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BRIEF COMMUNICATION

Naloxone-Induced Analgesia: Effects of the Benzodiazepine Antagonist Ro 15-1788

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CAPPELL, H , D M KNOKE, A D LÊ AND C X POULOS *Naloxone-induced analgesia Effects of the benzodiazepine antagonist Ro 15-1788* PHARMACOL BIOCHEM BEHAV **34**(1) 197–200, 1989 —Repeated exposure to pain under the influence of the opiate antagonists naloxone and naltrexone leads to the recruitment of substantial analgesia as measured by paw-lick latency on the hot-plate test (4,11) One hypothesis to explain this naloxone-induced analgesia (NIA) is that nociceptive stimulation in the face of opiate blockade becomes stressful enough to activate an analgesic adaptation that otherwise would not occur This hypothesis was examined in two experiments by the administration of a benzodiazepine antagonist with anxiogenic properties (Ro 15-1788, in a dose of 10 mg/kg) in conjunction with repeated administrations of naloxone (5 mg/kg) One experiment incorporated defecation as a relatively direct measure of stress Ro 15-1788 reliably augmented NIA Defecation was increased by naloxone alone and in combination with Ro 15-1788 Overall, the results were most consistent with the hypothesis that NIA is a form of stress-induced analgesia that is at least partly nonopiate in nature

Emotionality Naloxone Naloxone-induced analgesia (NIA) Ro 15-1788 Stress-induced analgesia

RECENTLY, there have been independent reports (4,11) of a form of analgesia that depends for its occurrence on the repeated *administration* of an opiate antagonist This naloxone-induced analgesia may be abbreviated as NIA Greeley *et al* (4) found that NIA had several stable features in their paradigm. It was not apparent on the first exposure to the hot-plate, but emerged only gradually with repeated exposure to nociceptive stimulation There was also no suggestion of tolerance to the effect

Since naloxone administered acutely can increase responsiveness to pain (5, 8, 9), it is possible that naloxone blockade increases the effective intensity of nociceptive stimulation, thereby making it more stressful Thus, NIA may be considered within the context of "stress-induced" analgesia, which can be mediated both by opiate and nonopiate mechanisms (7,12). In the face of blockade of endogenous opiate activity by naloxone, stress could engage a "collateral" adaptive mechanism to cope with nociceptive stimulation (1, 4, 11).

If NIA is a form of stress-induced analgesia, it should be possible to augment it with the coapplication of an anxiogenic manipulation One pharmacological method for manipulating anxiety is the administration of a benzodiazepine antagonist (3) Recently, Davidovich *et al* (2) hypothesized that the benzodiazepine antagonist Ro 15-1788 "should increase the anxiogenic effect of environmental manipulations resulting in an activation of the systems involved in analgesia (p 175) " In the experiments described below, we compared the effects of repeated administration of naloxone and Ro 15-1788 administered both separately and simultaneously If NIA is mediated by stress, Ro 15-1788 should enhance it To obtain an index of stress apart from analgesia itself, in one experiment we recorded defecation on test trials (6,13).

EXPERIMENT 1

METHOD

Subjects

The subjects were 53 male Sprague-Dawley rats weighing 290–340 g at the beginning of the experiment

Apparatus and Procedure

Hot-plate apparatus The apparatus was a water bath contained

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in a plastic tub covered by an aluminum plate (Grant Instruments Water Bath Model AB02) The temperature of the hot-plate was maintained at 49 5 ± 0 05 degrees C throughout testing Plate temperature was continuously monitored by a probe which was secured with clamps to the surface of the aluminum plate During testing, rats were confined to the surface of the plate by a Plexiglas chamber (27 × 16 5 × 9 cm) with a hinged top which was mounted atop the hot-plate

Screening trials Rats were handled 5 times over a 7-day period for purposes of taming Baseline pain sensitivity was then assessed in a series of 5 screening trials These trials and all subsequent experimental testing were conducted in the colony room between 1500 and 1800 hr, which was during the light phase of a 12-hr light/12-hr dark cycle On the first 3 screening trials, rats were removed from the home cage, weighed, and placed on the hot-plate until the first paw-lick was recorded If no paw-lick occurred during the maximum interval permitted (45 sec on the first trial and 30 sec thereafter) the rat was removed from the plate and assigned the maximum score On the last two screening trials, rats were weighed and then injected IP with physiological saline (1 ml/kg) 15 min prior to testing

