Synthesis and Biological Activity of Peptides Related to Bradykinin

Bradykinin has been obtained from plasma globulins by the action of various enzymes[1]. The amino acid composition[2,3], the structure[4], and the biological properties[3] of the pure product have recently been studied by ELLIOTT, HORTON, and LEWIS. We wish to report on the synthesis and the biological activity of several peptides related to bradykinin (Table).

Structure of peptides A–F

H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH A
H-L-Arg-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH . . B
H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Phe-L-Arg-OH . . C
H-L-Arg-L-Pro-L-Pro-L-Phe-Gly-L-Ser-L-Phe-L-Arg-OH . . D
H-L-Arg-L-Pro-Gly-L-Pro-L-Phe-L-Ser-L-Phe-L-Arg-OH . . E
H-L-Arg-L-Pro-Gly-L-Phe-L-Ser-L-Phe-L-Arg-OH . . . . . F

CBO-(nitro)Arg-Pro-OH (I) was condensed with H-Pro-Gly-NHNH-CTO (II)[5] by dicyclohexylcarbodiimid. By selective splitting of the CTO group and treatment with nitrous acid, CBO-(nitro)Arg-Pro-Pro-Gly-N_3 (III) was obtained. Condensation of CBO-Phe-Ser-N_3 (IV) with H-Pro-Phe-(nitro)Arg-OBzN (V)[6] and selective splitting of the CBO group yielded H-Phe-Ser-Pro-Phe-(nitro)Arg-OBzN (VI), which was condensed with III to CBO-(nitro) Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-(nitro)Arg-OBzN(VII). Catalytic hydrogenation of VII yielded A. By using CBO-(nitro)Arg-Pro-Gly-OH (VIII) (from I and H-Gly-OH) instead of III, B was obtained. By condensing IV with H-Phe-(nitro)Arg-OBzN (IX) and splitting the CBO group, H-Phe-Ser-Phe-(nitro)Arg-OBzN (X) was obtained; by condensation with III, followed by catalytic hydrogenation, this led to C. Condensation of IX with CBO-Gly-Ser-N_3 followed by splitting of the CBO group and condensation with CBO-Pro-Phe-OH gave a hexapeptide, which, after splitting of the CBO group, was condensed with I. Catalytic hydrogenation of the protected octapeptide gave D. Condensation of I with H-Gly-Pro-OH followed by condensation with X and catalytic hydrogenation gave E. Condensation of VIII with X, followed by catalytic hydrogenation gave F. All the intermediary and final products were obtained in an analytically pure state.

The biological activity of the peptides A–F was first determined on the isolated guinea pig ileum. The peptides C, D, E, and F were without effect in doses up to 2.5 µg/ml, whereas A and B produced a slow contraction in concentrations as small as 1 ng/ml and 100 ng/ml respectively. Boiling with concentrated HCl or incubation with guinea pig serum destroyed the activity, whereas pretreatment of the muscle with atropine and/or an antihistamine agent (Thenalidine) only slightly interfered with the activity.

The nonapeptide A was further investigated using tests known to be sensitive for bradykinin[1,3,6]. The following results were obtained: On the rabbit duodenum, the concentration required to elicit contraction was 1 ng/ml. To produce a depressor response in anesthetized rabbits, rats, guinea pigs, cats, and dogs, the doses ranged from 200 to 400 ng/kg. Bronchoconstriction in the guinea pig occurred with 200 ng/kg. An effect on capillary permeability to a circulating dye (Evan's Blue) in guinea pigs after intradermal injection was observed with 1 ng.

On a molar basis, the nonapeptide A was more active than histamine (ileum, bronchial muscle, and capillary permeability of the guinea pig) and acetylcholine (duodenum of the rabbit, blood pressure of the rabbit and dog).

The nonapeptide A is therefore one of the most active biological substances exerting a bradykinin-like effect. Further work will demonstrate if it is identical with or closely similar to pure bradykinin.

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Zusammenfassung

Die Synthese von 6 Polypeptiden aus den im Bradykinin vorkommenden Aminosäuren wird beschrieben. Das Nonapeptid H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH zeigte hohe Bradykinin-ähnliche Wirksamkeit in verschiedenen biologischen Testen.

