Proc. Natl. Acad. Sci. USA
Vol. 76, No. 10, pp. 5368–5371, October 1979
Neurobiology

Alterations in nociception and body temperature after intracisternal administration of neurotensin, β-endorphin, other endogenous peptides, and morphine

(peptide-induced analgesia/thermoregulation)

CHARLES B. NEMEROFF, ALBERT J. OSBAHR III, PAUL J. MANBERG*, GREGORY N. ERVIN†, AND ARTHUR J. PRANGE, JR.†‡

Biological Sciences Research Center, Departments of ‡Psychiatry and *Pharmacology, †The Neurobiology Program, University of North Carolina, School of Medicine, Chapel Hill, North Carolina 27514

Communicated by Carl W. Gottschalk, June 14, 1979

ABSTRACT **The antinociceptive and hypothermic effects of intracisternal administration of 11 endogenous neuropeptides and morphine were evaluated in mice. Of the substances tested, only neurotensin (NT) and β-endorphin exerted significant antinociceptive and hypothermic effects; NT was the most potent in inducing hypothermia whereas β-endorphin was the most potent antinociceptive agent via this route of administration. Both NT and β-endorphin were, on a molar basis, considerably more potent antinociceptive agents than morphine, [Met]enkephalin, or [Leu]enkephalin. NT-induced analgesia and hypothermia both were significantly dose-dependent. Substance P was found to produce significant hyperalgesia and hyperthermia. Bombesin produced a significant hypothermic effect, whereas somatostatin and luteinizing hormone-releasing hormone (luliberin) produced hyperthermia. None of the other peptides studied [bradykinin, thyrotropin-releasing factor (thyroliberin), melanocyte-stimulating hormone release-inhibiting factor (melanostatin), somatostatin, [Met]enkephalin, and [Leu]enkephalin] produced any significant alterations in colonic temperature or response to a noxious stimulus with the doses tested. These data demonstrate that NT and β-endorphin, two endogenous brain peptides, are potent in inducing hypothermia and in producing an antinociceptive state.**

In recent years, several peptides endogenous to brain have been found to produce various effects when administered either systemically or directly into the central nervous system (1, 2). Considerable attention has been focused on the endorphins and enkephalins which are endogenous opiate-like peptides that produce antinociception (3–5), hypothermia (6), and various other behavioral effects (7). In addition, they bind avidly to brain opiate receptors (8). Neurotensin (NT) and bombesin are potent hypothermic agents after intracerebroventricular administration in rats and mice (9, 10). Furthermore, peptides other than the endorphins and enkephalins have been reported to produce significant analgesia after direct intracerebral injection. These include NT, bradykinin, and substance P (11–14).

The purpose of the present study was to evaluate the antinociceptive properties and the effect on colonic temperature of 11 endogenous neuropeptides and morphine after intracisternal administration in mice. Of particular interest was the evaluation of the dose–response relationship of NT-induced antinociception.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Antinociceptive activity was measured in adult male albino Swiss–Webster mice (25–30 g) by the tail-immersion test developed for use in rats by Janssen and coworkers (15) as modified for use in mice by Sewell and Spencer (16). Groups of mice ($n \geq 6$ per group) were lightly anesthetized with ether and injected intracisternally with vehicle (0.9% NaCl, pH 7.5) or with one of the following peptides: NT, substance P, thyrotropin-releasing factor (thyroliberin), luteinizing hormone-releasing factor (luliberin), bradykinin, melanocyte-stimulating hormone-release inhibiting factor (melanostatin), somatostatin, β-endorphin, [Met]enkephalin, [Leu]enkephalin, and bombesin. Morphine sulfate was also tested. Thyroliberin (lot 31-330-AL) was provided by Abbott; NT (lot 14-296-10), β-endorphin (lot 28-111-30), and bombesin (lot 42-206-20) were generously supplied by Jean E. Rivier (The Salk Institute, La Jolla, CA). All the other peptides and morphine were purchased commercially: luliberin, [Met]enkephalin, and substance P (Beckman); melanostatin (U.S. Biochemical, Cleveland, OH); [Leu]-enkephalin (Calbiochem); bradykinin (Spectrum Medical Industries, Los Angeles, CA); somatostatin (Bachem, Torrence, CA); morphine sulfate (Eli Lilly). No mouse was utilized for more than one experiment.

