

Antinociceptive, hypothermic, hypotensive, and reinforcing effects of a novel neurotensin receptor agonist, NT69L, in rhesus monkeys

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Received 20 July 2004; received in revised form 2 December 2004; accepted 3 December 2004

Available online 30 December 2004

Abstract

Neurotensin (NT) is a tridecapeptide found in the nervous system, as well as elsewhere in the body. It has anatomic and functional relationships to dopaminergic neurons in brain. NT has been implicated in the actions of antipsychotic drugs and psychostimulants, and animal studies suggest that neurotensin directly injected into brain has reinforcing effects. Previously, we showed that one of our brain-penetrating analogs of neurotensin, NT69L (*N*-methyl-L-Arg, L-Lys, L-Pro, L-*neo*-Trp, L-*tert*-Leu, L-Leu), has many pharmacological effects in rats including antinociception, hypothermia, and blockade of the hyperactivity caused by psychostimulants (cocaine, D-amphetamine, and nicotine). Since these studies in rats suggest that this compound may have clinical use in humans, we were interested to know what effects NT69L had in primates. NT69L caused a potent antinociceptive effect against capsaicin (0.1 mg)-induced allodynia in 46 °C water in rhesus monkeys, inducing 40% of the maximal possible effect at an intravenous dosage of 0.03 mg/kg; its hypotensive effects precluded evaluation of higher dosages. Core temperature measured by rectal probe was modestly reduced at 0.01 and 0.03 mg/kg. In an intravenous self-administration procedure, NT69L was without reinforcing effects at any dose, including those that caused other pharmacological effects, and did not alter cocaine-maintained behavior when administered as a pretreatment.

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Keywords: Neurotensin; Self-administration; Opioids; Antinociception; Rhesus monkeys

1. Introduction

Neurotensin (NT) is a tridecapeptide that was discovered more than 30 years ago (Carraway and Leeman, 1973), and since then, interest in this compound and its receptors as targets for potential therapeutics (Kinkead et al., 1999; McMahon et al., 2002) has been considerable. Preclinical pharmacological studies, most of which involve direct injection of NT into the brain, strongly suggest that an agonist of neurotensin receptors would be clinically useful for the treatment of neuropsychiatric conditions including psychosis, schizophrenia, Parkinson's disease, pain, and the

abuse of psychostimulants. In this regard, three molecularly-cloned subtypes of the NT receptor have been identified thus far, although the functions of these recognition sites are not yet fully elucidated (Vincent et al., 1999).

The 8–13 fragment of neurotensin [NT(8–13)] causes all the known pharmacological effects of the full-length peptide, NT(1–13), pyroGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH, and in some cases, the 6-mer is more active than the parent compound. NT69L is an analog of NT(8–13). The amino acid sequence of this analog is *N*-methyl-L-Arg, L-Lys, L-Pro, L-*neo*-Trp, L-*tert*-Leu, L-Leu, where L-*neo*-Trp is a novel regio-isomer of L-Trp (Fauq et al., 1998). In binding studies, NT69L has high affinity for human NTR1 (Cusack et al., 2000; Tyler-McMahon et al., 2000) and NTR2 (unpublished observations). This compound is systemically active in rats, where, after intraperitoneal injection, a range of pharmacological

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effects are observed. This cluster of effects includes a potent induction of hypothermia and antinociception, attenuation of catalepsy induced by the dopamine receptor antagonist haloperidol (Cusack et al., 2000), suppression of climbing behavior induced by the dopamine receptor agonist apomorphine (Cusack et al., 2000), prevention of rotational behavior induced by either D-amphetamine or apomorphine in rats with unilaterally lesioned nigro-striatal pathways (Boules et al., 2001a), attenuation of the locomotor stimulant effects of cocaine, D-amphetamine (Boules et al., 2001b), and nicotine, and blockade of both the initiation and expression of sensitization to nicotine (Fredrickson et al., 2003b).

The psychomotor stimulant and reinforcing effects of drugs, such as D-amphetamine and cocaine, are linked to specific dopamine (DA) systems in the brain. The mesocorticolimbic DA system, to which NT co-localizes (Studler et al., 1988), appears to be the specific anatomical site for the reinforcing effects of cocaine (Roberts et al., 1977; Roberts et al., 1980) and other drugs (McBride et al., 1999). NT has modulating effects on these neurons, and many studies have linked NT with the behavioral effects of CNS stimulants.