[1] M. ROCHA e SILVA, *Biochemistry and Pharmacology of Bradykinin*, in M. SCHACHTER, *Polypeptides which Affect Smooth Muscles and Blood Vessels* (Pergamon Press, Oxford–London–New York–Paris 1960).

[2] D. F. ELLIOTT, G. P. LEWIS, and E. W. HORTON, Biochem. J. *74*, 15 P (1960).

[3] D. F. ELLIOTT, E. W. HORTON, and G. P. LEWIS, J. Physiol. *150*, 6 P (1960).

[4] D. F. ELLIOTT, paper read to the Biochemical Society (London) on April 8, 1960. We thank Dr. ELLIOTT for kindly giving us some of his results before communication.

[5] CBO = carbobenzoxy; CTO = carbo-ter-butoxy; OBzN = *p*-nitrobenzoxy.

[6] H. O. J. COLLIER, J. A. HOLGATE, M. SCHACHTER, and P. G. SHORLEY, J. Physiol. *149*, 54 P (1959).

Muscle Atrophy Following Section of the Spinal Cord in the Rat

FISCHER[1], SOLANDT and MAGLADERY[2] observed that the course of muscle atrophy, following transection of the spinal cord, is biphasic. During the first 10–14 days following complete transection of the cord and unilateral denervation, the gastrocnemius-soleus muscle was found to atrophy on the 'plegic' side (i.e. after lesion of the upper motor neuron) at a similar rate as on the side of denervation (i.e. after lower motor lesion). Later, however, the plegic muscles gained in weight, so that 56 days after the operation their weight returned almost to initial values. In neither of these reports, however, have changes in total body weight occurring in the course of the experiment been taken into account. It is plausible to assume that if there are any changes in body weight, they might influence the interpretation of these results.

For this reason experiments were performed in an identical manner as those of the above authors. The spinal cord was sectioned at Th_6 in female rats (190–205 g body weight) and at the same time the sciatic nerve was cut and its central stump ligated to prevent regeneration of nerve axons. The soleus and gastrocnemius muscles were then excised in different groups of animals 7, 14, 28 and, 56 days after the operation. The body weight of all animals was carefully registered. As can be seen from the Figure,

[1] E. FISCHER, Proc. Soc. exp. Biol. Med. *47*, 277 (1941).

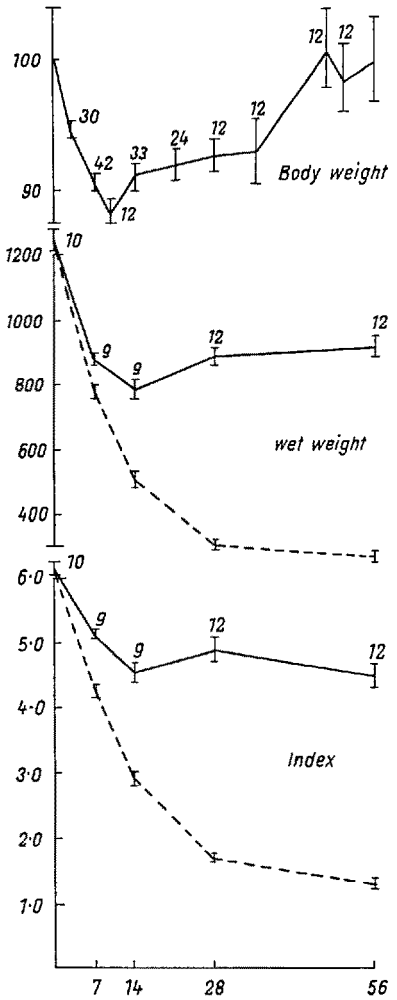
[2] D. Y. SOLANDT and J. W. MAGLADERY, J. Neurophysiol. *5*, 373 (1942).

there is a distinct loss of body weight during the first 7–9 days which is later followed by a gain in body weight, so that 56 days after the operation the initial body weight is regained.

It was found that during the first phase, the atrophy on the plegic side progressed at the same rate as that of the denervated muscles (Figure). No differences were noted between the wet and dry muscle weights.

Following this, there was a gradual but significant gain in weight in both the gastrocnemius and soleus 56 days after transection of the cord, as compared with their lowest values. These results are thus in accord with the findings of FISCHER[1], SOLANDT and MAGLADERY[2].

