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DEGRADATION OF VASOACTIVE INTESTINAL POLYPEPTIDE BY TISSUE HOMOGENATES

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SUMMARY: Extracts of liver, kidney and brain contain an enzyme that is highly specific for degradation of vasoactive intestinal polypeptide (VIP). The Michaelis constants (K_m's) appear to be nearly identical in all three tissues, averaging about 10^{-5} mol/liter. The V_{max} for kidney and liver are about the same but that for cerebral cortex is about two-fold lower. Since the relative V_{max} in the three organs differ for insulin and VIP, it is concluded that it is unlikely that the same enzyme is responsible for the degradation of both peptides.

INTRODUCTION

The degradation of radioiodine labeled insulin by liver and other tissues has been extensively studied at many laboratories since the first reports by Mirsky and associates (1). Similar degradatory systems have been described for other peptide hormones such as glucagon, growth hormone and ACTH (2). It has been reported that the rate of degradation of insulin by brain is considerably less than those of liver or kidney (3). The question to be addressed in this report is whether a peptide such as vasoactive intestinal polypeptide (VIP), whose concentrations in brain and in gastrointestinal mucosa are comparable (4), is more readily degraded by peripheral tissues such as kidney and liver or by brain and whether the Michaelis constant, K_m, of the degrading enzyme(s) varies in different regions.

MATERIAL AND METHODS

Preparation of Tissue Extracts: Extracts of rabbit liver, kidney and cerebral cortex were prepared as follows: A female Australian white rabbit was killed by exsanguination under light ether anesthesia. Immediately after death the liver, kidney and brain were removed, and the cortex was peeled from the cerebral hemispheres. The tissues were cut into multiple pieces, frozen on dry ice and stored at -70°C until used. For each study the frozen tissues were extracted at 4°C with a Teflon tissue grinder in 25 or 29 volumes of standard diluent consisting of 0.25M phosphate buffer (pH 7.4) fortified with 0.5 g/100

Abbreviations used are: VIP, vasoactive intestinal polypeptide; ACTH, adrenocorticotropic hormone.

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ml human serum albumin. The extracts were centrifuged at 3000 RPM for 15 minutes at 4°C. The supernatants were decanted and stored at 4°C for no more than 2 hours before use.

Substrate, Inhibitors and Separation Methods: Porcine vasoactive intestinal polypeptide (VIP) (gift of Dr. V. Mutt, Karolinska Institute, Stockholm) was iodinated by our minor modification of the chloramine-T technique (5) to a specific activity of ∿100 μCi/μg. Purification was by adsorption to and acid elution from QUSO G32 (6). The inhibition of degradation of ^{125}I-VIP by VIP, glucagon and insulin in the presence of the various tissue extracts was studied. Three methods to separate degraded from intact ^{125}I-VIP were evaluated: 1) During chromatoelectrophoresis (7), intact VIP binds to paper at the site of application; degraded fragments migrate anodally with serum proteins and radioiodide more anodally than the serum proteins. 2) When intact ^{125}I-VIP in 0.2 ml standard diluent is added to 2 ml of cold 10% trichloroacetic acid, about 86% of the peptide can be recovered in the precipitate. Degraded fragments do not precipitate. 3) When 50 mg talc is added to ^{125}I-VIP in 2.2 ml standard diluent and the mixture is vortexed to suspend the talc, about 93% of the intact peptide is adsorbed to the talc; fragments do not adsorb. For most studies of the kinetics of enzymatic degradation of ^{125}I-VIP the talc method was employed. Separation of talc-bound and unbound radioactivity was effected by centrifugation for 15 minutes at 5°C at 3000 RPM in an IEC-PR-6000 centrifuge. No correction was made for incomplete adsorption of intact ^{125}I-VIP to talc.