Importantly, NT directly injected into rodent brain can have opposite behavioral effects, depending upon where this peptide is injected. Specifically, NT directly injected into the ventral tegmental area (VTA), where cell bodies for DA neurons reside, has psychostimulant properties (Kalivas, 1994). The VTA is the origin of the DA system associated with mediating the reinforcing actions of drugs (reward behavior) and animals will self-infuse NT into this area (Glimcher et al., 1987). However, NT directly injected into a projection area for these neurons, the nucleus accumbens (NA), blocks the effects of psychostimulants, both D-amphetamine (Ervin et al., 1981) and cocaine (Robledo et al., 1993).

Since neurotensin agonists are currently being developed as potential therapeutics to treat a variety of neuropsychiatric disorders, we were interested to know what effects NT69L would have in primates. Here we report the first studies in primates of the brain-penetrating NT agonist NT69L. The effects of this compound on physiological variables (e.g., body temperature and blood pressure) and pain perception were as would have been predicted from the rat studies with this peptide. Unlike studies involving direct injection of neurotensin into brain, NT69L both lacked reinforcing effects in monkeys via the intravenous route, and did not alter the reinforcing effects of cocaine.

2. Methods

2.1. Subjects and apparatus—measurement of physiological variables

A single adult female rhesus monkey (*Macaca mulatta*) was studied for changes in blood pressure following several

intravenous doses of NT69L. Following training and adaptation to the restraint chair, chronic arterial and venous catheters were implanted as described in the self-administration section below. Instead of exiting the body, catheters were attached to plastic injection ports (DaVinci Biomedical, South Lancaster, MA) that were then sutured into subcutaneous pouches. Initiation of experimental conditions did not occur until the monkey had fully recovered from surgery (approximately 2 weeks).

Rectal temperature was measured in three adult male and two adult female chair-trained rhesus monkeys using standard thermometers. Before the initiation of the study, monkeys were habituated to the chairing procedure and insertion of the temperature probe over a period of several days. Temperature experiments were conducted in a temperature-controlled (72 ± 1 °F) room.

2.2. Subjects and apparatus—antinociception experiments

Three adult male rhesus monkeys were used in these studies. Animals were housed individually with free access to water and were fed approximately 25–30 biscuits (Purina Monkey Chow; Ralston Purina, St. Louis, MO) and fresh fruit daily. Individual weights did not significantly change throughout the duration of the study. All monkeys were previously trained in the warm water tail-withdrawal procedure and were drug-free for at least 1 month before the present experiments were initiated. Animals used in this study and in the self-administration experiments were all maintained in accordance with the University Committee on the Use and Care of Animals at the University of Michigan, and the Guide for the Care and Use of Laboratory Animals (7th ed.) by the Institute of Laboratory Animal Resources (Nat. Acad. Press, Washington DC, revised 1996).

2.3. Subjects and apparatus—self-administration experiments

Adult rhesus monkeys experienced with self-administration of alfentanil and saline, or with cocaine and saline, served as subjects. Animals were surgically prepared with indwelling silicone rubber catheters using 10.0 mg/kg im ketamine and 2.0 mg/kg im xylazine as anesthetics. Catheters were implanted in either a jugular (internal or external), femoral, or brachial vein as necessary. Catheters were passed subcutaneously to the mid-scapular region, exited the body, and continued through a hollow restraining arm to the outside rear of the cage. During these studies, each animal wore a Teflon mesh jacket (Lomir, Québec, Canada) connected to a flexible stainless steel spring arm attached to the rear of the cage. Animals were individually housed in 83.3×76.2×91.4 cm-deep stainless steel cages for the duration of these experiments. A side-mounted panel was present in each cage, equipped with a row of three stimulus lamps (red–green–red) across the top, and two response levers (one mounted under each red light.)

Animals were fed between 10 and 12 Purina monkey chows twice per day, and water was available ad libitum. Daily fresh fruit and other treats supplemented this diet. In accordance with IACUC requirements, environmental enrichment toys were also provided on a regular rotating basis.

2.4. Procedure—measurement of physiological variables

For blood pressure experiments, the subject was removed from the home cage and seated in a primate chair. Following habituation to the restraint chair (approximately 15 min), the arterial catheter was connected to a pressure transducer coupled to a Grass polygraph and data were recorded using Polyview software, Astro-Med (West Warrick, RI). Various doses of NT69L were injected through the previously-implanted subcutaneous intravenous access port and arterial blood pressure was continuously monitored and recorded every 60 s over a 1 h period.

