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RAPID COMMUNICATION

Nitric Oxide Synthase Inhibition Selectively Potentiates Swim Stress Antinociception in Rats

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SPINELLA, M. AND R. J. BODNAR. *Nitric oxide synthase inhibition selectively potentiates swim stress antinociception in rats.* PHARMACOL BIOCHEM BEHAV 47(3) 727–733, 1994. – Since nitric oxide (NO) has been implicated in nociceptive processing, the present study examined whether NO synthase inhibition with either N^{ω}-nitro-L-arginine (L-NA) or its methyl ester (L-NAME) would alter antinociception elicited by either continuous (CCWS) or intermittent cold-water swims (ICWS) on the tail-flick and jump tests. Whereas CCWS antinociception on both tests was significantly potentiated by a dose range of L-NA (0.1–4 mg/kg IP) and L-NAME (1 mg/kg IP), ICWS antinociception was largely unaffected by these manipulations. In contrast, administration of the less active D isomer (D-NAME) failed to alter CCWS antinociception and reduced ICWS antinociception. The ability of NO synthase inhibition to potentiate CCWS antinociception could not be explained by changes in CCWS hypothermia. Since ICWS antinociception is mediated by μ-opioid manipulations and CCWS antinociception is sensitive to δ-opioid and nonopioid manipulations, this indicates that NO synthase inhibition may be acting upon a selective form of pain inhibition.

Nitric oxide N^{ω}-Nitro-L-arginine Antinociception Continuous cold-water swims Intermittent cold-water swims Stress Opioid Nonopioid

ANTINOCICEPTION elicited by different parameters of cold-water swims are differentially sensitive to opioid manipulations [see review (2)]. Whereas continuous cold-water swim (CCWS) antinociception is insensitive to morphine cross-tolerance or naloxone antagonism (3,4), intermittent cold-water swim (ICWS) antinociception is sensitive to both manipulations (9,10). ICWS antinociception is also potentiated by either endopeptidase 24.11 or 24.15 inhibition (11), and thus appears to be an opioid-mediated stressor especially sensitive to manipulations involving the μ-opioid receptor. In contrast, CCWS antinociception is potentiated by either chronic naltrexone pretreatment or μ_1 antagonism, and is reduced by either endopeptidase 24.11 inhibition or simultaneous morphine treatment (5,12,25,30), indicating nonopioid modulation. However, CCWS antinociception is sensitive to reductions induced by either δ_2-opioid antagonists (28) or intrathecal combinations of μ, δ, and κ antagonists (29). Interestingly, although CCWS and ICWS antinociception dissociate from each other in their response to other physiological and pharmacological manipulations [see review (2)], both forms of swim antinociception display reciprocal cross-tolerance (22).

The *N*-methyl-D-aspartate (NMDA) receptor has been hypothesized to be one transmitter system that dissociates opioid and nonopioid antinociceptive responses. Whereas the NMDA antagonist MK-801 significantly reduces antinociception elicited by a nonopioid form of swim stress (17,18,27), it fails to alter antinociception elicited by either morphine (1,15,16,26) or an opioid form of swim stress (17,18). One effect of glutamate actions at NMDA receptors is the production of nitric oxide [NO (6,8)], which in turn has also been implicated in nociceptive processing [see review (20)]. Activation of NO release produces antinociception (7,21). NO production is blocked by the alternate substrate for the NO synthase enzyme N^{ω}-nitro-L-arginine (L-NA) and its methyl ester (L-NAME)

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(24). L-NAME blocks a number of hyperalgesic responses induced by NMDA or substance P administration, noxious cutaneous stimuli, or ligation of the sciatic nerve [e.g., (19,20,23)]. A recent role for NO in opioid function is supported by the observation that L-NA significantly reversed the development of tolerance to the μ-opioid agonist morphine, but not the κ_1-opioid agonist U50,488H or the κ_3-opioid agonist naloxone benzoylhydrazone (13,14). These latter studies failed to observe L-NA-induced effects upon any of these opioid forms of antinociception.

The present study examined whether CCWS and ICWS antinociception on the tail-flick and jump tests was affected by NO synthase inhibition following pretreatment with a dose range of L-NA and its methyl ester L-NAME. To examine specificity of drug effects, the less active isomer N^{ω}-nitro-D-arginine (D-NAME) was also tested. To examine specificity of nociceptive processes, the effects of L-NA, L-NAME, and D-NAME upon CCWS and ICWS hypothermia were also assessed.

