DEFINITION OF BRADYKININ AND OTHER KININS

M. ROCHA E. SILVA

Dept. of Pharmacology, Faculty of Medicine, U.S.P., Ribeirão Prêto, São Paolo, Brazil

Kinin-hormones

The name *kinin* suggested by the English authors, might be usefully applied to denote the whole group of pharmacologically active polypeptides acting upon the smooth muscle and producing changes in arterial tension, formed or released from stores in the blood or in tissues. In this sense, the kinins would belong to the class of *local* or *tissue hormones* and should be distinguishable from the neuro-hypophysis hormones.

Recently, I have suggested[1] the expression "kinin-hormones" to denote polypeptides, such as bradykinin, angiotensin and substance-P, with the exclusion of oxytocin and vaso-pressin. A characteristic of the kinin-hormones would be not to have special glands of secretion, being released from inactive precursors (bradykininogen, angiotensinogen) or from diffuse stores in the intestinal wall or the central nervous system (substance-P). I wish to make clear that I am not suggesting changes in traditionally accepted names, but simply suggesting adoption of the name kininhormone, in order to facilitate systematization of a complex class of substances.

Polypeptides of diverse origin, having some pharmacological or chemical resemblance with mammalian kinins, might as well be classified under the same generic connotation, with the obvious limitation that they are not hormones to the mammalian body. The suffix *kinin* annexed to the name of the material of origin could denote active polypeptides of diverse origin as urokinin (from urine), vespakinin (from wasps and the hornet), scorpiokinin (from the scorpion) and so-forth.

The name of plasmakinin has been proposed as a substitute for bradykinin. In that sense, and according to the comparative results obtained with pure or synthetic bradykinin, the name of plasmakinin is misleading and useless, since bradykinin itself "may be responsible for all the group of polypeptides which have been classified under the name of plasmakinin".[2]

According to our suggestion, the name "plasmakinins" (in the plural) would also indicate the polypeptide angiotensin, as one of the kinin-hormones present in plasma in the form of inactive precursors:

KININ-HORMONES:	
From plasma:	*Angiotensin, bradykinin, kallidin*
From intestinal wall and the C.N.S.:	*Substance P*
From other specified sources:	*Urokinin, colostrokinin, vespakinin, scorpiokinin, etc.*

Such a varied nomenclature might, of course, one day, be simplified as our knowledge of the chemical constitution of the different mentioned principles progresses.

Definition of Bradykinin

In the short time allowed to each speaker in this Symposium, it would be impossible to discuss more than a few points in relation to the definition of bradykinin as a chemical and pharmacological entity. To start this discussion I would like to present a small point of historical interest. I believe that the first clear cut demonstration that bradykinin is a polypeptide was given in 1956, by Andrade and Rocha e Silva[3] in a paper on the purification of bradykinin by using the ionic exchange resin, the Amberlite IRC50 (XE-64). As seen in Fig. 1, the final purified product appeared in a homogeneous and sharp peak of activity, coinciding exactly with the peak of ninhydrin colour of the hydrolysed material. A ceiling of activity of 5000 units per milligramme was then obtained, on rechromatography of the same material in a fresh Amberlite column. Since I am going to present data in which the purest preparations of bradykinin, including the synthetic one, appear to have *less* specific activity than the material obtained in 1956, I must go a little further in the question of the unitage adopted in our laboratory.

In 1948, when we came across bradykinin[4], we decided to avoid the use of "biological units" and to set as a "standard", the first stabilized preparation, "Pool I", adopting as a "unit", the activity contained in 1 mg of this material. In the subsequent years the advantage of such a procedure became obvious, since the material contained in our standard was perfectly stable at room temperature, even when in contact with air. Later on we adopted as a new standard, a material extracted with glacial acetic acid and precipitated with several volumes of ethyl ether. Each batch of this new standard was assayed against the "Pool I" and contained usually from 3 to 6 units per mg of activity. Therefore, some fluctuations in the specified activity of our standards could be expected in the course of time. Our present standard might be stronger than the one we had in 1956, by a factor of 1*5–2·0, which could explain the discrepancies between the activity of the bradykinin purified by the Amberlite IRC50 (XE-64), and the estimations of activity recently made in our laboratory and that I am going to present in continuation. Another factor might come into play to explain the low activity obtained with Elliot's pure material. The sample

which I received was for a long time retained in the Post-Office and had no preservative against destruction. However, the synthetic material received from Sandoz is very stable and we had the opportunity of rechecking the material received in Brazil, with a fresh sample supplied by Sandoz, Hannover, New Jersey and kept continuously on ice.* The activity