During temperature experiments, monkeys were removed from home cages and seated in restraint chairs. Each experimental determination began with two pre-drug control observations to obtain baseline temperatures approximately 15 min after seating each monkey. Saline or one of two doses of NT69L was then injected (iv) and subsequent temperatures were determined at 5, 15, 30, 45, 60, and 90 min after injection. Experimental sessions were conducted once per week.

2.5. Procedure—antinociception experiments

Nociceptive responses to thermal stimuli were measured by a warm water tail-withdrawal procedure that has been described previously (Ko et al., 1998). Briefly, monkeys ($n=3$) were seated in restraint chairs and the lower part of the shaved tail, approximately 15 cm, was immersed into warm water maintained at temperatures of 42, 46, or 50 °C. Tail-withdrawal latencies, up to a maximum of 20 s, were measured by a technician who was blind to the experimental conditions. Each experimental session began with control determinations at three temperatures in a varying order. Subsequent tail-withdrawal latencies were determined at 5, 15, 30, 45, and 60 min after injection. After capsaicin was administered subcutaneously in the tail, it dose-dependently induced thermal nociception, manifested as a reduced tail-withdrawal latency, from a maximum value of 20 s to approximately 2–3 s, in 46 °C water at 15 min after injection. The 15-min time point was the time of peak effects of capsaicin because there was no temperature- and capsaicin dose-dependency at the 5-min time point and there was a lesser degree of capsaicin effects at the 30-min and remaining time points. In addition, 46 °C water was chosen because there was a lesser degree of thermal allodynia/hyperalgesia in 42 °C water (Ko et al., 1998). Based on these previous results, we choose 100 µg of capsaicin in 46 °C water as a

standard noxious stimulus to evoke thermal allodynia/hyperalgesia for the present study. NT69L was administered intravenously through a saphenous vein from either leg immediately after capsaicin injection. A single dosing procedure was used in all test sessions. Experimental sessions were conducted once per week.

2.6. Procedure—self-administration experiments: alfentanil baseline

Two 130-min experimental sessions were conducted each day: a morning session starting at 10:00 AM and an afternoon session starting at 4:00 PM. Each session presented the opportunity to self-administer 4 discrete doses of alfentanil (iv). This schedule is quite similar to that previously described by Winger et al. (1989, 1992) and engenders stable alfentanil responding across the dose range tested. Each component of this multiple schedule was followed by a 10-min blackout period during which all stimulus lights were turned off and responses had no programmed consequences. The onset of each component was signaled by the illumination of the red stimulus light. In the presence of this light, the 30th response on the lever beneath it (FR 30) resulted in operation of the infusion pump. During the infusion, the red stimulus light was extinguished and the center green light was illuminated. Each component of this multiple schedule allowed a maximum of 20 self-injections, or the lapse of 25 min. Infusions were followed by a 45 s inter-injection timeout period (TO 45 s) during which all lights were turned off and responses had no programmed consequences.

The components differed from each other in the duration of infusion pump operation. The pumps were calibrated to deliver 1 ml of solution over a 5 s interval, thus, by lengthening the infusion interval, a greater amount of solution was administered, thereby providing a higher dose per infusion. Throughout these experiments the pump durations were 0.5, 1.7, 5.0, and 16.7 s. These durations corresponded to alfentanil doses of 0.00003, 0.0001, 0.0003, and 0.001 mg/kg/inj. Similarly, these pump durations delivered NT69L doses of 0.00003, 0.0001, 0.0003, 0.001, 0.003, and 0.01 mg/kg/inj. During saline substitutions, saline was available at injection volumes of 0.1, 0.33, 1, and 3.3 ml. Four different orders of pump durations were used: an ascending order, a descending order, and two mixed orders. One of these orders was selected randomly before each alfentanil or saline session; however, all results are based upon data obtained under the ascending order schedule. All alfentanil doses were infused in a constant injection volume per dose as described above. The expanded dose range for NT69L was assessed by providing two distinct concentrations—one to test the lower four doses, and another to test the higher four doses—during different sessions. Thus, the two intermediate NT69L doses were administered at distinct injection volumes depending on which range (low or high) was

being tested. As we have previously observed with this schedule, infusion volume did not alter responding for a given dose (Fantegrossi et al., 2002).

