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Bradykinin, Kallidin, and their Synthetic Analogues

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Introduction. Tissue hormones of polypeptide nature affecting smooth muscles and possessing vasoactive properties (hypo- and hypertensive) are called 'kinins' (kinin hormones) by ROCHA E SILVA[1–3]. These kinins are not secreted by specific glands but released from inactive precursors in blood plasma. Besides the angiotensins[4–6], compounds with hypotensive properties (properly called plasmakinins[7]), bradykinin, kallidin, and methionyl-lysyl-bradykinin have been chemically and pharmacologically characterized:

Bradykinin (kallidin I or kallidin-9)

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
1 2 3 4 5 6 7 8 9

Kallidin (kallidin II or kallidin-10)

H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
1 2 3 4 5 6 7 8 9 10

Methionyl-lysyl-bradykinin

H-Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
1 2 3 4 5 6 7 8 9 10 11

Apart from providing a brief summary of the most important pharmacological properties of the original kinins, this review will give a survey of the chemical modifications and the changes of biological activity connected therewith.

Isolation and structural investigations. Bradykinin, which was first described in 1949 by ROCHA E SILVA et al.[8] as a hypotensive substance stimulating smooth muscles, is released by the action of either snake venoms (*Bothrops jararaca*) or trypsin on plasma proteins. Isolation and structural clarification were achieved by ELLIOTT et al.[9–12] in conjunction with synthetic experiments carried out by BOISSONNAS et al.[13–15].

According to WERLE et al.[16] kallidin is obtained by the incubation of bovine serum with trypsin-free submaxillaris-kallikrein. The same authors also succeeded in elucidating the structure of kallidin. PIERCE and WEBSTER[17,18] established the same structure for a decapeptide released by incubating acid-treated human plasma with urinary kallikrein (human).

Methionyl-lysyl-bradykinin could be isolated by ELLIOTT et al.[19] after incubating an acid-treated pseudo-globulinfraction of bovine plasma at pH 7.5. The same authors were also able to determine its undecapeptide structure.

Formation and degradation. The mechanism of kinin formation from inactive precursors present in the α_2-globulin fraction of plasma in an enzymatic way is not clear as yet[20–23]. Several laboratories are working on the isolation of kininogenes[23–31].

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Kallidin and methionyl-lysyl-bradykinin are converted to bradykinin by aminopeptidases as well as by trypsin[18,19,32]. The C-terminal arginin is first removed by chymotrypsin in all three kinins and subsequently the phenylalanyl-seryl bond is split[11,13,33]. The C-terminal arginin and phenylalanin residues are removed by carboxypeptidases[11,13,33–36]. Specific inhibitors have not so far been ascertained[37–42].

Pharmacological activities. Apart from the chemical characteristics, extensive qualitative and quantitative pharmacological examinations with synthetic and natural bradykinin (trypsin- and snake venom-bradykinin) revealed the identity of all three products (however, see recent examinations by PEREIRA[43,44]). The activities of bradykinin may be briefly outlined as follows[3,21,45–51], concerning effects on smooth muscles, circulation, capillary permeability and local effects on tissues (pain and oedema).

The intravenous LD_{50} of synthetic bradykinin for white mice amounts to 16–20 mg/kg[45]. However, experiments of our own revealed that even quantities up to 70 mg/kg (injection time 1 min) are still tolerated.

An *in vitro* contracting action of bradykinin on the ileum of guinea-pigs, rats, rabbits, cats, and dogs has been described. The activity is exclusively myotropic[52]. Apparently only the longitudinal muscles are contracted[53]. The threshold dose for the guinea-pig ileum amounts to 1 ng/ml[45]. Some smooth muscle preparations like the rabbit duodenum and the rat colon are relaxed by doses of 1 ng/ml and then contracted by bradykinin[54,55]. The rat duodenum responds with relaxation only[54,56].

The rat uterus is the most sensitive organ with regard to bradykinin. The threshold dose is between 0.03 and 0.1 ng/ml[45,54,57]. The isolated human myometrium is influenced by very high doses only[58,59]. *In situ*, the uteri are less sensitive.

The isolated bronchial muscles of dog, rabbit, and man are not affected by bradykinin[60]. Intravenous doses of up to 0.2 μg/kg bradykinin, however, cause bronchoconstriction in urethan-anaesthetized guinea-pigs[54,55,61,62]. Bradykinin when inhaled from aerosols has a strong bronchoconstrictory effect in asthmatics[63].

No increased depletion of milk could be observed in lactating rabbits after intravenous administration of bradykinin in doses between 0.01 and 10.0 μg/animal[64].

Bradykinin causes a brief decrease in arterial blood pressure in all the mammals tested as well as in man and hen[54–56,65,66] (threshold doses: rabbits 0.05 μg/kg, dog and guinea-pig 0.2 μg/kg, hen 40–80 μg/kg). Pressor effects are frequently observed in nephrectomized or intact rats with subnormal blood pressure[67].

