SOME PROPERTIES OF SYNTHETIC BRADYKININ AND BRADYKININ-LIKE POLYPEPTIDES

H. KONZETT

I. *Comparison between Synthetic and Natural Material*

During the Symposium on Polypeptides held in London in March 1959 Elliott mentioned in the discussion the work on the purification of bradykinin and announced that the molecule of bradykinin contains one mole each of glycine and serine and two moles each of arginine, proline and phenylalanine (Elliott, Lewis and Horton, 1960*a*). This statement aroused our interest in bradykinin because the hope was now justified that its structure would be cleared up soon and its synthesis could then be accomplished. We, therefore, began to study the biological properties of natural trypsin-bradykinin which was prepared by J. F. Pechère from bovine plasma by incubation with trypsin and partly purified by a modification of the method of Hamberg and Deutsch (1958). In March 1960 Elliott precommunicated the sequence of the amino acids of bradykinin to Boissonnas who with his group in Basle immediately afterwards began its synthesis. I had at that time the full responsibility for the biological assay of these synthetized peptides. Valuable assistance was given me by E. Stürmer.

The octapeptide sequence first proposed by Elliott *et al.* (1960 *b*) for bradykinin (Table I, No. 1) was synthetized by several routes (Boissonnas *et al.*, 1960 *a*). We found no biological activity of the different preparations on the guinea-pig ileum and on the rat uterus in doses up to 10 μg/ml. (Konzett and Stürmer, 1960). It became probable that the proposed sequence was incorrect. Therefore the systematic synthesis of related polypeptides was undertaken (Boissonnas *et al.*, 1960 *a*, 1960 *b*). Table I contains these polypeptides in the chronological order of their

TABLE I

	No.
H — Arg — Pro — Pro — Gly — Phe — Ser — Phe — Arg — OH	1
H — Arg — Pro — Gly — Pro — Phe — Ser — Phe — Arg — OH	2
H — Arg — Pro — Gly — Phe — Ser — Phe — Arg — OH	3
H — Arg — Pro — Gly — Phe — Ser — Pro — Phe — Arg — OH	4
H — Arg — Pro — Pro — Phe — Gly — Ser — Phe — Arg — OH	5
H — Arg — Pro — Pro — Gly — Phe — Ser — Pro — Phe — Arg — OH	6

pharmacological investigation (Konzett and Stürmer, 1960 *a*). The following results were obtained: The peptides No. 2 and 5 were without effect on the above mentioned smooth muscle preparations in doses up to 10 µg/ml. The peptide No. 3 was only tested on the guinea-pig ileum; it had no action up to 2·5 µg/ml. The octapeptide No. 4 of Table I, however, produced a delayed slow contraction of the guinea-pig ileum in doses of 100 ng/ml or more and a contraction of the rat uterus in doses of 2 ng/ml or more. Besides that, the peptide No. 4 behaved like bradykinin in losing its effect after boiling with concentrated hydrochloric acid or after incubation with undiluted guinea-pig serum for 15 min. These properties of the peptide No. 4 incited the synthetic chemists to synthetize the nonapeptide No. 6 of Table I (Boissonnas *et. al.*, 1960 *b*). In a comprehensive pharmacological investigation (Konzett and Stürmer, 1960 *a*) using a series of different tests *in vitro and in vivo,* the activity of this nonapeptide (stimulation of plain muscles, depression of blood pressure, increase of capillary permeability) proved to be of the same qualitative and quantitative order as in the case of purified natural trypsin-bradykinin (Elliott, Horton and Lewis, 1960). A further direct comparison of the synthetic nonapeptide and of a purified natural trypsin-bradykinin in three different laboratories revealed no difference between the two preparations (Konzett and Stürmer, 1960 *b:* Lewis, 1960; Shorley and Collier, 1960).

In view of the results of our biological investigation of the synthetic nonapeptide, Elliott, Lewis and Horton (1960 c) had reinvestigated the structure of natural trypsin-bradykinin and after new degradation studies had presented evidence that natural bradykinin had the same structure as the nonapeptide No. 6 of Table I. Having therefore synthetic bradykinin in hand we did not investigate any further the much weaker octapeptide No. 4 of Table I but intended to clear up the action of synthetic bradykinin more thoroughly.