Under baseline conditions, animals were maintained on alfentanil following the above outlined schedule requirements, with periodic saline substitution sessions interspersed every 3 or 4 sessions, often for two consecutive sessions. Substitutions of NT69L occurred two to three times per week during AM sessions, and no substitutions were made on weekends. All NT69L substitutions followed an ascending dose order within-drug, and at least 4 recovery sessions occurred between substitution trials. The reinforcing effects of each dose range of NT69L were assessed at least twice in each animal.

2.7. Procedure—self-administration experiments: cocaine baseline

Two 60-min experimental sessions were conducted each day: a morning session starting at 10:00 AM and an afternoon session starting at 4:00 PM. Each session presented the opportunity to self-administer a single dose of cocaine (iv). The onset of each session was signaled by the illumination of the red stimulus light. In the presence of this light, the 10th response on the lever beneath it (FR 10) resulted in operation of the infusion pump. During the infusion, the red stimulus light was extinguished and the center green light was illuminated. Infusions were followed by a 60 s inter-injection timeout period (TO 60 s) during which all lights were turned off and responses had no programmed consequences. These TO periods contributed to the overall session time of 60 min.

Under baseline conditions, animals were maintained on 0.01 mg/kg/inj cocaine, with periodic saline substitution sessions interspersed every 3 or 4 sessions, often for two consecutive sessions. Substitutions of NT69L occurred two to three times per week during AM sessions, and no substitutions were made on weekends. All NT69L substitutions followed an ascending dose order, and at least 4 recovery sessions occurred between substitution trials. The reinforcing effects of each dose of NT69L were assessed at least twice in each animal.

2.8. Procedure—effects of NT69L pretreatments on cocaine-maintained behavior

The same three monkeys used in the cocaine baseline NT69L self-administration experiments were also used to assess the effects of non-contingent NT69L pretreatments on cocaine-maintained behavior. Monkeys were maintained on a cocaine dose of 0.01 mg/kg/inj, and were infused intravenously 15 min before the start of each test session with one of four doses of NT69L or its vehicle (saline). All NT69L pretreatments followed an ascending dose order, and at least 4 recovery sessions (cocaine or saline, no pretreatment) occurred between trials. The effects of each dose of

NT69L on cocaine-maintained behavior were assessed at least twice in each animal.

2.9. Drugs

NT69L was synthesized as described previously (Cusack et al., 2000). Alfentanil, cocaine, and NT69L were dissolved in physiological saline. Capsaicin (Sigma, St. Louis, MO, US) was dissolved in a solution of Tween 80/ethanol/saline in a ratio of 1:1:8 and was injected subcutaneously into the terminal 1 to 4 cm of the tail with constant 0.1 ml volume.

3. Results

3.1. Physiological effects

Pre-drug temperature determinations varied by approximately 1 °F across test days. As such, data (Fig. 1) are presented as change from baseline instead of as absolute temperatures. Following a control injection of equivolume saline, rectal temperature fluctuated during the observation period up to approximately 0.4 °F higher than the pre-infusion baseline value. In contrast, both 0.01 and 0.03 mg/kg NT69L induced a modest hypothermia that was not significantly different from saline control values at any time point post-injection (two-way repeated measures ANOVA, $p > 0.05$). This effect may suggest a trend towards dose-dependence as temperatures appeared to be returning to baseline by 90 min post-injection for the 0.01 mg/kg NT69L condition, but not at the 0.03 mg/kg condition. It should be noted, however, that at no point did absolute temperatures deviate outside the normal temperature range (98.6–103.1 °F) for an adult rhesus monkey (American Association for Laboratory Animal Science 2001/2002 Reference Directory).

Fig. 2 presents mean arterial blood pressure data (averaged over 5 min increments) as change from baseline

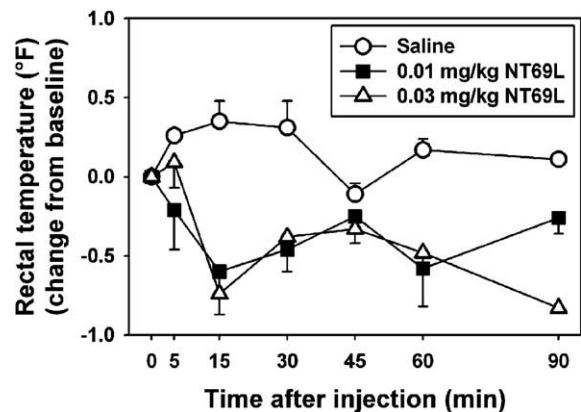


Fig. 1. Effects of systemic NT69L (iv) on rectal temperature. Each point represents mean temperature ($n=5$) and is presented as °F change from baseline.

