and across the two baseline sessions. On the first session, only the correlations between paw lick and jump were significant. ($r = .27$, $p < .05$) while on the second determination, none of the latencies correlated significantly. When correlations between the same measure were examined across days, only that for paw-lick latencies achieved signifance ($r = .62$, $p < .01$).

Animals separated on the basis of paw-lick latency were randomly assigned to naloxone and saline control conditions in the test for hyperalgesia. Two-way analyses of variance for each dependent measure revealed a significant effect of drug condition ($F = 6.55$ for tail flick, 5.44 for lick, and 5.82 for jump; $p < .05$ for each), but neither baseline tolerance nor drug-pain-tolerance interactions produced any effect.

The hyperalgesic effects of naloxone are illustrated in Figure 1. On all three measures, tail-flick, paw-lick, and jump, animals injected with naloxone responded more quickly than they did on the baseline trials (tail-flick, $t(19) = 3.81$, $p < .01$; paw-lick, $t(19) = 2.14$, $p < .05$; jump, $t(19) = 2.38$, $p < .001$) and, as noted earlier, more quickly than the control group receiving saline.

In the experiment which tested for stress-induced analgesia, both continuous and intermittent shock had antinociceptive properties when measured by some methods, but not by others. As seen in Figure 2, the results are complex. A three-way analysis of variance, assessing the effects of stress, tolerance, and drug condition on each of the response latencies, indicated a main effect of drug on paw lick ($F(1,32) = 6.79$, $p < .05$) and one approaching significance on tail flick ($F(1,32) = 3.68$, $p = .063$). Using continuous shock, significant increases in response times for the saline-treated animals were obtained with both tail-flick ($t(9) = 2.59$, $p < .05$) and paw-lick ($t(9) = 4.84$, $p < .001$) but not with the long-latency and high-variability jump measure. Intermittent shock caused a highly significant increase in latency for paw-lick ($t(9) = 5.57$, $p < .001$), but not for tail-flick or jump.

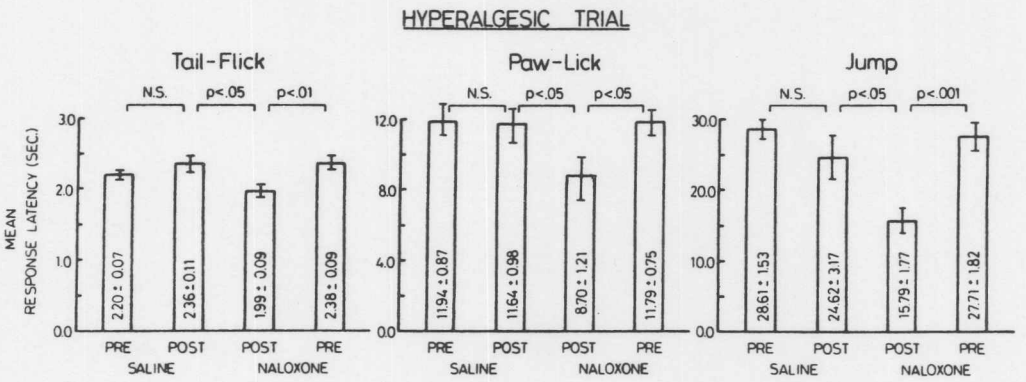

FIG. 1

Effects of naloxone on tail-flick, paw-lick, and jump tests of pain responsiveness. Pre shows the mean response latency on two baseline trials for the animals in that group; Post shows the latency obtained on a subsequent testing day 15 minutes after the injection of naloxone or saline. Comparisons are made within a treatment group (saline or naloxone pre-post) and across groups (saline-post - naloxone-post).

By the tail flick measure, a greater analgesic effect occurred after continuous shock than after intermittent ($F = 8.54$, $p < .01$), but no differences were obtained using the other indices. The analysis of variance failed to reveal any effect of baseline tolerance.