METHODS

Adult male albino Sprague–Dawley rats weighing 300–500 g (Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages on a 12-h light–dark cycle with ad lib access to rat chow and water. All rats were tested on the tail-flick and jump tests in that order. Tail-flick latencies were ascertained with a radiant heat source (IITC Analgesia Meter, Woodland Hills, CA) by which heat was applied to the dorsum of the rat's tail 3–8 cm proximal to the tip. Each session consisted of three latency determinations made at 10-s intertrial intervals. To avoid tissue damage, the determination was terminated if no response occurred after 12 s. Immediately thereafter, jump thresholds were ascertained in a chamber (30 × 24 × 26.5 cm) with 14 grid bars spaced 1.9 cm apart. Electric shocks (0.3 s) were delivered through the grids by a shock generator (BRS/LVE, Beltsville, MD) and shock scrambler (Campden Instruments, Chicago). An ascending method of limits procedure was employed for each of six trials with shock initially delivered at 0.1 mA and increased in 0.05-mA increments at 5-s intervals. The jump threshold was defined as either of the lowest of two consecutive intensities at which the rat simultaneously removed both rear paws from the grids or 1.2 mA, the cutoff.

After at least four days of baseline latency and threshold determinations to ensure stability, the rats were divided into two groups of six rats each which were matched for their baseline latencies and thresholds. The first group had the following experimental conditions at weekly intervals: 1) vehicle (1 ml/kg 0.9% normal saline IP)/no swim, 2) vehicle/CCWS, 3) L-NA (0.1 mg/kg)/CCWS, 4) L-NA (1 mg/kg)/CCWS, 5) L-NA (4 mg/kg)/CCWS, 6) L-NA (4 mg/kg)/no swim, 7) L-NAME (1 mg/kg)/CCWS, and 8) D-NAME (1 mg/kg)/CCWS. In the CCWS condition, rats were placed for 3 min in a 2°C bath in which the water was deep enough to prevent standing. L-NA, L-NAME, and D-NAME (Sigma Chemical Company, St. Louis) were dissolved in 0.9% normal saline and administered IP. All injections were administered 15 min prior to the swim or no swim conditions. Tail-flick latencies, jump thresholds, and core body temperatures were assessed in that order at 30, 60, and 90 min following the swim or no swim condition. Core body temperatures were ascertained by inserting a rectal probe (Sensortek, Clifton, NJ) until a stable reading (±0.1°C) was achieved. The second group was treated identically except that ICWS (2°C, ten 10-s swims, and ten 10-s recovery periods) was used as the stimulus condition. Latency, threshold, and core body temperature data were subjected to within-group analyses of variance (ANOVAs). Dunnett comparisons ($p < .05$) evaluated significant swim effects relative to the vehicle/no swim condition, and Dunn comparisons ($p < .05$) evaluated significant drug effects relative to the corresponding vehicle/CCWS or vehicle/ICWS conditions. Peak effects were examined at 30 min following each treatment. The duration of effects was examined by determining total analgesia, derived by subtracting the sum of latencies or thresholds following vehicle/no swim conditions from the sum of latencies or thresholds following a given experimental condition.

RESULTS

CCWS Antinociception

Significant differences were observed on the tail-flick and jump tests across conditions, across test times, and for the interaction between conditions and times ($ps < .0001$). CCWS significantly increased latencies (30–90 min) and thresholds (30 min) relative to vehicle/no swim control conditions. Significant and dose-dependent potentiations in the peak and total antinociceptive responses following CCWS on the tail-flick test were observed following L-NA doses of 1 (peak: 40%; total: 64%) and 4 (peak: 40%; total: 41%) mg/kg and following the 1-mg/kg dose of L-NAME (total: 55%) (Fig. 1, left panels). Significant and dose-dependent potentiations in the peak and total antinociceptive responses following CCWS on the jump test were also observed following L-NA doses of 0.1 (peak: 48%; total: 193%), 1 (peak: 100%; total: 255%) and 4 (peak: 63%; total: 234%) mg/kg and following the 1-mg/kg dose of L-NAME (peak: 101%; total: 294%) (Fig. 1, right panels). In contrast, a comparable dose of the less effective isomer, D-NAME, failed to significantly alter either the peak magnitude or total duration of CCWS antinociception on either nociceptive measure. The L-NA effects occurred despite the fact that L-NA (4 mg/kg) failed to alter baseline latencies and thresholds.