However, the evaluation of results in this type of experiment in absolute units only (mg) does not take into account the changes due to body weight which can influence the muscle weights. In order to elucidate the role played by the temporary loss of body weight in the above changes, all the muscle weights were calculated as an index: $\frac{\text{muscle weight in mg}}{\text{body weight in g}}$. It was found that during the first phase following section of the spinal cord, atrophy of the plegic muscles does actually occur, i.e. that the muscles lose weight at a faster rate than would correspond to the loss in body weight. But during the second part of the experiment, the index values do not exhibit a tendency to increase, i.e. the absolute gain in weight of the soleus and gastrocnemius is proportional to the gain in body weight (Figure).



The course of changes in body weight, muscle wet weight, and index of muscle to body weight in rats, 7, 14, 28, and 56 days after complete transection of the spinal cord and unilateral section of the sciatic nerve. Body weight expressed as percentage of initial values, muscle wet weight given in mg. Continuous line: lower motor neuron intact; dotted line: lower motor neuron transected. Vertical lines represent standard error of the mean, numerals give number of animals in each group. Abscissa: time after operation in days

It is thus possible to explain the restitution of weight of the gastrocnemius and soleus, i.e. muscles investigated by FISCHER, SOLANDT, and MAGLADERY, in the first place by the increase of body weight of the experimental animals.

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Zusammenfassung

Nach Myelotomie verläuft die Muskelatrophie in zwei Phasen. In der ersten Phase stimmt der Gewichtsverlust der Muskeln mit demjenigen nach Denervation überein. Dagegen erfolgt in der zweiten eine Gewichtszunahme, die durch die analoge Gesamtzunahme des Körpergewichts erklärt wird.

PRO EXPERIMENTIS

A Method of Determining Electrophoretic Mobilities of Antibodies

The localization of antibody activity after electrophoresis has been determined by several methods. It has been done using free electrophoresis[1], paper electrophoresis[2,3], and agar gel electrophoresis[4]. In the latter cases[2–4] the antibody activity has been determined in fractions of the separated serum obtained by elution from the paper or the agar after the electrophoretic run. A method has also been published, however, with which hemagglutinins can be localized after paper electrophoresis by the disposal of red blood cells directly on the paper after the electrophoretic run[5]. This method – although convenient – has been reported not to be very sensitive and does not seem to allow a very precise localization of the antibodies[6]. The present report discusses a technique in which antibodies can be accurately localized by antigen-antibody reactions in basins placed in direct contact with the electro-separated material after the run.

Technique: The electrophoretic separation is performed in agar as described by WADSWORTH and HANSON[7]. Buffered agar of 1% is poured on a 8·2 × 8·2 cm photographic glass to which is attached by adhesive tape two glass pieces 2 × 8·2 cm on opposing sides. The material to be analyzed is mixed with agar and placed in 3 mm wide cut basins. After the separation is complete, the glass end pieces, which provide contact with the buffer in the electrode vessels, are removed. A matrix (78 × 15 mm) of 3 mm thick plexiglass, in which basins 3 mm in diameter are formed, is placed on top of the agar over the separated material (Fig.). The basins in the matrix are placed so that they cover areas which overlap each other along the axis of separation. It is important to eliminate confluence of the material between these different basins. Therefore the matrix is provided with a piece of 0·5 mm thick waterproof tape at each end and melted agar of

[1] J. R. CANN, R. A. BROWN, J. G. KIRKWOOD, P. STURGEON, and D. W. CLARKE, J. Immunol. *68*, 243 (1952).

[2] B. D. JANKOVIC and H. W. KRIJNEN, Nature *171*, 982 (1953).

[3] R. PAYNE and Q. B. DEMING, J. Immunol. *73*, 81 (1954).

[4] R. FAURE, J.-M. FINE, M. SAINT-PAUL, A. EYQUEM, and P. GRABAR, Bull. Soc. Chim. biol. *37*, 783 (1955).

[5] G. BERG, W. FRENGER, and F. SCHEIFFARTH, Klin. Wschr. *33*, 767 (1955).

[6] A. SEMLOW, Z. Immun-forsch. *116*, 215 (1958).

[7] C. WADSWORTH and L. Å. HANSON, Int. Arch. Allergy, in press (1960).