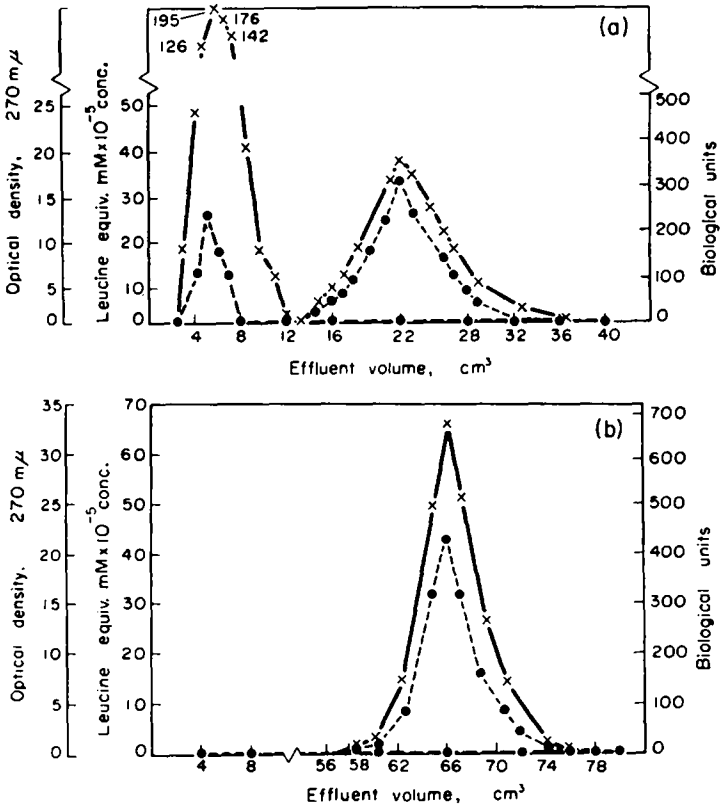

FIG. 1. Purification of bradykinin in a column of Amberlite IRC50 (XE-64). A. First passage through the Amberlite column. B. The same material collected above was submitted to a new column of Amberlite. A specific activity of 5000 units per mg of polypeptide (calculated as leucine), was obtained in both peaks of activity (A) and (B). Full lines: estimation of total amino acids by ninhydrin, in the hydrolysates of the eluates. Dotted lines: biological activity (in units of bradykinin). According to Andrade and Rocha e Silva.[3]

* Such a comparison was made at Baylor University, Houston, Texas, in collaboration with Dr. W. Krivoy. Our thanks are due to Dr. H. Busch for the facilities given to us at the Department of Pharmacology, and to Dr. R. Bircher of Sandoz, Hannover, New Jersey and Dr. A. Cerletti of Sandoz, Basel, for the samples of synthetic Bradykinin utilized.

was perfectly comparable and therefore the figure given in Table II, of 0·5 μmg per unit, appears to me to be significant.

Tables I and II show the relative activities of our standard and of the preparation supplied by Prof. Elliott (90% pure bradykinin) and the syn-

TABLE I

*Discrimination coefficients between 90% pure bradykinin and our standard**

Guinea-pig† ileum	Rat's† duodenum	Rat's† uterus	Cat's blood pressure
1·00	1·42	1·17	0·90

Cat's uterus (pregnant)	Rabbit's	
	Duodenum	Ileum
1·06	0·96	0·89

* According to Rocha e Silva, Antonio and Diniz (unpublished results).

† In these experiments, the comparison was done by utilizing a "2 × 2" design in 4 groups of 2 doses of each, pure and "standard" bradykinin.

TABLE II

Comparison between synthetic bradykinin (Sandoz) and standard bradykinin (3 units per mg)

Structure	Units/μg	1 Unit = μg	Discrimination index
Guinea-pig ileum*	2·0–2·5	0·5–0·4	1·00
Rat's duodenum	2·7	0·35	1·30
Cat's blood pressure*	1·5–1·7	0·70–0·60	0·70
Rat's uterus*	2·0	0·5	1·00

* 2 × 2 assay

thetic material from Sandoz (Synthetic Bradykinin, BRS 640, Sandoz). As mentioned above, I would not give too much emphasis to the absolute values obtained with Elliott's material, and they are not even mentioned in the Table. The figures presented in Table I are *discrimination coefficients*, in the sense of Gaddum[5] and therefore related to the activity obtained upon the guinea-pig ileum, taken as 1·00. We see that all figures fluctuate around the estimation obtained upon the guinea-pig ileum, with

the possible exception of the one obtained upon the duodenum of the rat; such a discrepancy, however, might depend upon the fact that this structure is not as reliable as the others utilized; on the other hand, the difference of 1·42–1·00 is not serious enough to suggest a difference in the active principles present in both materials. This confirms the data by Jaques and Meier[6] who have found no difference between bradykinin prepared with trypsin or with the venom of *Bothrops jararaca*. The small discrepancy indicated in Table II between the activity of our standard and synthetic bradykinin on the cat's blood pressure might be more significant, since it was rather persistent and obtained under a variety of conditions, in which our standard was consistently more active than a corresponding number of units of the synthetic material, as estimated in experiments with the smooth muscles (guinea-pig ileum and rat's uterus).

With all possible limitations indicated above, it seems reasonable to assume that the material obtained in 1956, at the Biological Institute in São Paulo, was near to chemical purity, or at least not very far from it. Therefore, the deductions about the extraordinary potency of the material then obtained[3] are still valid.

Recent developments in the field of bradykinin, such as its purification[7, 8] and synthesis[9, 10] confirmed the first hints that it might be one of the most potent auto-pharmacological agents existing in the body. Experiments with pure or synthetic bradykinin in comparison with other endogenous vaso-dilating principles, such as histamine or acetylcholine, have shown that weight by weight, bradykinin is as potent as them, and being a polypeptide of molecular weight around 1100, bradykinin is more powerful than acetylcholine on the rabbit's blood pressure[11] or in humans, when given intra-arterially.[12] Its effects upon the blood are more lasting than those produced by acetylcholine. The actions of bradykinin are also consistent. Thus in the rabbit, while histamine raises blood pressure, bradykinin induces a fall which is resistant to atropine. I will not insist upon details which are going to be dealt more extensively by the authoritative subsequent speakers, in this Symposium.

Bradykininogen

Bradykinin, as angiotensin, is released from a precursor (bradykininogen) contained in the α_2-globulin fraction of mammalian plasma. The following diagram (Fig. 2) gives an idea of the parallel origins of both kinin-hormones, when the globulin is submitted to the enzymatic action of renin and converting enzyme and of proteases having the specificity of trypsin.

