Central Pressor Action of Neurotensin in Conscious Rats

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SUMMARY The effects of neurotensin upon blood pressure in conscious rats were examined after intracerebroventricular (i.v.t.) or intravenous (i.v.) administration of this peptide. Whereas i.v. injected neurotensin (0.1-2.0 μg/kg) was depressor, i.v.t. injected neurotensin (1 μg and above) was pressor. Peripheral depressor responses could not be repeated in the same animal due to tachyphylaxis, but central pressor responses were repeatable without reduction in magnitude, showing that the two effects were separate entities. Thyrotropin-releasing hormone (TRH), which is reported to be a potent neurotensin antagonist, completely abolished the neurotensin depressor response, and attenuated the central pressor action. TRH did not alter the central pressor effect of another peptide, angiotensin II (All). The potent All receptor antagonist saralasin, while abolishing the central pressor effect of All, was completely without effect upon the neurotensin-induced pressor response. These results indicate that i.v.t. injected neurotensin and All stimulate a rise in blood pressure via different receptors. The alpha-adrenergic antagonists phentolamine, prazosin, or yohimbine (injected i.v.t.) were able to abolish or attenuate the pressor response due to i.v.t.-injected neurotensin, suggesting involvement of the sympathetic nervous system in this response. These results are discussed in relation to the central pressor actions of other neuropeptides. (Hypertension 4: 888-893, 1982)

KEY WORDS: blood pressure • neurotensin • angiotensin II • thyrotropin releasing hormone • alpha adrenergic antagonists

NEUROTENSIN is a basic tridecapeptide that was first isolated from bovine hypothalamus. This peptide is now known to be widespread in the central nervous system (CNS), and peripherally. Neurotensin has fulfilled many of the criteria for being recognized as a CNS neurotransmitter, and has many actions in the CNS. Such actions include its pronounced hypothermic effect after intracranial administration, and its marked enhancement of pentobarbital sleeping time. Peripherally, neurotensin modulates glucoregulatory systems, leading to hyperglycemia or hyperglucagonemia. In the blood it is a very potent hypotensive agent.

The findings that other neuroactive peptides such as angiotensin II (All), substance P, enkephalin, and bradykinin are pressor agents when administered centrally to conscious rats, and that bradykinin causes hypotension after peripheral administration, led us to investigate the effects of centrally administered neurotensin upon arterial blood pressure. These experiments were performed with the following aims: 1) to compare the blood pressure actions of neurotensin with those of other neuropeptides; 2) to determine whether the peripheral depressor effect of neurotensin contains a central component; and 3) to understand the mechanisms involved in the central vascular actions of neurotensin.

Methods

In all experiments, the animals used were male 250 to 300 g Sprague-Dawley rats. Animals were anesthetized with chloral hydrate (0.6 g/kg, i.p.), and a single stainless steel cannula was stereotaxically implanted into the right lateral cerebroventricle. After a recovery period of 5 to 7 days, the rats were anesthetized with ether, and both the right femoral artery and vein were catheterized with Silastic tubing; the catheters were kept patent with heparinized (100 U/ml) 0.9% saline. Both catheters were tunneled under the skin, excised on the back, and held in place with dental cement. Rats were used 24 hours later after recovery from ether anesthesia, and were free moving during all blood pressure recordings and injections. Any animals that looked unhealthy, or that were aggressive or unmanageable after either operation, were not used. Blood pressure was recorded from a femoral artery via a transducer and a Polygraph pen recorder (Grass Instru-
ment Company, Quincy, Massachusetts), and mean arterial blood pressure was calculated arithmetically in all cases. A femoral vein was used for intravenous (i.v.) injections in volumes of 0.1 to 0.2 ml 0.9% saline. Neurtensin (Sigma Chemical Company, St. Louis, Missouri, or Bachem, Torrance, California) or All (Hypertensin, Ciba Pharmaceutical Company, Summit, New Jersey) were injected intracerebroventricularly (i.v.t.) in volumes of 1 to 3 μl. Phentolamine (Regitine, Ciba), prazosin (Minipress, Pfizer, Sandwich, Kent, England), yohimbine HCL (Sigma), saralasin (Calbiochem, San Diego, California), or thyrotropin releasing hormone (TRH) (gift from Dr. A. J. Dunn) were injected i.v.t. in volumes of 1 to 4 μl. All drugs were dissolved in 0.9% saline, with the exception of yohimbine and prazosin, which were dissolved in 0.9% saline plus a drop of 0.01 N HCL, and buffered to pH 7.0 with NaOH. Rats were given a maximum of three i.v.t. injections of neurotensin. Results (mm Hg) were expressed as means ± SEM, using Student's unpaired t test.

