Pharmacology Biochemistry and Behavior, Vol. 44, pp. 381–386, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Naloxone Potentiation of Novelty-Induced Hypoalgesia: Characterization of the α-Noradrenergic Receptor Subtype

JOSEPH ROCHFORD,*†[1] PATRICIA DAWES* AND JANE STEWART*

**Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada*
†Douglas Hospital Research Center, Department of Psychiatry, McGill University, 6875 Boulevard LaSalle, Verdun, Quebec H4H 1R3, Canada

Received 28 April 1992

ROCHFORD, J., P. DAWES AND J. STEWART. *Naloxone potentiation of novelty-induced hypoalgesia: Characterization of the α-noradrenergic receptor subtype.* PHARMACOL BIOCHEM BEHAV **44**(2) 381–386, 1993. – Repeated daily administration of the opiate receptor antagonist naloxone (10 mg/kg) attenuates the habituation of novelty-induced hypoalgesia. This effect can be reversed by the α_2-noradrenergic receptor agonist clonidine and enhanced by the α_2-antagonist yohimbine. The present experiments were conducted to provide further support for the importance of the α_2-receptor and determine the possible influence of the α_1-receptor. Naloxone's effect on novelty-induced hypoalgesia was not affected by pretreatment with the specific α_1-receptor antagonist prazosin (0.2–1.0 mg/kg, SC) or the nonselective alpha antagonist phentolamine (2.0–10.0 mg/kg). In a second series of experiments, it was found that the potentiation of naloxone's effect by yohimbine (2 mg/kg) was reversed by clonidine (0.1 mg/kg) but was not influenced by prazosin or phentolamine. These results suggest that the α_1-noradrenergic receptor subtype does not mediate the effect of naloxone on novelty-induced hypoalgesia. They also reinforce the importance of the α_2-receptor subtype in the mediation of this effect.

Naloxone Novelty-induced hypoalgesia α_1-Receptor α_2-Receptor Noradrenaline Rat

ANIMALS exposed to a stimulus for the first time display lowered reactivity to nociceptive stimulation relative to animals that have been extensively exposed to the stimulus (1, 5,13,30). This result suggests that novel stimuli are capable of activating endogenous hypoalgesic substrates and the capacity of a stimulus to induce hypoalgesia habituates with repeated exposure to the stimulus. There is evidence to suggest that the habituation of novelty-induced hypoalgesia (NIH) can be attenuated by administration of the opiate receptor antagonist naloxone (12,26,27). In these studies, one group of rats is administered naloxone prior to exposure to a hot-plate apparatus. Control animals are exposed to the apparatus following saline administration; for these animals, naloxone is administered 2–4 h after exposure. The paw-lick latencies (PLLs) in control animals progressively decline over repeated exposures, an effect that suggests the habituation of NIH. The latencies in animals exposed following naloxone administration also decline. However, the magnitude of this reduction is attenuated relative to the decline observed in controls. Consequently, by the fourth or fifth hot-plate test animals exposed following naloxone administration display significantly longer PLLs than controls.

Because control animals receive the same quantity of naloxone, the longer latencies observed in naloxone-exposed animals cannot be attributed to repeated opiate receptor blockade alone. Rather, the effect is dependent upon opiate receptor blockade at the time when animals are exposed to the plate apparatus. Moreover, it has been shown that this effect occurs if animals are repeatedly exposed to a nonfunctional, ambient temperature plate and then tested once on the functional plate (12,27). These results demonstrate that exposure to noxious thermal stimulation is not necessary for the development of the effect [but, cf. (15–17,35–37)].

The inhibitory effect of naloxone on the habituation of NIH can be attenuated by pretreatment with the α_2-noradrenergic receptor agonist clonidine and enhanced by the α_2-receptor antagonist yohimbine (26). These ligands do not significantly alter PLLs in controls. These results suggest that naloxone's inhibition of the habituation of NIH is at least in part mediated through noradrenergic substrates. Moreover, they suggest the importance of the α_2-receptor subtype in the mediation of this effect.