Unanaesthetized dogs with an exposed loop of the carotis communis and carotis interna showed an increased blood flow in both these spheres after intravenous (10–80 μg) or intra-arterial (5–20 μg) administration of bradykinin[68]. By means of registration of the intra-cranial venous pressure and the intra-cranial venous blood flow, a dilatation of the cerebral vessels[69] could be observed in dogs under chloralose-anaesthesia after the intravenous administration of 0.5–3 μg/kg.

The isolated hearts of guinea-pigs (threshold doses 0.1–1 ng/ml[70]), rabbits, cats, dogs, and rats respond to bradykinin with a considerably increased coronary flow. Spiral strips of the coronary arteries of sheep respond to 0.001–0.1 μg/ml[71].

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In the perfused lungs of dogs, a dilatation of the vessels and a dropping of the resistance of the pulmonary artery could be observed[72,73] after the injection of 0.5–5 μg/kg bradykinin.

After 5 μg/kg bradykinin unanaesthetized and unrestrained dogs showed an increased heart frequency and cardiac output and a decreased peripheral resistance of the vessels[74,75] in the course of hypotension. Intravenous doses of 1–8 μg/kg bradykinin cause a considerable but only briefly increased rate of respiration in guinea-pigs and rabbits under urethan-anaesthesia. High doses (20–40 μg/kg i.v.) cause a cessation of breathing in guinea-pigs lasting for 10–30 sec[76,77].

In man, bradykinin causes a dilatation of the peripheral arteries and thus diminution of the systolic and diastolic pressure, increase of the cardiac output and the pulse rate[68,78,79]. Flushing in the face, a feeling of oppressed respiration, palpitation, and a chill or feeling of heat over the whole body are observed after intravenous administration. The haemodynamics of the kidneys (0.07 μg/kg per min) are influenced by bradykinin. The considerable hypotension after high doses causes a decrease of the diuresis[80,81].

The capillary permeability in guinea-pigs is increased by intracutaneous injection of 0.001–0.01 μg/0.1 ml[54–56,65,82]. After subplantar injection (rat, 1–100 μg/0.1 ml) a dose-dependent oedema develops at the paw within 30–60 min[83,84].

Concerning the physiological role of kinins, see [2, 21, 45, 85–90].

Kallidin does not differ qualitatively from bradykinin in its pharmacological properties. Quantitative differences could, however, be found in different assays by STÜRMER and BERDE[91–94]. As an example, kallidin possesses one-third of the bradykinin activity in the guinea-pig ileum and ca. two-thirds in the rat uterus. Kallidin is three times more active than bradykinin on the blood pressure of the rat and twice as active on the blood pressure of the rabbit.

Methionyl-lysyl-bradykinin has about one-quarter the activity of bradykinin on the isolated ileum of guinea-pigs, the rat uterus, colon, and duodenum, as well as on bronchoconstriction in guinea-pigs, vasodilatation in cats and pain production in man[19]. A quantitative comparison of the synthetic methionyl-lysyl-bradykinin and bradykinin on the blood pressure of rabbits revealed that the undecapeptide has twice the activity of bradykinin[95].

Synthesis. BOISSONNAS et al.[13] (Figure 1) succeeded in carrying out the first synthesis of bradykinin with complete biological activity. Other syntheses were described by GUTTMANN et al.[96] (Figure 2), NICOLAIDES and DEWALD[97] (Figure 3), and afterwards by SIEMION[98] (Figure 4). The syntheses of kallidin which were carried out by PLESS et al.[99], NICOLAIDES et al.[100], and SCHRÖDER and GIBIAN[101] respectively, can be seen from Figures 5, 6, 7, and 8.

Methionyl-lysyl-bradykinin has been prepared by SCHRÖDER[95] from tert.-butyloxycarbonyl-L-methionyl-N^{ε}-tert.-butyloxycarbonyl-L-lysinhydrazide and bradykininhydrochloride, and removal of the protecting groups with trifluoracetic acid.

Relations between chemical structure and biological activity. Particularly BODANSZKY et al.[102], NICOLAIDES et al.[103], and SCHRÖDER[104] have synthesized numerous analogues of bradykinin and kallidin in an attempt to discover relationships between structure and activity. The compounds listed in Table I have been subdivided into 'analogues by modified functional groups', 'analogues by replacement of amino acids', 'analogues by shortening of the peptide chain' and 'aminoacyl-bradykinins (kallidin analogues)'. Since different test objects (rat uterus, guinea-pig ileum, blood pressure of rat, guinea-pig, or rabbit, and bronchoconstriction of guinea-pig) were employed by

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the different research groups, a comparison is sometimes possible to a limited degree only.