Table II summarizes the main results. These, although being mainly self-explanatory, may be commented on as follows: Whereas the guinea-pig ileum and the rat uterus *in vitro* are very sensitive to bradykinin and therefore provide valuable means for its assay it seems that bradykinin does not influence these structures *in vivo* in the same pronounced fashion. There where e.g. no signs of an increased activity of the small intestine in experiments on anaesthetized guinea-pigs in which the tidal air was measured with an overflow-method (Konzett and Rössler, 1940) when bradykinin in doses of 0·2 µg/kg–0·8 µg/kg was given. And the rat uterus *in situ* was contracted only after doses as high as 10 or 100 µg/kg (Berde and Saameli, 1961).

The importance of the species for the bradykinin action is well illustrated by the difference in doses necessary to decrease the tidal air in guinea-pigs and cats. In the guinea-pig, bradykinin as a bronchoconstrictor agent is 50 times more active than in the cat.

TABLE II

Threshold doses of synthetic bradykinin in producing effects on different structures and systems

Structures or systems	Doses	Effect	Reference
Plain muscles *in vitro:*			Konzett and
Guinea-pig ileum	1 ng/ml.	Contraction	Stürmer, 1960 *a*
Rat uterus	0·03 ng/ml.	Contraction	,,
Tidal air (Plain muscles *in vivo*):			
Anaesthetized guinea-pig	0·2 μg/kg	Decrease	,,
Spinal cat	10 μg/kg	Decrease	,,
Blood pressure:			
Anaesthetized rabbit	0·05 μg/kg	Fall	,,
Anaesthetized guinea-pig	0·2 μg/kg	Fall	,,
Anaesthetized rat	0·2 μg/kg	Fall	,,
Anaesthetized dog	0·2 μg/kg	Fall	,,
Anaesthetized cat	0·5 μg/kg	Fall	,,
Spinal cat	0·5 μg/kg	Fall	,,
Anaesthetized cock	50 μg/kg	Fall	,,
Permeability:			
Intracutaneous injoction (Guinea-pig)	0·1 ng/0·1 ml.	Increase	,,
Intraplantar injection (Rat)	1 μg/0·1 ml.	Increase	Stürmer and Cerletti, 1961

With regard to the action of bradykinin on the blood-pressure, the differences between the different species are not so pronounced. It is, however, noteworthy that the rabbit was more sensitive than the guinea-pig, rat, dog and cat. Even in the cock bradykinin produced a fall of blood-pressure sometimes followed by an increase, particularly after high doses.

An increase of the capillary permeability occurred in guinea-pigs and rats. The difference in the methods used does not allow definite conclusions with regard to species differences.

The high activity of bradykinin is evident if a comparison is made with the threshold doses of naturally occurring substances as e.g. histamine and acetylcholine on the same structure or system. On a molar basis, bradykinin is much more potent than histamine in decreasing the tidal air and in increasing the capillary permeability in guinea-pigs and also more potent than acetylcholine in producing a fall of blood-pressure in guinea-pigs and rabbits. On cats, however, acetylcholine was a stronger depressor agent than bradykinin.

The acute toxicity of bradykinin is remarkably low (Konzett *et al.*, 1961). The LD_{50} (intravenous injection) in white mice lay between 16 and 20 mg/kg. Rats tolerated the intravenous injection of 10 mg/kg well. After the rapid intravenous injection (within 10 sec) of 3 mg/kg into a dog vocalization, a short-lasting respiratory arrest, vomiting and defaecation was observed; but the animal survived.

II. *Antagonistic Properties towards Naturally Occurring Pressor Agents*

Since bradykinin is formed from plasma-proteins and belongs to the most active depressor agents so far known it seemed of interest to study its antagonistic properties towards naturally occurring pressor agents e.g. noradrenaline, angiotensin and vasopressin.

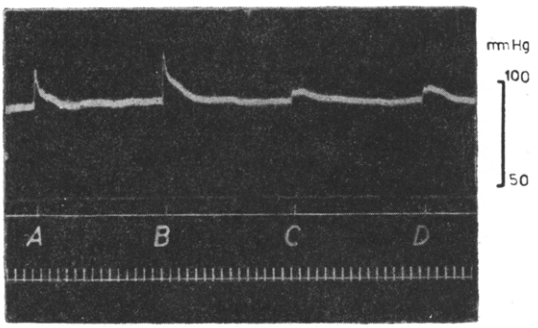

FIG. 1. Blood pressure of a rat, anaesthetized with urethane. Inhibition of the rise in blood pressure produced by noradrenaline when bradykinin is added (see text). A: 0·25 μg noradrenaline; B: 0·5 μg noradrenaline; C: 0·25 μg noradrenaline plus 0·1 μg bradykinin; D: 0·5 μg noradrenaline plus 0·2 μg bradykinin. Time in minutes.