ICWS Antinociception

Significant differences were observed on the tail-flick and jump tests across conditions, across test times, and for the interaction between conditions and times ($ps < .001$). ICWS significantly increased latencies and thresholds across the time course relative to vehicle/no swim control conditions. Small but significant reductions in the peak antinociceptive response following ICWS were observed for both nociceptive measures following the 0.1-mg/kg dose of L-NA (tail-flick: 39%; jump: 35%) and the 1-mg/kg dose of D-NAME (tail-flick: 31%; jump: 25%) (Fig. 2, top panels). Neither the other doses of L-NA nor the 1-mg/kg dose of L-NAME significantly altered the magnitude of duration of ICWS antinociception on either nociceptive measure (Fig. 2).

CCWS and ICWS Hypothermia

Significant differences were observed for core body temperatures across conditions, across test times, and for the interaction between conditions and times ($ps < .0001$). Core body temperatures were significantly decreased following both CCWS (30 min) and ICWS (30–90 min) relative to the corresponding vehicle/no swim control conditions. The magnitude of CCWS hypothermia was significantly reduced after 30 min

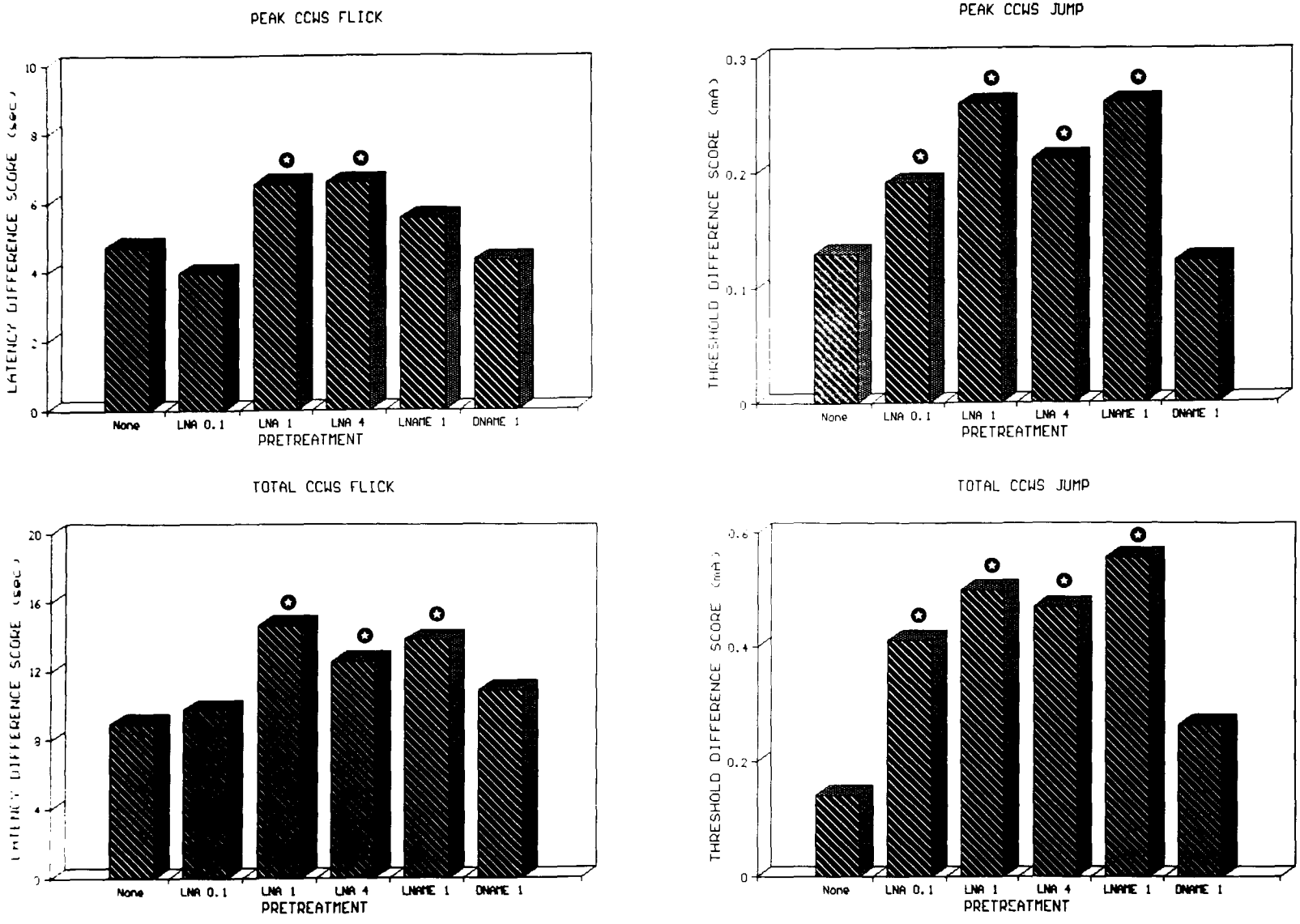

FIG. 1. Alterations in continuous cold-water swim (CCWS) antinociception on the tail-flick (left panels) and jump (right panels) tests following pretreatment with either N^{ω}-nitro-L-arginine (L-NA), its methyl ester (L-NAME), or its less active dextro-isomer (D-NAME). Peak (upper panels, 30 min) and total (lower panels, 30–90 min time course) magnitudes of CCWS antinociception were calculated by subtracting baseline latencies and thresholds from each experimental score. The enclosed stars indicate significant differences in CCWS antinociception relative to the no-pretreatment condition (Dunnett comparison, $p < .05$).

