STUDY OF KININ-LIKE PROPERTIES OF A NEW PEPTIDE ISOLATED FROM BOVINE BRAIN

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A new undecapeptide NRP-11 (Neurotensin Resembling Peptide) has recently been isolated from an acid extract of bovine brain homogenate [2]. Among natural physiologically active peptides, those most closely resembling it in structure are neurotensin and kallidin:

1. pGlu—Leu—Tyr—Clu—Asn—Lys—Pro—Arg—Arg—Pro—Tyr—Ile—Leu
2. Tyr—Lys—Lys—Arg—Pro—Tyr—Ser—Lys—Arg—Thr—Ala
3. Tyr—Lys—Lys—Arg—Pro—Tyr—Ser
4. Lys—Arg—Pro—Pro—Gly—Phe—Ser—Pro—Phe—Arg

The structures of neurotensin (1), kallidin (4), NRP-11 (2), and P7 (3) are compared above.

This paper describes a comparative study of the biological activity (kinin-like properties) of NRP-11 and its P7 fragment, and also of neurotensin and kallidin. The myotropic activity of the compounds and their effect on histamine secretion were studied in experiments in vitro, and their effect on blood pressure (BP) in experiments in vivo.

METHODS

Myotropic activity of the substances was studied on the isolated ileum of guinea pigs weighing 450-500 g, by the method in [6]. The preparation was incubated in Tyrode solution at 37°C, with aeration and an attached load of 1 g. Contractions were recorded under isometric conditions on an RM-6000 polygraph (Nihon Kohden, Japan). The agonistic activity of the substances was studied within the concentration range of 0.1-100 nM. Parameters characterizing interaction of the substances with receptors were calculated from cumulative "concentration—effect" curves: α) internal activity, pD_2) an indicator of specific affinity [6].

This histamine-releasing activity of the substances was studied within a concentration range of 0.01-10 μM on isolated rat peritoneal mast cells by the method in [3]. The histamine released was determined fluorometrically [5]. Activity of the substance was assessed in terms of $EC_{50(30)}$ values — the concentration of the compounds at which release of 50(30)% of the total quantity of histamine in the experimental sample takes place. In a concentration of 100 μM of the peptides, their cytotoxic activity was determined by measuring release of the enzyme lactate dehydrogenase LDH) [3]. LDH activity was expressed as a percentage of the control, which was taken to be LDH activity released as a result of destruction of the cells with 0.05% Triton X-100.

The effect of the substances on BP of rats anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) was studied by injecting them into the femoral vein in doses of between 0.49 and 490 μg/kg. BP was recorded in

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TABLE 1. Myotropic Activity of Test Substances in Experiments on Isolated Guinea Pig Ileum (M ± m)

Substances	Myotropic activity	
	α	pD_2
Kallidin	1,00	7,22±0,17
Neurotensin	0,50±0,10	8,68±0,15
NRP-11	0	0
P7	0,34±0,067	5,86±0,20

TABLE 2. Effect of Substances on Histamine Secretion and LDH Activity in Experiments on Isolated Rat Peritoneal Mast Cells (M ± m)

Substances	Histamine secretion, EC_{50} (g/l)	LDH activity, %
Kallidin	$(2,54\pm0,63)\cdot10^{-6}$	—
Neurotensin	$(3,63\pm1,54)\cdot10^{-5}$	19±3
NRP-11	$(3,22\pm0,62)\cdot10^{-6}$	37±7
P7	$(9,08\pm2,42)\cdot10^{-7}$	29±5

the common carotid artery on a "Gemini" two-channel automatic writer, by means of a Bentley Trantic Physiological Pressure Transducer (Italy).

The NRP-11 and P7 used in the experiments were obtained by solid-phase synthesis; neurotensin was obtained from Protein Research Foundation (Japan) and kallidin from Reanal (Hungary). During statistical analysis of the results values of activity were determined as the arithmetic mean of six experiments ± the mean-square error of the arithmetic mean (σ).

RESULTS

Contractile activity is a common property both of kallidin and neurotensin, which most closely resemble NRP-11 in their structure, and also of virtually all peptides belonging to the neurotensin group, such as neuromedin N and xenopsin [1]. As Table 1 shows, the greatest "internal" activity in experiments on the isolated guinea pig ileum was possessed by kallidin, although its specific affinity for receptors is an order of magnitude less than that of neurotensin. NRP-11 has no myotropic activity, and activity of P7 averages 34.33% of that of kallidin; the affinity of P7 for receptors is three orders of magnitude lower than that of neurotensin. It can be tentatively suggested that the reason for this weak interaction with receptors of the smooth muscle of the guinea pig ileum in the case of P7 and the absence of such interaction in the case of NRP-11 is that, despite the presence of homology with neurotensin fragment 8—13, responsible for manifestation of its myotropic activity [4], the homologous region in peptides NRP-11 and P7 has a different conformation.

The results of the experiments in vitro on rat peritoneal mast cells (Table 2) indicate that NRP-11 and P7 have well-marked histamine-releasing activity, similar to that of kallidin, and twice as strong as the activity of neurotensin. The study of the effect of the substances on LDH activity showed that in a concentration of 100 μM NRP-11 and P7 can release LDH. This is evidence that, in high concentrations, the histamine-releasing activity of NRP-11, P7, and neurotensin is connected with irreversible destruction of the cell, whereas kallidin does not possess cytotoxicity.

In response to its intravenous injection neurotensin is known to induce a histamine-mediated fall of BP [1]. Despite the fact that the histamine-releasing activity of peptides NRP-11 and P7 is more than an order of magnitude higher than the activity of neurotensin, these peptides have virtually no effect on BP. For instance, NRP-11 within a dose range of 0.075-75 μg/kg, did not affect BP, injection of peptide P7 in doses of 0.49-49 μg/kg likewise had no effect, but in the maximal dose (490 μg/kg) it lowered BP on average by 17 mm Hg in the course of 1 min. Meanwhile, threshold doses for neurotensin and kallidin are 0.07 and 0.7 μg/kg, respectively; in a dose of 7 μg/kg neurotensin lowered BP by 50 mm Hg in the course of 1 h, whereas kallidin in the same dose lowered it by 40 mm Hg in the course of 2 min.

Thus the endogenous peptide NRP-11 and its P7 fragment, which have structural similarity with neurotensin and kallidin, also possess well-marked histamine-releasing activity, characteristic of kallidin and neurotensin. Meanwhile, the myotropic and hypotensive activity of P7 was much weaker than that of neurotensin, and NRP-11 had no such activity whatever. The results are evidence that with respect to peripheral activity, NRP-11 and P7 differ considerably from neurotensin and kallidin.

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