A VASODEPRESSOR PEPTIDE IN COHN FRACTION III-0 OF HUMAN PLASMA PROTEINS

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Previous reports (1,2) have described the ability of human plasma to cause constriction in an isolated perfused vein from the rabbit ear. The activity emerges as a single peak from a Sephadex G200 column when high molality buffer is used, in a fraction suggesting a molecular weight of approximately 100,000. The original plasma and the eluate containing venoconstrictor activity are also vasodilator in the intact vascular beds of the guinea pig heart and the dog hind limb and on several other preparations behave very like bradykinin. Similar activity is also found in several Cohn fractions of human plasma proteins, the richest source being fraction III-0 the major components of which are partly denatured β-lipoproteins. The activity from this source is however readily dialysable and this paper describes the partial isolation of an active peptide from it.

MATERIALS AND METHODS

Cohn fraction III-0 was obtained from Commonwealth Serum Laboratories, Parkville, Australia. This had been prepared by standard cold ethanol prescription but had not been dialysed before lyophilization.

The columns used were: Sephadex CM25 43 x 3 cm and 50 x 1 cm (Gradient column) and Sephadex G25 90 x 1.75 cm. Buffers were as described with the results. Counter-current distribution was carried out in a Gallenkamp counter-current apparatus (Model Ev810/820) with 1% acetic acid as the lower phase and sec-butanol as the upper.
High voltage electrophoresis was performed on a Savant apparatus with a voltage gradient of 50 V/cm and 0.041 M pyridine/3.3% acetic acid pH 3.5 as buffer.

Amino acid analysis after 20 hours acid hydrolysis employed a Beckman 120B auto-analyser.

The assay preparation was the isolated perfused vein of the rabbit ear. This has been described in detail elsewhere (3).

RESULTS

In preliminary studies lyophilized Cohn fraction III-0 was dissolved in 5% acetic acid and applied to a Sephadex G25 column. The majority of material absorbing at 280 nm eluted in the void volume. Activity emerged in two peaks, the smaller in the void volume and the other, representing some 80%, was retarded to a degree suggesting a molecular weight of approximately 1500. (Fig. 1).

Fig. 1 Passage of III-0 in 5% acetic acid through Sephadex G25 column 90 x 1.75 cm

- = Venoconstrictor activity
●●●●● = Absorbance (280 nM)