Male rats, weighing 400–500 g, anaesthetized with urethane were used. In most experiments a small dose of dibenamine (100–200 μg/100 g body weight) was injected at the beginning to stabilize the blood-pressure (Dekanski, 1952). This dose, according to our experience, is too small to interfere markedly with the pressor action of the doses of noradrenaline used. In four-point assays two submaximal doses of the pressor substances without and with bradykinin (mixed immediately before the intravenous injection) were compared.

The pressor effect of 0·25 and 0·5 μg noradrenaline bitartrate (per rat) was reduced to the half by adding 0·05 and 0·1 μg synthetic bradykinin, respectively. The addition of 0·1 and 0·2 μg bradykinin to these doses of noradrenaline diminished greatly the pressor action (see Fig. 1) where as the addition of 0·2 and 0·4 μg bradykinin inhibited completely the effect of 0·25 and 0·5 μg noradrenaline, respectively.

Higher doses of bradykinin were necessary to antagonize the pressor action of angiotensin. 0·2 and 0·4 μg of bradykinin diminished by about 50 per cent the rise in blood pressure due to 0·1 and 0·2 μg angiotensin, respectively. There was a good dose-response relationship of the inhibitory action of bradykinin towards the pressor action of angiotensin (see Fig. 2). To inhibit the pressor action of these doses of angiotensin nearly completely, 0·8 and 1·6 μg of bradykinin were necessary.

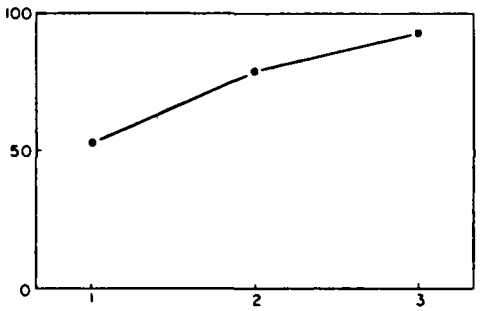

FIG. 2. Inhibition of the pressor effect of angiotensin (0·1 and 0·2 μg) in the rat by the addition of different doses of bradykinin (see text). Ordinate: Inhibition in per cent. Abscissa: Doses of bradykinin added. 1 : 0·2 and 0·4 μg; 2 : 0·4 and 0·8 μg; 3 : 0·8 and 1·6 μg, respectively.

Still higher doses of bradykinin were needed to counteract the increase of blood pressure due to synthetic lysine-vasopressin. With 0·8 and 1·6 μg of bradykinin the pressor effect of 5 and 10 mu vasopressin was only reduced by about 40 per cent.

From these experiments it results that bradykinin unspecifically antagonizes the pressor effect of the different vasopressor substances formed in the body. On the other hand, it may be concluded that on a weight-for-weight basis to antagonize the depressor action of bradykinin, vasopressin would be more active than angiotensin and the latter more active than noradrenaline. Such an antagonism may become important when the rôle of bradykinin under pathological conditions is better understood.

III. *Sensitivity Differences in Human Beings*

The species differences in the sensitivity to bradykinin already described prompted us to study its effect on human beings as well. In collaboration with H. Ehringer and P. Herzog (1961) we investigated the changes of blood flow in the hand, foot, calf and forearm under the influence of bradykinin using the venous occlusion plethysmography (Barcroft and Swan, 1953). In the feet and hands skin-vessels prevail, whereas in the calf and in the forearm the skeletal muscle-vessels are dominant.

Bradykinin was infused intra-arterially or intravenously in doses from 0·2 μg/min up to 50 μg/min during 2–4 min. The blood flow increased after the intra-arterial infusion of bradykinin into the femoral artery in the foot more regulary than in the calf. The sensitivity to the dilator action of bradykinin is therefore greater in the skin-vessels than in the skeletal muscle-vessels. The blood flow in the calf showed sometimes even a diminution after the intra-arterial infusion of bradykinin.

After intravenous infusion of bradykinin the increase in blood flow was more pronounced in the hand and foot than in the forearm and calf.

Besides these differences in sensitivity of skin-vessels and muscle-vessels there were also remarkable variations in the sensitivity of different individuals. Further it was noticed that the responses due to bradykinin showed no clear-cut dose-response relationship. Tachyphylactic reactions occurred quite regulary.

Our results regarding the effect of bradykinin on the blood flow in human beings differ only partly from those obtained by Fox *et al.* (1961). It remains to clear up the discrepancies. We already have some hints.

The correlation of facts established in experiments on animals with the findings in human beings will clear up further the importance of bradykinin under physiological and pathophysiological conditions.

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