It has been shown in biologically active polypeptides of which many synthetic analogues are so far known (angiotensintype[4–6], oxytocin-vasopressintype[105, 106], bradykinin-kallidin-type), that the change of the biological activity, which accompanies the replacement of amino acids in a single position, is highly dependent upon the character of the amino acid being exchanged.

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Syntheses of bradykinin

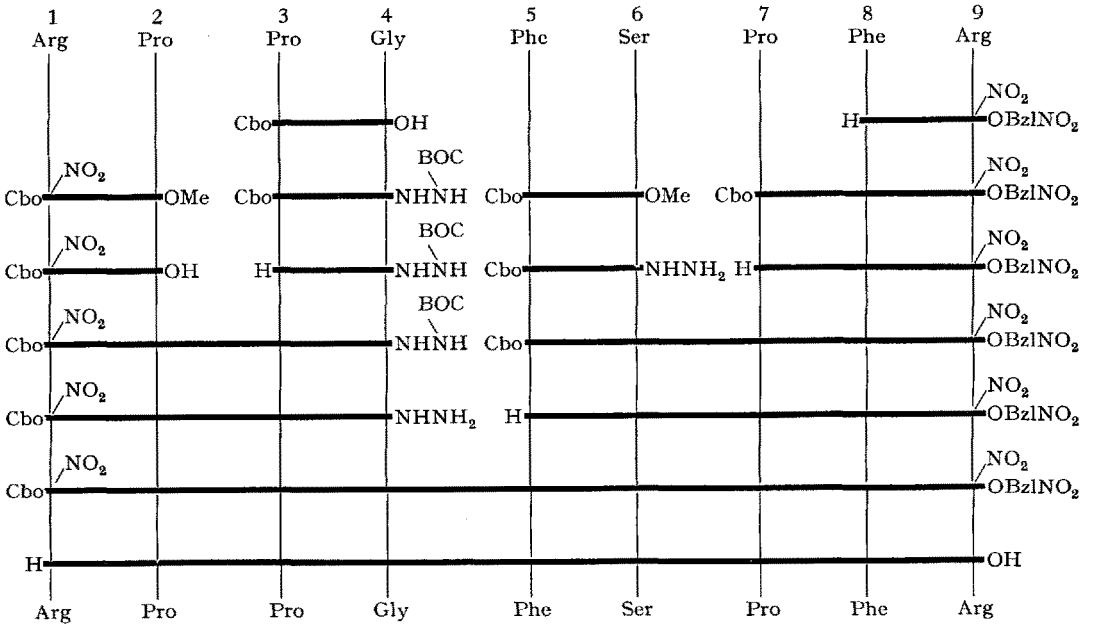

Fig. 1. BOISSONNAS et al.[13]

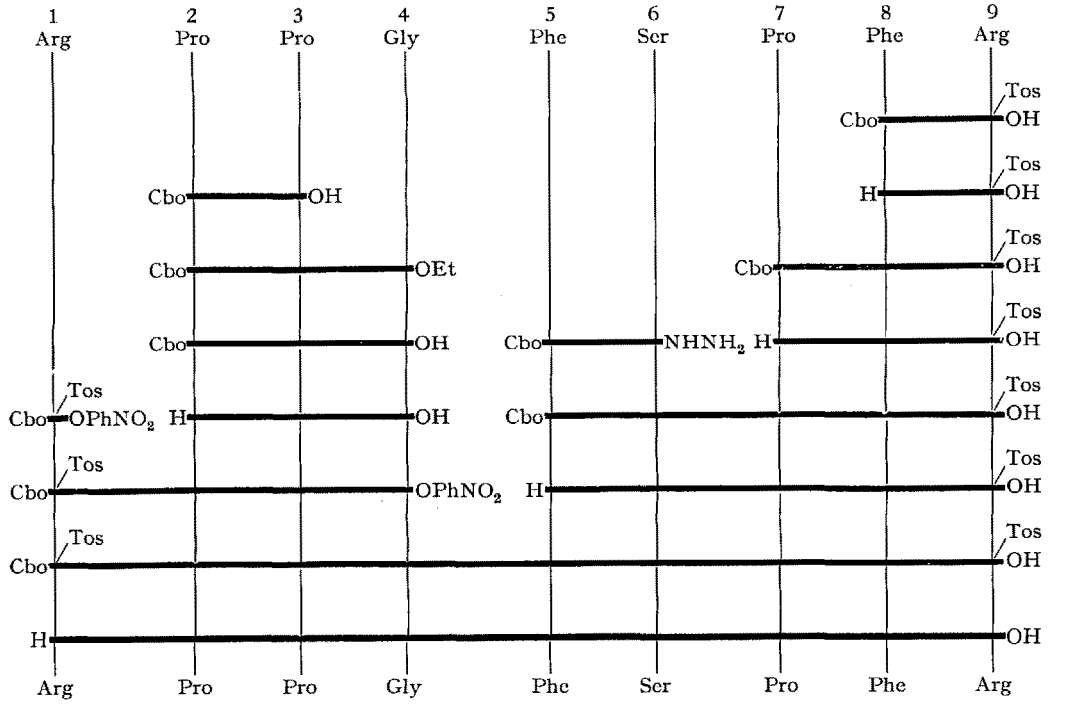

Fig. 2. GUTTMANN et al.[96]