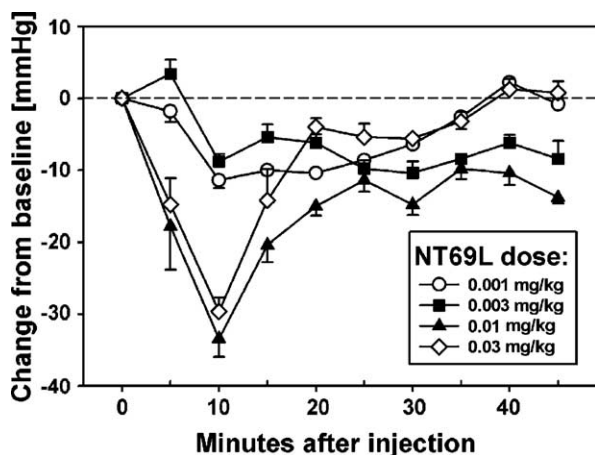


Fig. 2. Effects of systemic NT69L (iv) on mean arterial blood pressure. Each point represents mean arterial pressure (in 5 min intervals) in a single monkey, presented as change from baseline.

following several doses of NT69L (iv). The smaller two doses (0.001 and 0.003 mg/kg) each produced a 10 mm Hg drop in mean arterial pressure between 5–10 min after delivery, while the two larger doses (0.01 and 0.03 mg/kg) led to more dramatic blood pressure reductions of 30–35 mm Hg. Although the absolute pressor effects of NT69L were dose-dependent, the time-courses of these effects were similar across all four doses tested.

3.2. Antinociception

Monkeys used in this study displayed behavior consistent with standard tail-withdrawal responses, that is, they maintained their tails in 42 °C and 46 °C water for 20 s (maximal latency cutoff) and removed their tails rapidly from 50 °C water (within 1–3 s). Subcutaneous injection of 100 µg capsaicin into the tail evoked a nociceptive response, thermal allodynia/hyperalgesia, which was manifested as a reduced tail-withdrawal latency in 46 °C water. These responses were similar to those we have reported previously in different groups of monkeys (Ko et al., 1998).

Fig. 3 illustrates antinociceptive effects of NT69L against capsaicin-induced allodynia/hyperalgesia in 46 °C water. Intravenous administration of NT69L attenuated capsaicin-induced nociception in a dose-dependent manner [$F(3,8)=78.7; p<0.05$]. Post hoc comparisons indicated that both 0.01 and 0.03 mg/kg NT69L (0.01 and 0.03 mg/kg) significantly attenuated capsaicin-induced nociception ($p<0.05$).

3.3. Self-administration—alfentanil baseline

Six doses of NT69L were evaluated in four rhesus monkeys. Each animal was tested at least twice per dose. This compound engendered very low response rates across the dose range tested (Fig. 4), and was not self-

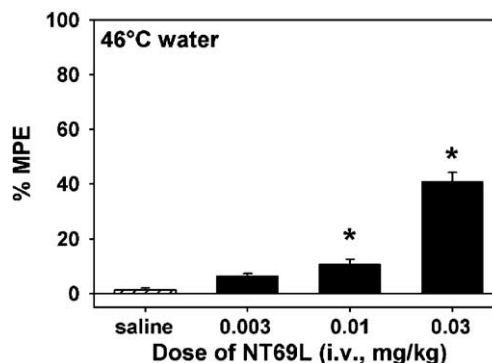


Fig. 3. Antinociceptive effects of systemic NT69L (iv, 0 min PT) against capsaicin (0.1 mg)–induced allodynia in 46 °C water ($n=3$). Each data point was obtained 15 min after capsaicin injection. MPE—maximum possible effect (20 s).

administered by any animal studied. Fig. 4 shows the mean dose–effect curve (\pm S.E.M.) for NT69L self-administration, aggregated across all four animals and plotted as percent of alfentanil-maintained responding. Rates of response for NT69L were low across the entire dose range. Previous studies conducted in this lab (ca. 1999) assessed the behavioral effects of this compound administered non-contingently via the intravenous route across a dose range between 0.001–0.03 mg/kg. The highest dose tested produced several behavioral and physiological effects, including pale face and increased defecation in all subjects (unpublished observations). These findings, coupled with the presently reported results for the physiological and antinociception measures, indicate that the dose range tested for self-administration should have been sufficiently wide to capture doses with behavioral effects. NT69L thus appears to lack reinforcing