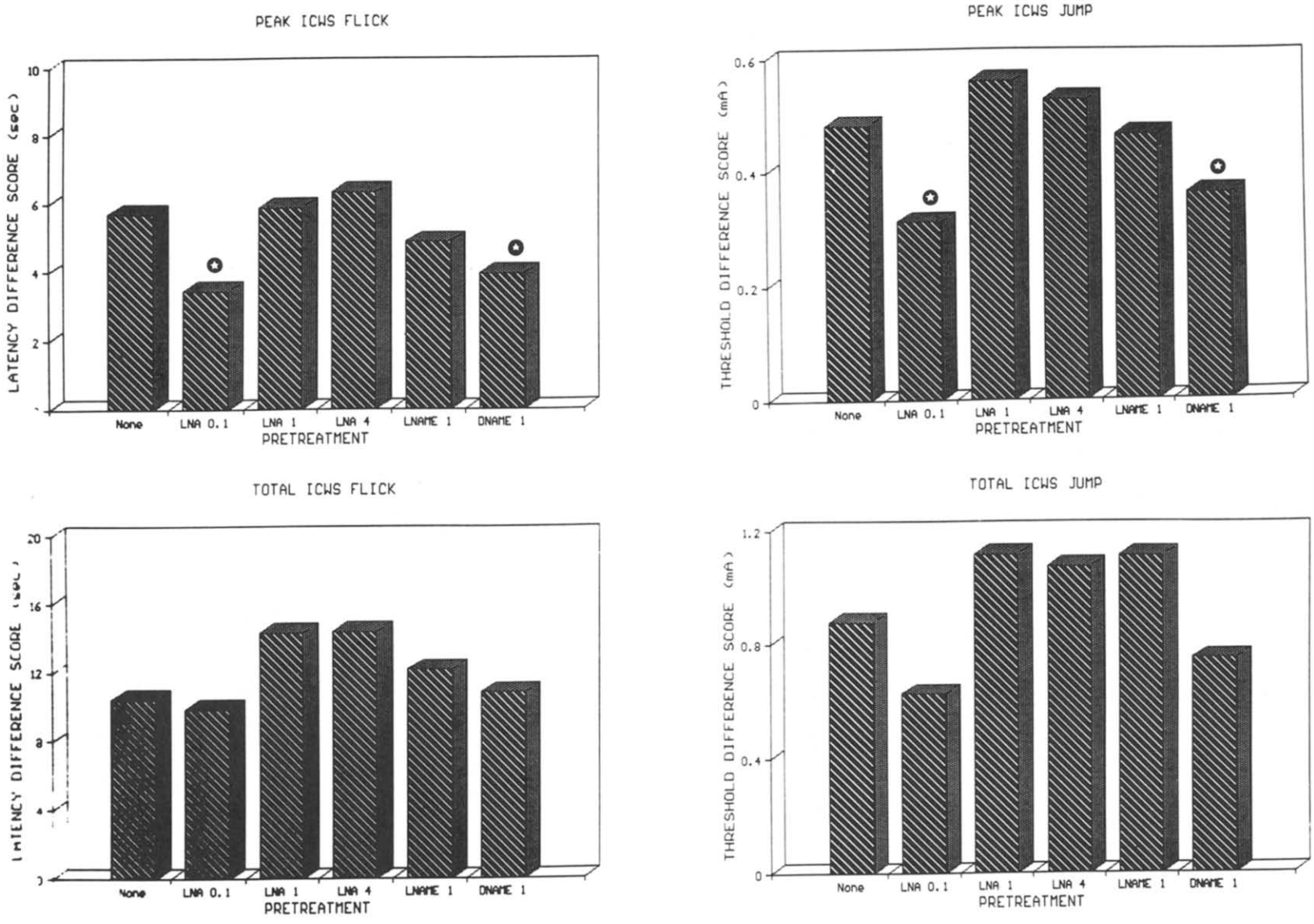

FIG. 2. Alterations in intermittent cold-water swim (ICWS) antinociception on the tail-flick (left panels) and jump (right panels) tests following pretreatment with either N^{ω}-nitro-L-arginine (L-NA), its methyl ester (L-NAME), or its less active dextro-isomer (D-NAME).