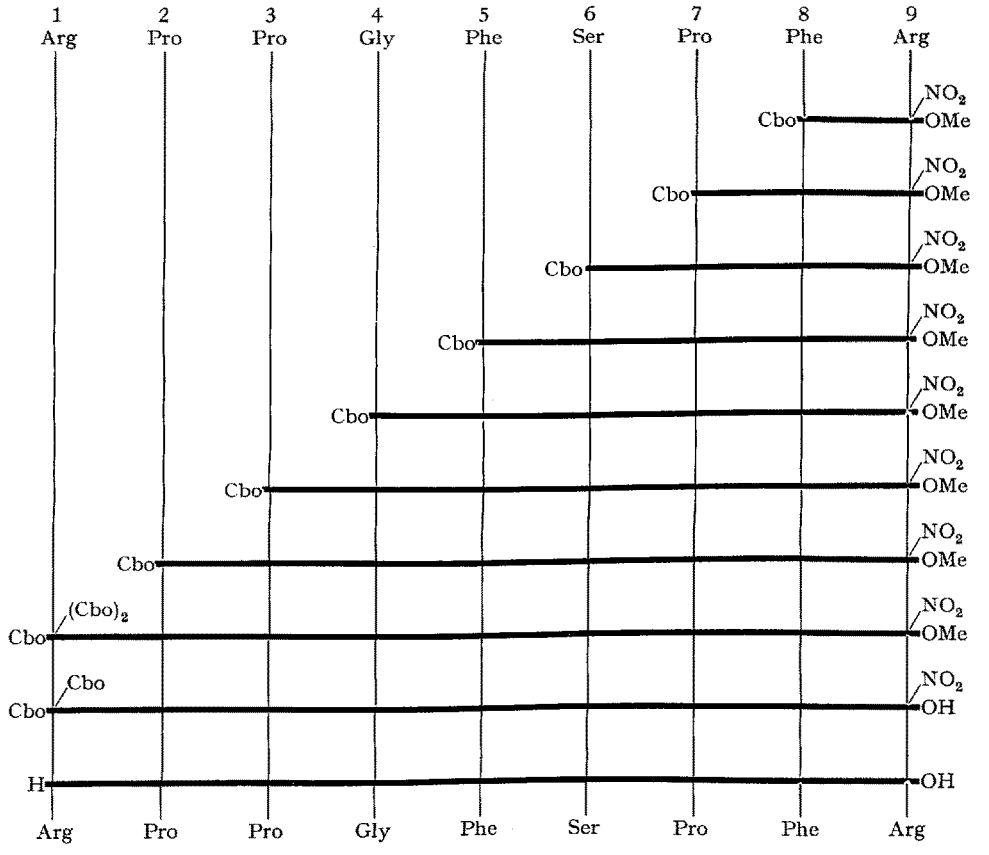

Fig. 3. NICOLAIDES and DEWALD[97]