Figure 2 also shows the differential effects of naloxone on the two forms of stress-induced analgesia. Both tail-flick and paw-lick latencies were lower for the animals receiving naloxone prior to intermittent shock than for their similarly-stressed saline controls ($t(18) = 2.67$, $p < .05$ and $t(18) = 2.75$, $p < .05$ for tail-flick and paw-lick respectively). The analgesic effects of continuous shock, however, were independent of whether saline or naloxone were administered.

DISCUSSION

Four major conclusions stem from the results obtained in this study: 1) differential measures of pain sensitivity show parallel alterations in tests for hyperalgesia but do so only sometimes in response to manipulations influencing analgesia; 2) baseline pain sensitivity is not generally consistent across measures nor is it a good predictor of a pure-bred rat's response to naloxone or to stress; 3) naloxone given subcutaneously significantly decreases the response latency to intense thermal stimulation, and 4) by some measures, stress-induced analgesia occurs after both continuous and intermittent shock, although the underlying neural mechanisms are not equivalent since the latter is reversible by naloxone whereas the

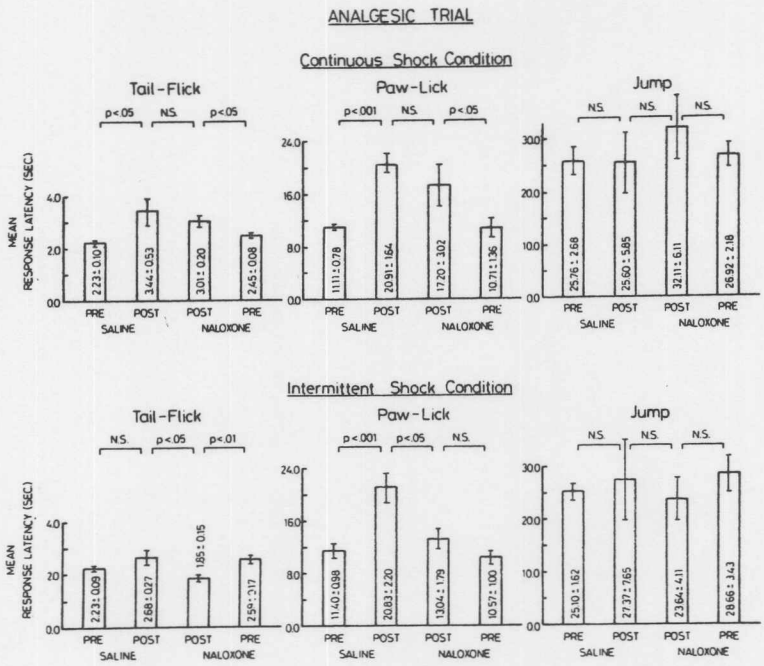

FIG. 2

Effects of naloxone on the influences of continuous and intermittent shock on tail-flick, paw-lick, and jump tests of pain responsiveness. Pre shows the mean response latency for the animals in that group; Post shows the latency obtained on a subsequent testing day 15 minutes after the injection of naloxone or saline and the presentation of continuous or intermittent shock. Comparisons for each stress condition are made within and across treatment groups for analgesia (saline or naloxone pre-post), hyperalgesia (naloxone pre-post), and naloxone-reversal of analgesia (saline-post - naloxone-post).

former effect is not.

The three measures are not equivalent tests of some general pain response; their neural and motivational properties are likely to vary considerably. Consequently, analgesic studies must look at multiple indices of pain (18, 19), in order to identify the specific mechanisms and avoid unjustifiably suggesting that a treatment is without effect.

The jump measure is a particularly difficult one. First, it shows high variability both across and within animals. Second, although it generally occurs after tail-flick and paw-lick responses, stressors which involve foot-shock and produce vigorous jump responses may predispose the animals to jump in later hotplate tests (frequently prior to the paw lick), increasing the variability yet further. The jump test did not reveal any effects of either stressor or drug, although, interestingly, the standard error increased by two to four times following the stress while the mean remained constant.

Tail-flick, despite its short latency and presumptive spinal mediation, is often sensitive to opiates (15). However, since the rats would frequently squeal and struggle vigorously immediately following their tail withdrawl, this quite rapid response may tap higher centers as well.