Test trials For assignment to experimental groups, rats were matched on mean paw-lick latencies based on the last two screening trials Four groups were formed based on combinations of treatments with physiological saline (SAL) or naloxone hydrochloride (NAL) combined with Ro 15-1788 (RO) or a vehicle (VEH) The dose of naloxone was 5 mg/kg administered SC in solution with physiological saline, injection volumes of naloxone and saline were 1 ml/kg Ro 15-1788 was administered in a dose of 10 mg/kg in a vehicle consisting of 10 ml of distilled water to which 2 drops of Tween 80 were added Injections were IP in a volume of 5 ml/kg Injections were given 15 min apart The groups thus formed, with the injections indicated in temporal order (e g , RO-NAL means RO followed by NAL), were as follows RO-NAL (n = 14), RO-SAL (n = 13), VEH-NAL (n = 13), VEH-SAL (n = 13)

There were single acquisition trials on each of 7 consecutive days Fifteen min after the second injection, rats were placed on the hot-plate On the first three trials, if no paw-lick was observed within a maximum latency of 30 sec, a score of 30 sec was assigned and the rats were removed from the hot-plate Thereafter, the maximum latency was increased to 45 sec

EXPERIMENT 2

Subjects

The subjects were 69 male Sprague-Dawley rats weighing 325–400 g at the beginning of the experiment Housing and maintenance conditions were as described in Experiment 1

Apparatus and Procedure

The apparatus and procedure for assessing analgesia were as in Experiment 1, with the exception that there were only three screening trials on the hot-plate, 6 acquisition trials, and the maximum latency recorded before removal from the hot-plate was always 45 sec On each of the last 5 acquisition trials, the number of fecal boli deposited by each rat during the 45-sec interval on the hot-plate was recorded Boli were removed and the apparatus was thoroughly cleaned between tests The experimental groups, with the treatments given in order, were RO-NAL (n = 17), RO-SAL (n = 17), VEH-NAL (n = 18), VEH-SAL (n = 17)

RESULTS

The mean paw-lick latencies for all acquisition trials of Experiment 1 are shown in Fig 1 Increasing latencies were evident in groups that received naloxone, but as with previous findings (4), this change emerged only over repeated trials on the hot-plate A 2 × 2 × 7 analysis of variance (ANOVA) was conducted with between groups factors of Drug Treatments (RO or NAL), and a within groups factor of Trials A glance at the figure indicates that there were no differences among the groups in paw-lick latency on Trial 1 Differences emerged progressively Paw-lick latencies were low and stable in rats treated with saline or with Ro 15-1788 alone When naloxone was injected, latencies increased gradually over trials. Finally, paw-lick latencies involving naloxone in combination with Ro 15-1788 grew progressively and were longer than those with naloxone alone The pattern of data suggests that the most critical analyses to consider are the interactions The interaction of NAL × Trials, $F(4,294) = 16\ 32$, $p < 0\ 001$, indicates that paw-lick latencies became larger over trials in rats injected with naloxone compared to those that were not Tukey tests showed that paw-lick latency among rats injected with naloxone exceeded those of vehicle controls ($p < 0\ 05$ or less) from the second trial onward The interaction of RO × Trials was weaker ($F = 2\ 06$, $p < 0\ 06$) because treatment with Ro 15-1788 was not invariably associated with an increase in latency over trials However, the critical interaction of RO × NAL × Trials was significant, $F(6,294) = 2\ 18$, $p < 0\ 05$ The interpretation of this interaction is that over trials, paw-lick latencies became relatively longer when naloxone was given than when it was not, but that this tendency was even stronger when Ro 15-1788 and naloxone were given together Tukey tests showed that the augmentation effect (RO-NAL vs VEH-NAL) became significant by Trial 4 ($p < 0\ 01$) and thereafter