Results
Effects of Intraventricular and Intravenous Injections of Neurotensin on Arterial Blood Pressure
Neurotensin, in doses ranging from 1 to 30 μg, caused a dose-related increase in arterial blood pressure after i.v.t. administration (fig. 1). The threshold dose was approximately 1 μg, and the response reached a maximum at 20 to 30 μg. The latency of the response was 20 to 30 seconds, as judged by the time when there was a significant difference from control mean arterial blood pressure, and the duration was 5 to 8 minutes. Repeat responses could be obtained in the same animal, although at least 30 minutes to 2 hours were needed between each injection to prevent tachyphylaxis and overlap with neurotensin's central hypothermic action. No measurements of core body temperature were made in the present study, but a similar dose of neurotensin given i.v.t. does not cause significant hypothermia until 10 to 15 minutes after injection.11 There is little overlap, therefore, between the pressor and hypothermic effects of i.v.t.-injected neurotensin, and both actions appear to be different entities.

Neurotensin injected i.v. in doses of 0.1 to 2.0 μg/kg caused a marked depressor effort (fig. 2), sometimes preceded by a slight hypertensive effect. This lasted for up to 5 minutes at 1 μg (fig. 2), 30 to 60 minutes at 5 μg, and could not be repeated in the same animals due to tachyphylaxis. Doses of neurotensin above 5 μg/kg were often lethal when given via this route. With all i.v. neurotensin injections there was no apparent hypothermia. The slight initial hypertensive effect was possibly an injection artifact since it did not occur in all animals.

Injections of 0.9% saline (1 to 3 μl i.v.t. or 0.1 to 0.2 mls i.v.) produced no changes in blood pressure.

To test whether the central and peripheral actions of neurotensin were completely independent, neurotensin...
Effects of Thyrotropin-Releasing Hormone and Saralasin upon the Blood Pressure Actions of Neurotensin

Thyrotropin-releasing hormone (TRH) (pGlu-His-Pro-NH$_2$) has been reported as a potent neurotensin antagonist, e.g., it blocks neurotensin-induced hypothermia and antinociception.\textsuperscript{11,12} The bar graphs in figure 4 show that TRH (2 or 5 µg, i.v.t.) given 1 minute prior to the tridecapeptide attenuated the rise in blood pressure due to neurotensin (20 µg), but did not alter basal blood pressure in the doses used here. The block was not complete at 5 µg TRH, but larger doses of TRH were not used since they cause a rise in blood pressure and also hyperactivity.

To examine whether neurotensin was acting centrally at the same receptor as the potent pressor agent All, the neurotensin pressor response was tested in the presence of the All antagonist saralasin, given 5 to 10 µg i.v.t. Saralasin did not alter the pressor effect of neurotensin in either amplitude or duration, and had no pressor activity of its own. Conversely, 1 µg saralasin given i.v.t. abolished the pressor activity of 100 µg All injected i.v.t., but 5 µg TRH (i.v.t.) did not alter this All response. Results of these experiments are summarized as bar graphs in figure 5.

TRH given i.v. at 2 to 5 µg/kg completely abolished the fall in arterial blood pressure caused by 1 µg/kg neurotensin given i.v. (fig. 6) while not altering basal blood pressure. However, TRH at 5 µg/kg i.v. did not alter the pressor effect of 20 µg neurotensin given i.v.t. (21 ± 2.4 mm Hg, n = 6, compared with 23.4 ± 2.5 mm Hg, n = 15 in control).

Effects of α-Adrenergic Antagonists upon the Pressor Effect of Intraventricularly Injected Neurotensin

The central pressor actions of All and other peptides are thought to be mediated by α-adrenergic receptors,\textsuperscript{13,14} so we tested whether the central pressor effect could be inhibited by prior i.v.t. administration of α-adrenergic antagonists.

Phentolamine, which is active at both α$_1$ and α$_2$ receptors, inhibited the neurotensin response in a dose of 10 µg (fig. 7), similar to its action upon the central pressor effect of angiotensin II.\textsuperscript{13} A dose of 1 µg phentolamine caused approximately a 30% block of the pressor effect due to 20 µg neurotensin, i.e., the rise in arterial blood pressure after neurotensin plus phentolamine was 15 ± 1.9 mm Hg (n = 6), compared with 24.2 ± 2.5 mm Hg (n = 17) in control animals. Yohimbine, which is more selective for α$_2$ than α$_1$ receptors, attenuated the neurotensin pressor response at a dose of 10 µg (fig. 7). At a larger dose, yohimbine was toxic to the animal. Prazosin, a selective α$_1$ antagonist, was more potent than either phentolamine or yohimbine since at 1 µg it abolished the neurotensin pressor effect.
Discussion

In this study, the blood pressure actions of a naturally occurring peptide, neurotensin, have been examined. While confirming the fact that i.v.-injected neurotensin is a potent depressor agent, the novel finding is reported that i.v.t.-administered neurotensin causes a rise in blood pressure in conscious rats. The central pressor effect of a single dose of this peptide lasted 5 to 8 minutes, and the threshold dose for this response (1 μg) was 10 to 20 times greater than that needed for the depressor effect of i.v.-injected neurotensin. Comparisons of the relative potencies of i.v.- and i.v.t.-applied neurotensin cannot be made, however, due to probable differential metabolism of this peptide in the brain and periphery.

