Pharmacology Biochemistry and Behavior, Vol. 45, pp. 507–511, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

BRIEF COMMUNICATION

Effects of Calcitonin on CNS Monoamines Following Carrageenan-Induced Inflammation in Rats

KENNETH J. SUFKA,*[1] AND DEAN A. HOGANSON†

*Department of Psychology, University of Mississippi, Oxford, MS 38677
†Department of Biology, Drake University, Des Moines, IA 50311

Received 8 September 1992

SUFKA, K. J. AND D. A. HOGANSON. *Effects of calcitonin on CNS monoamines following carrageenan-induced inflammation in rats.* PHARMACOL BIOCHEM BEHAV 45(2) 507–511, 1993.– The present study examined the effects of systemically administered calcitonin (CT, 10 IU/0.25 ml, SC) on changes in CNS monoamines (MAs) following unilateral carrageenan (CARRA)-induced inflammation in the rat hindpaw. High-performance liquid chromatography with electrochemical detection (HPLC-EC) for MAs was performed on the whole brain and rostral spinal cord. Carrageenan-evoked inflammation significantly increased brain serotonin [5-hydroxytryptamine (5-HT)], norepinephrine (NE), and dopamine (DA) levels. CT significantly reduced these CARRA-induced elevations in brain MAs. Elevated spinal cord 5-HT and NE levels were observed in CARRA-treated animals. CT administration increased 5-HT and NE in both the CARRA-treated animals and their saline controls. Spinal cord DA levels were not affected by either CARRA or CT administration. These findings suggest the involvement of CNS monoaminergic substrates in CT-induced hypoalgesia in inflammatory nociception.

Rats Inflammatory nociception Pain Hypoalgesia Carrageenan Calcitonin Monoamines Dopamine Serotonin Norepinephrine

CONSIDERABLE research has shown the 32 amino acid peptide calcitonin (CT) to possess hypoalgesic effects in a variety of experimental (1,2,8,13) and clinical (10,12,15,23) settings. Systematic evaluation of the hypoalgesic action of CT in several nociceptive assays has shown this peptide to be more effective against inflammatory than thermal or mechanical noxious stimuli in rats (20). Inflammatory processes have been shown to evoke changes in central serotonergic [5-hydroxytryptamine (5-HT)] and noradrenergic (NE) but not dopaminergic (DA) systems (3). A role for 5-HT involvement in CT hypoalgesia has been suggested by the observation that the 5-HT receptor antagonist methysergide has been shown to attenuate the antinociceptive effects of centrally administered CT (4). However, it is unclear whether this effect reflects changes in central or peripheral 5-HT-mediated activity. Noradrenergic involvement in CT hypoalgesia has been suggested by the observations that 6-hydroxydopamine, phentolamine, and propranolol treatment reduced CT antinociception (5). While evidence exists for distinct neuroanatomic and biochemical mechanisms that subserve the processing of different nociceptive stimuli (3,7), direct characterization of CT effects on CNS monoamine (MA) systems subserving inflammatory nociception remains incomplete. Thus, the present study examined the effects of CT on whole-brain and spinal cord MA levels following carrageenan (CARRA)-induced inflammation in rats.

METHOD

Male Sprague-Dawley rats (300–350 g, Dominion Laboratories, Omaha, NE) were housed in pairs in suspended stainless steel cages (360 cm^2) at an ambient temperature of 23 ± 1°C and maintained on a 12 L : 12 D cycle. Animals were allowed food, water, and conspecific contact ad lib prior to experimental use. All experimental protocols were conducted during the middle third of the light cycle.

Unilateral hindpaw inflammation was produced by injection of 100 μl CARRA (1% suspension in 0.9% NaCl; Sigma Chemical Co., St. Louis, MO) into the plantar aponeurosis (n = 4–5). Control animals received IPL administration of

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

the saline vehicle (n = 4). Maximal CARRA inflammation was observed at 2 h postinjection (8,19). This interval (i.e., peak CARRA effects) served as the time point for animal sacrifice and tissue harvest. Salmon CT (Sandoz Pharmaceuticals, East Hanover, NJ) was administered SC in a concentration of 10 IU/0.25 ml 60 or 120 min before peak CARRA effects. Control animals received SC injection of the saline vehicle at only the 60-min interval.

