DE-GLYCINAMIDE^9-VASOPRESSIN AND DE-LYSINE^8, GLYCINAMIDE^9-VASOPRESSIN: SOME PHARMACOLOGICAL PROPERTIES

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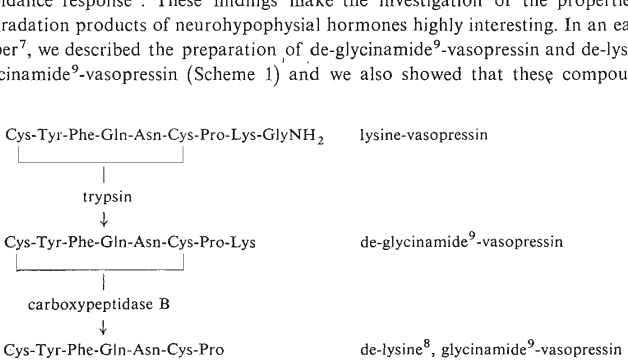
The uterotonic, pressor and antidiuretic activities of de-glycinamide^9-vasopressin and de-lysine^8, glycinamide^9-vasopressin were lower by two orders of magnitude than those of lysine-vasopressin.

Shortening the linear peptide chain of the neurohypophysial hormones oxytocin and vasopressin results in a striking decrease of the typical biological activities of the substances[1]. Products of the enzymic degradation affecting the linear peptide chain of neurohypophysial hormones were also inactive in typical biological assays[2-4]. However, some products of enzymic cleavage had unexpected biological effects. Degradation products of oxytocin, *i.e.* H-Pro-Leu-GlyNH_2 and the hexapeptide ring, may participate in the regulation of MSH liberation[5]. De-glycinamide^9-vasopressin was found to restore the ability of hypophysectomized rats to acquire a conditioned avoidance response[6]. These findings make the investigation of the properties of degradation products of neurohypophysial hormones highly interesting. In an earlier paper[7], we described the preparation of de-glycinamide^9-vasopressin and de-lysine^8, glycinamide^9-vasopressin (Scheme 1) and we also showed that these compounds



SCHEME 1

did not differ from lysine-vasopressin in their affinity to neurophysin. In this paper we present the basic biological properties of the compounds.

EXPERIMENTAL

Materials

Synthetic lysine-vasopressin and oxytocin were commercial preparations of Spofa, Prague. De-glycinamide[9]-vasopressin was prepared by tryptic cleavage of lysine-vasopressin[7]. The reaction mixture containing N-tosyl-L-phenylalanyl chloromethyl keton-treated-trypsin, glycinamide and de-glycinamide[9]-vasopressin was freeze-dried after the reaction had been stopped; the individual components were separated by high voltage paper electrophoresis. De-lysine[8], glycinamide[9]-vasopressin was obtained by the incubation of de-glycinamide[9]-vasopressin with carboxypeptidase B. The products were separated by high voltage paper electrophoresis.

Methods

Antidiuretic assay. The assay was performed with male rats anaesthetized with ethanol and hydrated with a water load of 8% of body weight[8]. The urine flow was measured by means of a drop divider; the number of drops was registered automatically. The potency of the compounds studied was estimated by comparing the threshold doses of lysine-vasopressin with those of its degradation products.

Uterotonic activity in vitro. The assay was performed with uterine strips[9] placed in Van Dyke — Hasting's solution (pH 7·4). The Ca^{2+} concentration was 0.5 mM. The solution was aerated with a mixture of 95% O_2 and 5% CO_2, and its temperature was maintained at 30°C. The isometric contractions of the uterus were registered electromagnetically[10], and the potency of the individual compounds was determined by the four-point test in comparison with oxytocin.

Pressor activity. The assay was performed with pithed male rats maintained by artificial respiration[11]. The comparison with lysine-vasopressin was made by the four-point test.

RESULTS AND DISCUSSION

The pressor, uterotonic and antidiuretic activities of the two compounds are presented in Table I. In all the assays, the analogues had less than 1% of the activity of the parent hormone, with the exception of the antidiuretic potency of de-glycinamide[9]-vasopressin. The consequences of the shortening of the linear peptide chain and of the presence of the free carboxyl group in the degradation products of lysine-vasopressin are comparable with changes in the activities of analogues or degradation products of the oxytocin series[1,3].

The possibility of preparing de-glycinamide[9]-vasopressin by treatment with trypsin became obvious after finding that lysine-vasopressin was inactivated by trypsin[13]. Further attention was paid to de-glycinamide[9]-vasopressin after it had been isolated from porcine hypophyses and after observation that it restores the ability of hypophysectomized rats to acquire a conditioned avoidance response[6]. De-glycinamide[9]-

TABLE I
Pharmacological Activities (IU/mg)

Compound	Antidiuretic	Uterotonic	Pressor
Lysine-vasopressin	250[a]	4[a]	280[a]
De-glycinamide[9]-vasopressin	3	<0·01	1·0
De-lysine[8], glycinamide[9]--vasopressin	0·29	<0·01	<0·1

[a] Ref.[12].

-vasopressin was prepared by tryptic cleavage of lysine-vasopressin[14] in order to study its effect on the consolidation of memory traces[14,15]. Under physiological conditions, de-glycinamide[9]-vasopressin can be formed in the hypothalamus by trypsin-like enzymic cleavage which may be followed by the release of the C-terminal lysine residue. Enzymic systems capable of releasing glycinamide and the terminal dipeptide (*i.e.* lysyl-glycinamide or arginyl-glycinamide) from vasopressins have been found in the hypothalamus[16]. It seems most probable that de-lysine[8], glycinamide[9]-vasopressin is present in the hypothalamus under physiological conditions. It might be interesting to investigate the role of the compound in the consolidation of the memory trace.

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