Pharmacology Biochemistry & Behavior, Vol. 22, pp. 189–193, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Motor Hypoactivity Induced by Neurotensin and Related Peptides in Mice

GERHARD MEISENBERG

Department of Biochemistry, Ross University School of Medicine
Portsmouth, Commonwealth of Dominica (West Indies)

AND

WILLIAM H. SIMMONS

Department of Biochemistry and Biophysics, Loyola University Stritch School of Medicine
2160 S. First Ave. Maywood, IL 60153

Received 21 June 1984

MEISENBERG, G. AND W. H. SIMMONS. *Motor hypoactivity induced by neurotensin and related peptides in mice.* PHARMACOL BIOCHEM BEHAV 22(2) 189–193, 1985.—The tridecapeptide neurotensin (NT) induces a variety of behavioral changes in animals. The present study characterizes the behavioral hypoactivity observed after intracerebroventricular (ICV) injection in mice. At doses higher than 25 ng, NT induced a reduction of general motor activity and increases in immobility which lasted for about one hour. The NT-related amphibian skin peptide xenopsin was about 70-fold more potent than NT itself. After repeated NT-injections, tolerance developed within 2–4 days and disappeared within 2–4 days after cessation of the treatment. The motor hypoactivity induced by NT was not attenuated by pretreatment with naloxone (5 mg/kg, SC). Furthermore, amphetamine-induced locomotor activity was not blocked by NT or xenopsin. These results suggest that the NT-effect is not mediated by a stimulation of opioid mechanisms or attenuation of dopamine-mediated events.

Neurotensin . Xenopsin Motor activity Sedation Time-course Tolerance

NEUROTENSIN (NT) is a neurotransmitter candidate with wide, but distinct distribution in the central nervous system [17, 18, 19]. It induces a variety of behavioral and physiological effects after central, but not peripheral, administration in animals. These include hypothermia [1,12], antinociception [5, 6, 12], the potentiation of ethanol and barbiturate anesthesia [13], changes in spontaneous motor activity [8, 9, 10, 11], and apparent "neuroleptic-like" properties [7, 14, 15]. The mechanisms and physiological significance of these effects are poorly understood, and the possible interdependence of the different effects has rarely been investigated. The present study describes the behavioral and pharmacological characteristics of NT's effects on "spontaneous" behavior after intracerebroventricular (ICV) injection in mice.

METHOD

Animals

Male Swiss-Webster mice, weighing 30–35 g, were used. The animals were kept—5/cage—at 23°C and 60% relative humidity in a 12×12 hour light-dark cycle with food and water available ad lib.

Injection

The animals were injected intracerebroventricularly (ICV) under light ether anesthesia with a Hamilton microsyringe. The injection site was 1.0–1.2 mm lateral of the midline. Vehicle for the injection was artifical cerebrospinal fluid containing 128 mM NaCl, 10 mM $NaHCO_3$, 2.8 mM KCl, 1.25 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1 mM D-glucose, and HCL to pH 7.3. The injection volume was 20 μl. Control injections with 1% methylene blue had shown that this procedure results in a distribution of the injected solution throughout the ventricular system. Naloxone and D-amphetamine-sulfate were injected subcutaneously (SC) 20–25 minutes and 30–35 minutes, respectively, before the start of behavioral testing. Vehicle for the SC injections was 5 ml/kg saline.

Behavioral Testing

Spontaneous behavior was determined in a rectangular, transparent plastic cage, 16×27 cm. In most experiments, behavior was determined for 5 minutes, 5–10 minutes after the ICV injection. Immobility (the time spent motionless) and grooming were determined cumulatively in seconds per test session. Running was quantified as the number of crossings of two imaginary lines within the cage.

