POSSIBLE MECHANISMS OF THE TRIPHASIC EFFECTS
OF NEUROTENSIN ON THE RAT BLOOD PRESSURE

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Accepted September 2, 1981

Abstract—Neurotensin administered intravenously in a dose of 1 nmole/kg
produced triphasic blood pressure responses in anesthetized rats: the
first depressor, second pressor and third depressor responses. The first
depressor response was significantly suppressed by treatment of animals with
a mixture of diphenhydramine and metiamide or chronic administration of
compound 48/80, but was not modified by treatment with atropine, phentol-
amine, yohimbine, propranolol, sulpiride and adrenalectomy. The second
pressor response was abolished by phentolamine, yohimbine and adren-
alectomy. The second phase response was also markedly reduced by
diphenhydramine in reserpinized rats and chronic administration of com-
 pound 48/80. The third depressor response was blocked by treatment of
animals with diphenhydramine or chronic administration of compound
48/80. These results suggest that neurotensin may produce an immediate
depressor response (the first phase) partly through a histamine-mediated
process, and the second pressor response is produced by catecholamines
released from the adrenal medulla through a histamine-mediated process.
The third depressor response appears to be mediated mainly by histamine.
The participation of mast cells as an origin of histamine which mediates
these processes is suggested.

Neurotensin (NT), a tridecapeptide, found
in the central nervous system, gastrointestinal
tract and circulating blood in mammals, has
been reported to exert potent cardiovascular
actions including production of hypotension.
Carraway and Leeman (1, 2) first demon-
strated an immediate decrease in blood
pressure after intravenous administration of
NT, and Rosell et al. (3) and Quirion et al.
(4, 5) also have refered to the NT-induced
depressor response. These investigations,
however, have been limited to a rapid and
transient fall of blood pressure induced by
NT. Our present work demonstrated that NT
produced a triphasic change in the rat blood
pressure, and experiments were undertaken
to elucidate pharmacologically possible
mechanisms for the triphasic effect of NT.

MATERIALS AND METHODS

Male Wistar rats weighing between 250
and 350 g were anesthetized with pen-
tobarbital sodium (50 mg/kg i.p.), and the
unilateral carotid artery and femoral vein
were cannulated. The catheters inserted into
the artery and vein were used for systemic
blood pressure recording and drug injections, respectively. The blood pressure was measured by a pressure transducer connected to a polygraph (Nihon Kohden, RM-50).

NT was synthesized and purified by a conventional method, as described previously (6, 7). The following drugs were used: acetylcholine chloride (Daiichi Seiyaku), atropine sulfate (Tanabe), compound 48/80 (Sigma), diphenhydramine HCl (Kowa Kogyo), isoproterenol HCl (Nihon Chemicals), methysergide bimaleate (Sandoz), metiamide (kindly provided by Dr. H. Azuma), noradrenaline HCl (Sankyo), phentolamine HCl (Ciba-Geigy), reserpine (Daiichi Seiyaku), sulpiride (Fujisawa) and yohimbine HCl (Sigma). Metiamide was dissolved in a small volume of 0.1 N HCl, neutralized by addition of 0.1 N NaOH, and made up to volume with saline. All other drugs were dissolved or diluted in saline. The animal was examined to determine responsiveness to intravenous (i.v.) injection of acetylcholine (2.5 μg/kg), noradrenaline (4.0 μg/kg) and isoproterenol (0.2 μg/kg). Then the animal was treated with appropriate amounts of the following antagonists: 2 mg/kg of atropine, 5 mg/kg of phentolamine, 0.3 mg/kg of propranolol, 2 mg/kg of sulpiride, 4 mg/kg of diphenhydramine or 4 mg/kg of metiamide administered i.v., and 3 mg/kg of yohimbine or 1 mg/kg of methysergide given intraperitoneally (i.p.). One nmole/kg (1.67 μg/kg) of NT was injected i.v. 15 or 30 min after i.v. or i.p. administration of the antagonists, respectively. Two mg/kg of reserpine were injected i.p. 16 hr before experiments. Bilateral adrenalectomy was performed 3 days before experiments. Compound 48/80 was administered i.p. once or twice a day for 8 days, with increasing doses according to the method of Feldberg and Talesnik (8).

Briefly, the schedule of injections was as follows (in μg/animal): 1st day, 150 and 200; 2nd day, 200; 3rd day, 200 and 250; 4th day, 300; 5th day, 350 and 350; 6th day, 400; 7th day, 450; 8th day, 500. When two doses were given in a day, one was administered in the morning and the other in the afternoon. The animal was used for experiments 24 hr after the last injection of compound 48/80. Only one dose of NT was administered to each animal to avoid tachyphylaxis.

The statistical significance of differences was evaluated by the Student's t-test and the differences with P values of 0.05 or less were considered to be significant.