At the time of peak CARRA effects, animals were sacrificed by rapid decapitation. Whole-brain and rostral spinal cord segments (approximately C1-T6) from each animal were rapidly dissected on dry ice, weighed to 0.1 mg, placed in 1.5-ml polyethylene tubes, and stored at −70°C until use. A total of 22 brain and 21 spinal cord samples were available for MA analyses. High-performance liquid chromatography with electrochemical detection (HPLC-EC) of CNS MAs was performed according to methods described elsewhere (17, 21). Data were analyzed using one-way analysis of variance (ANOVA) and power-adjusted Student's t-test (9). Significance was considered at $p < 0.05$ in all cases.

RESULTS

The effects of CT on CARRA-induced changes in brain MAs are summarized in Fig. 1. Calcitonin produced a 14% increase in 5-HT levels in noninflamed animals; CARRA-treated animals that received saline exhibited a 46% increase in 5-HT. Inflamed animals in the CT 60 and 120 groups, however, exhibited only a 25 and 24% increase in brain 5-HT, respectively (see Fig. 1B). In support of these observations, ANOVA of the brain 5-HT data revealed a significant treatment effect, $F(4, 17) = 6.252$, $p < 0.003$. Further analyses demonstrated that CARRA produced a significant 5-HT elevation in saline-treated animals, $t(21) = 4.84$, $p < 0.001$, and that these CARRA-induced increases in brain 5-HT were significantly attenuated by CT administration at both the 60- and 120-min intervals, $t(21) = 2.35$ and 2.38, respectively, $p < 0.05$.

The pattern of CT and CARRA effects on brain NE was like that for the 5-HT data. Calcitonin produced a 30% increase in NE levels in saline controls; CARRA produced a 57% increase in NE. Inflamed animals in the CT 60 and 120 groups, however, exhibited only a 6 and 8% increase in brain NE, respectively. ANOVA of brain NE data revealed a significant treatment effect, $F(4, 17) = 6.263$, $p < 0.003$. Further analyses demonstrated that CARRA produced a significant NE elevation in saline-treated animals, $t(21) = 4.21$, $p < 0.001$, and that these CARRA-induced increases in brain NE were significantly attenuated by CT administration at both the 60- and 120-min intervals, $t(21) = 3.99$ and 3.80, respectively, $p < 0.005$.

As well, the pattern of CT and CARRA effects on brain DA was like that for the 5-HT and NE data. Calcitonin produced a 13% increase in DA levels in saline controls; CARRA produced a 36% increase in DA. Inflamed animals in the CT 60 and 120 groups, however, exhibited only an 8 and 7% increase in brain DA, respectively. ANOVA of brain DA data revealed a significant treatment effect, $F(4, 17) = 3.062$, $p < 0.05$. Further analyses demonstrated that CARRA produced a significant DA elevation in saline-treated animals, $t(21) = 3.20$, $p < 0.005$, and that these CARRA-induced increases in brain DA were significantly attenuated by CT administration at both the 60- and 120-min intervals, $t(21) = 2.59$ and 2.76, respectively, $p < 0.01$.

The effects of CT on CARRA-induced changes in spinal cord MAs are summarized in Fig. 2. Calcitonin increased spinal 5-HT levels in noninflamed animals by 39% and CARRA-treated animals that received saline exhibited a 41% increased in spinal 5-HT. However, the greatest increase in spinal 5-HT was observed in inflamed animals that received CT (129 and 122% in the CT 60 and 120 groups, respectively; Fig. 2B). ANOVA of the spinal 5-HT data revealed a significant treatment effect, $F(4, 16) = 5.118$, $p < 0.01$. Further analyses demonstrated a significant elevation in spinal 5-HT in the CT 60 and 120 CARRA-treated groups compared to their saline-CARRA controls, $t(20) = 2.30$ and 2.12, respectively, $p < 0.05$. However, the increase in spinal 5-HT in the saline-CT and CARRA-saline groups failed to reach statistical significance ($p > 0.05$).