Experimental Design and Procedure

All experiments were performed in a completely randomized design. In the experiment on the time-courses of the peptide-effects, the animals were placed in the observation cage at repeated intervals during which immobility was de-

TABLE 1

BEHAVIORAL EFFECTS OF NEUROTENSIN AND XENOPSIN

N	Treatment			Immobility	Grooming	Running	Rearing
24	CSF			76.8 ± 15.2	23.9 ± 6.0	36.7 ± 5.2	9.5 ± 2.4
12	Neurotensin	25	ng	145.6 ± 24.1	6.8 ± 3.2	32.0 ± 6.2	4.3 ± 1.5
8		125	ng	218.1 ± 40.5†	0.5 ± 0.5*	19.3 ± 8.6	0.9 ± 0.8*
6		625	ng	261.3 ± 12.3†	0.0*	9.5 ± 3.4	0.5 ± 0.4*
10	Xenopsin	0.32	ng	115.6 ± 30.1	17.7 ± 7.0	50.7 ± 22.0	8.4 ± 3.5*
8		1.6	ng	226.1 ± 15.6†	25.6 ± 13.3	5.6 ± 2.6	0.5 ± 0.5*
8		8	ng	260.5 ± 7.6†	1.8 ± 1.2*	6.1 ± 1.2	0.1 ± 0.1*
6		40	ng	233.5 ± 49.4†	0.0*	13.5 ± 10.1	0.3 ± 0.4*
6		200	ng	252.8 ± 18.9†	0.0*	15.0 ± 7.5	0.0*
6		1000	ng	279.8 ± 9.6†	0.0*	4.0 ± 2.0	0.0*

*$p<0.05$; †$p<0.01$; ANOVA followed by Duncan's multiple-range test between individual drug and CSF groups.

N=number of mice per group. Data is mean ± S.E.M.

termined cumulatively for 9 minutes. The mice were kept in their home cages in between test sessions. In the experiment on tolerance formation, the animals were subjected to ICV pretreatment injections with CSF, 500 ng NT, or 5.0 μg NT twice daily for 4 days. Every other day they received a challenge-dose of 200 ng NT ICV, followed by behavioral testing 5–10 minutes later. During the pretreatment phase, the challenge-dose was administered 6–8 hours after the last pretreatment-injection. In this experiment, each mouse received a total of 13 ICV injections.

Peptides

Neurotensin (NT), NT(8–13) and [D-Phe^{11}]NT were obtained from Bachem and xenopsin and [D-Trp^{11}]NT from Peninsula Laboratories. The cyclic analogs L-363,847-01P01 and L-363-799-01J01 were generous gifts from Dr. D. F. Veber at Merck, Sharp and Dohme Research Laboratories in West Point, PA [5].

RESULTS

Structure-Activity Relationships

NT caused reductions of grooming behavior and, to a lesser extent, running and rearing activity, at doses of 125 or 625 ng, with a concomitant increase in immobility. The effect was of considerable magnitude, with periods of immobility typically exceeding 75% of the test session. Xenopsin induced qualitatively similar effects, but was about 70-fold more potent than NT (Table 1). Table 2 shows the relative potencies of some other NT-analogs. The effects of all these peptides were qualitatively similar.

Duration of Action

The structurally dissimilar peptides (see Fig. 1), NT, xenopsin and L-363,799 showed very similar time-courses, with duration of action of about 1 hour (Fig. 2). Because the control animals are highly active, having immobility scores close to zero, mild "sedative" effects insufficient to produce immobility would go undetected in this experimental paradigm, possibly underestimating the duration of action. In addition, the ability to observe the protracted action of these peptides is dependent upon returning the animals to their home cage between measurement sessions. If the animals are kept in the observation cage throughout the experiment, the CSF-injected mice also develop extended periods of immobility, thus rendering the determination of the peptide-effect impossible (data not shown).

