THE SYNTHESIS OF A BIOLOGICALLY ACTIVE DECAPEPTIDE HAVING THE STRUCTURE PROPOSED FOR KALLIDIN II

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In a recent report, Pierce and Webster (1961) have described the isolation of two kallidins from the incubation of human urinary kallikrein with acid-treated human plasma. Kallidin I could not be distinguished from bradykinin whereas kallidin II was assigned the structure of a decapeptide L-Lys-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg.

In order to unequivocally establish the structure of kallidin II as that proposed, we were asked by the above investigators to undertake the synthesis of the decapeptide. This communication describes our synthetic approach to the problem and the pharmacological comparison of our synthetic material with kallidin II and synthetic bradykinin.

The protected decapeptide, dicarbobenzoxy-L-Lys-nitro-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-nitro-L-Arg methyl ester was obtained by the reaction of the octapeptide L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-nitro-L-Arg methyl ester (Nicolaides and DeWald, 1961) with the p-nitrophenyl ester of dicarbobenzoxy-L-lysyl-nitro-L-arginine (Hofmann *et al.* 1956). The latter substance was not isolated but was formed *in situ* and used as such. Found: C, 53.66; H, 6.44; N, 15.80. Calc. for $C_{73}H_{97}N_{19}O_{20}\cdot 4H_2O$: C, 53.70; H, 6.48; N, 16.30. The methyl ester was removed from the decapeptide by hydrolysis with 1N sodium hydroxide giving the free acid, m.p. 155-160°, $[\alpha]_D^{23}$ -49° (c 1.65, dimethylformamide). Found: C, 55.25; H, 6.31; N, 17.11. Calc. for $C_{72}H_{95}N_{19}O_{20}\cdot H_2O$: C, 55.27; H, 6.25; N, 17.01. Hydrogenation in glacial

acetic acid/methanol using palladium black catalyst gave the tetraacetate salt of L-Lys-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg. Found: C; 53.39; H, 7.28; N, 16.57. Calc. for $C_{84}H_{107}N_{17}O_{20}$: C, 53.81; H, 7.13; N, 16.67.

Paper electrophoresis in pH 5.6 acetate buffer revealed the decapeptide as a single component migrating towards the cathode at a faster rate than pure synthetic bradykinin, the spots being detected with bromphenol blue, ninhydrin and Sakaguchi reagent. When compared to kallidin II no differences could be detected in their behavior on paper electrophoresis. The two compounds appeared to be identical.

The synthetic approach used to obtain the decapeptide has a distinct advantage in that contamination with bradykinin is not possible as indeed there might have been if lysine were coupled to the bradykinin nonapeptide. Any possible contamination by the bradykinin octapeptide which was used would not interfere with activity since this substance has been shown to be inactive (Elliott, Lewis and Horton, 1960).

The synthetic decapeptide and kallidin II were compared with pure synthetic bradykinin *in vitro* and *in vivo*. Isolated guinea pig uterus suspended in Tyrodes solution containing 0.01μg/ml. of atropine sulfate and 0.02μg/ml. tripelennamine. HCl was used as the *in vitro* test object. The synthetic decapeptide was about 70% as active as kallidin II which was found to have 25-35% the activity of bradykinin.

The dog perfused hind quarters (Beck, 1958) served as a sensitive *in vivo* test object. Mongrel dogs of either sex, weighing more than 10kg., anesthetized with 30mg/kg. sodium pentobarbital I.V were used. The peptides were introduced into the perfused vascular bed by injecting 0.1 ml. of the appropriate concentration in 0.9% saline. Vasodilation as indicated by a fall in perfusion pressure was observed following total doses of as little as 0.005γ bradykinin in some animals. Pure synthetic bradykinin gave responses which were quantitatively and qualitatively indistinguishable from kallidin II, whereas the synthetic decapeptide was only 60% as active.

The failure of the decapeptide to exhibit the full biological activity of kallidin II is believed to be due to extensive racemization occuring during the coupling reaction of the dipeptide with the octapeptide. Marked racemization of the arginine moeity during a peptide synthesis has been noted before (Hofmann et al. 1958). While the possibility that the decapeptide and kallidin II have slightly different structures cannot be completely excluded it appears reasonable from the available chemical and biological data that the decapeptide is indeed kallidin II. The preparation of a pure, unracemized sample of the decapeptide is under investigation.[1]

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1) In a private communication Dr. Webster and Dr. Pierce reported that they have obtained by carboxymethyl cellulose chromatography of the synthetic decapeptide a small fraction which appears to have the full biological activity of kallidin II. Other fractions obtained were progressively less active. These preliminary results are being repeated on a larger scale.