After the intracisternal injection, the mice were placed in restraining cages as described by Janssen *et al.* (15). Their tails were immersed in a constant-temperature water bath set at 48°C. The time that elapsed for the "tail-jerk" or "tail-withdrawal" was estimated to the nearest 0.5 second. This procedure was performed every 15 min over a 2-hr period. One experimental and one control mouse were paired. Comparison between the mean areas under the curves for the experimental group and the control group allows for evaluation of whether the treatment produced a significant alteration in the response of the mice to the noxious stimulus. Furthermore, the difference between the area under the curve calculated by planimetry for the experimental mouse and that for the saline-treated mouse produced a measure representing both the magnitude and the duration of the analgesic effect of the substance under study. The mean of the areas for the six experimental mice minus that for control mice represents a measure of the antinociceptive effect which allows comparison of the *relative* potency of different peptide treatments in inducing antinociception. Dunnett's test for multiple comparisons was utilized for this analysis (17).

Abbreviation: NT, neurotensin.

Rectal temperature was measured by using a thermistor probe as described (9). The magnitude and duration of the hypothermic or hyperthermic effect were evaluated by planimetric comparisons between the experimental and their respective control groups (Student's *t* test, two-tailed). Comparisons between different treatment groups was performed with Dunnett's test for multiple comparisons (17).

The relationship among antinociceptive effect, temperature alteration, and dose–response effects of NT was estimated by using a least squares multiple regression technique.

RESULTS

In the first series of experiments the effect of various doses of NT (0.003–300 μg), administered intracisternally, on the latency to withdrawal from the noxious stimuli (48°C water) over a period of 2 hr was studied. One microgram of NT produced a 4- to 6-sec delay in tail removal from the water bath throughout the 2-hr test period, whereas saline-treated mice removed their tails with a latency of 1–2 sec (Fig. 1). NT produced a dose-dependent antinociceptive effect; all doses of NT >300 ng induced significant antinociception. Least squares multiple regression analysis and analysis of variance revealed the increase in response time to be significantly related to the dose of NT ($P < 0.01$). Similarly, as previously described (9), NT-induced hypothermia was dose-related (Fig. 2), the minimal effective dose being 30 ng; 1 ng of NT produced a 3–4°C decrease in rectal temperature over the 2-hr test period compared with saline-treated controls.