In order to have an idea of the magnitude of the stores of bradykinin in normal plasma and its possible variations under physiological or pathological conditions, we have developed a method in our laboratory for the estimation of bradykininogen in plasma.[13] This work is being directed

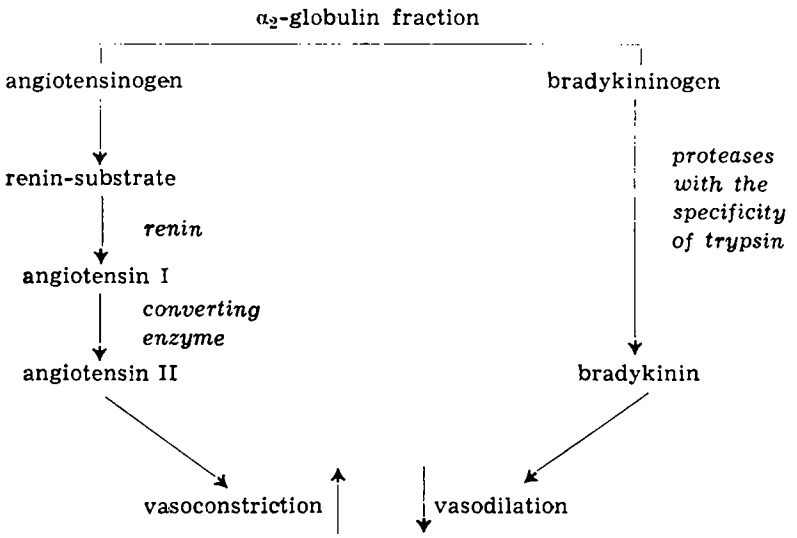

FIG. 2. Parallel origins of bradykinin and angiotensin from precursors in the same globulin fraction and their possible antagonism upon the circulatory apparatus.

by Dr. Diniz, in collaboration with the Clinics at our Faculty. The method, as now established, is a development of previous methods described by van Arman[14] and Hamberg and Rocha e Silva.[15] Heparinized blood is collected in siliconized centrifuge tubes or in polyethylene tubes. After centrifugation, 0·2 ml of plasma are transferred with a siliconized pipette to a 18×150 mm glass tube containing 1·8 ml of a 0·2% solution of acetic acid. The mixture is heated in a boiling water bath during 30 min, cooled to room temperature and the pH adjusted to 7·30–7·80 with normal NaOH and then buffered to pH 7·8 with a 0·2 molar Tris buffer. The suspension thus formed is incubated with 0·2 mg of crystalline trypsin during 30 min. At the end of this period, the enzyme in the clear digest is inactivated by adding 5 ml of boiling alcohol, heated for 5 min in a water bath at 70°C and the whole fluid evaporated under reduced pressure and the residue taken up in 4 ml of saline for the bioassay upon the guinea-pig ileum, using a "4-point" assay as described previously.[16] Table III gives a survey of all comparative estimations in humans as well as in several animal species. As seen in Table III, quantities of the order of 21·2 units equivalent to 10 μg of synthetic bradykinin could be found in each ml. of human plasma. To have an idea of how considerable those quantities are, one has to consider the total amount which might be contained in an individual, by multiplying by 2500, the volume of circulating plasma. No less than 25,000 – 50,000 units or the equivalent of 12·5–25 mg of the synthetic bradykinin are present in the human body. If such quantities could be released at once, they would produce certainly serious

disturbances in the haemodynamic equilibrium. However, we have to consider that the relase of part of it, say 2 units per ml, as it was found during labour, in our laboratory by Prof. Martinez and Dr. Diniz, could certainly be of significance if one multiplies by the total volume of circulating plasma.

TABLE III

Determination of the total bradykininogen content per ml of normal plasma (units per ml)

According to Diniz *et al.*[13]

Origin of material	Average	Range
Human*	21·2	14·8–28·5
Ox	28·9	23·7–33·9
Horse	18·9	17·6–19·7
Sheep	12·7	12·0–13·8
Pig	8·1	5·5–11·6
Dog	12·3	12·0–13·0
Rabbit	20·1	17·5–22·7
Guinea-pig	17·7	15·8–20·0
Rat	4·0	2·5– 5·0

* Data from 10 normal men and 5 normal women; other results are of 3 individuals in duplicate samples.

On the other hand, the plasma contains all the enzymatic system necessary for its release, part of it being the plasminogen-plasmin system itself and part a still unknown protease which can be activated by trypsin and blocked by the soya bean trypsin inhibitor. Activation by contact with glass, as shown by Armstrong *et al.*,[17] or by dilution,[18] or by a previous heating of the plasma with 0·1 N HCl[19] is consistently inhibited by soya bean trypsin inhibitor, which is a good indication that the releasing enzyme is a protease exerting a similar action as trypsin or plasmin on synthetic substrates such as BAME or TAME. To call this enzyme "plasmakallikrein" appears to me an unnecessary complication in nomenclature. We have suggested the name "kininogenins" to denote this class of enzymes releasing bradykinin from its precursor.

Bradykinin versus Angiotensin

Since bradykinin is a vasodilating substance released from a precursor in plasma (bradykininogen) in a manner very similar to angiotensin, which displays vasoconstrictor activity, and is released from a similar globulin fraction (angiotensinogen), this coincidence might induce one to think of a sort of equilibrium or balance, between both principles to regulate blood pressure, as indicated in Fig. 2.

In the earliest days of bradykinin we had observed[20] that the simultaneous injection of it with angiotensin, in the rabbit, results in cancellation of the effect on the arterial blood pressure. We have assumed that this antagonism is of the "physiological" type, and in fact there was no reason to believe any true competition between bradykinin and angiotensin for the same receptors. Now that both substances have been isolated and synthesized and their chemical structure determined (Fig. 3) it would be even more difficult to think of any competitive antagonism between such dissimilar structures:

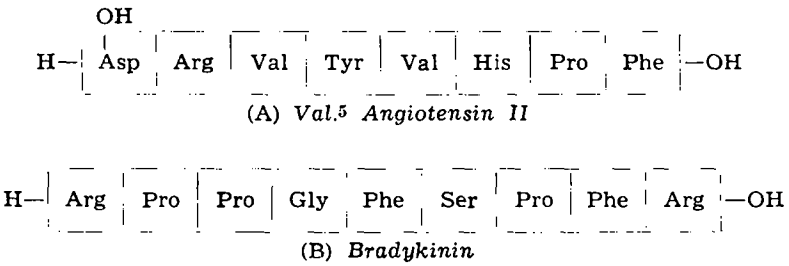

FIG. 3. Structural formulas of angiotensin (A) and bradykinin (B).