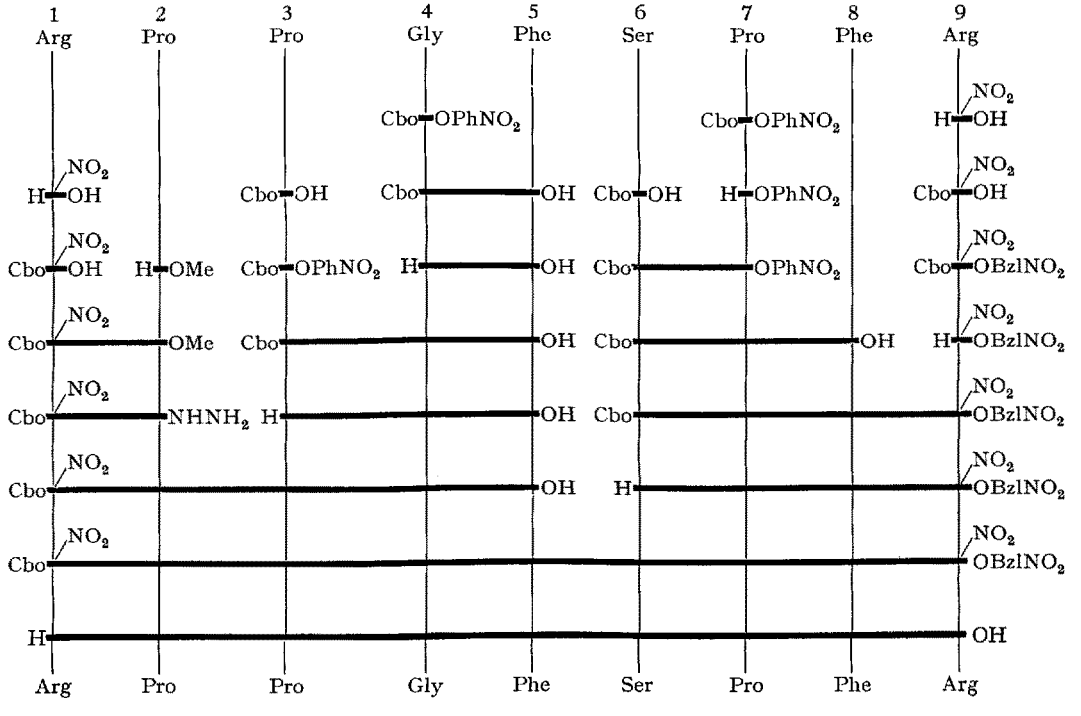

Fig. 4. SIEMION[98]

Syntheses of kallidin

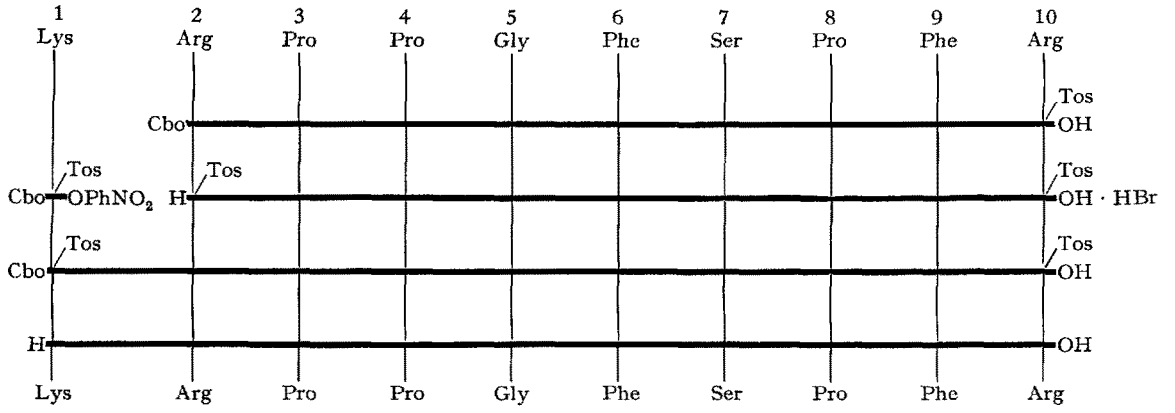

Fig. 5. PLESS et al.[99]

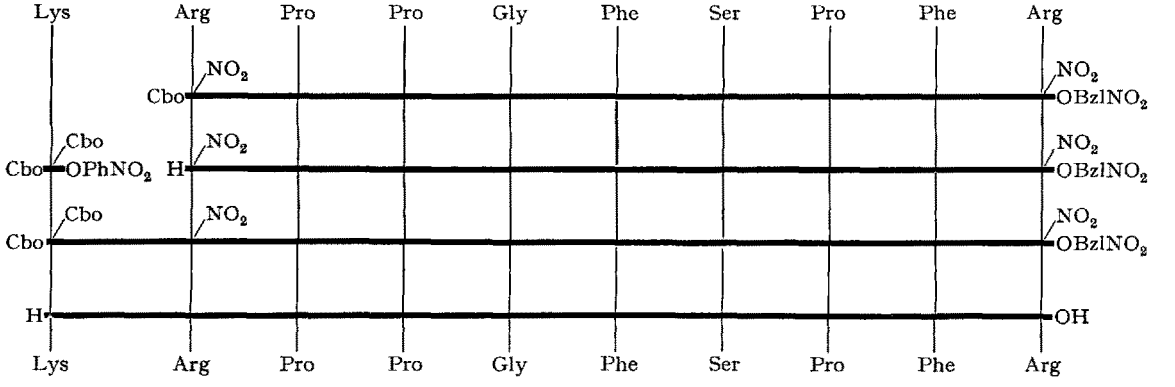

Fig. 6. PLESS et al.[99]