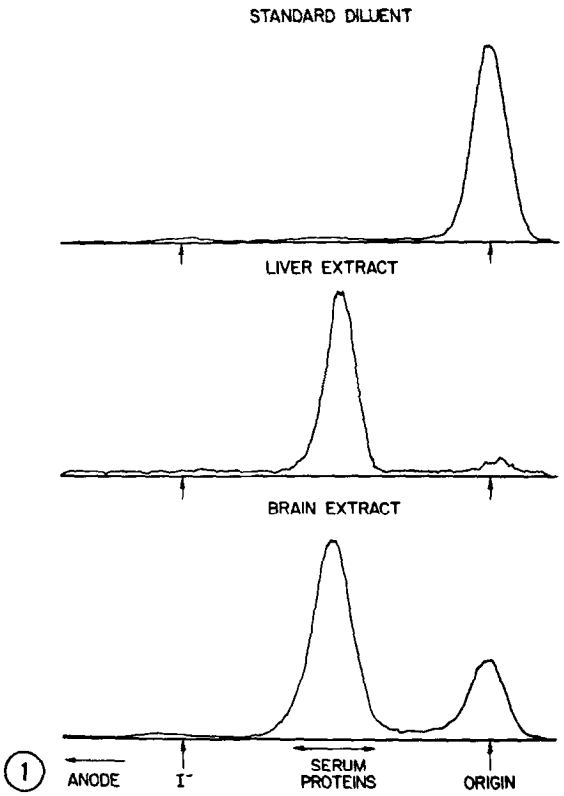
Enzymatic Degradation Studies: Solutions of standard diluent containing ^{125}I-VIP and concentrations of unlabeled VIP ranging from 0.1 μg/ml to 25 μg/ml were mixed with equal volumes of standard diluent alone or of tissue extracts in standard diluent, each preheated to 37°C. Final concentrations of tissue extracts were 0.020 g/ml, 0.020 g/ml and 0.017 g/ml for liver, kidney and brain respectively. At times ranging from 1 minute to 90 minutes, 0.2 ml portions were removed and added to 2 ml standard diluent in a water bath at 100°C. After 1 minute, which was sufficient to inactivate enzymatic activity, 50 mg talc was added; the mixture was vortexed and stored at 4°C until the end of the experiment at which time all samples were centrifuged for 15 minutes at 5°C at 3000 RPM. The supernatant was decanted and the radioactivities adsorbed to talc and in the supernatant were determined by counting in an automatic well-type scintillation counter.

Similar studies were performed with other hormones. In place of unlabeled VIP, unlabeled insulin at concentrations up to 9×10^{-5} mol/liter and a mixture of insulin and glucagon, each 7×10^{-5} mol/liter, were used to inhibit the degradation of ^{125}I-VIP by liver extracts.

The maximum velocity (V_{max}) and Michaelis constant (K_m) for enzymatic degradation of VIP, assuming identical behavior of labeled and unlabeled VIP were determined from Lineweaver-Burk plots (8).

RESULTS

^{125}I-VIP when incubated in standard diluent for 30 minutes remains unaltered and during chromatoelectrophoresis binds to paper at the site of application (Fig. 1). However when incubated with tissue extracts, the radioactivity associated with the degraded fragments migrates in whole or in part with the serum proteins. There is no evidence for deiodination even during prolonged exposure to the degrading enzyme (Fig. 1).



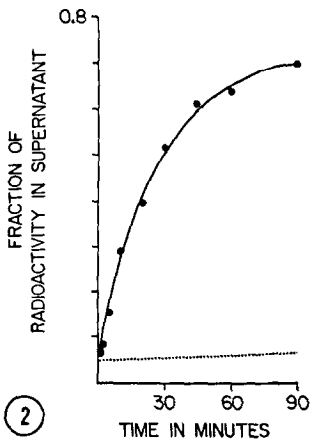
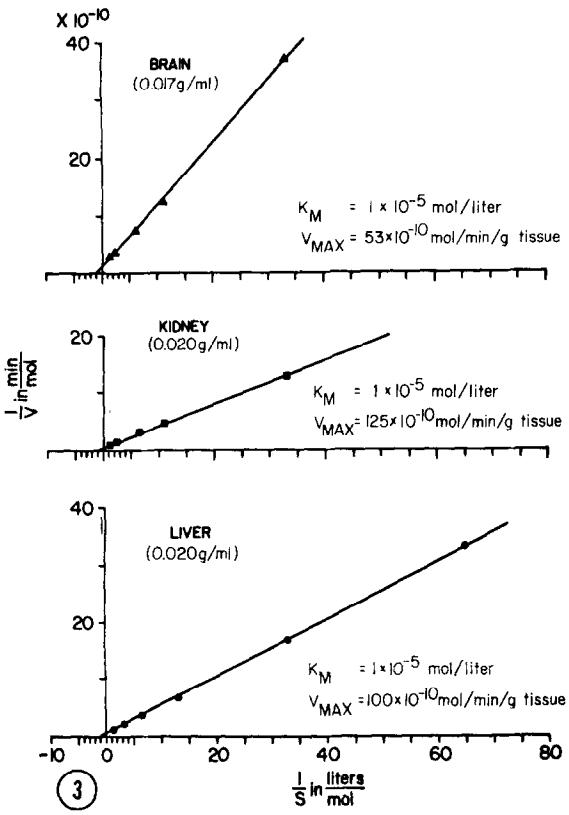


Fig. 1 - Paper chromatoelectrophoretograms of ^{125}I-VIP incubated for 30 minutes in standard diluent alone (top), in liver extract (middle) and in brain extract (bottom). Intact ^{125}I-VIP binds to paper at the site of application (origin) and damaged fragments migrate anodally with the serum proteins. There is no deiodination during degradation.