On the contrary, upon many biological structures, such as the guinea-pig ileum or the rat's uterus, both principles work synergistically as stimulating principles. Upon the rat's duodenum or the rabbit's intestine, both principles behave in a quite distinct way, since the typical effect of bradykinin is to relax the rat's duodenum or produce an initial fall in tonus of the rabbit's intestine, while angiotensin produces in both cases a stimulating effect.

It became interesting to see whether bradykinin would reduce the contraction produced by angiotensin upon the rabbit's intestine. The result was definitely negative, as shown in Fig. 4. In some preparations of rabbit intestine, bradykinin produces a predominant fall in tonus as shown in Fig. 4 (left) and angiotensin produces always a sharp increase in tonus. When both polypeptides are mixed and added together to the bath, after a prolonged fall in tonus due to bradykinin, the contraction due to angiotensin develops as before. In the duodenum of the rat (Fig. 4, right) bradykinin produces the known fall in tonus and angiotensin a sharp rise; when both are mixed, we can see first the fall due to bradykinin and then the rise due to angiotensin, and the end position of the lever gives an almost perfect algebraic summation of both effects.

Upon the coronary flow of the isolated heart (Langendorff's method) the antagonism eventually seen between bradykinin and angiotensin is also of the physiological type. It was found in our laboratory, that bradykinin is very potent in producing an increased flow in the coronary circulation, when the mammalian hearts (guinea-pig, rabbit, cat and dog)

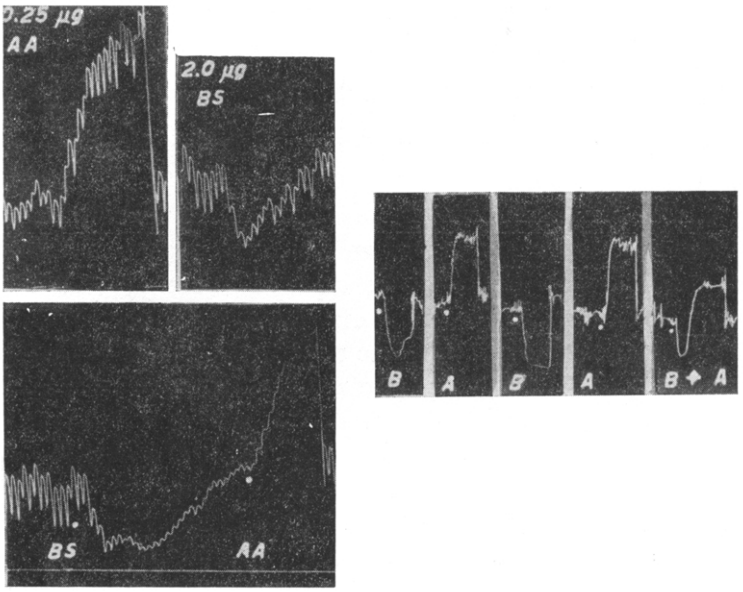

FIG. 4. A. Rabbit's duodenum. Upper tracings; effect of angiotensinamide (AA) and synthetic bradykinin (SB) when given separately. Lower tracings; AA given while bradykinin is still in the bath. B. Rat's duodenum. Opposite effects produced by bradykinin (B) and angiotensinamide (A). When both are mixed (B + A) the effect observed is the algebraic sum of the individual effects.

are perfused with Tyrode solution[21] Fig. 5 shows a typical effect of the injection of 1 µg of the synthetic bradykinin (BS) upon the isolated guinea-pig heart. In other experiments we have found that the effective concentration of synthetic bradykinin producing a sharp rise in coronary flow could be as low as 10^{-9}–10^{-10}. Therefore, the guinea-pig coronary circulation can be as sensitive to bradykinin as the rat's uterus, consid-

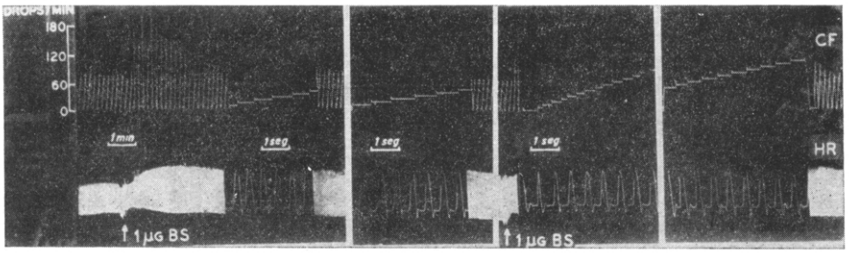

FIG. 5. Isolated guinea-pig heart. Effect of the injection of 1 µg of synthetic bradykinin (BS) on the coronary flow as measured by the number of drops/min. Note the change in velocity of the drum from 1 min to 1 sec, to show that the effect of bradykinin upon the dynamics of the heart was small or trivial, in contrast to the sharp rise in coronary flow. According to Antonio and Rocha e Silva.[21]

ered so far the most sensitive preparation for the biological action of bradykinin. Upon the isolated heart, angiotensin has an opposite effect[22, 23] in some of the preparations, but even here we could not detect more than a "physiological antagonism", since when both polypeptides were injected together, there was no visible competition but simply an algebraic sum of their effects.

