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Effect of Dopamine Agonists and Antagonists on Neurotensin-Induced Antinociception

DANIEL E. HERNANDEZ,*‡[1] FILIPPO DRAGO,† GEORGE A. MASON,‡ DONALD A STANLEY‡ AND ARTHUR J PRANGE, JR ‡§

North Carolina State University, School of Veterinary Medicine, Raleigh, NC 27606*
Institute of Pharmacology,† University of Catania Medical School, Catania, Italy
and Biological Sciences Research Center,‡ Department of Psychiatry,§
University of North Carolina School of Medicine, Chapel Hill, NC 27514

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HERNANDEZ, D E , F DRAGO, G A MASON, D A STANLEY AND A J PRANGE, JR *Effect of dopamine agonists and antagonists on neurotensin-induced antinociception* PHARMACOL BIOCHEM BEHAV **24**(3) 425–428, 1986 —In previous reports, we have demonstrated that intracisternal (IC) administration of neurotensin (NT), an endogenous tridecapeptide, produces significant antinociception in a variety of analgesic tests, including the hot-plate test In addition, many of the central nervous system effects of NT (i e , hypothermia, gastric cytoprotection) appear to be mediated by brain dopamine (DA) systems In this study, we evaluated the effect of selected DA agonists and antagonists on NT-induced antinociception in the hot-plate test with mice Doses, route of administration, and pretreatment interval were determined from the available literature to significantly affect the incidence of DA-dependent behaviors Pretreatment with chlorpromazine but not haloperidol significantly potentiated NT-induced antinociception This potentiating effect of chlorpromazine appears not to be due to any intrinsic antinociceptive activity of this agent, chlorpromazine had no significant effect on hot-plate latencies when administered alone The involvement of DA on NT-induced antinociception was further substantiated by the findings that pretreatment with several DA receptor agonists, including methylphenidate, apomorphine, and d-amphetamine, significantly antagonized the antinociceptive response to IC NT None of these agents significantly altered the animal's response to the hot-plate when administered alone The data furnished in the present report suggest that central DA circuits may be involved in the expression of NT-induced antinociception

Neurotensin Antinociception Dopamine

ONE area that has produced a substantial development in our understanding of reception and processing of pain is the novel discovery that antinociception can be affected by several endogenous brain peptides (see [11] for review) One such peptide is neurotensin (NT) Immunohistochemical and radioimmunoassay techniques have revealed that NT is distributed in regions of the rat brain implicated in the animal's response to noxious stimuli These include the amygdala, periaqueductal gray, and thalamus [19] Neurotensin-containing terminals and NT receptors have also been described in areas implicated in modulation of pain, most notably the substantia gelatinosa of the spinal cord [19,21] The original hypothesis that NT may induce spinal and supraspinal analgesia has been widely confirmed [2, 3, 4, 6, 12]

Intracisternal (IC) administration of NT in mice and rats has been shown to produce dose-dependent antinociception in a variety of tests, including the hot-plate, tail-flick, tail-immersion, and acetic-acid writhing tests. Of interest is the finding that NT-induced antinociception has been shown to be more potent than morphine, but not β-endorphin, in some analgesic tests [2,12] More recently, it has been demonstrated that intrathecal administration of NT produces a dose-related antinociceptive response in the hypertonic saline paradigm in mice [9] The mechanisms by which central (IC) or peripheral (intrathecal) NT induces antinociception are still unclear Neurotensin-induced antinociception is apparently not due to motor impairment Doses of NT that produce significant antinociception do not affect coordinated motor control on a rotating rod However, spontaneous locomotor activity is reduced [16]

Although, many aspects of NT distribution also coincide with the location of enkephalinergic cell bodies and terminals [8], recent evidence indicates that NT-induced antinociception is not mediated by opiate receptors [6, 11, 17] In more recent work, we have found that co-administration of NT and leucinal, an aminopeptidase inhibitor which potentiates leu-enkephalin and β-endorphin-induced antinociception, does not affect the antinociceptive response to IC NT in the hot-plate test in mice [3] This recent observation, coupled to the fact that NT produces a naloxone-insensitive

[1]Requests for reprints should be addressed to Dr Daniel E Hernandez, North Carolina State University, School of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606