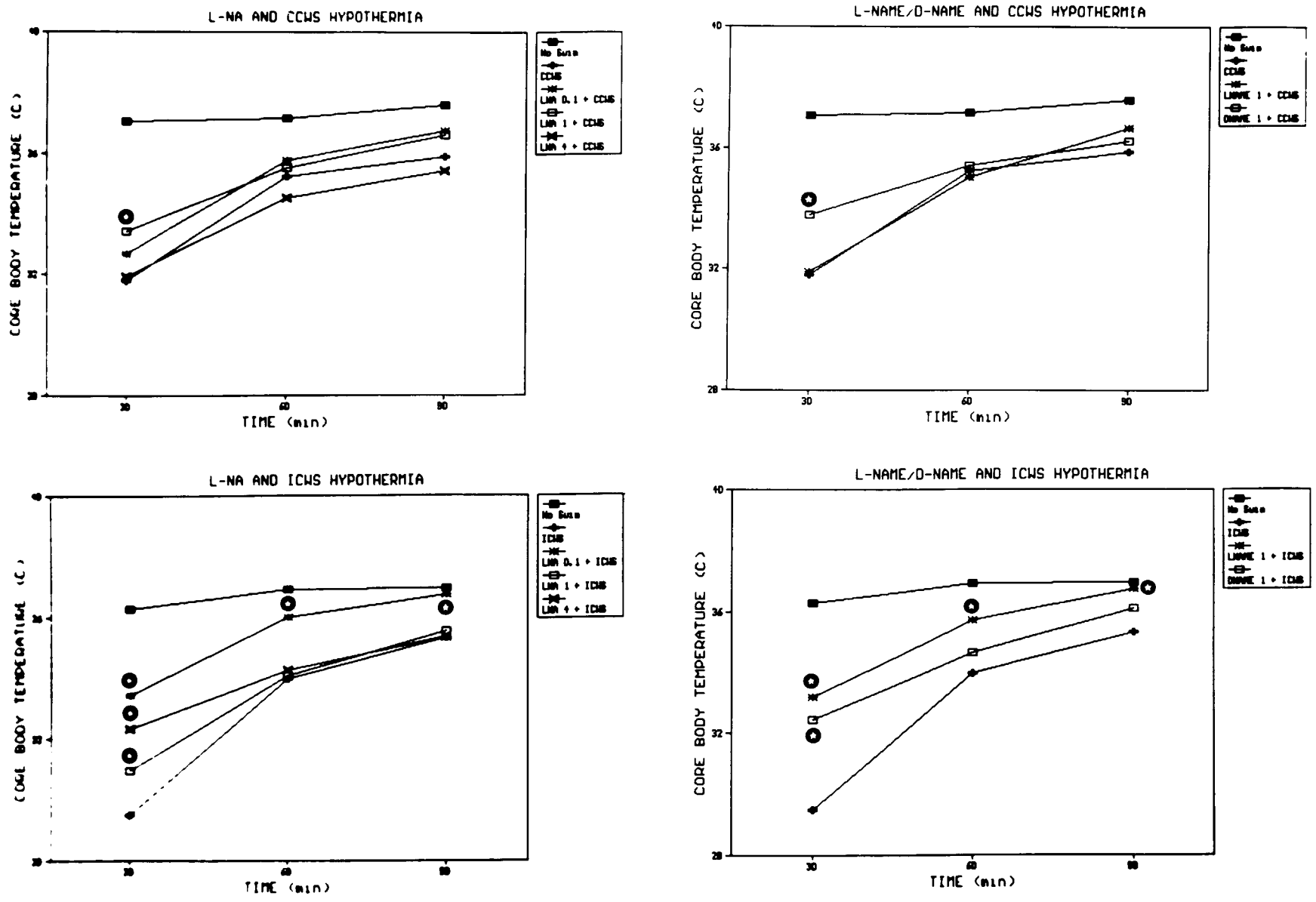

FIG. 3. Alterations in core body temperatures (°C) following CCWS (upper panels) and ICWS (lower panels) in rats pretreated with N^{ω}-nitro-L-arginine (L-NA) (left panels) or its methyl ester (L-NAME) or its less active dextro-isomer (D-NAME) (right panels). The enclosed stars indicate significant differences in CCWS and ICWS hypothermia relative to its corresponding control condition (Dunnett comparison, $p < .05$).

by either L-NA (1 mg/kg) or D-NAME (1 mg/kg) (Fig. 3, upper panels). The magnitude of ICWS hypothermia was significantly reduced by L-NA doses of 0.1 (30–90 min), 1 (30 min), and 4 (30 min) mg/kg; the 1-mg/kg dose of L-NAME (30–90 min); and the 1-mg/kg dose of D-NAME (30 min) (Fig. 3, lower panels).

DISCUSSION

The present study found that L-NA and its methyl ester, L-NAME, significantly potentiated the magnitude and duration of CCWS antinociception on the tail-flick and jump tests. In contrast, the less active isomer, D-NAME, failed to exert significant effects. The potentiation of CCWS antinociception by L-NA and L-NAME occurred independently of changes in CCWS hypothermia, since only low L-NA doses and the less active isomer actually reduced the magnitude of CCWS hypothermia. While NO synthase inhibition potentiated CCWS antinociception, it generally failed to alter ICWS antinociception. ICWS antinociception and hypothermia were reduced by either low doses of L-NAME or D-NAME. Thus, the present study reveals selective, facilititory effects of NO synthase inhibition upon one form of stress-induced antinociception, but not another form.

A major mechanism by which NO is implicated in nociceptive processes is through NMDA receptor activation [see review (6,8,20)]. If this is the presumed linkage between NO and nonopioid swim stress antinociception, one would expect similar results upon antinociceptive processes if there was interference with either NMDA receptor activation (e.g., antagonism by MK-801) or NO synthase inhibition (e.g., administration of L-NA or L-NAME). The present results indicate differential actions for NMDA receptor