The central and peripheral blood pressure effects of neurotensin appear to be different entities, controlled by different populations of receptors. Evidence for this is twofold. First, it was still possible to obtain the central pressor effect of neurotensin in animals that had undergone tachyphylaxis to i.v. peptide. Second, the central pressor response to neurotensin was unaltered in rats where the i.v. depressor effect had been extinguished by TRH injection. The results demonstrate a tight compartmentalization between the brain and periphery with respect to neurotensin administration.

TRH, which antagonizes many of the brain effects of neurotensin,11,15,16 antagonized the central and peripheral vascular effects of this peptide, but did not alter mean arterial blood pressure itself. This is surprising since TRH is known to be pressor when given i.v. or intracisternally.17,18 Differences between these results and the present study may be due to dose (we have used smaller doses) or anesthesia. How TRH antagonizes neurotensin actions is not understood, but it does not inhibit neurotensin binding to its brain receptors,19 and so a direct receptor interaction may be excluded.

The central pressor and hypothermic actions of neurotensin appear to be different entities. No measurements of core body temperature have been made in this study, but other work has shown that there is no significant hypothermia in the first 5 to 8 minutes after central neurotensin injection.11 Maximum hypothermia is not reached until 30 to 45 minutes after central neurotensin injection. Since the central neurotensin pressor response was never longer than 8 minutes, there can be little overlap between this and the hypothermic action.

Experiments were also performed to compare the central pressor actions of neurotensin with those of
All. Central injection of All caused a rise in blood pressure, which was completely antagonized by saralasin but not affected by TRH. Conversely, the central pressor action of neurotensin was attenuated by TRH, but not altered by saralasin. These results indicate that neurotensin and All act at separate peptide receptors in the brain to stimulate an increase in arterial blood pressure.

Another laboratory has reported that central injections of neurotensin elicit hypotension in rats. There were some differences between these and the present studies. First, in the other studies neurotensin was injected i.v.t. in volumes of 10 to 20 μl/injection. In view of the fact that the cerebroventricular volume of a rat is only 150 to 200 μl, repeated injections of such large volumes may have had an adverse effect upon the integrity of the blood-brain barrier, leading to leakage of the neurotensin into the periphery where it is certainly hypotensive. The second difference is that most of the other studies were performed in pentobarbital anesthetized rats, while the present work was carried out in conscious unrestrained rats. It has been shown that peripheral blockade of α-adrenoceptors leads to a reversal of the blood pressure effects (from pressor to depressor) of another neuroactive peptide, substance P. Injections of substance P into the nucleus tractus solitarius of anesthetized rats resulted in hypotension, whereas central injections of the peptide in conscious rats are normally pressor. In our experiments with conscious animals, neurotensin always gave a pressor response, whereas, in the studies where neurotensin was depressor, animals were pentobarbital anesthetized and so sympathetic activity was reduced. In the anesthetized rats, it is possible that neurotensin does not appear pressor since the activity of the sympathetic nervous system is so reduced.

The present results show the importance of the sympathetic nervous system in the central pressor action of neurotensin. The potency of α-adrenergic antagonists in blocking this response was prazosin > phentolamine > yohimbine; these results imply that α-receptors are more important in the response, since prazosin is a selective α-receptor antagonist. Yohimbine is more selective for α-adrenergic receptors, but does have α-blocking activity. This may explain the effects of yohimbine in the present study, but involvement of α-receptors in the central pressor action of neurotensin cannot be ruled out.

In summary, we hypothesize that centrally injected neurotensin acts at a central receptor, which can then influence sympathetic (noradrenergic) neurons and cause a pressor effect. From the present work, there is no indication as to which neurotensin receptors are influenced, since all central peptide injections were into the brain ventricles. There is evidence that neurotensin interacts with brain catecholamine-rich vascular control centers, e.g., neurotensin fibers innervate the locus ceruleus, and i.v.t. injections of neurotensin cause increased turnover of norepinephrine in brain stem. Neurotensin and dopamine are also known to interact in the brain.

Similar studies on the pressor effects of All after i.v.t. injection have led to the hypothesis that it is involved in hypertension, especially in the spontaneously hypertensive model. Comparison of the doses of All and neurotensin shows that neurotensin is 1000 times less potent than All in causing a rise in blood pressure. Since this study shows that both peptides act on different receptors, however, it is possible that neurotensin plays an independent role in blood pressure control in as yet undefined conditions.

References
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