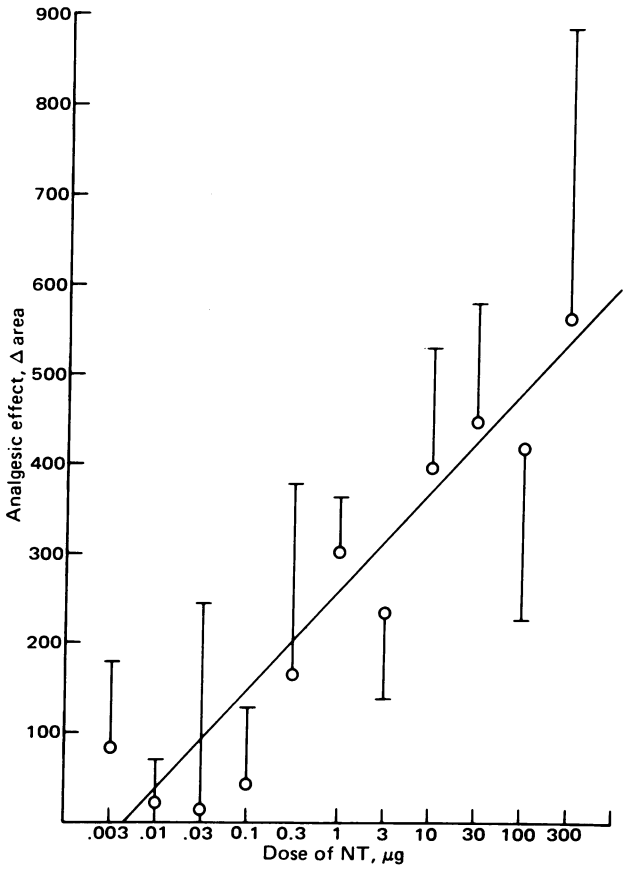

FIG. 1. Effect of various doses of NT (0.003–300 μg intracisternally on the tail withdrawal response of mice (48°C water bath). Mice were tested every 15 min after injection over a 2-hr period. The ordinate represents the area under the curve (±SEM) described by the duration and magnitude of the antinociceptive effect after the scores from control mice were subtracted from those of the NT-treated animals. All doses >300 ng produced a significant effect ($r = 0.3397$).

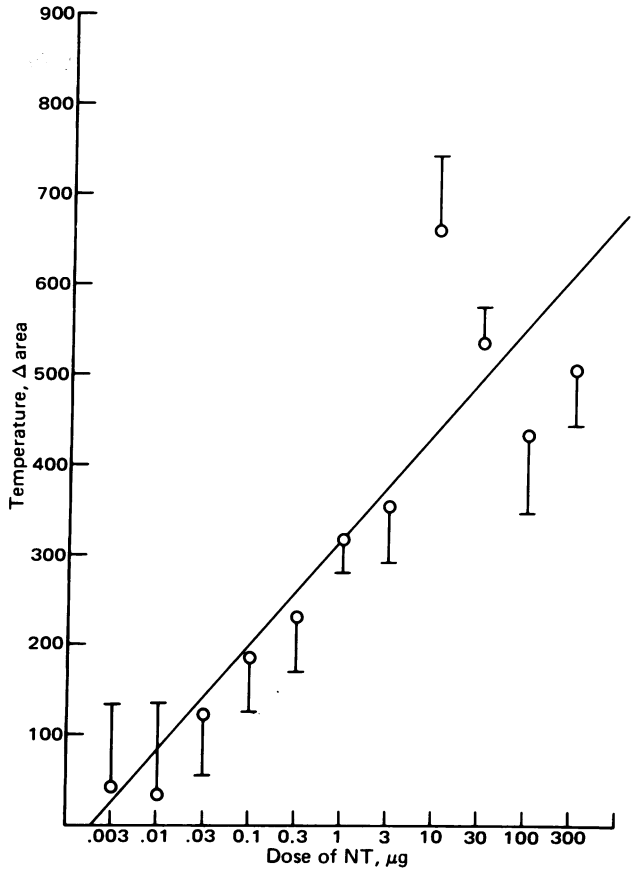

FIG. 2. Effect of various doses of NT (0.003–300 μg) intracisternally on the colonic temperature of mice at an ambient temperature of 23°C. The ordinate represents the area under the curve (±SEM) described by colonic temperature measured every 15 min after injection over a 2-hr period and is determined by both the magnitude and duration of the *hypothermic* response. All doses of NT >10 ng produced a significant hypothermic effect. The area under the curve was calculated by planimetry, and the scores of the saline-treated control mice were subtracted from the NT-treated animals ($r = 0.5910$).