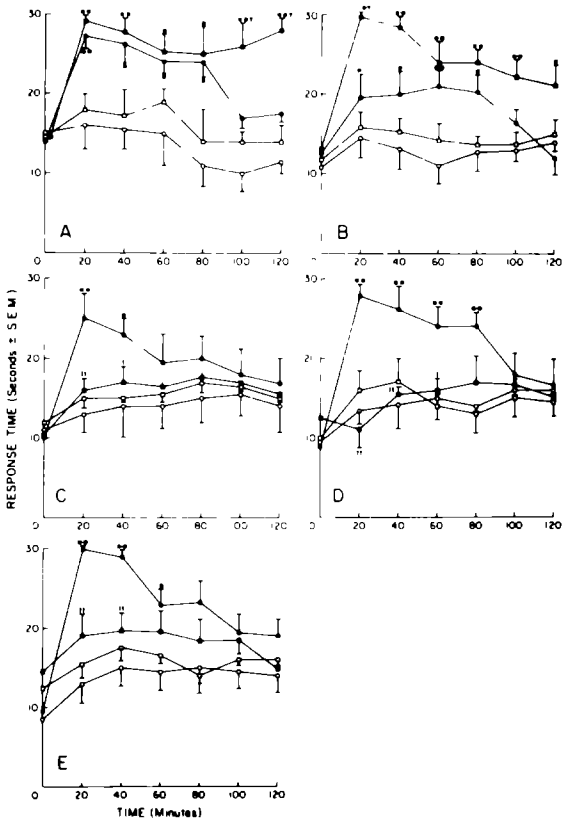

FIG 1 Effect of pretreatment with selected dopamine receptor agonists and antagonists on neurotensin (NT)-induced antinociception in the hot-plate test in mice Groups of mice (n=8/group) were pretreated (−30 min) with haloperidol (A), chlorpromazine (B), methylphenidate (C), d-amphetamine (D) and apomorphine (E) For doses and route of administration, see text Mice were then injected IC with NT (10 μg) or vehicle (10 μl of 0 9% NaCl) Treatment categories were as follows (○) saline IC + vehicle (IP or SC, −30 min), (●) NT IC (10 μg) + vehicle (IP or SC, −30 min), (□) saline IC + drug (IP or SC, −30 min), and (■) NT (10 μg) IC + drug (IP or SC, −30 min) *$p<0$ 05, **$p<0$ 01 when compared to saline-treated controls (Dunnett's test) †$p<0$ 05, ††$p<0$ 01 vs NT-treated mice (Dunnett's test)

antinociception [17], suggests that the antinociceptive response to central NT is not mediated by endogenous opiate systems

Central (IC) NT produces a variety of behavioral effects [16], and many of these actions of NT are antagonized by thyrotropin-releasing hormone (TRH) Simultaneous IC administration of NT and TRH and several TRH congeners, including 3-methyl-His-TRH, MK-771, β-ala-TRH, and RX-77368, significantly antagonize NT-induced hypothermia and antinociception in the hot-plate test in mice [6,17]

A growing body of evidence suggests that many of the central nervous system (CNS) effects of NT are mediated by central dopamine (DA) circuits [15,18] Depletion of brain catecholamines with 6-hydroxydopamine, or pretreatment with haloperidol (a DA receptor blocker), significantly potentiates NT-induced hypothermia. Dopamine receptor agonists, on the other hand, block the hypothermic response to NT [13] In addition, NT also reduces locomotor activity induced by several DA agonists [15] Neurochemical studies have shown that IC administration of NT increases DA turnover and DA metabolite concentrations in several brain regions, including nucleus accumbens, olfactory tubercle, and striatum [20] This study was designed to evaluate the effect of DA receptor agonists and antagonists on the antinociceptive responses to IC NT in the hot-plate test with mice

METHOD

Adult, male Swiss-Webster mice (25–35 g) were purchased from Flow Laboratories (Dublin, VA) and were group housed (6 mice/cage) in a controlled environment animal facility (12 hr light, 12 hr dark) with laboratory chow and water available ad lib The mice were housed for at least one week prior to experimentation All experiments were conducted between 0800 and 1200 at ambient temperatures of 22–24°C