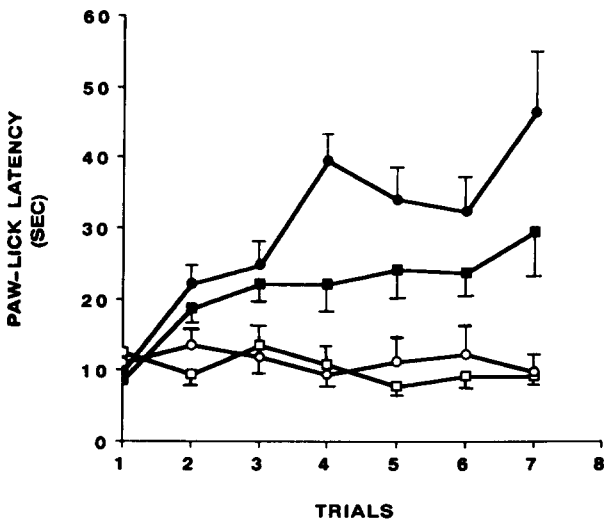

FIG 1 Mean paw-lick latencies in Experiment 1 The legend depicts the treatments as follows (RO, Ro 15-1788, NAL, naloxone, SAL, saline in which naloxone was dissolved, VEH, the vehicle for Ro 15-1788) The symbols give the order in which injections were given (i e , RO-NAL means Ro 15-1788 followed by naloxone) Standard errors are indicated on one side of each data point ● RO-NAL, ○ RO-SAL, ■ VEH-NAL, □ VEH-SAL

Paw-lick latencies for the four groups of Experiment 2 are shown in Fig 2 The ANOVA was the same as that indicated for Experiment 1 Once more, the effect of principal interest was the interaction of RO × NAL × Trials, $F(5,325) = 3\ 94$, $p < 0\ 002$ Although the main finding of Experiment 1 concerning the combination of Ro 15-1788 and naloxone was confirmed, the

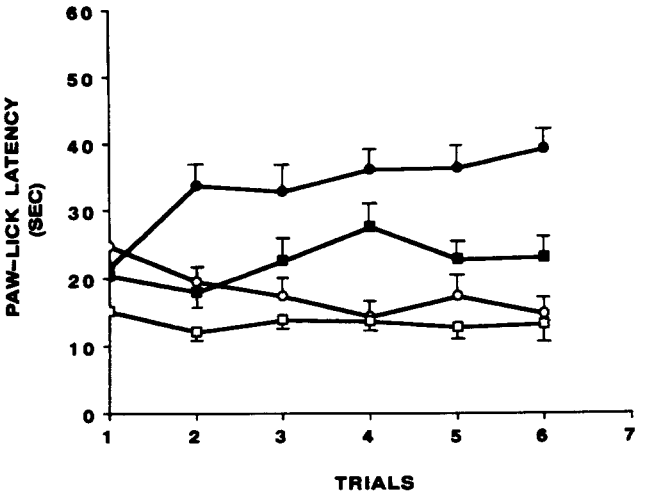

FIG 2 Mean paw-lick latencies in Experiment 2 Symbols are explained in Fig 1 Although RO by itself increased paw-lick latency in this experiment, the effect was no longer significant by Trial 3 When combined with naloxone, the augmentation of NIA by RO persisted as it did in Experiment 1

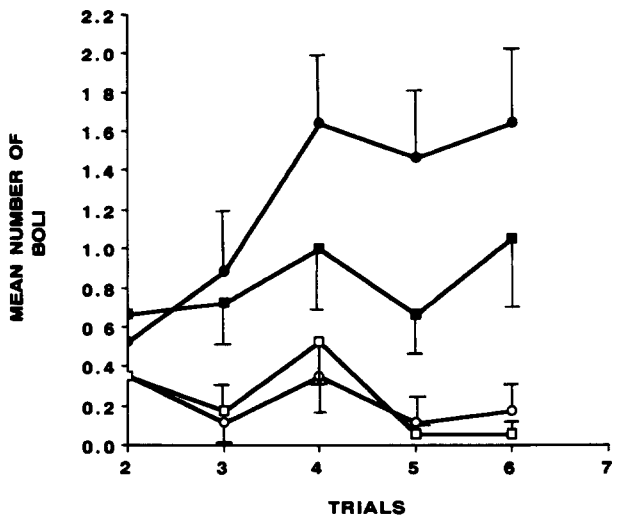

FIG 3 Mean number of boli deposited during analgesic testing in Experiment 2 The symbols are explained in Fig 1

pattern in the data was somewhat different from that in Experiment 1 The groups in Experiment 2 did not all begin with similar latencies in the first trial. Ro 15-1788 alone had an effect on Trial 1, but it diminished over trials In contrast, Ro 15-1788 was involved in a steadily increasing effect when it was administered in combination with naloxone The interaction was explored in more detail with individual comparisons based on Tukey tests. When Ro 15-1788 was administered alone, it resulted in significantly increased paw-lick latencies (comparing RO-SAL vs. VEH-SAL) on Trial 1 ($p<0$ 01) and Trial 2 ($p<0.05$); however, by Trial 3 and thereafter, this difference was no longer significant When given in combination with naloxone, Ro 15-1788 exerted no differential effect (RO-NAL vs. VEH-NAL) on Trial 1, but contributed to an increased paw-lick latency on Trial 2 and thereafter ($p<0.01$ on each trial) Naloxone by itself (VEH-NAL vs VEH-SAL) exerted a significant effect by Trial 2 ($p<0$ 05) and thereafter ($p<0$ 01 on each trial)