antagonism and NO synthase inhibition in that the former reduced nonopioid forms of swim stress antinociception (17,18,27) and the latter potentiated CCWS antinociception in the present study. Typically, opioid and nonopioid mediation of swim stress antinociception was defined by morphine cross-tolerance and naloxone/naltrexone antagonism studies with ICWS antinociception sensitive to these manipulations (9,10) and CCWS antinociception insensitive (3,4). Inhibition of enkephalin-degrading enzymes potentiates ICWS antinociception (11) and reduces CCWS antinociception (5). Further, CCWS antinociception is reduced by simultaneous morphine treatment (25) and potentiated by either chronic naltrexone treatment (30) or acute μ_1 receptor antagonism (12). Thus, ICWS analgesia is an opioid-mediated stressor especially sensitive to manipulations involving the μ-opioid receptor and, like morphine (13,14), is insensitive to NO synthase inhibition. This lack of effect is similar to the failure of NMDA antagonism to affect analgesia elicited by either morphine (1,15,16,26) or opioid forms of swim stress (17,18).

The different patterns of effects by NMDA receptor antagonism and NO synthase inhibition upon nonopioid swim stress analgesia may alternatively reflect the definition of "nonopioid." Recently, some forms of swim analgesia that were insensitive to such μ-mediated manipulations as morphine cross-tolerance or naloxone/naltrexone antagonism were found to be sensitive to either δ_2 opioid antagonists (28) or intrathecal combinations of μ, δ, and κ antagonists (29). Further studies are currently exploring the potential relationships between sensitivity to NO synthase inhibition, NMDA receptor antagonism, and differential responses to opioid receptor subtype antagonists using central microinjection studies. Whether NO synthase inhibition is potentiating CCWS antinociception by opioid (non-μ) or nonopioid mechanisms is not known at present, but these data provide further evidence for the selective activation of different pain-inhibitory systems.

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