We thought that an obvious preparation to study the antagonism between bradykinin and angiotensin would be the isolated strips of rabbit's aorta suspended in a smooth muscle chamber, according to the method described by Furchgott *et al.*[24] This effect can be ascribed to angiotensin II and therefore is probably related to the hypertensive effect of the polypeptide.[25] Noradrenaline also contracts this structure. Using this *in vitro* preparation, I recently studied the combined effects of angiotensinamide (AA), noradrenaline (NA) and bradykinin (BS) and was surprised to find that bradykinin had no effect whatsoever upon this preparation. Not only did it have no antagonistic effect towards noradrenaline or angiotensinamide, but even when the tonus of the preparation was naturally high to start with, bradykinin had no effect at all. Neither natural bradykinin

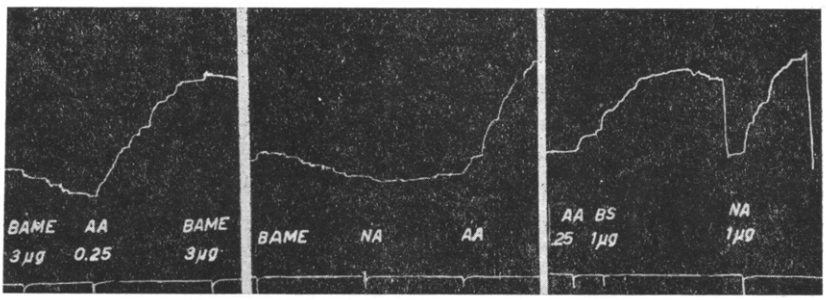

FIG. 6. Stimulation of an isolated strip of rabbit's aorta by angiotensinamide (AA) and noradrenaline (NA). Bradykinin (BS) did not alter the effect of AA nor that of NA (not shown in the figure). BAME blocked the effect of NA but not that of AA.

nor the synthetic material (BS) had any spasmolytic effect upon strips of rabbit's aorta whatever the cause of the spasm. Fig. 6 shows a typical experiment. We did not represent here the negative results with many preparations on the action of bradykinin upon th tonus of the aortic strips, but thought it more interesting to show the effect of benzoyl-L-arginine methyl ester (BAME) inhibiting the effect of noradrenaline but not that of angiotensinamide (first and second panels of Fig. 6).

Another pertinent fact about the difference in locus of action of bradykinin and angiotensin is derived from experiments by Fasciolo *et al.*[26] They have shown in certain preparations of angiotensin the presence of a vasodilating material, almost indistinguishable from bradykinin and

to which they have given the name of "substance V". I would not argue here about the identity of "substance V" and bradykinin, since both materials could not be distinguished from each other in any biological preparation; therefore any deduction about "substance V" would also be valid for bradykinin and vice-versa. But the interesting fact lies in the preparation used to study "substance V", namely, the intra-arterial injection into a perfused dog's paw. In such a preparation bradykinin, as "substance V", is very active in inducing vasodilatation and angiotensin is much less active in producing rise in blood pressure than it is when injected intravenously in the intact animal. The differences are so marked that the authors could, as a routine, use the perfused legs of the dog to study "substance V" in presence of angiotensin. The proposed interpretation that bradykinin or "substance V" would be preserved from destruction and their effects accentuated could not tell the whole story, since pure angiotensin, in equipotent doses induces considerably more effect when injected intravenously to the intact animal than by the intra-arterial route, to the perfused paw.

It seems reasonable to conclude that the *loci* of action of both principles, bradykinin and angiotensin, are entirely different and that the only possible antagonism between these principles upon the blood pressure would be of the physiological type. Angiotensin, according to the above evidence, would act predominantly by modifying the calibre of larger vessels, though bradykinin is a more specific dilator for the small arterial vessels, and possibly for the capillary bed. It is also interesting that the coronary vessels are no exception to the action of bradykinin, as in the perfused isolated heart bradykinin produces a powerful vasodilating effect, as shown in Fig. 5. The effects of angiotensin upon the isolated heart are somewhat variable. After an initial small decrease in flow, a late increase can take place, and for a certain range of doses, no effect could be detected. These experiments are being carried out in our laboratory by Dr. A. Antonio.[23]

This interpretation about the locus of action of bradykinin as residing in the peripheral small vessels and the capillary bed, is in agreement with the recent experiments done in humans, by Fox *et al.*,[12] which, I hope, will be further discussed in this Symposium. By the intra-arterial route, doses of 0·1 μg of pure bradykinin were already enough to increase blood flow in the hand and forearm of about 5–33ml/100ml/min. In such conditions, weight by weight, bradykinin was as potent as histamine and was 10–100 times more potent than acetylcholine. When injected intravenously, however, the amounts of bradykinin had to be increased much more than those of histamine and was as effective as acetylcholine, judging also from experiments by Konzett and Stürmer[11] in the intact rabbit. From these evidences one might draw the conclusion that

bradykinin might be a factor in the maintenance of the blood supply to tissues counteracting vaso-constrictor agents acting normally upon these areas. Since angiotensin appears to be acting in other areas of the circulatory system, we have to turn our views strongly to a possible interplay between bradykinin and other vaso-constrictor agents, and the next step should be to discuss any possible antagonism between bradykinin and catechol amines.