The present experiments were conducted to more fully characterize the pharmacological substrate through which

[1] To whom requests for reprints should be addressed.

noradrenaline mediates the effect of naloxone on the habituation of NIH. Specifically, they were designed with two primary goals in mind: a) to provide further evidence implicating the α_2-receptor subtype; and b) to determine the possible influence of the α_1-receptor subtype. β-Noradrenergic receptors were not considered because of their limited involvement in antinociception (18). In the first series of experiments, we assessed whether naloxone's effect on NIH is affected by pretreatment with the selective α_1-receptor antagonist prazosin and the nonselective α-receptor antagonist phentolamine (29). In the second series of experiments, we attempted to replicate our previous demonstration (26) that yohimbine enhances the longer PLLs observed in animals exposed to the plate following naloxone administration. More importantly, we assessed the ability of prazosin, phentolamine, and clonidine to reverse this enhancement.

METHOD

Subjects

Experimentally naive, male Wistar rats (275–300 g) were obtained from Charles River Breeding Farms (St. Constant, Quebec). Rats were individually housed with free access to food and water. The colony room was maintained on a 12 L : 12 D cycle (light on 0800–2000 h). All procedures were conducted during the light phase of the cycle.

Apparatus and Drugs

Pain reactivity was assessed by the hot-plate test. The hot plate apparatus consisted of a 20.3 × 38.1 × 20.3 clear Plexiglas chamber mounted on a 0.6-cm thick piece of sheet metal. A hinged, wire mesh top prevented animals from escaping. Plate temperature was controlled by immersing the sheet metal in a water bath heated by a Haake E2 Immersion/Open Bath Circulator. The apparatus was located in a test room illuminated by two 25-W red light bulbs. During the interval between injection in the test room and analgesic testing, animals were isolated in separate 30 × 20 × 15-cm wooden boxes lined with Beta-Chip and covered by steel grid tops.

Drugs used were: naloxone HCl (duPont de Nemours & Co., Wilmington, DE), yohimbine HCl, clonidine HCl, prazosin HCl (Research Biochemicals, Inc., Natick, MA), and phentolamine mesylate (Ciba Geigy, Basel, Switzerland). Naloxone and clonidine were dissolved in physiological saline; all other ligands were dissolved in distilled water. The injection volume for each ligand was 1 ml/kg.

Procedure

Series 1: Effects of prazosin and phentolamine.
Naloxone treatment phase. During the 8 days of the naloxone treatment phase, rats in group NAL (n = 8 per experiment) were administered 10 mg/kg naloxone in the test room. Rats in group SAL (n = 8 per experiment) were administered saline. Test room injections were administered SC in the dorsal neck area. Thirty minutes following injection, each animal was tested for pain sensitivity on the hot plate. The latency to lick a hind paw-(PLL) was taken as the measure of pain threshold. The water temperature of the hot-plate bath was 48.5°C. We found this temperature optimal for the expression of NIH and effects of naloxone on NIH (27). If no response was observed within 90 s, the test was terminated and a PLL of 90 s was recorded. Following the hot-plate test, animals were returned to the colony room where, 2–4 h later, group NAL rats were administered saline and group SAL rats 10 mg/kg naloxone.
Test phase. The test phase consisted of 2 days. Within each experiment, half the animals in groups NAL and SAL were preadministered (IP) drug (0.2 or 1.0 mg/kg prazosin; 2.0 or 10.0 mg/kg phentolamine) on the first test day and vehicle (saline or distilled water) on the second. The other half received the reverse sequence: Vehicle was administered on the first test day and drug on the second. Thus, the sequence of these injections was counterbalanced within groups.

These injections were administered in the colony room. Fifteen minutes after injection, animals were transported to the test room, where group NAL rats were injected with naloxone and those in group SAL received saline. Thirty minutes later, hot-plate tests were administered. Animals were then returned to the colony room, where posttest injections were administered as described above.

Series 2: Effects of prazosin, phentolamine, and clonidine on yohimbine's potentiation of naloxone's effect.
Naloxone treatment phase. This phase was identical to that in the previous experiments with the exception that all animals were allocated to group NAL. Group SAL animals were not included on the basis of previous work demonstrating that yohimbine does not alter PLLs in these animals (26).
Test phase. The test phase consisted of 2 days. On both test days, animals were preadministered 2 mg/kg yohimbine (IP) in the colony room. Immediately following this injection, half the animals were administered (IP) a second injection of drug (0.2 or 1.0 mg/kg prazosin; 0.05 or 0.1 mg/kg clonidine; 10 mg/kg phentolamine) on the first test day and vehicle on the second test day. The remaining animals received the reverse sequence. All other procedural details were identical to those followed for the first series of experiments.