Groups of mice (n=8/group) were selected for each experiment, no animal being used in more than one experiment Intraperitoneal (IP) pretreatments included methylphenidate (5 mg/kg), d-amphetamine (2 mg/kg), and haloperidol (1 mg/kg) Subcutaneous (SC) pretreatments included apomorphine (5 mg/kg) and chlorpromazine (2 mg/kg) All the dopamine agonists (e g , d-amphetamine, methylphenidate, and apomorphine), and the dopamine antagonists (e g , haloperidol and chlorpromazine) were administered 30 min before IC NT (10 μg) or vehicle (10 μl of 0 9% NaCl) Doses, route of administration, and pretreatment interval were determined from the available literature to significantly affect the incidence of dopamine-dependent behaviors [7, 13, 15] Intracisternal injections were performed under light ether anesthesia as previously described [5] All drugs were dissolved in 0 9% (w/v) NaCl, except apomorphine, which was dissolved in 0 5% (w/v) ascorbic acid, and haloperidol, which was dissolved in 0 3% (w/v) tartaric acid All drugs were calculated as salts

The basic experimental design included four groups of mice (n=8/group) each one receiving one of the following treatments (1) vehicle (IC) + vehicle (IP or SC), (2) NT (IC) + vehicle (IP or SC), (3) vehicle (IC) + DA agonist or antagonist (IP or SC), and (4) NT (IC) + DA agonist or antagonist (IP or SC) The dose of IC NT (10 μg) utilized in these experiments was chosen because it has been previously described to reliably produce antinociception in mice [2, 3, 6, 12]

Antinociception was assessed by using the hot-plate test [1] In this test, mice are placed with all four paws on a heated copper plate and the time to the nearest tenth second for the mice to either lick their paws or jump is considered as a response to the noxious stimulus The temperature in the hot-plate was set at 50–52°C This hot-plate temperature permits repeated testing of the response of an individual mouse to the noxious stimulus without inflicting injury to the animals An arbitrary cut-off was used to score animals not responding to the noxious stimulus within 30 sec Each animal was tested every 20 min for 2 hr Time 0 refers to the first reading of the hot-plate latencies after the IC injection This is performed routinely in our laboratory between 1–3 min after the injections Mice normally regain the writhing reflex after 10–15 sec after exposure to light ether anesthesia There is probably some effect of ether on the initial hot-plate latencies However, comparisons of latencies between nonanesthetized controls and mice exposed to light ether anesthesia have failed to reveal significant differences (unpublished observations)

Neurotensin was purchased from Bachem (Torrance, CA), haloperidol from McNeil Laboratories (Fort Washington, PA) and chlorpromazine from Elkins Sinn (Cherry Hill, NJ) Methylphenidate was a gift from Ciba-Geigy Corporation (Summit, NJ) Apomorphine HCL was purchased from Merck and Sharp Company, Inc (Rahway, NJ) and d-amphetamine sulphate from Sigma Chemical Co (St Louis, MO) A significant increase in the response time for experimental compared to control mice was defined as antinociception One-way analysis of variance followed by Dunnett's test for multiple comparisons was used for this analysis A p value of 0 05 or less was considered to represent significant differences between groups

RESULTS

The results illustrated in Fig 1 show the effect of pretreatment with selected DA receptor agonists and antagonists on the antinociceptive response to IC NT in the hot-plate test with mice In confirmation of previous findings [2, 3, 4, 6, 12], NT (10 μg) produced significant antinociception after IC administration (Fig 1A–E) As indicated in Fig 1A, haloperidol (1 mg/kg, IP) did not significantly affect NT-induced antinociception Only at 100 and 120 min a significant effect of haloperidol on NT's effect was observed Chlorpromazine (2 mg/kg, SC), however, significantly ($p<0$ 01) potentiated NT-induced antinociception (Fig 1B) The effect of this agent on the antinociceptive properties of NT was long-lasting Significant antinociception was observed after 2 hr, a time at which the antinociceptive activity of NT alone had already disappeared In confirmation of previous work [14], the two neuroleptic drugs tested in this study (haloperidol and chlorpromazine), unlike NT, did not induce antinociception (Fig 1A, 1B) Of interest were our findings with the DA receptor agonists on the antinociceptive response to IC NT (10 μg) As indicated in Fig 1C–D, indirect stimulation of DA receptors with methylphenidate (5 mg/kg, IP) or d-amphetamine (2 mg/kg, IP) significantly blocked NT-induced antinociception The antagonism of NT-induced antinociception by methylphenidate or d-amphetamine is apparently not due to an intrinsic hyperalgesic property of these agents, as evidenced by their inability to significantly affect hot-plate latencies when administered alone (Fig 1C–D) Finally, as indicated in Fig 1E, apomorphine (5 mg/kg, SC), a directly acting DA receptor agonist, also produced a significant ($p<0$ 01) antagonism of NT-induced antinociception Apomorphine did not have any significant effect when administered alone