Bradykinin versus *Catechol Amines*

There seems to occur a still mysterious relationship between the actions and release of bradykinin and discharge of catechol amines. When injected intravenously, sympatholytic agents have a strong potentiating effect upon the vaso-dilation produced by bradykinin.[27] Not only dibenzyline (phenoxybenzamine), chlorpromazine and dibenamine increase the duration of the hypotensive effect of bradykinin, but also reserpine potentiates it, when given acutely half an hour before bradykinin. This potentiation could not be explained simply by a summation of the hypotensive effect of adrenaline, for instance, after the sympatholytics, with that of bradykinin, since the previous injection of DCI (dichloroisoproterenol) will not prevent the described potentiation of bradykinin by dibenzyline, for example, in spite of the fact that the inversion of adrenaline effects have been corrected by DCI. If there is any effect of DCI, it is rather in the same direction as that obtained with the mentioned sympatholytic agents. These experiments are still being continued in our laboratory by Drs. Antonio and Corrado. We still do not have a complete picture of the mechanism by which these agents potentiate bradykinin, but it seems obvious that to a certain extent the release of catechol amines by bradykinin would play a role in its pharmacodynamic effects, as has been recently demonstrated by Lecomte.[28]

But what seems even more interesting, is the possibility that catechol amines might activate the mechanism of release of bradykinin. It has been demonstrated that the release of bradykinin depends upon enzymes which display the specificity of trypsin, splitting a bond next to a basic amino acid, such as arginine or lysine. It does not seem essential that the releasing enzyme should have a proteolytic activity of the conventional type, since we can destroy completely the capacity of the venom of *Bothrops jararaca* to digest casein or plasma proteins, and still have its full capacity of releasing bradykinin.[15, 29, 30] However, it seems essential that the enzyme displays the capacity of rupturing the ester bond of BAME or TAME. This is quite important to know when one has to study the enzymatic factors which might be responsible for the release of bradykinin. Many enzymes splitting BAME or TAME can be found in the body. Among the best known is plasmin, but also thrombin, cathepsin II, and probably the protease in plasma responsible for a fast release of brady-

kinin, blocked by SBI, to which the Germans gave the name of plasma-kallikrein, are of the same type. Therefore, one might expect to find in any tissue or in the interstitial fluid, proteases which might release bradykinin.

We have recently[31] described a sort of oedema produced in the rat's paw by immersion in a water bath at 44–45°C, for 25–30 min. We have called this oedema, which strongly resembles the one produced by egg white or dextran, a "thermic oedema", to stress the fact that it is produced in a range of temperature (44–45°C), lower than that required to produce a "burn". We could say we are in a range of "quasi-physiological" temperatures, and surely such temperatures can be found in certain regions of the globe. The occurrence of marked changes in blood supply and capillary permeability at such temperatures, might induce one to think that the mechanism underlying such changes might be operative in the regulation of the peripheral circulation. Another reason induced us to give emphasis to the study of such an oedema. This "thermic oedema" was observed in the course of experiments to show the effect of a previous heating at 45°C on the ulterior release of histamine by perfusion with such a potent releaser as 48/80. We had shown[32] that this heat treatment applied to the intact rat, before starting the perfusion, prevented, partially or totally, the release of histamine by 48/80. Since the heat treatment was applied to the intact anaesthetized animal, and the subsequent experiment was done at 37°C, this finding would extend, under *in vivo* conditions, the observation by Mongar and Schild[33] that the anaphylactic release of histamine from slices of the guinea-pig lung is totally abolished when the fragments are previously kept for 30 min at 45°C, and also the findings of Högberg and Uvnäs[34] that the mast cells of the rat mesentery become stabilized against disruption provoked by 48/80, if previously maintained at 45°C for 20–30 min. Antonio[35] has also shown that the amounts of histamine contained in the paws are not significantly altered if the heated paw is compared with the non-heated one. Therefore, from the start, histamine and possibly also 5-HT, could be ruled out as potential mediators of that kind of oedema. It became urgent, however, to find out which endogenous substances might be concerned with the formation of the "thermic oedema 44–45°C" in the rat's paw.

In a series of preliminary tests we could exclude once more histamine and 5-HT (serotonin), since by treating the rats with a series of inhibitors such as anti-histaminics, anti-serotonin agents, such as LSD-25 and BOL-148 in doses which would counterbalance any effect of the mediators, no change in the intensity or severity of the oedema could be found. The data presented in Table IV summarize the experiments done with many different agents previously given to the animal before the heating treatment. So far, reserpine, when given twice, has been the best drug

to counteract to a large extent the "thermic oedema". Fig. 7 gives a picture of the reduction of the oedema induced by one or two treatments with reserpine. Also dibenzyline and less so, dibenamine and chlorpromazine, as shown in Table IV, significantly decreased the intensity of the oedema.

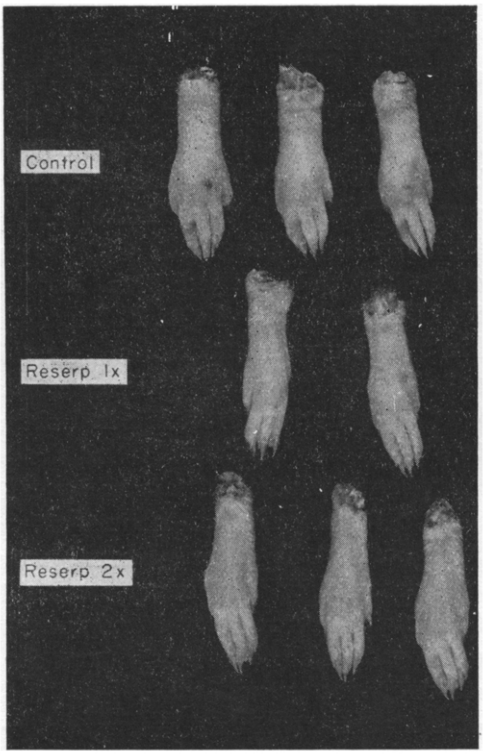

FIG. 7. "Thermic oedema" produced in the rat's paw by immersion in a water bath at 44-45°C for 25-30 min. Controls (above) and the paws of animals treated 1 × with reserpine (medium row) and 2 × with reserpine (lower row). According to Rocha e Silva and Antonio.[31]

These facts would point to the participation of catechol amines in the intimate mechanism of production of this oedema. However, the fact that no complete abolition could be obtained with such a potent sympatholytic agent as dibenzyline would indicate a rather subsidiary role of the amines and prompted us to look for a more specific capillary active endogenous agent to explain the plasma filtration leading to the local oedema observed. It has been demonstrated that bradykinin increases capillary permeability in the guinea-pig, and in our laboratory, Dr. A. Rothschild found that also the capillaries of the rat are extremely sensitive to the permeability increasing effect of bradykinin.