NT at 1 μg was found to consistently induce significant antinociceptive and hypothermic effects. To evaluate the effects of other endogenous peptides and morphine, doses of these substances equimolar to 1 μg of NT were injected intracisternally as described above. The results are shown in Fig. 3. NT was the most potent hypothermic substance; β-endorphin and bombesin were next. These substances were found to differ significantly from each other as well as from the other peptides in regard to hypothermic potency (Dunnett's test for multiple comparison). Somatostatin, luliberin, and substance P all produced a significant hyperthermia. [Leu]Enkephalin, melanostatin, morphine, bradykinin, [Met]enkephalin, and thyroliberin produced no effect on thermoregulation in the mouse. β-Endorphin and NT were the only substances, in the doses utilized, that produced a significant antinociceptive effect although the former was clearly more potent (Dunnett's test for multiple comparisons). Although morphine in a dose equimolar to 1 μg of NT did not produce a significant antinociception, a dose 100-fold higher produced a marked effect (data not shown). Substance P produced a significant hyperalgesia; none of the other peptides studied significantly altered the response of the mice to the noxious stimulus.

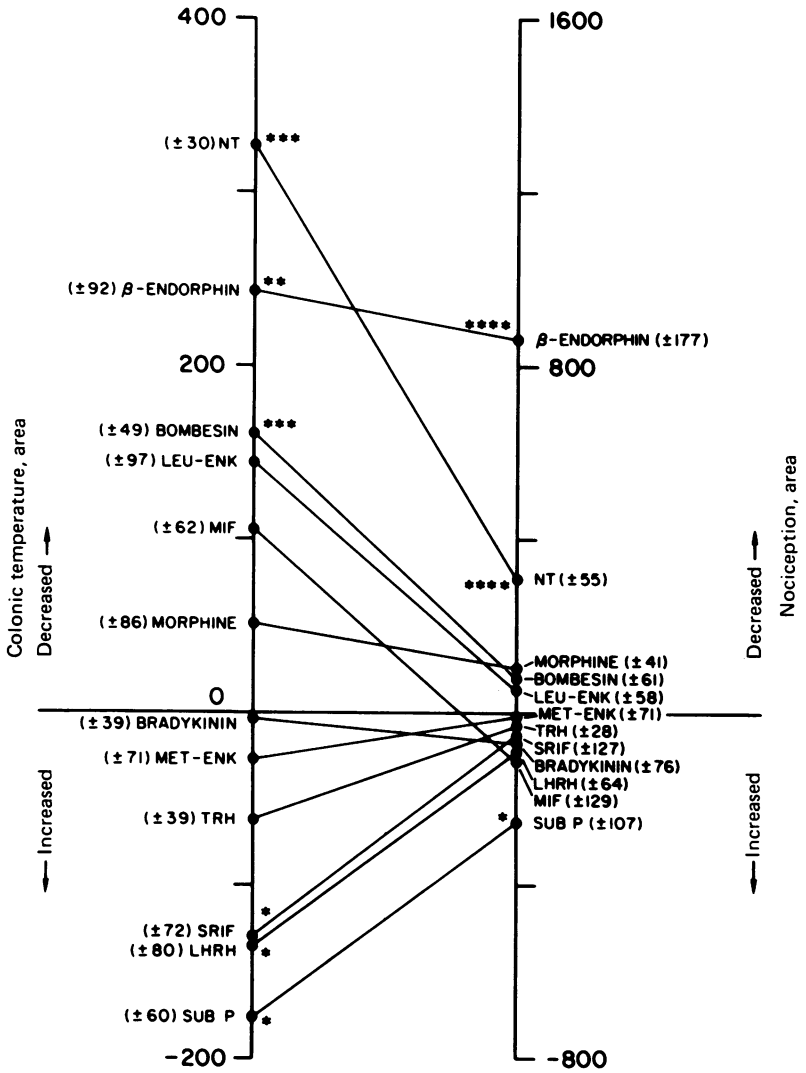

FIG. 3. Effects of 11 endogenous peptides and morphine on response latency to a noxious stimulus and on alterations of body temperature after intracisternal administration to mice. NT was administered in a dose of 1 μg; all other substances were administered in an equimolar dose to allow direct comparison of potency. SEM is shown in parentheses. By Dunnett's *t* test for multiple comparisons, NT was the most potent hypothermic substance and β-endorphin was the most potent antinociceptive substance. Asterisks indicate significant antinociceptive, hyperalgesic, hypothermic, or hyperthermic responses when compared to the controls (Student's *t* test, two-tailed; *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.01$; ****, $P < 0.001$). LEU-ENK, [Leu]enkephalin; MIF, melanostatin; MET-ENK, [Met]enkephalin; TRH, thyroliberin; SRIF, somatostatin; LHRH, luliberin; SUB P, substance P.