Statistical Analysis

Data from the experiments in Series 1 were analyzed by analysis of variance (ANOVA). Significant interactions were analyzed by F-tests for simple main effects (38). Data from Series 2 experiments were analyzed by the Wilcoxon signed-rank test (6) due to the fact that yohimbine administration provoked the 90-s cutoff criterion in over 90% of animals. Regardless of the type of analysis, the level of statistical significance adopted was $p < 0.05$.

RESULTS

Series 1

Figures 1A–1D portray the results from the experiments assessing the effects of prazosin and phentolamine. The left panel displays the results from the naloxone treatment phase. In each experiment, the PLLs in group SAL declined markedly over repeated hot-plate tests. Although the magnitude of the effect tended to vary across experiments, in each case it can be observed that the PLLs in group NAL did not decline as dramatically as those in group SAL. This differential reduction was such that the mean PLLs for group NAL were significantly longer than those for group SAL beginning on the fourth or fifth day of the naloxone treatment phase.

The results from the test phase are shown in the right panels of Fig. 1. The PLLs for group NAL continued to be significantly longer than those for group SAL. Of more importance was the finding that prazosin and phentolamine failed to influence PLLs in group NAL. These ligands also were without effect in control animals.

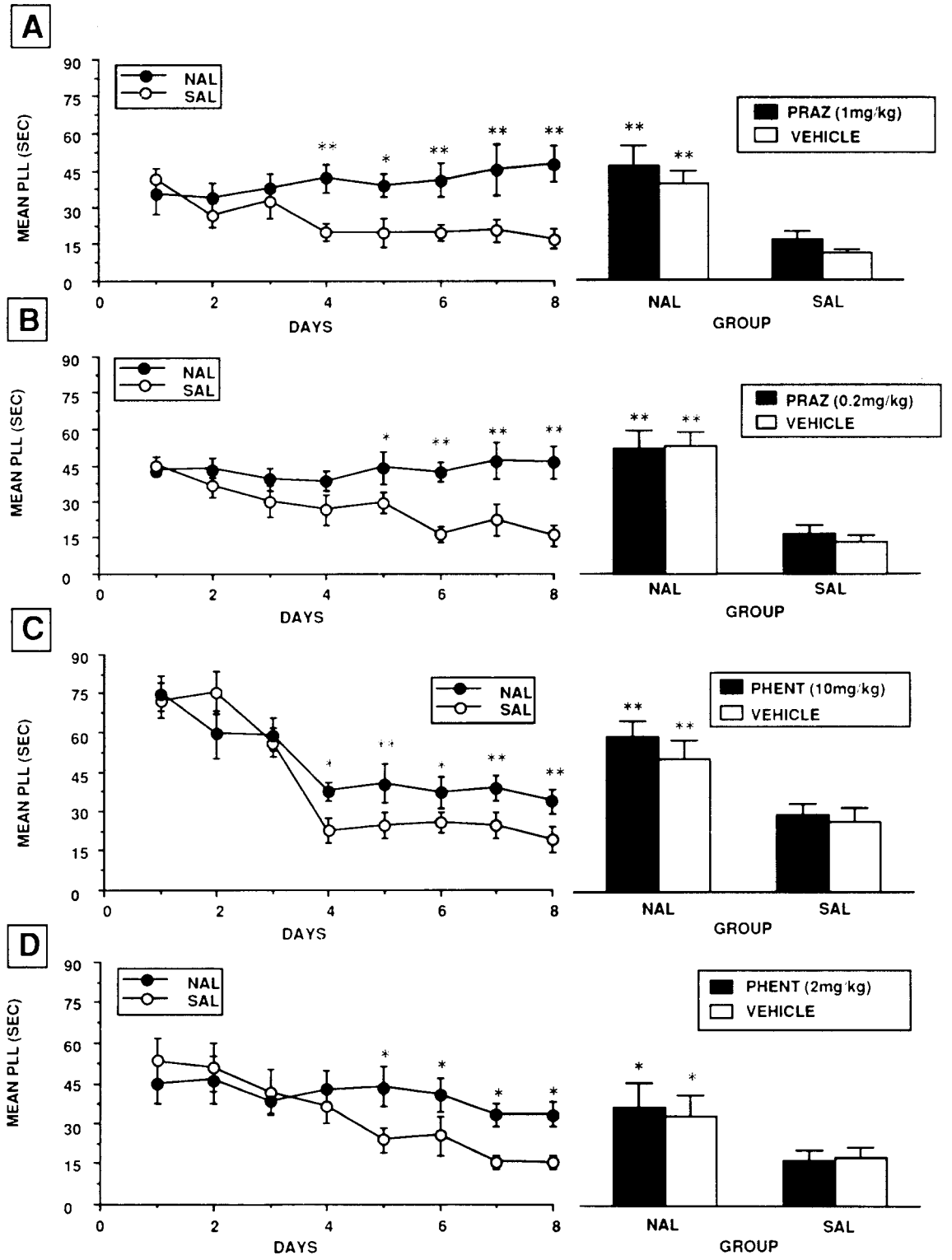

FIG. 1. Mean paw-lick latencies (PLLs) during the naloxone treatment phase (left) and the test phase (right) of the experiments in Series 1. Figures 1A and 1B display the results from the experiments assessing the effects of prazosin (1.0 mg/kg in A, 0.2 mg/kg in B). Figures 1C and 1D portray the results from the phentolamine experiments (10 mg/kg in C, 2 mg/kg in D). Error bars represent ±SEM. Asterisks reflect significant differences from the respective mean PLL in the SAL control group, $*p < 0.05$, $**p < 0.01$.

Series 2

Figures 2A–2E present the results from the test phase of the experiments in Series 2. To demonstrate the effects of yohimbine, the PLLs observed following yohimbine preadministration were contrasted with the mean PLLs calculated over the last 2 days of the naloxone treatment phase (i.e., when naloxone was administered alone). Preadministration of 2 mg/kg yohimbine significantly prolonged PLLs in each case. This enhancement was significantly reduced by administration of 0.1 mg/kg clonidine. Administration of 0.05 mg/kg clonidine also tended to reverse the