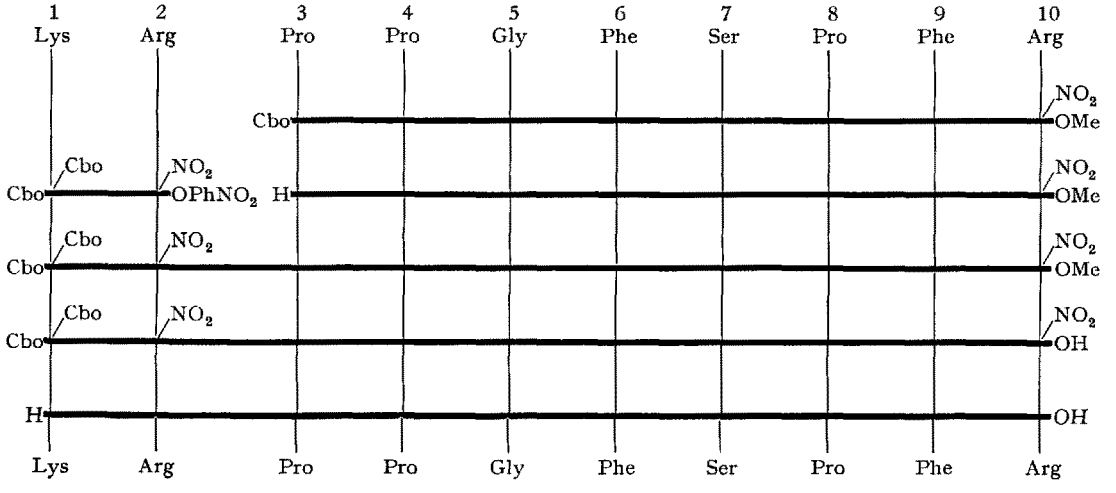

Fig. 7. NICOLAIDES et al.[100]

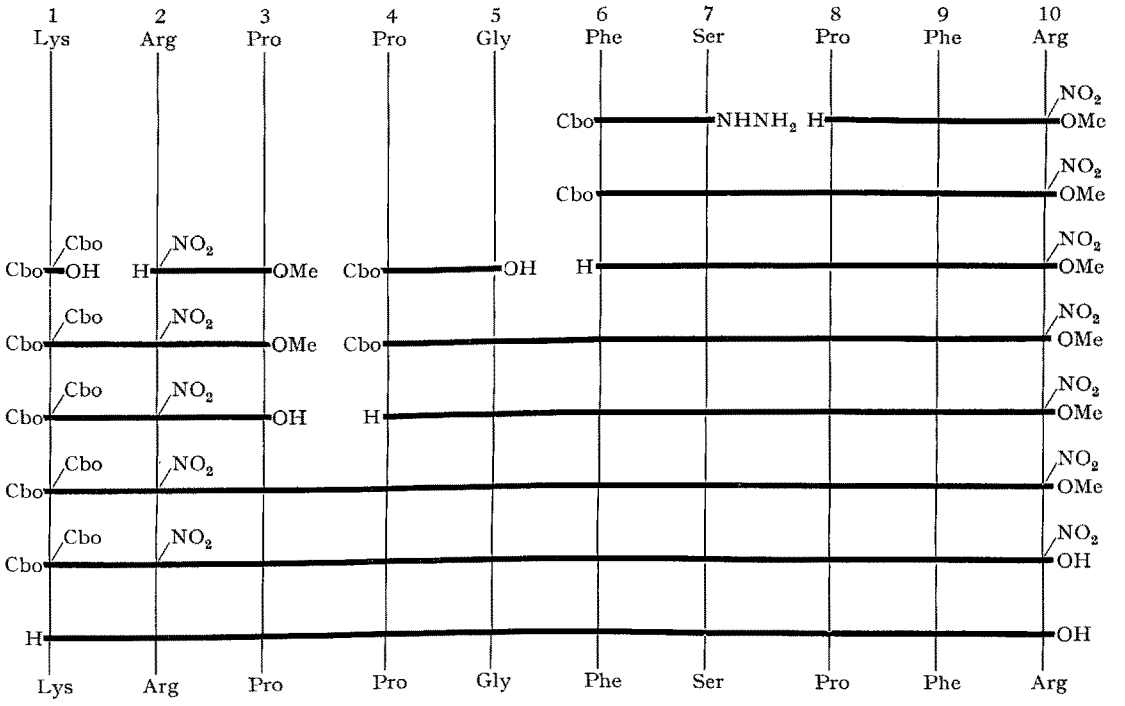

Fig. 8. SCHRÖDER and GIBIAN [101]

Depending upon the amino acid used, both ineffective and also highly effective analogues may result from a change in one single position (e.g. the inactive tyr^3-oxytoxin or the active val^3-oxytocin). Therefore, the different positions cannot in every case be compared *per se* with regard to their importance for biological activity.

For modifications of biologically active polypeptides by replacing amino acids, the following principles were taken into consideration: exchange of amino acids related in structure [e.g. Arg/Cit, Ser/Thr, Lys/Orn, Glu/Asp, Glu(NH_2)/Asp(NH_2), Ileu/Leu/Val], or related in polarity (e.g. Arg/Lys/Orn, Glu/Asp). Furthermore, exchanges were made of complementarily charged amino acids [e.g. Lys/Glu], or by the omission or substitution of functional groups [e.g. Ser/Ala, Tyr/Phe, Lys/Lys(Form), Arg/Arg(NO_2), Glu/Glu(NH_2)]. Finally it was attempted to detect an influence of the length of the chain by shortening or lengthening the peptide chain at the N- or C-terminal or in the middle, respectively. The information available on principles of the primary structure of polypeptides and proteins is also frequently used as a guide for the experimental exchange of amino acids. In this respect, the fact may be mentioned that frequently only one or a few amino acids are exchanged in the amino acid sequences of polypeptides or proteins of functional equality. This refers also to polypeptides and proteins of the same organs of different species and possibly even only of different individuals. Furthermore, changes of the position, insertions or omissions of amino acids occur[107–109].