**RESULTS**

**Effects of NT on blood pressure:** Following i.v. administration of 1 nmole/kg of NT, the blood pressure changed in a triphasic pattern, i.e. the first depressor response (1st phase) with the maximum effect 20–30 sec after the injection, the second pressor response (2nd phase) with the maximum effect approximately 1 min after the injection, and the third depressor response (3rd phase) with the maximum effect approximately 10 min after the injection (Fig. 1A). The 3rd phase lasted for about 1 hr and tachyphylaxis of the responses was observed during this phase. The dose-response curve for change in blood pressure (Δ B.P.) induced by NT is shown in Fig. 1B.

The responses in the 1st and 3rd phases were expressed as Δ B.P. from the initial level at 20 sec and 10 min after the injection, respectively, and the response in the 2nd phase was expressed as Δ B.P. at 1 min by measuring the difference from the maximum depressor point of the 1st phase to the peak pressor point at 1 min. The responses in these three phases developed with increasing NT doses over 0.1 nmole/kg. The maximum responses were reached at 1, 2 and 1 nmole/kg of NT for the 1st, 2nd and 3rd phases,
Transient decrease in the heart rate was observed only in the IInd phase after NT in doses over 10 nmole/kg had been administered.

**Effects of antagonists on the IInd phase:**
The IInd pressor response was completely blocked by phentolamine. This phase was also inhibited significantly by yohimbine and slightly by diphenhydramine. No other drugs tested altered the IInd phase response (Table 1).

**Effects of antagonists on the IIIrd phase:**
The IIIrd depressor response was significantly inhibited by treatment of animals with all antagonists examined except methysergide. Diphenhydramine especially produced a complete inhibition of the IIIrd phase (Table 1).

**Reserpinization and adrenalectomy:** As the IInd pressor response to NT was completely blocked by phentolamine, this phase was considered to be mediated by catecholamines released from the sympathetic nerve terminals innervating blood vessels and/or from the adrenal medulla, or direct stimulation of α-adrenergic receptors in the vascular system. In reserpinized animals, the initial blood pressure level was lowered to 82.0±2.7 mm Hg (n=4). When 1 nmole/kg of NT was administered, neither ISt nor IIIrd depressor response was observed. The IInd pressor response, however, was retained and rather augmented, as demonstrated in Fig. 2.

After adrenalectomy, the IInd pressor phase was markedly suppressed both in the normal and reserpinized animals. Diphenhydramine also brought about a distinguishable inhibition of the IInd pressor response in the reserpinized rats (Table 2 and Fig. 2).

**Pretreatment with compound 48/80:** The above-mentioned results suggested the participation of histamine in the action of NT on blood pressure. In the rats pretreated with compound 48/80, which is known to be a histamine releaser from mast cells, the ISt depressor response was significantly (P<0.05) reduced. The IInd pressor response was never found and the IIIrd depressor response was markedly suppressed (Table 2).
Table 1. Effects of antagonists on triphasic blood pressure responses induced by neurotensin (1 nmole/kg i.v.)

| Antagonists     | Doses (mg/kg) | Route | N | Initial level | Changes in blood pressure (JmmHg) | 1st phase | 2nd phase | 3rd phase |
|-----------------|---------------|-------|---|---------------|----------------------------------|----------|----------|----------|
| None            |               |       | 9 | 134.6± 4.3    |                                  | -43.0± 4.0 | +21.2±3.4 | -79.4± 5.3 |
| Atropine        | 2             | i.v.  | 5 | 103.0± 6.5    |                                  | -38.4± 4.8 | +12.0±3.2 | -23.8± 6.6*** |
| Phentolamine    | 5             | i.v.  | 5 | 108.6± 7.9    |                                  | -35.0± 4.6 | -3.6±1.1*** | -47.8± 7.4** |
| Yohimbine       | 3             | i.p.  | 4 | 95.5± 8.5     |                                  | -30.2± 3.6 | +7.2±2.6*  | -27.5± 4.8*** |
| Propranolol     | 0.3           | i.v.  | 4 | 121.7± 8.9    |                                  | -30.5±10.5 | +28.7±6.5 | -55.0± 3.5* |
| Sulpiride       | 2             | i.v.  | 4 | 110.0± 8.4    |                                  | -45.7± 9.5 | +16.7±5.6 | -18.7± 4.8*** |
| Methysergide    | 1             | i.p.  | 4 | 132.5±11.5    |                                  | -62.7± 5.6* | +40.5±9.8 | -59.7±15.9 |
| Diphenhydramine (D) | 4       | i.v.  | 5 | 113.0±10.9    |                                  | -33.4± 7.6 | +7.2±7.3  | -4.4± 1.9*** |
| Metiamide (M)   | 4             | i.v.  | 4 | 129.0± 5.8    |                                  | -64.0± 6.0* | +30.2±4.5 | -42.7±14.4* |
| (D) + (M)       | 4 + 4         | i.v.  | 4 | 106.0± 5.8    |                                  | -26.0± 4.0* | +19.0±4.4 | -3.5± 1.7*** |