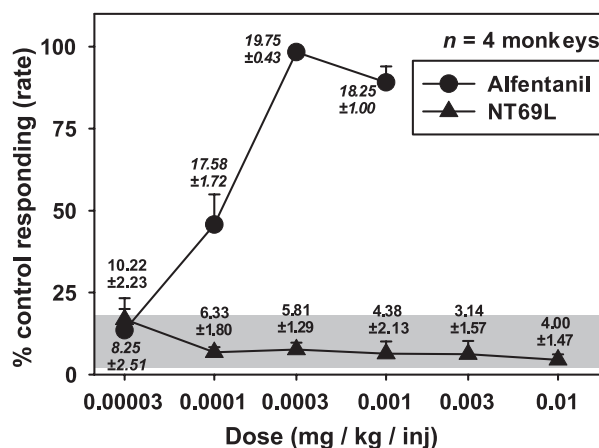


Fig. 4. Aggregated dose–response curves for NT69L in rhesus monkeys ($n=4$), presented as percent of alfentanil-maintained responding (response rate). Grey region represents mean (\pm S.E.M.) response rates engendered by contingent saline. Numbers adjacent to each point represent mean (\pm S.E.M.) number of injections earned per component (max=20). Normal text delineates injections of NT69L, italicized text applies to alfentanil. Fewer than 8 injections per component were obtained when contingent saline was available for self-administration.

effects in rhesus monkeys with a history of opioid self-administration.

3.4. Self-administration—cocaine baseline

Four doses of NT69L were evaluated in three rhesus monkeys. Each animal was tested at least twice per dose. As before, this compound engendered very low response rates across the dose range tested (Fig. 5), and was not self-administered by any animal studied. Fig. 5 presents the mean dose–effect curve (\pm S.E.M.) for NT69L self-administration, aggregated across all three animals and plotted as percent of cocaine-maintained responding. As mentioned above, this dose range tested for self-administration should have been sufficiently wide to capture doses with behavioral effects. NT69L thus also appears to lack reinforcing effects in rhesus monkeys with a history of cocaine self-administration.

3.5. Effects of NT69L pretreatments on cocaine-maintained behavior

Four doses of NT69L were evaluated against the reinforcing effects of 0.01 mg/kg/inj cocaine in three rhesus monkeys. Each animal was tested at least twice per dose. NT69L did not alter cocaine-maintained responding at any dose presently tested (Fig. 6). Fig. 6 presents the mean dose–effect curve (\pm S.E.M.) for NT69L pretreatments, aggregated across all three animals and plotted as percent of cocaine-maintained responding. At a dose of 0.3 mg/kg NT69L, several direct effects were observed in these subjects, including slouched posture and pale face, indicating that the doses tested for effects on cocaine-maintained behavior were likely active. NT69L thus appears not to alter the reinforcing

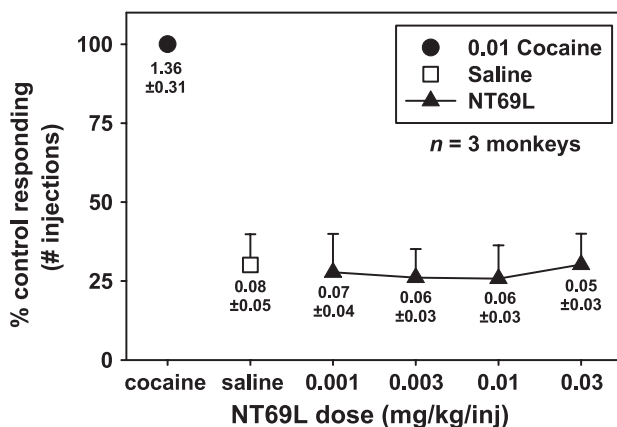


Fig. 5. Aggregated dose–response curves for NT69L in rhesus monkeys ($n=3$), presented as percent of cocaine-maintained responding (# of injections earned). Numbers adjacent to each point represent mean (\pm S.E.M.) response rates (responses per second) for cocaine, saline, or NT69L.