For the first time in the case of the bradykinin molecule, it was attempted to replace one by one the position by a single amino acid (alanin)[104,110]. The result of this systematic replacement is shown in Figure 9. Based on additional results with replacement by other amino acids, the following relationship of structure-activity is obtained: the bradykinin analogues are randomly divided into three groups: effective, medium effective and slightly effective (i.e. practically inactive) analogues with 1–1/50 or 1/50–1/500 and $<1/500$ of the original bradykinin activity.

A replacement of amino acids by alanin in the positions 1, 5, 7, 8 and 9 (compounds III, XIX, XXX, XXXIV, XLII) causes a decrease of activity to 1/1000 or less compared with bradykinin on the blood pressure of rabbits, i.e. inactive derivatives are obtained. Analogues such as Gly^1-bradykinin (VI), Gly^9-bradykinin (XLIV), Asp(NH_2)^7-bradykinin (XXXI) and His^7-bradykinin (XXXIII) are also ineffective, and confirm the importance of positions 1, 7, and 9. In the case of Ala^7- a considerably lower loss of activity (1/100) is noted with smooth muscle preparations [see

[107] H. GIBIAN, Z. Naturforsch. *16b*, 18 (1960).
[108] F. ŠORM, Coll. Czech. Chem. Commun. *27*, 1604 (1962); *26*, 1180 (1961); *24*, 3169 (1959).
[109] B. KEIL and F. ŠORM, Coll. Czech. Chem. Commun. *27*, 1310 (1962).
[110] E. SCHRÖDER, Liebigs Ann., in press.

Table I

Nr.	Variation in position	Substance	Amino acid sequences
		Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH 1 2 3 4 5 6 7 8 9
			(α) Bradykinin analogues with modified functional groups
I	1+9	Arg(NO_2)[1]-Arg(NO_2)[9]-Bradykinin	H-*Arg*(NO_2)-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Arg*(NO_2)-OH
II	6	(O-Carbamyl-L-Ser)[6]-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-*Ser*($CONH_2$)-Pro-Phe-Arg-OH
			(β) Bradykinin analogues by replacing of amino acid
III	1	Ala[1]-Bradykinin	H-*Ala*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
IV	1	Cit[1]-Bradykinin	H-*Cit*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
V	1	Glu[1]-Bradykinin	H-*Glu*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
VI	1	Gly[1]-Bradykinin	H-*Gly*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
VII	1	Lys[1]-Bradykinin	H-*Lys*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
VIII	1	Orn[1]-Bradykinin	H-*Orn*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
IX	2	Ala[2]-Bradykinin	H-Arg-*Ala*-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
X	2	Val[2]-Bradykinin	H-Arg-*Val*-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
XI	1+2	Pro[1]-Arg[2]-Bradykinin	H-*Pro-Arg*-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
XII	3	Ala[3]-Bradykinin	H-Arg-Pro-*Ala*-Gly-Phe-Ser-Pro-Phe-Arg-OH
XIII	3	Pip[3]-Bradykinin	H-Arg-Pro-*Pip*-Gly-Phe-Ser-Pro-Phe-Arg-OH
XIV	3	Val[3]-Bradykinin	H-Arg-Pro-*Val*-Gly-Phe-Ser-Pro-Phe-Arg-OH
XV	4	Ala[4]-Bradykinin	H-Arg-Pro-Pro-*Ala*-Phe-Ser-Pro-Phe-Arg-OH
XVI	4	Pro[4]-Bradykinin	H-Arg-Pro-Pro-*Pro*-Phe-Ser-Pro-Phe-Arg-OH
XVII	4	Sar[4]-Bradykinin	H-Arg-Pro-Pro-*Sar*-Phe-Ser-Pro-Phe-Arg-OH
XVIII	4	Ser[4]-Bradykinin	H-Arg-Pro-Pro-*Ser*-Phe-Ser-Pro-Phe-Arg-OH
XIX	5	Ala[5]-Bradykinin	H-Arg-Pro-Pro-Gly-*Ala*-Ser-Pro-Phe-Arg-OH
XX	5	Tyr[5]-Bradykinin	H-Arg-Pro-Pro-Gly-*Tyr*-Ser-Pro-Phe-Arg-OH
XXI	6	Ala[6]-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-*Ala*-Pro-Phe-Arg-OH
XXII	6	Gly[6]-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-*Gly*-Pro-Phe-Arg-OH
XXIII	6	Sar[6]-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-*Sar*-Pro-Phe-Arg-OH
XXIV	6	D-Ser[6]-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-D-*Ser*-Pro-Phe-Arg-OH
XXV	6	Thr[6]-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-*Thr*-Pro-Phe-Arg-OH
XXVI	3+6	Ala[3]-Gly[6]-Bradykinin	H-Arg-Pro-*Ala*-Gly-Phe-*Gly*-Pro-Phe-Arg-OH
XXVII	4+6	Ser[4]-Gly[6]-Bradykinin	H-Arg-Pro-Pro-*Ser*-Phe-*Gly*-Pro-Phe-Arg-OH
XXVIII	5+6	Ala[5]-Gly[6]-Bradykinin	H-Arg-Pro-Pro-Gly-*Ala-Gly*-Pro-Phe-Arg-OH
XXIX	5+6	Pro[5]-Gly[6]-Bradykinin	H-Arg-Pro-Pro-Gly-*Pro-Gly*-Pro-Phe-Arg-OH