TABLE IV

Action of drugs on the formation of the "thermic oedema" in the rat's paw
According to Rocha e Silva and Antonio.[31]

Drugs*	Number of animals	Doses (mg/kg)	Per cent increases in weight (averages)	Test of significance†
Chlorpromazine	12	10, 20, 35	32·9; 39·2; 36·9	$P<0{\cdot}05>0{\cdot}01$
Dibenzyline	16	20	16·5	$P<0{\cdot}01$
Dibenamine	5	10	59·7	N.S.
Dibenamine	2	24	60·0	N.S.
Dibenamine	6	42	26·0	$P<0{\cdot}05>0{\cdot}01$
Priscol	2	20, 100	52·8; 51·1	N.S.
Serpasol	12	2×10	28·1	$P<0{\cdot}05>0{\cdot}01$
Controls	47	—	49·8; 51·7; 63·2	—
BOL-148	4	10	51·2	N.S.
Iproniazide	8	76,100,200	51·6; 56·3; 55·7	N.S.
Cocaine	6	40. 80	60·1; 62·7	N.S.
Controls	22	—	63·2; 51·7	—

Other drugs assayed, with no significant effect upon the intensity of the "thermic oedema": Phenergan (5 mg/kg); Neo-antergan (24 mg/kg); LSD-25 (10 mg/kg); atropine sulphate (250 mg/kg); sodium salicylate (50 mg/kg); Pendiomid (5 mg/kg); Andantol (24 mg/kg); Eupaverin (150 mg/kg); Neozine (24 mg/kg).

* With the exception of cocaine given around the thigh, by infiltration, all the drugs were given intraperitoneally, 30 min before the experiment. With serpasol, 2 treatments, 24 hr and 30 min before, were applied.

† The tests of significance were performed upon the differences of weight and not upon the percentages; therefore, the SE of the means are not indicated in the table.

To demonstrate the release of bradykinin under the conditions of production of the "thermic oedema", a new technique of *coaxial perfusion* was developed, as shown in Fig. 8. After anaesthetizing the animal, a polythene tubing of about 4 mm diameter was introduced under the skin through a small incision at the thigh and pulled forward with occasional insufflation of air, until it went across the ligaments of the tibio-tarsic articulation. A thinner polythene tubing of about 2 mm diameter was then introduced through the lumen of the larger one, until it protruded a few millimeters below the end. The thinner tubing was then connected with a reservoir of Tyrode through a serpentine immersed in the bath. After starting the perfusion, the larger tubing would serve as an outlet to the fluid bathing the area of oedema. The perfusion fluid was collected at a rate of 3–4 drops per minute into a test tube immersed in ice water. To regulate the temperature inside of the paw at the area of per-

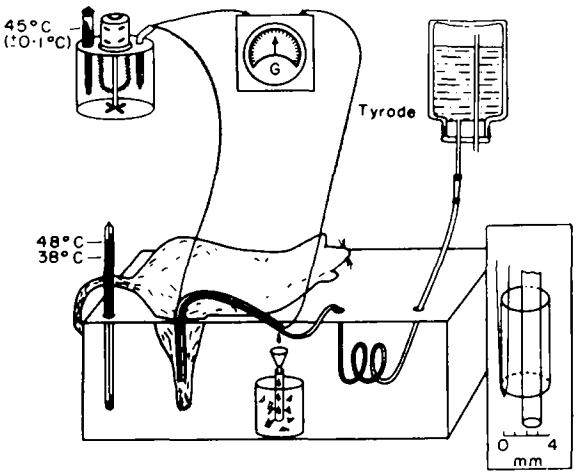

FIG. 8. Coaxial perfusion of the areas of the "thermic oedema" with two polythene tubings (details in the inset at the right corner). G, galvanometer connected with two thermocouples to indicate the temperature inside of the paw in reference to a precision thermostat (45°C ± 0·1°C). According to Rocha e Silva and Antonio.[31]

fusion and to correct for any differences in temperature outside and inside, two thermocouples were used as indicated in Fig. 8. When no current would flow through the galvanometr, it was an indication that the temperature inside was exactly that of the precision thermostate shown in the diagram. A calibration of the apparatus indicated that the inside temperature was about 1·2°C below the outside temperature, and such a correction is indicated in the diagrams of Fig. 9 and 10.

The perfusion fluid collected was immediately tested upon a rat uterus preparation set in a smal 1 ml smooth muscle chamber. The preparation was sensitive to 2–4 m u (1–2 n g) of synthetic bradykinin. In the perfusates collected at lower temperatures (37–40°C) only occasionally and when the introduction of the polythene tubing was laborious, some activity could be detected in the first samples. Thereafter, the control samples would remain inactive upon the rat's uterus, until the temperature of the outside bath reached 44–45°C, corresponding to an inside temperature of 43·8–44·8°C. Then, a definite release of bradykinin takes place and goes on until the temperature of the outside bath reaches 48–49°C, when there was a tendency of the activity to decrease in intensity. In parallel experiments upon the guinea-pig ileum, no histamine or 5-HT could be detected in that range of temperature. Only at 57°C the histamine started to be released. As shown in Fig. 9 and 10, there is a very sharp point (44–45°C) at which bradykinin starts to come out. There was also a good agreement on time-relation and the conditions for production of the oedema and the

release of bradykinin. Since we could exclude other possible mediators as histamine and serotonin, it appears that this kind of oedema is predominantly, if not exclusively, produced by the release of bradykinin. As for the identification of the released material with bradykinin, we have to