DISCUSSION

This report confirms and extends the recent observation (11) that NT, a peptide endogenous to the central nervous system, produces significant antinociception and hypothermia (9) after intracisternal administration in mice. NT-induced antinociception and hypothermia clearly are dose-related. Of special interest is the finding that, on a molar basis, the tridecapeptide is a more effective antinociceptive agent than is [Met]enkephalin, [Leu]enkephalin, or morphine. Thus, of the endogenous opiate ligands tested, only β-endorphin is more potent than NT. The findings that the relatively low doses of the enkephalins and morphine utilized in this study do not produce a significant antinociceptive effect confirms previous studies in the mouse (18) and rat (4). It is possible, of course, that differential degradation by peptides contributed to this array of findings. These results along with previous findings (9, 11) raise the possibility that NT plays a physiological role in mediating pain sensibility or temperature regulation.

Our results demonstrating hyperalgesia after substance P administration are consistent with previous findings (13) that microgram doses of the peptide are hyperalgesic whereas nanogram doses are antinociceptive.

Bradykinin has been reported to produce antinociception in the rabbit after intracisternal administration of 3 μg or more (12). Our data show no evidence of such an effect in the mouse in the dose studied (0.63 μg, equimolar to 1 μg NT).

These results provide new information concerning central nervous system effects of NT. This endogenous peptide is distributed heterogeneously in the brain (19); it is selectively localized in the synaptosomal fraction after density gradient centrifugation of brain homogenates (19) and binds to brain membranes in a saturable, high-affinity manner (20–22). In addition, centrally administered NT decreases locomotor activity in rats (9), potentiates barbiturate sedation (9), and produces hypothermia (9), muscle relaxation (23), and analgesia in mice (11) and rats (unpublished observations). Recently, administration of NT directly into the nucleus accumbens of rats has been shown to block certain behavioral effects of *d*-amphetamine (24). These data indicate that NT shares several characteristics with both neuroleptic agents such as haloperidol

and chlorpromazine on the one hand, and with opiates such as morphine and β-endorphin on the other hand. The importance of these observations is underscored by the recent hypotheses postulating a role for endorphins and other opiate peptides in the regulation of behavioral states including mental disorders (25–28). The precise role and action of NT in the modulation of neural transmission and its subsequent expression in alterations of physiological and behavioral processes remain obscure.

We acknowledge the generous gift of neurotensin, β-endorphin, and bombesin from Dr. Jean E. Rivier of the Salt Institute (La Jolla, CA). We are grateful to Dorothy Yarbrough for preparation of this manuscript. This research was supported by grants from the National Institute of Mental Health (MH22536, MH32316) and the National Institute of Child Health and Human Development (HD03110).