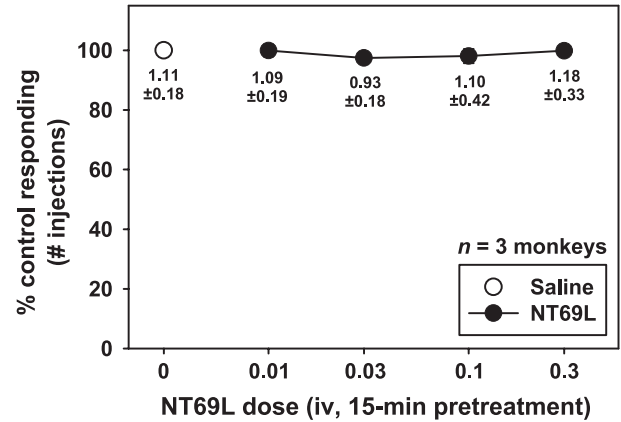


Fig. 6. Aggregated dose–response curves for the effects of NT69L pretreatments on cocaine-maintained responding in rhesus monkeys ($n=3$), presented as percent of cocaine-maintained responding following saline pretreatment (# of injections earned). Numbers adjacent to each point represent mean (\pm S.E.M.) response rates (responses per second) for cocaine following pretreatment with saline or various doses of NT69L.

effects of cocaine under the conditions presently described.

4. Discussion

This report presents the first description in primates of the effects of a neurotensin agonist, NT69L, that has previously been shown to be centrally active in rats following peripheral administration. In rhesus monkeys, NT69L produced a potent antinociceptive effect in the warm water tail-withdrawal assay, induced a profound state of hypotension and a modest hypothermia, but was without reinforcing effects in an intravenous self-administration procedure, and did not alter the reinforcing effects of a single cocaine unit dose. The effects of peripherally administered NT69L on temperature, blood pressure, and antinociception were similar to those previously reported following administration in rats. However, unlike rodent studies involving direct injection of neurotensin into the brain, NT69L lacked reinforcing effects in monkeys via the intravenous route.

There have been a few reports describing peripheral administration of NT in humans (Feurle et al., 1986; Pedersen et al., 1989). However, these studies have not documented any changes that could be attributed to central effects of NT. Although we have not yet shown the physical presence of NT69L in rodent brain after extracranial administration, several previous studies have indicated that this neurotensin receptor agonist has effects on various behavioral variables that could not occur in the absence of blood–brain-barrier penetration. Specifically, likely CNS effects of NT69L in rats after intraperitoneal injection include hypothermia, antinociception (Cusack et al., 2000; TylerMcMahon et al., 2000), and reduced food intake (Boules et al., 2000). Additionally, pretreatment of rats with

NT69L blocks the catalepsy caused by the dopamine receptor antagonist haloperidol (Cusack et al., 2000); blocks the climbing behavior caused by the dopamine receptor agonist apomorphine (Cusack et al., 2000); blocks the turning behavior caused by indirect (D-amphetamine) and direct (apomorphine) dopamine agonists in rats with super-sensitive dopamine receptors after unilateral lesioning of the nigro-striatal pathway (Boules et al., 2001a); and blocks the locomotor stimulatory effects of the psychostimulants cocaine (Boules et al., 2001b), D-amphetamine (Boules et al., 2001b), and nicotine (Fredrickson et al., 2003a). Interestingly, NT69L also blocks the behavioral sensitization to nicotine when it is administered 30 min before nicotine once per week for 5 weeks in rats (Fredrickson et al., 2003b). Based upon these data, it seems safe to conclude that NT69L does indeed pass the blood–brain-barrier following peripheral administration, at least in rodents.

In the present study, we showed that NT69L given intravenously to rhesus monkeys had potent effects on antinociception. Although iv NT69L did not produce full antinociception against capsaicin, the effect presently reported is comparable with the degree of antinociception produced by μ opioid receptor agonists in monkeys. Nonsteroidal anti-inflammatory drugs (NSAIDs) are not effective in the warm water tail-withdrawal model of capsaicin-induced allodynia (Ko et al., 1998), suggesting that the antinociceptive efficacy of NT69L may be somewhere between that of opioid analgesics and NSAIDs in primates.

With regard to the profound effect of NT69L on blood pressure, it should be noted that hypotensive effects have not previously been observed in rodents following central administration of NT. However, only with central administration of this peptide were hypothermia (Bissette et al., 1976) and antinociception (Nemeroff et al., 1979) observed. Indeed, NT was named based on the combined facts that the compound was isolated from brain and that it caused hypotension when it was peripherally administered to rats (Carraway and Leeman, 1973). Thus, the effect of NT69L on blood pressure in rhesus monkeys is likely a peripheral effect of this compound.

