SCALING-UP OF THE PROCEDURE FOR THE PURIFICATION OF HUMAN URINARY KALLIKREIN URINARY KALLIKREIN

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The purification of human urinary kallikrein described by Hial et al. (Biochemistry 13, 4311, 1974) is being scaled up for the preparation of gramquantities of enzyme. In a first approach, 125 liters of male human urine were filtered, diluted to 250 1 with deionized water, adjusted to pH 7.0, and passed through a column of DEAE-Sephadex (13 x 48cm) with 4.84 1 of gel bed. The kallikrein was adsorbed onto the gel in this process. The column was washed with 200 1 ammonium acetate 0.10M, pH 7.0 at a flow rate of 0.48 1/hr, then with 4.5 1 of the same buffer containing 0.20M NaC1.
The active fractions had kinin-liberating activity over human kininogen, hydrolized carbobenzoxy-L-tyrosine p-nitrophenyl ester and had practically no hydrolytic activity toward tosyl-L-arginine methyl ester, as previously reported (Diniz et al., Biochem. Biophys. Res. Commun. 21, 448, 1965).
The pooled fractions were filtered through Sephadex G-25 and lyophilyzed. The active material obtained was then twice chromatographed on a 4.6 x 120 cm column of Sephadex G-150, whereby a large amount of pigment was removed.
The final lyophilyzed product was homogeneous upon acrylamide electrophoresis (7.5%) but was microheterogeneous on electrofocusing, as reported by Hial et al. (loc. cit.).
The initial 125 1 of human urine yielded 1.58g of a lyophilyzed powder containing 0,51 g protein, with a specific activity similar to that previously reported.
The process is now being dimensioned for the desalting of 1250 1 of human urine, with the use of Sephadex columns of the KS-370 type.
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INHIBITORS OF KININ-FORMING ENZYMES

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The inhibitory effect of esters of aminocaproic acid, guanidinocaproic acid and trans-4-aminomethylcyclohexane carboxylic acid, and other inhibitors such as

trasylol and leupeptin on trypsin-like proteolytic enzymes such as kallikrein, Clr, tissue activator, pancreatin, plasmin and thrombin were studied. The derivatives of phenyl ester of these acids showed potent inhibitory effect on the trypsin-like enzymes. The effect of these esters on inflammation induced by croton oil and on experimental pancreatitis in animals will be reported.
Cl esterase activity and the conversion of Cl to Cl esterase were competitively inhibited by aromatic esters of guanidinocaproic acid. However, p-aminophenyl- phenylpropionate, chymotrypsin inhibitor, had no effect on Cl esterase activity. Such synthetic inhibitors as described above reversibly inhibited trypsin, while derivatives of p-guanidinobenzoic acid inhibited trypsin irreversibly (E. Shaw; 1969).

THE ACTION OF U.V. RAYS ON EXPERIMENTAL CUTANEOUS INFLAMMATION INDUCED BY CANTHARIDIN

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In previous research, we observed that histamine, administered intravenously in microdoses (40 μg) in man, activates a self-extinguishing localized cutaneous inflammation.
This experimental inflammation in man is represented by a cantharidin blister on the lateral surface of the forearm.
Histamine intravenous administration is followed by: 1), an increase in the turgescence of the blister; 2), a sensation of burning pain; 3), an extension of the primary hyperalgesia (erythralgia) and of the secondary hyperalgesia; and 4), an increase of kininogen in the blister fluid.
We suggest that histamine acts specifically on the altered microvessels of the local inflammation, thus inducing the passage of the kininogen from the vascular to the interstitial space. Here, there are conditions apt to activate the polypeptide.
We carried out another experiment with the same biological meaning. In these subjects, instead of injecting histamine intravenously, we have induced an erythema with U.V. rays (2900-3200 A°) on a cutaneous area (dorsal section of the thorax) far from the experimental blister. After a mean of 8-10 hours from U.V. irradiation, the above-mentioned signs of reactivation of the cantharidin blister appeared, including the burning pain.
Since many active substances of the inflammation are released after U.V. erythema, and among them histamine, we advance the hypothesis that the blister reactivation is mainly due to histamine.
This experiment represents a model of a non-specific reactivation of the experimental inflammation in man.