1. Prange, A. J., Jr., Nemeroff, C. B., Lipton, M. A., Breese, G. R. & Wilson, I. C. (1978) in *Handbook of Psychopharmacology*, eds. Iversen, L. L., Iversen, D. S. & Snyder, S. H. (Plenum, New York), Vol. 13, pp. 1–117.
2. Nemeroff, C. B. & Prange, A. J., Jr. (1978) *Arch. Gen. Psychiatry* **35,** 999–1010.
3. Li, C. H., Yamashiro, D., Tseng, L. F. & Loh, H. H. (1977) *J. Med. Chem.* **20,** 325–328.
4. Beluzzi, J. D., Grant, N., Garsky, V., Sarantakis, D., Wise, C. D. & Stein, L. (1976) *Nature (London)* **260,** 625–626.
5. Terenius, L. (1978) *Annu. Rev. Pharmacol. Toxicol.* **18,** 189–204.
6. Li, C. H., ed. (1978) in *Hormonal Proteins and Peptides: Lipotropin and Related Peptides* (Academic, New York), Vol. 5, pp. 35–73.
7. Watson, S. J., Akil, H., Berger, P. A. & Barchas, J. D. (1979) *Arch. Gen. Psychiatry* **36,** 35–41.
8. Miller, R. J., Chang, K. J., Cuatrecasas, P., Wilkinson, S., Lowe, L., Beddell, C. & Follenfent, R. (1978), in *Centrally Acting Peptides*, ed. Hughes, J. (Macmillan, London), pp. 195–213.
9. Nemeroff, C. B., Bissette, G., Prange, A. J., Jr., Loosen, P. T. & Lipton, M. A. (1977) *Brain Res.* **128,** 485–496.
10. Brown, M. R., Rivier, C. & Vale, W. (1977) *Science* **196,** 998–1000.
11. Clineschmidt, B. V. & McGuffin, J. C. (1977) *Eur. J. Pharmacol.* **46,** 395–396.
12. Ribeiro, S. A., Corrado, A. P. & Graeff, F. G. (1971) *Neuropharmacology* **10,** 725–731.
13. Frederickson, R. C. A., Burgus, V., Harrell, C. E. & Edwards, J. D. (1978) *Science* **199,** 1359–1362.
14. Stewart, J. M., Getto, C. J., Neldner, K., Reeve, E. B., Krivoy, W. A. & Zimmerman, E. (1976) *Nature (London)* **262,** 784–785.
15. Janssen, P. A. J., Niemqeers, C. J. E. & Dony, J. C. (1963) *Arzneim.-Forsch.* **13,** 502–507.
16. Sewell, R. D. E. & Spencer, P. S. J. (1976) *Neuropharmacology* **15,** 683–688.
17. Dunnett, C. W. (1964) *Biometrics* **20,** 482–491.
18. Büscher, H. H., Hill, R. C., Römer, D., Cardinaux, F., Closse, A., Hauser, D. & Pless, J. (1976) *Nature (London)* **261,** 423–425.
19. Kobayashi, R. N., Brown, M. & Vale, W. (1977) *Brain Res.* **126,** 584–588.
20. Uhl, G. R. & Snyder, S. H. (1977) *Life Sci.* **19,** 1827–1832.
21. Lazarus, L. H., Brown, M. R. & Perrin, M. H. (1977) *Neuropharmacology* **16,** 625–629.
22. Kitabgi, P., Carraway, R., van Rietschoten, J., Granier, C., Morgat, J. L., Menez, A., Leeman, S. & Freychet, P. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1846–1850.
23. Osbahr, A. J., III, Nemeroff, C. B., Manberg, P. J. & Prange, A. J., Jr. (1979) *Eur. J. Pharmacol.* **54,** 299–302.
24. Lipton, M. A., Ervin, G. N., Birkemo, L. S., Nemeroff, C. B. & Prange, A. J., Jr. (1979) in *Catecholamines: Basic and Clinical Frontiers*, eds., Usdin, E., Kopin, I. J. & Barchas, J. D. (Pergamon, New York), pp. 657–662.
25. Verebey, K., Volovka, J. & Clovet, D. (1978) *Arch. Gen. Psychiatry* **35,** 877–888.
26. Guillemin, R., Ling, N., Lazarus, L., Burgus, R., Minick, S., Bloom, F., Nicoll, R., Siggins, G. & Segal, D. (1977) *Ann. N. Y. Acad. Sci.* **297,** 131–157.
27. Goldstein, A. (1978) *Ann. N. Y. Acad. Sci.* **311,** 49–58.
28. Watson, S. J., Akil, H., Berger, P. A. & Barchas, J. D. (1979) *Arch. Gen. Psychiatry* **36,** 35–41.