TABLE 2

RELATIVE POTENCIES OF SOME NEUROTENSIN-ANALOGS

Peptide	potency (NT=100)
neurotensin	100
xenopsin	7,000
[D-Trp^{11}]-NT	250
[D-Phe^{11}]-NT	0.9
NT(8–13)	1.0
L-363,799	45
L-363,847	45

Tolerance Formation

The degree of immobility induced by a challenge dose of NT did not change significantly over time when animals were pretreated with control injections of CSF (Fig. 3). However, animals pretreated with either 500 ng or 5.0 μg NT twice daily showed significantly reduced immobility in response to the challenge-dose of 200 ng NT. This effect was maximal after the 8th pretreatment-injection. In the animals pretreated with the 5μg-dose of NT, tolerance-formation may have been partially masked by a residual effect of the pretreatment-injection 6–8 hours before the behavioral test, particularly after the 4th pretreatment-injection. The tolerance was reversible and dissipated completely within 2–4 days after cessation of the pretreatment-injections. All 33 mice survived this experiment.

Pyr-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu

Neurotensin (NT)

Pyr-Gly-Lys-Arg-Pro-Trp-Ile-Leu

Xenopsin

D-Lys-Pro-Lys-Lys-Pro-Tyr-Ile-Leu

L-363,847

D-Lys-Pro-Orn-Orn-Pro-Tyr-Ile-Leu

L-363,799

FIG. 1

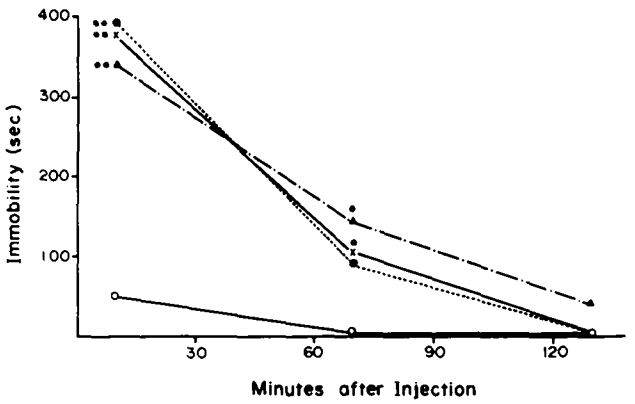

FIG. 2. The mice were tested repeatedly for 9 minutes each at different times after the injection. The durations of action for the three peptides are very similar in spite of their considerable structural differences. ○——○ CSF; ●— — —● NT, 300 ng; ▲—·—▲ xenopsin, 5 ng; x———x L-363, 799, 800 ng. *$p<0.05$, **$p<0.01$; ANOVA and Duncan's multiple-range test. Compared with the CSF-injected group.

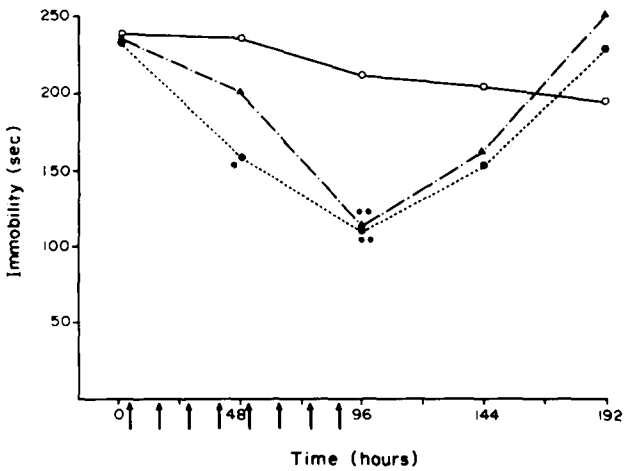

FIG. 3.The mice were pretreated twice daily with CSF, 500 ng NT, or 5.0 μg NT (arrows). Every other day they received a challenge-dose of 200 ng NT, and immobility was determined 5–10 minutes after the injection. ↑ Pretreatment injections; ○———○ animals pretreated with CSF; ●— — —● pretreated with NT, 500 ng; ▲—·—▲ pretreated with NT, 5.0 μg. *$p<0.05$: **$p<0.01$.