enhancement, but this effect was not significant. Prazosin and phentolamine did not influence the enhancement. This conclusion is supported by the findings that: a) PLLs observed following concurrent administration of yohimbine and either prazosin or phentolamine were not significantly different from those observed following administration of yohimbine; and b) PLLs observed following yohimbine and either prazosin or phentolamine were significantly longer than those displayed following administration of naloxone alone.

DISCUSSION

The results from the naloxone treatment phase of the experiments in Series 1 replicate those previously reported (26, 27). The PLLs of control animals declined markedly over repeated plate exposures, whereas the latencies of group NAL animals remained relatively stable or declined less rapidly. These results suggest that repeated plate exposures provoke the habituation of NIH and naloxone retards this habituation.

The results from the test phase of the experiments in Series 2 also replicate our previous demonstration that yohimbine augments PLLs in naloxone-treated animals (26). More importantly, this augmentation was reversed by coadministration of clonidine but unaffected by either prazosin or phentolamine. These results, therefore, reinforce the hypothesis that the α_2-receptor is involved in the mediation of naloxone's effect on the habituation of NIH.

One objection to this conclusion could be that clonidine and yohimbine may have exerted their effects through the recently discovered imidazoline binding site, which possesses significant affinity for a variety of purported α_2-specific ligands. While our data do not rule out this possibility, we believe it unlikely. The imidazoline binding site possesses relatively low affinity for yohimbine and related alkaloids (7–9,18). Further, the affinity of clonidine for the α_2-site is greater than that for the imidazoline site (22). Thus, these data suggest that the effects provoked by clonidine and yohimbine are most likely mediated through the α_2-receptor.

The results of the present experiments failed to confirm a role for the α_1-receptor subtype in the mediation of naloxone's effect on the habituation of NIH. First, during the test phase of the experiments in Series 1 the specific α_1-receptor antagonist prazosin and the nonselective α-antagonist phentolamine did not significantly influence PLLs in animals exposed to the plate following naloxone administration. Moreover, in the test phase of Series 2 experiments these ligands did not reverse the enhancement of PLLs provoked by yohimbine.