Calcitonin increased spinal NE levels in noninflamed animals by 62% and CARRA-treated animals receiving saline exhibited a 60% increased in spinal NE. Spinal NE levels were further elevated in inflamed animals that received CT administration (92 and 74% in the CT 60 and 120 groups, respectively). An ANOVA of these data, however, failed to reveal a significant treatment effect, $F(4, 16) = 2.136$, $p > 0.05$. Exploratory analyses demonstrated a significant elevation of NE in both saline-CT- and CARRA-saline-treated animals compared to saline-saline controls, $t(20) = 1.80$ and 2.68, respectively, $p < 0.05$. All other relevant comparisons (saline vs. CT 60 and 120 in CARRA animals) failed to reach statistical significance ($p > 0.05$).

Detectable DA data were available in 19 of 21 spinal samples tested; only these 19 data points were included in the analyses. Calcitonin increased spinal DA levels in noninflamed animals by 9% and CARRA-treated animals receiving saline exhibited an 85% increased in spinal DA. Spinal DA levels were further elevated in inflamed animals that received CT administration (143 and 66% in the CT 60 and 120 groups, respectively). While spinal DA levels appeared to be greatly influenced by CARRA and CT, these changes were accompanied by large within-group variability (see Fig. 2A). In support of this observation, an ANOVA of the spinal DA data failed to reveal a significant treatment effect, $F(4, 14) = 0.853$, $p > 0.05$.

DISCUSSION

Monoaminergic systems have been shown to modulate pain at various sites within the nociceptive neuraxis. However, these MA systems may function oppositionally at spinal and supraspinal loci; bulbospinal MA systems mediate antinociception (6,14,16,24), while evidence suggests that supraspinal MA systems, particularly NE, may be involved with nociceptive processing (19).

The present study demonstrated that CARRA-induced inflammation produced elevated MAs (i.e., 5-HT, NE, and DA) levels in the brain. These results are consistent with earlier reports that inflammatory processes potentiate supraspinal MA function (4). These changes may reflect activation of ascending circuitry mediating both sensory and affective dimensions of nociception (21). While CT administration reduced CARRA-induced increases in brain MAs, it is unlikely that CT would exert direct and uniform effects upon these chemically distinct systems. The calcitrophic action of CT has been suggested as a mechanism by which this agent produces analgesia (2,16). Thus, it is possible that CT may affect Ca^{++} utilization in nocisponsive afferent units activated by inflammation. The CT reductions in brain MAs following inflammation may, therefore, reflect a change in the extent of nociceptive afferent activity within this neuraxis.