DISCUSSION

The results of this investigation confirm and extend previous observations that IC administration of NT produces significant antinociception in the hot-plate test in mice [3, 4, 6, 12] The antinociceptive response to central (IC) NT was significantly potentiated by prior blockade of DA receptors with chlorpromazine This interesting observation suggests that NT-induced antinociception may be exaggerated by diminished DA neurotransmission within the CNS These findings resonate with previous observations Depletion of brain catecholamines with 6-hydroxydopamine and desmethylimipramine, or pretreatment with haloperidol, but not atropine or naloxone, significantly potentiates the hypothermic response to IC NT [13] In addition, IC administered NT, like haloperidol (a neuroleptic drug), reduces spontaneous locomotion or forward locomotion induced by indirect DA agonists [15] It would appear that NT and neuroleptics (all of which are DA blockers) share a very similar neuropharmacological profile This is evidenced by the fact that NT and neuroleptics produce a variety of behavioral effects including hypothermia, potentiation of barbiturate- and ethanol-induced sedation, muscle relaxation and catalepsy [14] However, haloperidol and chlorpromazine, unlike NT, do not induce antinociception In this study, the lack of effect of neuroleptic drugs on nociceptive processes is further documented These findings, taken together, indicate that the potentiation of NT-induced antinociception by chlorpromazine may be the result of decreased DA activity at relevant brain sites, but not the consequence of an additive effect between NT and neuroleptic drugs In addition, it is entirely possible that the differences observed on the effects of chlorpromazine and haloperidol on NT-induced antinociception reflect preferential activation of D_1 receptors by chlorpromazine Considering the dose of chlorpromazine used in this study, indirect involvement of cholinergic, serotonergic and histaminergic pathways can not be ruled out

The involvement of DA on the antinociceptive effect of NT was further substantiated by our findings with the directly- or indirectly-acting DA agonists As indicated in results (vide supra), pretreatment with methylphenidate, d-amphetamine, or apomorphine significantly antagonized NT-induced antinociception These agents did not affect hot-plate response by themselves These results indicate that increased presynaptic release of DA, or direct stimulation of postsynaptic DA receptors is an equally effective mechanism for blocking the antinociceptive activity of central NT

As mentioned above, a growing body of evidence suggests a role for NT in modulation of pain transmission Our recent neuroanatomical and neurochemical studies indicate that NT acts not only on classical nociceptive pathways in the brain stem, but may also interfere with more integrated behavioral and affective responses to painful stimuli [11,16]

Bilateral stereotaxic microinjections of NT (2 5 μg/site) have been shown to elicit significant antinociception in several brain loci, including the central amygdaloid nucleus, caudal diagonal band of Broca, rostral preoptic area, ventromedial thalamus, and the rostral half of the mesencephalic reticular formation [10] All of these sites contain endogenous NT [19] Another interesting observation is that NT and DA systems are located in proximity to one another in several brain regions, including the nucleus accumbens, ventral tegmentum (VTA), median eminence, and substantia nigra Thus, it would appear that this distinct neuroanatomical overlapping between NT and brain DA systems subserves a major role in the expression of the CNS effects elicited by NT [15,18]

This original hypothesis has been confirmed for a number of the brain effects of NT including hypothermia, locomotion, and gastric cytoprotection [7, 13, 15] Although the work described in the present report provides evidence for the involvement of DA on the antinociceptive response to central NT, a full description of this interaction awaits future investigation

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