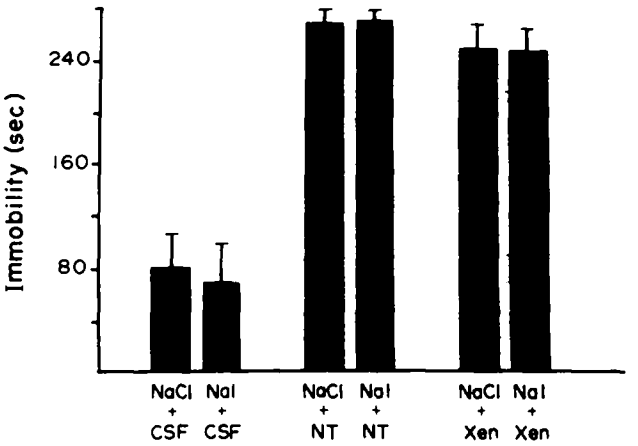

FIG. 4. The immobility induced by 250 ng NT or 4.0 ng xenopsin ICV was not attenuated by pretreatment with naloxone (5 mg/kg, SC 25–30 minutes before the test).

Interactions with Naloxone and Amphetamine

The immobility induced by NT or xenopsin was not modified by a dose of 5 mg/kg naloxone (Fig. 4). When doses of NT or xenopsin that reduced spontaneous motor activity by themselves were administered to animals pretreated with an amphetamine-dose that enhanced locomotion, the amphetamine-effect was only slightly modified at the peptide doses used in this experiment (Table 3). This result suggests that amphetamine-induced locomotion is not specifically antagonized by these peptides.

DISCUSSION

NT and related peptides cause a "sedative" effect of considerable magnitude after ICV injection in mice. This effect appears to be dose-dependent, and no toxic effects were evident after doses far exceeding those that cause maximal motor hypoactivity. This was particularly evident with the amphibian skin peptide xenopsin, which proved to be about 70-fold more potent than NT. Xenopsin, as well as NT, appears to be present in amphibian brains [2], but it is not known whether xenopsin or a closely related peptide is also present in mammalian brains.

In contrast to the results shown in Table 1, [D-Trp^{11}]NT and [D-Phe^{11}]NT have been reported to act opposite to NT in rats by enhancing locomotor activity after ICV injection [8,9]. NT itself induces hypoactivity after ICV injection in rats [8,9], but microinjection of NT into the ventral tegmental area has been reported to induce hyperactivity in rats [10,11]. Thus, distinct brain sites seem to exist at which NT

TABLE 3
INTERACTION BETWEEN NT, XENOPSIN, AND AMPHETAMINE

N	Treatment SC	ICV	Immobility	Grooming	Running	Rearing
11	NaCl	CSF	60.5 ± 11.9	29.5 ± 9.9	45.0 ± 8.6	6.5 ± 2.8
11	NaCl	NT	196.6 ± 22.3†	2.4 ± 1.2†	25.0 ± 10.4	0.9 ± 0.3
10	NaCl	Xen	191.0 ± 19.6†	3.7 ± 1.9*	24.7 ± 7.8	3.4 ± 1.0
12	Amph 2.5 mg/kg	CSF	52.7 ± 23.2	4.3 ± 1.6	90.8 ± 19.3	4.9 ± 2.9
10	Amph 5.0 mg/kg	CSF	3.2 ± 2.0	4.0 ± 1.2	148.7 ± 13.0	4.8 ± 0.9
11	Amph 5.0 mg/kg	NT	7.4 ± 3.9	2.6 ± 2.0	129.8 ± 18.3	12.3 ± 4.7
10	Amph 5.0 mg/kg	Xen	35.6 ± 14.7	4.4 ± 2.3	127.8 ± 23.6	14.5 ± 9.7

N=number of mice per group.
Doses: NT=50 ng; Xen=5 ng.
*$p<0.05$; †$p<0.01$ compared with NaCl-CSF. The 5.0 mg/kg amphetamine groups did not differ significantly from one another in any of the parameters measured (ANOVA and Duncan's multiple-range test).

can induce either hypo- or hyperactivity, and these sites may either be differentially accessible to NT depending on the method of administration, or they may be differentially sensitive to its analogs and metabolites in different species.

The behavioral potencies of the various NT-analogs correlate poorly with their reported potencies in a receptor binding assay using rat brain sections [16]. This suggests that factors other than receptor affinity, such as the metabolic inactivation of the peptides, are important determinants for their potency.

