New Test for Biological Identification of Bradykinin

In 1961, Boisonnas, Guttmann and Jaquenoud[1] first synthesized bradykinin and other syntheses have since been recorded[2–4]. In these studies the contracting effect of this substance on the isolated uterus of a rat or the ileum of a guinea-pig has, among other tests, been taken as evidence of identity with bradykinin which was isolated from plasma, and as a measure of its biological activity. As will be shown here, however, this sole criterion may not suffice to characterize the peptide.

Recently, we determined the biological identity and activity of bradykinin which was synthesized by Fridkin, Patchornik and Katchalsky according to a new method (presented at the International Symposium on the Pharmacology of Hormonal Polypeptides and Proteins, Milan, September 14, 1967; results to be published). The ileum of a guinea-pig which was suspended in a 5 ml. organ bath of Tyrode solution at 35° C was used as the test object. Contractions were recorded on a kymograph by means of a frontal writing lever. Drugs were allowed to act for 1 min and then the preparation was washed twice. The time cycle was 5 min. Solutions of all substances were made up in saline. Bradykinin Sandoz and a sample prepared by Sakakibara and Inukai[4] served as reference compounds.

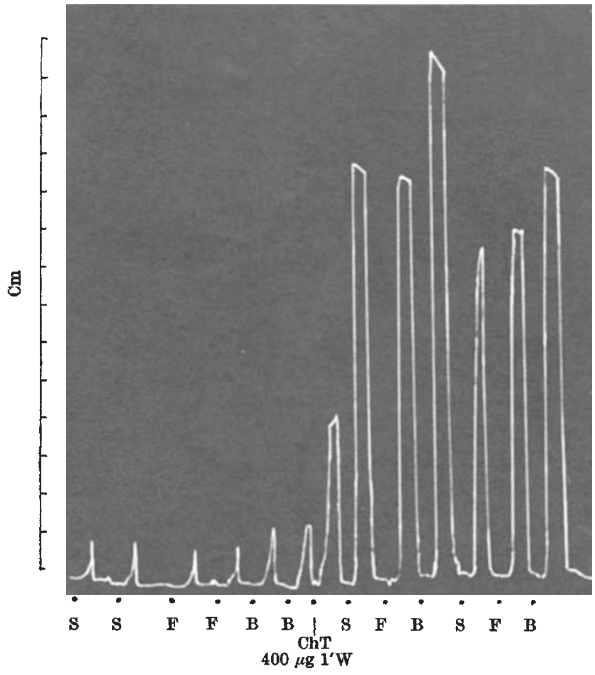

Fig. 1. Responses of the isolated ileum of a guinea-pig to three different specimens of bradykinin (20 μg); namely that of Sakakibara and Inukai (S); Fridkin, Patchornik and Katchalsky (F) and Sandoz (B). After each contraction the preparation was washed twice. Note that after the presence of 400 μg of chymotrypsin for 1 min (ChT 400 μg 1'W), responses were about ten times higher than that of control.

The material which was obtained at the final step of the new synthesis caused contraction of the isolated gut in doses of 7 to 15 ng. The magnitude of the responses closely compared with those elicited by corresponding amounts of bradykinin Sandoz. When 500 μg of chymotrypsin was introduced into the bath, however, the subsequent contractions elicited by the test compound did not increase but in fact decreased. In contrast, those provoked by bradykinin Sandoz were five times higher than those of the control. These experiments indicated that the test substance was not identical with the reference substance. Re-examination of the material revealed that the *p*-nitrobenzyl group which was attached to the terminal arginine had not been removed after treatment with hydrofluoric acid[5]. The material was therefore reduced in the presence of palladium and barium sulphate; it was purified in a column of 'Amberlite *IRC*-50' and lyophilized. The product was tested on the isolated ileum in doses of 5 to 20 ng and elicited responses similar to those of the two reference bradykinins. Furthermore, after the presence of chymotrypsin, all three compounds behaved identically: they elicited contractions which were eight to ten times larger than those of control. Thus the identity of all three specimens of the peptide could be established with certainty. Fig. 1 illustrates a typical experiment.

This work supports a previous suggestion[6] on the usefulness of employing chymotrypsin as a specific sensitizer of the isolated ileum of a guinea-pig for the biological identification of bradykinin. It should be remembered that no specific antagonist of bradykinin is at present available. Moreover, it can also be inferred that the present biological test for identification of bradykinin, which consists of the disappearance of its contracting activity on smooth muscle on incubation with chymotrypsin, might be misleading. In the present work, for example, Phe-Arg bond of the *p*-nitrobenzyl blocked bradykinin could readily be split by chymotrypsin, rendering it inactive on smooth muscle, even though it is not identical with the pure peptide.

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H. Edery

Israel Institute for Biological Research,
Ness-Ziona,
Israel.

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Changes in Iron Metabolism in Natives of 13,000 ft. brought down to Sea Level

Iron metabolism in natives of high altitudes (14,900 ft.) decreased when they were brought down to sea level[1,2]. The values for the plasma iron and the plasma iron turnover rate obtained by Huff *et al.*[1] were higher than those reported by other workers, but this may have been caused by storage and shipment of the samples.

In experiments carried out in the north of Argentina from 1960 to 1963, we studied the iron metabolism of high altitude dwellers during the first 13 days after descent to sea level. We had a greater number of volunteers and a more homogeneous population than in previous work. Thirty-two high school students born and living at 13,000 ft. (Mina aguilar, Argentina) served as volunteers. None had ever worked in the mines and all played football or basketball. Chest X-rays and physical and laboratory examinations confirmed their good health. The average weight was 58 kg; height, 164 cm; and age, 18 yr. Erythrokinetic studies were performed at high altitudes and afterwards the volunteers were flown to sea