It could be argued that the failure of prazosin and phentolamine to influence naloxone's effect on NIH can be attributed to the pharmacokinetic properties of these ligands. For instance, prazosin does not readily cross the blood–brain barrier (19). However, there is evidence to suggest that, within the dose range employed in the present studies, penetration into the CNS does occur (34) and can provoke effects that are centrally mediated (14,23).

Although the present results add to previous findings implicating noradrenergic substrates in the effects of naloxone on NIH, they do not allow us to make any firm conclusion as to how noradrenaline is involved. Electrophysiological and biochemical studies have demonstrated that naloxone enhances the stress-induced increase in firing rate and release in noradrenergic neurons, suggesting that noradrenergic substrates are under inhibitory opioid control (2,3,20,24,33). This raises the possibility that naloxone may retard the habituation of NIH by preventing opioid inhibition of noradrenergic neurotransmission. Moreover, because noradrenergic firing rate and release is augmented by α_2-receptor blockade (3,10,20,25) and inhibited by α_2-receptor stimulation (4,21,31,32), this hypothesis could also account for the observed effects of yohimbine and clonidine, that is, yohimbine may potentiate the effects of naloxone by enhancing naloxone's facilitory effect on noradrenergic neurotransmission. Clonidine would attenuate the effect of naloxone, and reverse the potentiating effect of yohimbine, by inhibiting noradrenergic neurotransmission. The unifying theme of this hypothesis is that naloxone, yohimbine, and clonidine exert their effects by influencing the efficacy of noradrenergic neurotransmission. If it is correct, then the effects of each of these ligands should be abolished by specific noradrenaline depletion. We are currently exploring this possibility in our laboratory.

The present results contrast with those recently reported by Foo and Westbrook (11), who reported that the longer PLLs provoked by naloxone are insensitive to pretreatment with clonidine or yohimbine and are enhanced by pretreatment with 1 mg/kg prazosin. Thus, the pharmacological profile obtained by Foo and Westbrook is completely at odds with that reported here.

The resolution to this issue may come from considering the differences in protocol between the present study and that of Foo and Westbrook, who used a 50.5°C hot plate and exposed animals to the plate for a full 60 s, independent of when animals paw licked. Under these conditions, animals exposed to the hot plate following naloxone administration displayed a profound increase in PLLs over hot-plate exposures [see also (15–17,35–37)]. This increase implies that naloxone is not simply preventing the habituation of NIH. Rather, it suggests that when animals are exposed to more intense levels of noxious thermal stimulation naloxone augments the acquisition of a hypoalgesic response via a Pavlovian conditioning mechanism. The fact that this effect is observed when animals are exposed to more intense noxious thermal stimulation is also consistent with this hypothesis in that it has been shown that the ability of noxious heat to promote Pavlovian conditioning is proportional to its intensity (17,28,36).

Current evidence, therefore, suggests that naloxone can affect pain reactivity by modulating two forms of learning: Pavlovian conditioning and habituation. Evidence has also implicated noradrenergic substrates in the mediation of each effect. However, the disparate results obtained by us and by Foo and Westbrook raise the intriguing possibility that noradrenaline's involvement in the mediation of these effects may be distinct. The α_1-receptor subtype may be of primary importance for Pavlovian conditioning, whereas the α_2-subtype preferentially mediates habituation.

ACKNOWLEDGEMENTS

Research for this study was supported by grants from the FCAR (JR) and the MRC (JS).