Our results on the duration of action of NT are in accordance with those reported by others for centrally-mediated NT-effects in mice [1,4] and rats [15]. The finding that two structurally dissimilar peptides show a very similar duration of action as NT itself may be due to a similar rate of metabolic inactivation or removal from the site of action. It is however, also compatible with the possibility that the duration of action is determined by the properties of the NT-sensitive structures rather than the removal or degradation of the peptide. Unfortunately, no information is available about the *in vivo* metabolism of NT after ICV injection. The observation that tolerance is more pronounced after 8 than after 4 pretreatment-injections (Fig. 3) suggests that short-term desensitization (tachyphylaxis) is not of major importance in determining the duration of action. This is in contrast to certain peripheral NT-effects [3].

Tolerance after repeated injections of NT seems to develop gradually within a few days and to dissipate completely after cessation of the pretreatment. Twice daily injections of 500 ng NT each were sufficient to induce tolerance, but the test-injections of 200 ng NT every other day did not induce appreciable tolerance-formation. Tolerance-formation to central nervous NT-effects has not been described so far, and it would be important to determine whether other central nervous NT-effects show this phenomenon as well. Differential tolerance-information to different NT-effects may change the spectrum of central nervous NT-actions considerably after repeated or chronic exposure, and tolerance-formation may be a valuable tool for the study of the mechanisms of central nervous NT-effects. The formation of tolerance suggests that the receptors mediating this effect are not normally exposed to very high NT-concentrations. However, the apparent lack of tachyphylaxis and the lack of significant tolerance-induction by the administration of 200 ng NT every other day is compatible with the view that the behavioral effects of exogenous NT mimick a physiological role of endogenous NT in the regulation of motor activity. Tolerance formation is also of importance for the development of centrally-acting analogs which has been pursued in at least one major pharmaceutical company [5].

The sites mediating the "sedative" effect of NT are unknown. NT-doses comparable to those inducing motor hypoactivity in mice are sufficient to cause peripheral effects after intravenous injection [3]. The peripheral effects are, however, very short-lasting due to tachyphylaxis and the rapid degradation of the peptide, whose plasma half-life is less than one minute [2]. Also, the structure-activity relationships for the peripheral effects are different from those for the induction of motor hypoactivity after ICV injection [9]. This suggests that the behavioral effects observed after ICV injection are centrally-mediated and not due to leakage of the peptide into the bloodstream.

The inability of naloxone to antagonize NT-induced hypoactivity suggests that this effect, like NT-induced antinociception [5, 6, 12], is not mediated by the release of endogenous opioids. The inability of NT and xenopsin to block amphetamine-induced locomotion suggests that the reduction of motor activity induced by these peptides is not related to antidopaminergic properties. NT had been proposed to possess the properties of an "endogenous neuroleptic" by virtue of an antidopaminergic action at the level of the nucleus accumbens [7, 11, 14, 15]. However, it also appears to stimulate the activity of the mesolimbic dopamine-system at the level of the ventral tegmental area [10,11], an effect which might, after ICV injections, counterbalance these antidopaminergic properties. Since "neuroleptic-like" properties of NT were generally described only after local microinjection of microgram doses in the nucleus accumbent of rats [7, 11, 14, 15], the doses used in our experiment may have been too low to demonstrate an effect.

The considerable magnitude of the "sedative" effect also suggests that it might contribute to some other reported actions of NT. Thus, a nonspecific reduction of behavioral responsiveness may contribute to NT's actions in analgesia-tests. This possiblity is supported by the observation that both NT-induced analgesia [6] and hypoactivity (Meisenberg

and Simmons, unpublished) are more pronounced in mice than in rats. In addition, a reduction of motor activity may possibly contribute to NT-induced potentiation of ethanol and barbituate anesthesia [13] and NT-induced hypothermia [12].

ACKNOWLEDGEMENT

The authors wish to thank Dr. D. F. Veber for his generous gifts of the cyclic neurotensin analogs. This work was supported by USPHS grants AM30970, HI28710 and NS20252.