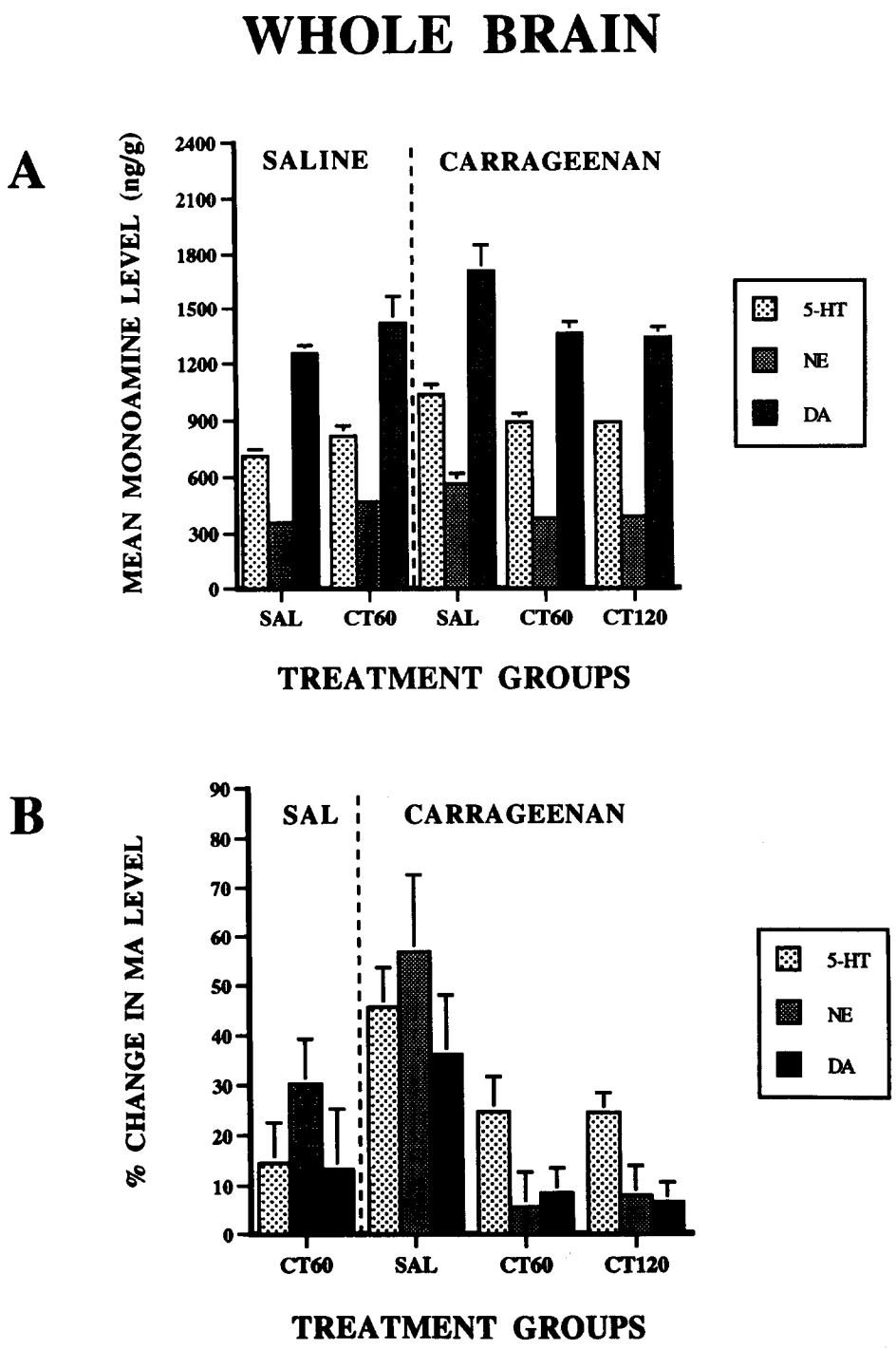

FIG. 1. Effects of unilateral hindpaw carrageenan (CARRA)-inflammation (100 μl 1% suspension) and systemically administered calcitonin (CT) (10 IU, SC, 60 or 120 min before sacrifice) on whole-brain monoamine (MA) levels. (A) Bars represent mean ng/g wet weight tissue. (B) Bars represent % change in MA levels as compared to noninflamed saline control animals. Vertical lines represent SEM.

The pattern of CT and CARRA effects on spinal MAs are less clear. While CT potentiated spinal 5-HT levels in CARRA-treated animals, CT and CARRA alone failed to significantly elevate spinal 5-HT. Both CT and CARRA produced significant increases in spinal NE levels. However, CT did not alter CARRA-induced changes of spinal NE. No alterations in DA functioning were observed with either CT or CARRA treatment. Collectively, CARRA tended to increase spinal 5-HT