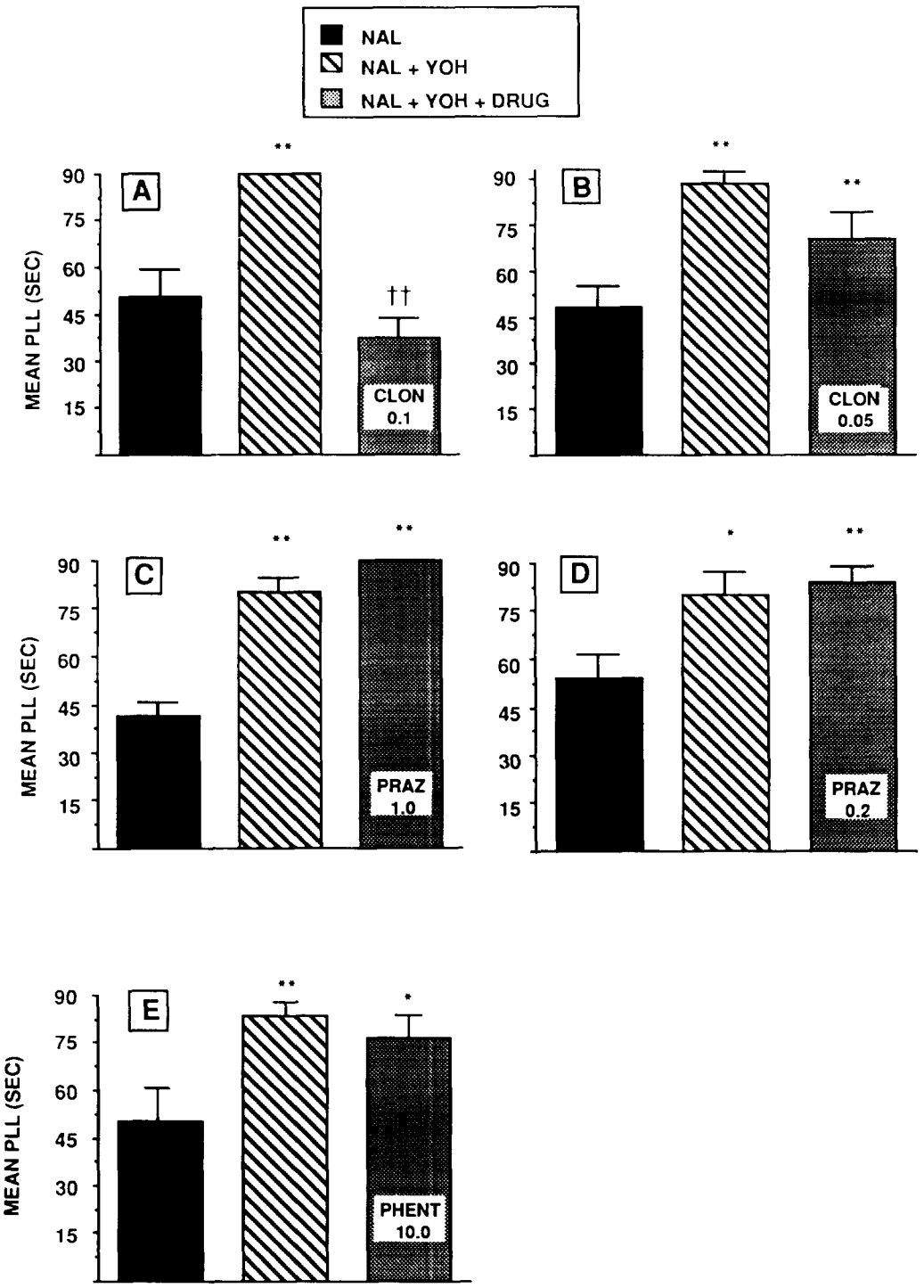

FIG. 2. Mean paw-lick latencies (PLLs) from the test phase of the experiments in Series 2. Solid bars reflect the latencies observed following naloxone (10 mg/kg) administration alone. Diagonally lined bars represent the effects of yohimbine (2 mg/kg) administered prior to naloxone. Stippled bars portray the latencies observed following administration of naloxone, yohimbine, and either clonidine (0.1 mg/kg in A, 0.05 mg/kg in B), prazosin (1.0 mg/kg in C, 0.2 mg/kg in D), or phentolamine (10 mg/kg, E). Error bars reflect 1 SEM. The absence of error bars reflects the fact that all animals achieved the 90-s cutoff. Asterisks represent significant differences from the mean PLL following naloxone administration alone, $*p < 0.05$, $**p < 0.01$. Daggers represent significant differences from the mean PLL observed following naloxone and yohimbine administration, $\dagger\dagger p < 0.01$.