On the other hand, the changes observed on body temperature and antinociception are likely central effects of NT69L. However, the NT69L-induced hypothermia presently observed in rhesus monkeys was not as pronounced as that previously seen in rats (Cusack et al., 2000; Katz et al., 2001; Tyler-McMahon et al., 2000). Additionally, our present observation of increased defecation in monkeys after injection of NT69L is consistent with the presence of neurotensin receptors in the gut (Mazella et al., 1987) and the stimulatory effects of neurotensin on intestinal smooth muscle (Crocì et al., 1999; Stapelfeldt and Szurszewski, 1989).

Based on our previously mentioned studies in rats, we have been interested in testing NT69L in humans for possible use in the treatment of several neuropsychiatric

diseases, including abuse of psychostimulants. However, as mentioned in the Introduction, some of the rodent literature suggests that endogenous NT may actually contribute to the abuse potential of psychostimulants. In this regard, an early study in rats (Glimcher et al., 1984) dubbed NT the “reward peptide” based on the observation that rats would self-infuse it into the VTA (Glimcher et al., 1987). However, NT has different behavioral effects, depending upon which area of the brain receives the injection: psychostimulant-like effects in the VTA; blockade of behavioral effects of psychostimulants in the nucleus accumbens. Our neurotensin receptor agonist, NT69L, when injected outside the brain also blocks the behavioral effects of psychostimulants. In addition, NT69L has no properties suggestive of psychostimulant effects, either with single or with multiple injections. Clearly, this would suggest that NT69L's effects on NT receptors in the DA terminal regions of the nucleus accumbens predominate over its possible effects on NT receptors on DA cell bodies in the VTA.

These different effects of NT may be explained by the different biochemical effects of NT in the VTA and nucleus accumbens. NT applied directly into the VTA causes enhanced release of DA in the nucleus accumbens (Kalivas and Duffy, 1990), while NT injected into the nucleus accumbens terminal region of these neurons opposes the effects of dopamine at its autoreceptors (Legault et al., 2002). Thus, this increase of DA release caused by NT applied to the VTA may be the mechanism for its rewarding behavior, rather than a direct effect of neurotensin on reward circuitry. Direct injection of NT69L into the VTA would be a way to investigate the anatomical aspect of NT actions on rewarding behaviors.

Further evidence that endogenous NT may enhance the abuse potential of psychostimulants comes from studies investigating the role of NT in the initiation of sensitization to psychostimulants (Horger et al., 1994; Panayi et al., 2002; Rompre and Perron, 2000). In studies of this sort, sensitization is manifested as an enhanced locomotor response to the stimulant after repeated administrations. Sensitization is argued to underlie the craving associated with abuse of psychostimulants and may lead to relapse following a period of abstinence from these drugs. The studies suggesting that endogenous NT is involved in sensitization to psychostimulants have used the NT receptor antagonist SR48692 in combination with either cocaine (Horger et al., 1994) or D-amphetamine (Panayi et al., 2002; Rompre and Perron, 2000). In general, this antagonist has no acute effects on activity induced by either of the psychostimulants, however, SR48692 has been shown both to delay the development of sensitization to cocaine (Horger et al., 1994) and to block its development to D-amphetamine (Panayi et al., 2002; Rompre and Perron, 2000).

In this regard, we have previously shown that the NT receptor agonist NT69L blocks the acute locomotor effects of cocaine and D-amphetamine (Boules et al., 2001b) in rats. Furthermore, NT69L also blocks the acute locomotor effects

of nicotine (Fredrickson et al., 2003a), as well as the initiation and expression of sensitization to nicotine (Fredrickson et al., 2003a,b). Despite these disparate findings with NT agonists and antagonists, NT69L did not alter the reinforcing effects of cocaine in the monkeys presently described. The majority of data regarding NT agonists and antagonists has been collected in rodents, and the reinforcing effects of these drugs have not been previously assessed in primates. Therefore, we were interested to know whether NT69L would have any rewarding effects in rhesus monkeys. The data presented in this report indicate that it does not have rewarding effects when it is presented in discrete substitution trials to alfentanil- or cocaine-trained monkeys. Thus, if an NT receptor agonist were to find clinical utility in humans, it is likely that such a drug would have low abuse potential.

Acknowledgements

This work was funded by Grant MH 27692 from the National Institute of Mental Health, DA00254 from the National Institute of Drug Abuse, the Forrest C. Lattner Foundation, and the Mayo Foundation for Medical Education and Research. Expert technical help was provided by Erich Doser, Deborah Huntzinger, and Mark Johnson.

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