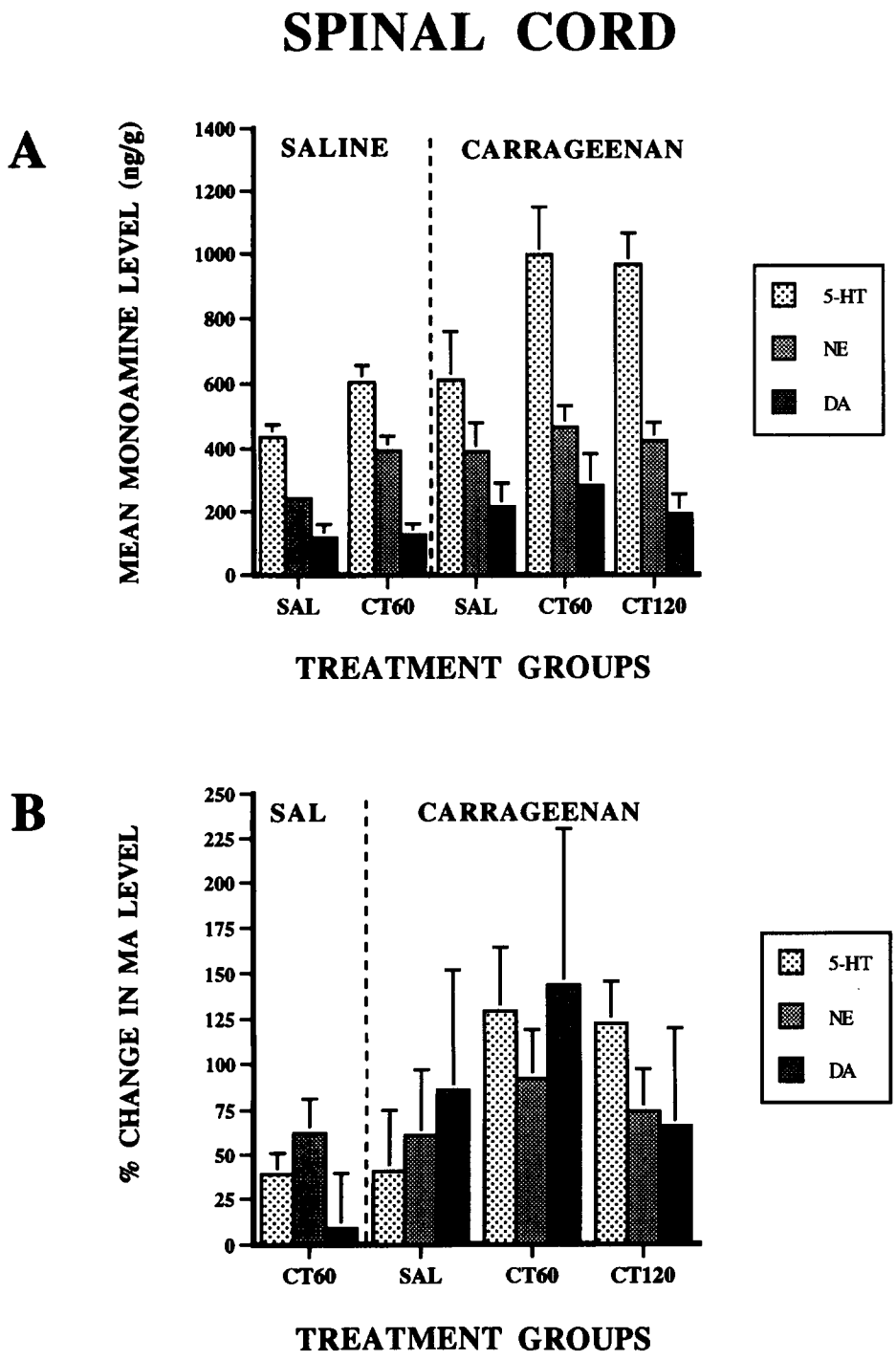

FIG. 2. Effects of unilateral hindpaw carrageenan (CARRA)-inflammation (100 μl 1% suspension) and systemically administered calcitonin (CT) (10 IU, SC. 60 or 120 min before sacrifice) on rostral spinal cord monoamine (MA) levels. (A) Bars represent mean ng/g wet weight tissue. (B) Bars represent % change in MA levels as compared to noninflamed saline control animals. Vertical lines represent SEM.

and NE and CT tended to potentiate these MA changes. It is possible that this pattern of effects reflects the action of CT to disinhibit descending MA systems, particularly 5-HT and NE, that mediate antinociception (14,23).

It is also possible that the pattern of CT and CARRA effects on brain and spinal MAs reflects the activity of systems unrelated to nociceptive processing. For example, the increased MA levels in the CNS may reflect neuroendocrine

system activation in response to inflammation stress. The increase in brain MAs induced by CARRA is consistent with this notion. However, CT has been shown to potentiate rather than attenuate anterior pituitary activity (11). Thus, the CT attenuation of CARRA-induced increase in brain MAs does not support this hypothesis. While nonnociceptive MA systems (e.g., motor) exist within the spinal cord, their interaction with spinal cord nociceptive and antinociceptive systems that subserve inflammation and hypoalgesia, respectively, remains to be determined.

ACKNOWLEDGEMENTS

The authors thank Fritz Schomburg, Keith Stewart, and Deepak Kumar for their assistance in data collection.

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