REFERENCES

1. Abbott, F. V.; Franklin, K. B. J.; Connell, B. The stress of a novel environment reduces formalin pain. Eur. J. Pharmacol. 126:141–144; 1986.
2. Abercrombie, E. D.; Jacobs, B. L. Systemic naloxone administration potentiates locus coeruleus noradrenergic neuronal activity under stressful but not nonstressful conditions. Brain Res. 441: 362–366; 1988.
3. Aghajanian, G. K.; Cedarbaum, J. M.; Wand, R. Y. Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. Brain Res. 136:570–577; 1977.
4. Anden, N. E.; Corrodi, H.; Fuxe, K.; Hokfelt, T.; Rydin, C.; Svensson, T. Evidence for a central NA receptor stimulation by clonidine. Life Sci. 2:513–523; 1970.
5. Bardo, M. T.; Hughes, R. A. Exposure to a nonfunctional hot plate as a factor in the assessment of morphine-induced analgesia and analgesic tolerance. Pharmacol. Biochem. Behav. 10:481–485; 1979.
6. Bradley, J. V. Distribution-free statistical tests. Englewood Cliffs, NJ: Prentice Hall; 1968.
7. Bricca, G.; Dontenwill, M.; Molines, A.; Feldman, J.; Belcourt, A.; Bousquet, P. The imidazoline preferring receptor: Binding studies in bovine, rat and human brainstem. Eur. J. Pharmacol. 162:1–9; 1989.
8. Brown, C. M.; MacKinnon, A. C.; McGrath, J. C.; Spedding, M.; Kilpatrick, A. T. Alpha-2 adrenoceptor subtypes and imidazoline-like binding sites in the rat brain. Br. J. Pharmacol. 99: 803–809; 1990.
9. Clark, R. D.; Berger, J.; Garg, P.; Weinhardt, K. K.; Spedding, M.; Kilpatrick, A. T.; Brown, C. M.; MacKinnon, A. C. Affinity of 2-(tetrahydroisoquinolin-2-methyl)- and 2-(isoindolin-2-ylmethyl) imidazolines for the [3H]yohimbine-labeled site. J. Med. Chem. 33:596–600; 1990.
10. Dubocovich, M. L. Presynaptic alpha-2 receptors in the central nervous system. Ann. NY Acad. Sci. 420:7–25; 1984.
11. Foo, H.; Westbrook, R. F. Naloxone-induced hypoalgesia: Effects of noradrenergic antagonists and agonist. Pharmacol. Biochem. Behav. 39:795–797; 1991.
12. Foo, H.; Westbrook, R. F. Naloxone-induced hypoalgesia: Effects of heat, cold and novelty. Q. J. Exp. Psychol. 43B:137–156; 1991.
13. Gamble, G. D.; Milne, R. J. Repeated exposure to sham testing procedures reduces reflex withdrawal and hot-plate latencies: Attenuation of tonic descending inhibition? Neurosci. Lett. 96:312–317; 1989.
14. Gonzalez-Mariscal, G.; Beyer, C. Blockade of LHRH-induced lordosis by alpha and beta adrenergic antagonists in the ovariectomized, estrogen primed rat. Pharmacol. Biochem. Behav. 31:573–577; 1989.
15. Greeley, J. D. Pavlovian conditioning of pain regulation: Insights from pharmacological conditioning with morphine and naloxone. Biol. Psychol. 28:41–65; 1989.
16. Greeley, J. D.; Le, A. D.; Poulos, C. X.; Cappell, H. "Paradoxical" analgesia induced by naloxone and naltrexone. Psychopharmacology (Berl.) 96:36–39; 1988.
17. Greeley, J. D.; Westbrook, R. F. Some effects of exposure to a heat stressor upon the rat's subsequent reactions to that stressor. Q. J. Exp. Psychol. 42B:241–265; 1991.
18. Hamon, M. D.; Collin, E.; Chantrel, D.; Verge, D.; Bourgoin, S. The contribution of monoamines and their receptors to pain control. In: Basbaum, A. I.; Besson, J. M., eds. Towards a new pharmacotherapy of pain. New York: Wiley & Sons; 1991:83–102.
19. Hess, H. J. Biochemistry and structure-activity studies with prazosin. In: Krogsgaard-Larsen, P.; Scheel-Kruger, J.; Kofood, H., eds. Prazosin-evaluation of a new antihypertensive. New York: Academic Press; 1974:3–15.