There was some other evidence of acute effects that did not occur in Experiment 1 The combination of Ro 15-1788 and naloxone had a significant effect compared to no drug treatment on Trial 1 (RO-NAL vs. VEH-SAL, $p<0$ 05), but naloxone alone did not (VEH-NAL vs VEH-SAL), this was despite the approximately equal latencies in the RO-NAL and VEH-NAL groups on Trial 1

The defecation scores from Experiment 2 are shown in Fig 3, which gives the mean number of fecal boli on each of the trials (2–6) on which defecation was recorded The ANOVA for these data was a $2\times2\times5$ analysis of RO × NAL × Trials Again, the interactions are of greatest interest The interaction of NAL × Trials was significant, $F(4,260)=3.84$, $p<0$ 01 This occurred because rats injected with naloxone defecated more than those that were not, and because these differences grew larger with trials

Although the pattern in Fig 1 suggests that the combination of Ro 15-1788 and naloxone produced more defecation than naloxone alone, the interaction of RO × NAL × Trials failed to confirm this difference statistically To pursue this matter further, an additional analysis was done in which animals' defecation scores were summed over the last three trials, during which the differences had emerged most strongly. When this was done, there was an interaction of RO × NAL, $F(1,65)=3$ 52, $p=0.06$, which can be attributed to an augmentation of naloxone's effect by Ro 15-1788

DISCUSSION

There were three main findings in these experiments beyond the replication of the basic phenomenon of NIA First, NIA by itself was associated with heightened emotionality as measured by defecation Second, Ro 15-1788 augmented NIA Third, this augmentation of NIA was also associated with an augmentation of emotionality as measured by defecation

Considered together, the data on defecation and paw-lick latency provide corroboration for the hypothesis that NIA is a form of stress-induced analgesia The overall pattern in the defecation scores was quite consistent with the pattern seen in paw-lick latency scores NIA per se was associated with increased defecation, and Ro 15-1788 combined with naloxone to produce an augmentation of defecation in parallel to its augmentation of analgesia That both measures developed in parallel supports the suggestion that both were a reflection of a common process It might be suggested that the effects on defecation reflected a direct effect of either naloxone or Ro 15-1788 on gastrointestinal activity and is not indicative of stress Although opiate agonists have well-established effects on gastrointestinal motility, we are not aware of any effects of opiate antagonists on defecation in the absence of stress or opiate dependence In any event, an alternative hypothesis based on direct gastrointestinal disturbance has difficulty in explaining why maximal differences in drug-induced defecation were not evident on early trials, it is equally unclear how such an hypothesis could explain why defecation increased progressively over trials in such close correspondence with paw-lick latency

Analysis of the implications of our data for the details of the mechanisms underlying NIA and its interaction with Ro 15-1788 presents something of a challenge At a neural level, the mere fact that NIA occurs with naloxone in the system immediately suggests a nonopiate mechanism, as does the fact that no tolerance appears to develop [e g., (5,10)] At a strictly behavioral level, our data seem clear in implicating heightened emotional reactivity in NIA

and in the ability of Ro 15-1788 to augment NIA

Our experiments were limited to combinations of single doses of naloxone and Ro 15-1788 The dose of naloxone was based upon dose-response determinations that have shown NIA to be highly reproducible at 5 mg/kg The dose of Ro 15-1788 has been commonly used to produce anxiogenic actions in rats (3) In any event, the purpose in these experiments was not to explore parameters but to apply an effective anxiogenic dose of Ro 15-1788 that would permit a test of our hypothesis concerning a putative behavioral mechanism in NIA Studies manipulating dose and other parameters would be of interest, but the absence of dose-response determinations does not appear to compromise the conclusions that can be drawn from our data about the role of stress in NIA

NIA is a new phenomenon, and at this stage of knowledge it is raising many intriguing questions With regard to the role of stress, the data from these experiments are most consistent with the hypothesis that NIA involves a form of stress-induced analgesia, and that it is mediated by a nonopiate system

ACKNOWLEDGEMENTS

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