Each figure represents the mean±standard error. N: Number of rats.
Each antagonist was administered i.v. or i.p. 15 or 30 min respectively before injection of neurotensin.
Significant difference * at P<0.05, ** at P<0.01 and *** at P<0.001, as compared with each control.
The lst depressor response to NT was not affected by treatment of animals with cholinergic, adrenergic and serotonergic antagonists tested. These results coincided with findings of Carraway and Leeman (1, 2). Recently, Quirion et al. (5) have reported that the acute and chronic administration of compound 48/80 completely abolishes the transient hypotensive effect of NT (0.4 nmole/kg). In the present study, repetitive administration of compound 48/80 for 8 days significantly depressed the lst depressor responses, however, the response did not completely disappear. The discrepancy may be due to the differences in rat strains and doses of NT administered. These data suggest that the lst depressor response is, at least partly, mediated by histamine, though the participation of other components besides histamine could not be excluded. The response in the lst phase was slightly augmented by methysergide, a serotonin antagonist, and metiamide, an H2-histamine antagonist, but the mechanism involved herein was not clear.

The llnd pressor response was completely blocked by phentolamine, an a-adrenergic antagonist. This phase was augmented in the reserpinized animals, but was significantly suppressed after adrenalectomy. These findings strongly suggest that the llnd phase was mainly mediated by catechol-

**DISCUSSION**

Table 2. Effects of pretreatment with reserpine, adrenalectomy and compound 48/80 on triphasic blood pressure responses induced by neurotensin (1 nmole/kg i.v.)

| Treatments           | N  | Initial level | Change in blood pressure (mmHg) |
|----------------------|----|---------------|---------------------------------|
|                      |    | (mmHg)        | lst phase          | IIId phase          | llnd phase          |
| None                 | 9  | 134.6± 4.3    | −43.0± 4.0          | +21.2± 3.4          | −79.4±5.3           |
| Reserpine (R)        | 4  | 82.0± 2.7     | 0***               | +36.1±1.7*          | −2.5±1.9***         |
| Adrenalectomy (A)    | 4  | 103.5±12.6    | −41.0±10.8          | + 5.2±1.6*          | −47.2±8.0***        |
| (A)+ (R)             | 4  | 42.6± 3.4     | −7.6± 4.0***       | + 1.3±0.5***       | −1.6±6.6***         |
| (R)+ diphenhydramine | 4  | 89.5± 3.6     | −8.5± 2.7***       | + 3.0±1.3**        | + 0.5±2.9***        |
| Compound 48/80       | 8  | 122.5± 3.7    | −22.5± 6.2*        | n.d.               | −7.0±3.3***         |

Each figure represents the mean±standard error. N: Number of rats. n.d.: not detectable. Reserpine (2 mg/kg i.p.) was injected 16 hr before experiments. Bilateral adrenalectomy was performed 3 days before experiments. Diphenhydramine (4 mg/kg i.v.) was administered 15 min before injection of neurotensin. Compound 48/80 was administered for 8 days according to the schedule shown in text. Significant difference *at P<0.05, ** at P<0.01 and *** at P<0.001, as compared with each control.
amines released from the adrenal medulla. It seems unlikely that release of noradrenaline from the peripheral adrenergic nerve terminals contributes to the IInd phase. The augmentation of this phase observed in reserpinized animals may indicate that catecholamines released from the adrenal medulla acted on supersensitized α-adrenergic receptors in the vascular system. The IInd pressor response was also blocked by diphenhydramine in reserpinized animals, which suggested that the release of catecholamines from the adrenal medulla might be caused by histamine.

The IIIrd depressor response was completely suppressed by diphenhydramine and compound 48/80. These results raised the possibility that this phase was mediated mainly by histamine released from mast cells. There are several pieces of evidence for the contribution of histamine in the effects of NT. The hyperglycemic response to systemically administered NT is partially depressed by diphenhydramine and promethazine, and the hyperglucagonemic response to NT is completely blocked by H₁-histamine antagonists (9). Diphenhydramine also inhibits the elevation of plasma prolactin and growth hormone levels induced by NT in rats (10). Specific and reversible binding of NT to rat mast cells has been reported by Lazarus et al. (11). Selbekk et al. (12) have demonstrated degranulation of human jejunal mast cells after the treatment with NT. Thus, our hypothesis on histamine mediation of the triphasic blood pressure response to systemically administered NT is in line with the findings reported by these investigators.

Rioux et al. (13) have reported that intracerebroventricular (i.c.v.) injection of NT produces a monophasic fall of blood pressure in the rats. However, the doses of NT which induced the hypotensive effect by i.c.v. injection were much higher (5.4-16.2 nmole/kg) than those by i.v. injection. Furthermore, the hypotensive effect of centrally administered NT was not altered by a chronic treatment of animals with compound 48/80. Therefore, the fall of blood pressure induced by i.v. administration of NT is thought to be the peripheral action, but not the central action.

Acknowledgements: We are grateful to Sandoz LTD and Dr. H. Azuma, Tokyo Medical and Dental University for the gifts of methysergide and metiamide, respectively. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Health and Welfare, from the Ministry of Education, Science and Culture, Japan, and from Japanese Medical Women's Association.

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