Fig. 2 - Fraction of radioactivity in the supernatant as a function of time of incubation of ^{125}I-VIP in standard diluent (---) and in a diluted liver extract (●—●). Degraded fragments of ^{125}I-VIP appear in the supernatant; intact ^{125}I-VIP is adsorbable to talc. Even in the absence of unlabeled VIP, the rate of degradation over the first 15 minutes appears to be linear.

The fraction of radioactivity not adsorbed to talc, i.e., in the supernatant, is associated with the degraded fragments and increases with time of exposure to the tissue extracts. Shown in Fig. 2 is a typical curve of radioactivity in supernatant as a function of time of exposure to tissue extracts. Even in the absence of unlabeled VIP, this curve appears linear over the first 15 minutes. Therefore the degradation velocity for each substrate concentration was determined from the product of the mean degradation rate over the first 15 minutes and the substrate concentration. A Lineweaver-Burk plot of the data for



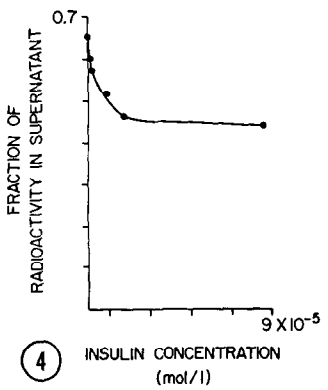


Fig. 3 - Graphs of 1/V as a function of 1/S (Lineweaver-Burk plot). The degradation velocity (V) for each substrate concentration was determined from the product of the mean degradation rate over the first 15 minutes and the substrate concentration(S).

Fig. 4 - Inhibition of degradation of ^{125}I-VIP in the presence of increasing concentrations of insulin. The specificity of the enzyme is evidenced by the inability of a marked excess of insulin to inhibit completely the degradation of ^{125}I-VIP by liver extract.

each tissue and the calculated K_m and V_{max} for each tissue are shown in Fig. 3. The K_m's appear to be nearly identical, averaging about 10^{-5} mol/liter. The V_{max} for kidney and that for liver were virtually identical but that for cerebral cortex was about two-fold lower.

Although insulin does inhibit to some extent the degradation of ^{125}I-VIP by liver extracts, concentrations of insulin as high as 9 X 10^5 mol/liter do not inhibit degradation at 30 minutes by more than 30% of control values (Fig. 4). Glucagon at equivalent molar concentrations is somewhat less inhibitory than insulin and a combination of 7 x 10^{-5} mol/liter glucagon and 7 x 10^{-5} mol/liter

insulin is no more effective an inhibitor than the insulin alone. The ineffectiveness of these peptides in inhibiting the degradation of ^{125}I-VIP is evidenced by the observation that the same inhibition can be achieved with only 3×10^{-9} mol/liter VIP. Thus the degrading enzyme appears to be highly specific for VIP.

DISCUSSION

The finding that the K_m's for the enzymes degrading VIP in liver, kidney and brain were virtually identical suggests that the same enzyme is involved in each of these organs. The K_m (1×10^{-5} mol/liter) which is a measure of the relative affinity of this enzyme for VIP, is comparable to that reported (9) for the glucagon degrading enzyme ($K_m = 4.5 \times 10^{-6}$ mol/liter) and much larger than that reported (10) for insulin ($K_m = 1.6 \times 10^{-7}$ mol/liter).

Duckworth et al (9) have purified an insulin-degrading enzyme from rat skeletal muscle and have shown that glucagon-degrading activity paralleled that of insulin degrading activity at all stages of purification. They therefore concluded that insulin and glucagon are degraded by the same enzyme. Since insulin does inhibit the degradation of VIP, it is quite likely that the VIP degrading enzyme can also degrade insulin. However the question of interest is whether the same enzyme does degrade these peptides in vivo. If so one would expect that the relative V_{max} would be the same in the different organs. Katabchi and Stentz (3) observed that the rate of degradation of insulin per gram of tissue homogenate by the liver was two-fold greater than the degradation by kidney and ten-fold greater than by brain. In the current study the V_{max}'s for kidney and liver were virtually identical within the range of experimental error and that for brain was only about two-fold lower. It is therefore unlikely that the same enzyme is responsible for degradation of both insulin and VIP. Furthermore the finding that the VIP degrading enzyme in the brain is present in relatively higher concentration than is the insulin degrading enzyme may well be related to the observation that brain VIP is quite comparable to that found in the gut (4) but brain insulin is not (11). Although both insulin

and glucagon inhibit the degradation of VIP by the VIP-protease they are not completely inhibitory even in ten thousand-fold excess. The enzyme therefore is quite specific for VIP in spite of the rather considerable resemblance in structure between glucagon and VIP.

ACKNOWLEDGEMENT

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