The paw-lick response was the only one which demonstrated a significant test-retest reliability, and it, therefore, was selected for blocking the animals on subsequent tests. The proposal (10, 16) that paw-licking behavior is uninfluenced by endorphins and that it reflects the sensory properties of pain behavior but not the affective ones was not supported; indeed, the paw-lick response was particularly naloxone sensitive, showing both hyperalgesia and the reversal of analgesia induced by intermittent shock. Paw-lick is not simply a sensory index; twitching and lifting of the limbs as well as greatly increased exploratory behavior often precede it.

The tolerance for pain, as measured by the paw-lick response, was not a significant factor in the hyperalgesic and analgesic trials. In fact, the data obtained from saline-treated animals did not reflect the different latencies they exhibited on the baseline trials. Consequently, the animal data failed to follow the findings of Buchsbaum et al (13) in which high tolerance human subjects described electric shock as being significantly more painful after naloxone injection, whereas low tolerance subjects did not. However, individual differences in endogenous opiate levels are unlikely to manifest themselves significantly within a selective genetic strain. The subjects in this study were Sprague-Dawley rats; other research (20) has demonstrated that inter-strain differences in pain responsiveness can be quite large.

Naloxone caused hyperalgesia on all three nociceptive tests. Past inconsistencies cannot be attributed simply to differences in the dependent variable. Although mice given naloxone demonstrated reduced jump latencies without changes in time preceding paw-lick (10, 11), the outcome of the present study challenges the view that only long-latency pain response involve opiate-related systems. Naloxone may block endorphins which are released in response to affective pain properties; it may also, as Goldstein (21) has suggested, release a hyperalgesic substance which is normally under the inhibitory control of endorphins.

The effects of continuous and intermittent stress are also relevant to this distinction. Lewis et al (17), using tail-flick in the rat, found that both stressors produced analgesia, but only the second effect was naloxone-reversible. In this study, a similar pattern of results was obtained when pain tolerance was measured by the paw-lick test. Saline-treated animals exposed to either stressor showed significantly increased response times; only those receiving intermittent shock had their analgesia reversed by naloxone.

The paw-lick data are compatible with a model of pain inhibition involving both opioid and non-opioid systems: intermittent shock causes analgesia because of its naloxone-reversible actions on the opioid system; continuous shock involves non-opioid mechanisms in producing its analgesic outcome. The possible neural mechanisms responsible for these outcomes are discussed by Watkins and Mayer (22).

The tail-flick data, however, point to an alternative explanation for at least some forms of naloxone-reversible effects. With that measure, animals receiving both intermittent shock and naloxone had significantly shorter latencies than those exposed to the same stressor after saline injections. The naloxone group responded faster after treatment than on their baseline trial. However, there was no significant pre-treatment - post-treatment difference for the saline animals; that is, no analgesia. Accordingly, it appears that by this measure, the difference between the naloxone and the saline groups after treatment is not due to the reversibility of stress-induced opioid-mediated analgesia but, rather, to a hyperalgesic effect of the drug.

This conclusion could apply even in cases where analgesia is produced; reversal of analgesia by naloxone does not provide conclusive evidence that the elevation in pain tolerance is mediated by an opioid system. The stress could influence a non-opioid system, causing higher response latencies and the naloxone could appear to reverse that effect because of its hyperalgesic effects on an opioid mechanism. Moreover, naloxone, despite its role as a narcotic antagonist, can have nonopioid effects (23). If the opioid and non-opioid systems have inhibitory influences on each other as well (24), if spinal opiates serve as neuromodulators of postsynaptic activity (22), and if ongoing pain serves to activate an endogenous analgesia system (22), highly complex interactions of stress, drug, and pain measures can occur, and the disparate results in the literature should not appear surprising. Since naloxone administration can produce results which give rise to erroneous conclusions about the mechanisms of analgesic procedures, appropriate controls (such as tests for cross-tolerance with morphine (23, 25)) and appropriate cautions seem to be in order.

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