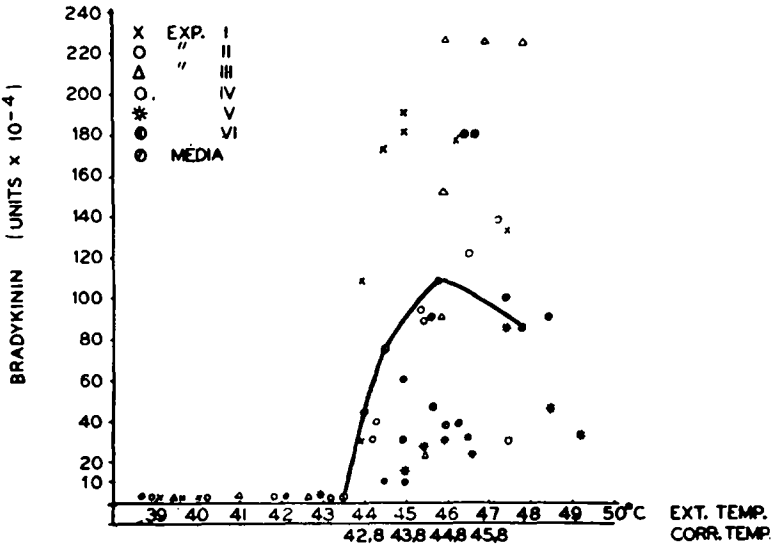

FIG. 9. Release of a uterus stimulating principle (bradykinin-like), estimated in units $\times$ 10^{-4} of bradykinin; the lower scale of temperature as corrected by the thermocouple device indicated in Fig. 8. Summary of 6 experiments of coaxial perfusion; the release takes place around 43°C (internal temperature). According to Rocha e Silva and Antonio.[31]

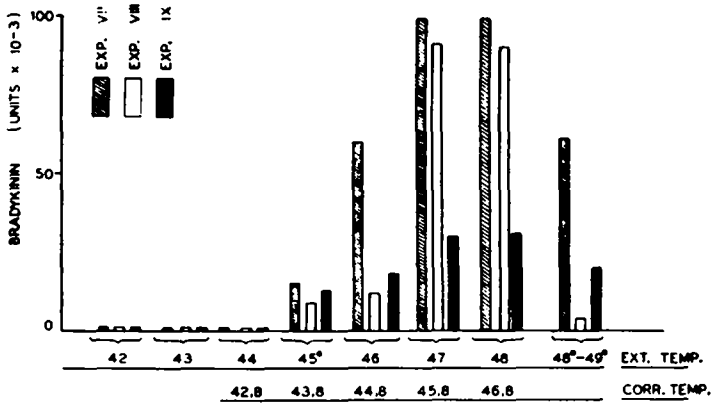

FIG. 10. Release of a uterus stimulating principle (bradykinin-like) by coaxial perfusion of an area of the "thermic oedema". The active material released is estimated in units $\times$ 10^{-3} of bradykinin. Summary of 3 experiments, showing again the same critical point of 43°C for the beginning of the release of bradykinin. According to Rocha e Silva and Antonio.[31]

say that is was destroyed by chymotrypsin, produced a fall in tonus of the rat intestine and was progressively inactivated if kep in the unboiled perfusates, at room temperature.

But how to reconcile the apparently contradictory facts that catechol amines appear to contribute to the formation of this kind of oedema, since sympatholytic agents decrease its intensity, and that bradykinin appears to be potentiated by sympatholytic agents when injected into the circulation. The explanation is that we have two entirely different situations here. When bradykinin is injected intravenously, catechol amines might be released and therefore sympatholytic agents have a potentiating effect, although when a local injury or stress is inflicted to the rat's paw, catechol amines might first be released and then start the process of bradykinin formation, by activating proteases which would act upon bradykininogen. A vicious circle, or maybe *two* vicious circles might be established under such conditions of local stress. By increasing permeability of the capillary walls more bradykininogen would cross the capillary barrier and more bradykinin would be released. In Fig. 11 we try to visualize the possibility of a double feed back mechanism working continuously in the formation of the "thermic oedema":

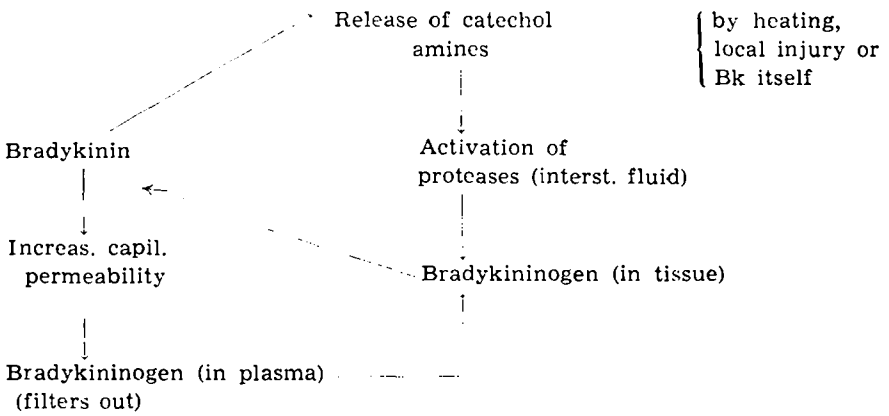

FIG. 11.

This vicious circle would start, possibly, by a release of catechol amines under the stress due to increased local temperature. Activation of proteolytic enzymes and proteases have been demonstrated under such conditions of stress.[36, 37, 38] These proteases acting upon tissue or interstitial fluid bradykininogen, could release bradykinin which, by affecting capillary permeability, might filter off more plasma proteins and therefore more bradykininogen to react with the proteases and more bradykinin would come into the picture.

I have to insist that these possibilities are only speculative at the moment.

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