REFERENCES

1. Bissette, G., D. Luttinger, G. A. Mason, D. E. Hernandez and P. T. Loosen. Neurotensin and thermoregulation. *Ann NY Acad Sci* **400**: 268–282, 1982.
2. Carraway, R. E. A critical analysis of three approaches to radioimmunoassay of peptides: Applications to the study of the neurotensin family. *Ann NY Acad Sci* **400**: 17–36, 1982.
3. Carraway, R. and S. E. Leeman. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *J Biol Chem* **248**: 6854–6861, 1973.
4. Clineschmidt, B. V., J. C. McGuffin and P. B. Bunting. Neurotensin: Antinocisponsive action in rodents. *Eur J Pharmacol* **54**: 129–139, 1979.
5. Clineschmidt, B. V., G. E. Martin and D. F. Veber. Antinocisponsive effects of neurotensin and neurotensin-related peptides. *Ann NY Acad Sci* **400**: 283–306, 1982.
6. Cowman, A. and D. E. Gmerek. Defining the antinocisponsive actions of neurotensin. *Ann NY Acad Sci* **400**: 438–439, 1982.
7. Ervin, G. N., L. S. Birkemo, C. B. Nemeroff and A. J. Prange Jr. Neurotensin blocks certain amphetamine-induced behaviors. *Nature* **291**: 73–76, 1981.
8. Jolicoeur, F. B., A. Barbeau, F. Rioux, R. Quirion and S. St. Pierre. Differential neurobehavioral effects of neurotensin and structural analogues. *Peptides* **2**: 171–175, 1981.
9. Jolicoeur, F. B. and A. Barbeau. Pharmacological evidence for a heterogeneity of receptors underlying various central and peripheral effects of neurotensin. *Ann NY Acad Sci* **400**: 440–441, 1982.
10. Kalivas, P. W., C. B. Nemeroff and A. J. Prange, Jr. Increase in spontaneous motor activity following infusions of neurotensin into the ventral tegmental area. *Brain Res* **229**: 525–529, 1981.
11. Kalivas, P. W., C. B. Nemeroff and A. J. Prange, Jr. Neuroanatomical site-specific modulation of spontaneous motor activity by neurotensin. *Eur J Pharmacol* **78**: 471–474, 1982.
12. Kalivas, P. W., L. Jennes, C. B. Nemeroff and A. J. Prange, Jr. Neurotensin: Topographical distribution of brain sites involved in hypothermia and antinociception. *J Comp Neurol* **210**: 225–238, 1982.
13. Luttinger, D., G. D. Frye and G. Bissette. Effects of neurotensin on the actions of barbiturates and ethanol. *Ann NY Acad Sci* **400**: 259–267, 1982.
14. Nemeroff, C. B. Neurotensin. Perchance an endogenous neuroleptic? *Biol Psychiatry* **15**: 283–302, 1980.
15. Nemeroff, C. B., D. E. Hernandez, D. Luttinger, P. W. Kalivas and A. J. Prange, Jr. Interactions of neurotensin with brain dopamine systems. *Ann NY Acad Sci* **400**: 330–344, 1982.
16. Quirion, R., P. Gaudreau, S. St.-Pierre, F. Rioux and C. B. Pert. Autoradiographic distribution of [^{3}H] neurotensin receptors in rat brain: Visualization by tritium-sensitive film. *Peptides* **3**: 757–763, 1982.
17. Uhl, G. R. Distribution of neurotensin and its receptor in the central nervous system. *Ann NY Acad Sci* **400**: 132–149, 1982.
18. Young III, W. Scott and M. J. Kuhar. Neurotensin receptor localization by light microscopic autoradiography in rat brain. *Brain Res* **206**: 273–285, 1981.
19. Zennes, L., W. E. Stumpf and P. W. Kalivas. Neurotensin: Topographical distribution in rat brain by immunocytochemistry. *J Comp Neurol* **210**: 211–224, 1982.