20. Illes, P.; Norenberg, W. Blockade of alpha-2 adrenoreceptors increases opioid mu-receptor mediated inhibition of firing rate of rat locus coeruleus neurons. Nuanyn Schmiedeberg's Arch. Pharmacol. 342:490–496; 1990.
21. Karege, F.; Gaillard, J. M. Metabolism time-course of monoamines in the rat brain after low dose of clonidine. Biogenic Amines 7:37–48; 1990.
22. Lachaud, V.; Coupry, I.; Podevin, R. A.; Koenig, E.; Parini, A. Selectivite des agonistes alpha 2-adrenergiques pour les recepteurs imidazolinique-guanidiniques et alpha 2-adrenergiques. Arch. Mal. Coeur 82:1135–1137; 1989.
23. Leaton, R. N.; Cassella, J. V. The effect of clonidine, prazosin and propranolol on short-term and long-term habituation of the acoustic startle response in rats. Pharmacol. Biochem. Behav. 20: 935–942; 1984.
24. Montel, H.; Starke, K.; Weber, F. Influence of morphine and naloxone on release of noradrenaline from rat brain cortex slices. Nuanyn Schmiedeberg's Arch. Pharmacol. 283:357–369; 1974.
25. Rasmussen, K.; Jacobs, B. L. Single unit activity of locus coeruleus neurons in the freely moving cat. II. Conditioning and pharmacologic studies. Brain Res. 371:335–344; 1986.
26. Rochford, J.; Dawes, P. Clonidine and yohimbine modulate the effect of naloxone on novelty-induced hypoalgesia. Psychopharmacology (Berl.) 107:575–580; 1992.
27. Rochford, J.; Stewart, J. Activation and expression of endogenous pain control mechanisms in rats given repeated nociceptive tests under the influence of naloxone. Behav. Neurosci. 101:87–103; 1987.
28. Rochford, J.; Stewart, J. Morphine attenuation of conditioned autoanalgesia: Implications for theories of situation-specific tolerance to morphine analgesia. Behav. Neurosci. 101:690–700; 1987.
29. Ruffolo, R. R., Jr.; DeMarinis, R. M.; Wise, M.; Hieble, J. P. Structure-activity relationships for alpha-2 adrenergic agonists and antagonists. In: Limbird, L. M., ed. The alpha-2 adrenergic receptors. Clifton, NJ: Humana Press; 1988:155–186.
30. Sherman, J. E. The effects of conditioning and novelty on the rats' analgesic and pyretic response to morphine. Learn. Motiv. 10:381–418; 1979.
31. Starke, K.; Altman, K. P. Inhibition of adrenergic neurotransmission by clonidine: An action on prejunctional alpha receptors. Neuropharmacology 12:339–347; 1973.
32. Svensson, T. H.; Bunney, B. S.; Aghajanian, G. K. Inhibition of both noradrenergic and serotonergic neurons on brain by the alpha-adrenergic receptor agonist clonidine. Brain Res. 92:291–306; 1974.
33. Tanaka, M.; Kohno, Y.; Nakagawa, R.; Ida, Y.; Iimori, K.; Hoaki, Y.; Tsuda, A.; Nagasaki, N. Naloxone enhances the stress-induced increases in noradrenaline turnover in specific brain regions in rats. Life Sci. 30:1663–1669; 1982.
34. Taylor, J. A.; Twomey, T. M.; Schach von Wittenau, M. The metabolic fate of prazosin. Zenobiotica 7:357–364; 1977.
35. Westbrook, R. F.; Greeley, J. D.; Some effects of the opioid antagonist, naloxone, upon the rat's reaction to a heat stressor. Q. J. Exp. Psychol. 42B:1–40; 1990.
36. Westbrook, R. F.; Greeley, J. D. Nabke, C. P.; Swinbourne, A. L.; Harvey, A. Effects of morphine and naloxone upon the reactions of rats to a heat stressor. Q. J. Exp. Psychol. 43B:323–346; 1991.
37. Westbrook, R. F.; Greeley, J. D.; Nabke, C. P.; Swinbourne, A. L. Aversive conditioning in the rat: Effects of a benzodiazepine and of an opioid agonist and antagonist on conditioned hypoalgesia and fear. J. Exp. Psychol. Anim. Behav. Proc. 17:219–230; 1991.
38. Winer, B. J. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill; 1971.