Table I

Biological activities					References
Rat uterus (isolated)	Guinea-pig ileum (isolated)	Rabbit blood pressure	Guinea-pig blood pressure	Guinea-pig broncho-constriction	
$1 \cdot 10^{-10}$ g/ml = 1	$1 \cdot 10^{-9}$ g/ml = 1	$5 \cdot 10^{-8}$ g/kg = 1	$2 \cdot 10^{-7}$ g/kg = 1	$2 \cdot 10^{-7}$ g/ml = 1	CERLETTI et al.[45] NICOLAIDES and DEWALD[97] BODANSZKY et al.[57]
1/100	—	—	—	—	BODANSZKY et al.[57, 102]
—	—	—	—	< 1/2000	NICOLAIDES et al.[103]
—	—	—	1/75	< 1/500	DEWALD et al.[111]
< 1/1500	< 1/1500	~ 1/1000	—	—	SCHRÖDER[104, 110]
1/600	—	—	—	—	BODANSZKY et al.[57, 102]
1/400	—	—	≤ 1/100 (rat)	—	ONDETTI[112]
—	—	—	1/250	< 1/2000	NICOLAIDES et al.[103, 113]
—	—	—	1/300	< 1/2000	NICOLAIDES et al.[113]
< 1/1500	—	< 1/1000	—	—	SCHRÖDER[104]
—	—	—	1/10	1/62	NICOLAIDES et al.[103, 113]
1/500	1/150–1/300	1/250	—	—	SCHRÖDER[104, 114]
—	—	—	1/50	1/1000	NICOLAIDES et al.[103, 113]
1/300	1/150	1/200	—	—	SCHRÖDER[104, 110]
1/100	1/500	1/200	—	—	SCHRÖDER (unpublished)
~ 1/500	~ 1/1500	~ 1/1500	—	—	SCHRÖDER et al.[104, 115]
1	1	1	—	—	SCHRÖDER[104, 110]
—	—	—	1/2000	< 1/2000	NICOLAIDES et al.[103]
—	~ 1/1500	1/125	—	—	SCHRÖDER (unpublished)
< 1/1500	< 1/1500	1/300	—	—	SCHRÖDER et al.[115] SCHRÖDER[104]
—	< 1/800	~ 1/200	—	—	SCHRÖDER (unpublished)
~ 1/800	1/500	1/125			SCHRÖDER (unpublished)
—	< 1/800	~ 1/200	—	—	SCHRÖDER (unpublished)
1/1000	< 1/1500	~ 1/1000	—	—	SCHRÖDER[104, 116]
1/500	1/150	1/300	—	—	SCHRÖDER[104] SCHRÖDER et al.[115]
—	—	—	—	—	NOWAK[117]
1/10	1/5	1/10	—	—	SCHRÖDER[104, 110]
1	—	—	—	—	BODANSZKY et al.[102, 118]
1/10	1/3–1	1/2–1	—	—	SCHRÖDER[104, 116]
< 1/1500	1/1500	1/1000	—	—	SCHRÖDER[104]
—	—	—	1/2,5	1/40	NICOLAIDES et al.[103] DEWALD et al.[111]
—	—	—	1/2	< 1/500	DEWALD et al.[111]
1	1/3	1/10	—	—	SCHRÖDER (unpublished)
1/50	1/5	1/6	—	—	SCHRÖDER (unpublished)
—	1/100	1/100	—	—	SCHRÖDER et al.[115] SCHRÖDER[104]
—	< 1/1500	1/200	—	—	SCHRÖDER (unpublished)
—	—	< 1/1000	—	—	SCHRÖDER (unpublished)

Nr.	Variation in position	Substance	Amino acid sequences
XXX	7	Ala^{7}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-*Ala*-Phe-Arg-OH
XXXI	7	Asp(NH$_2$)^{7}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-^{NH_2}*Asp*-Phe-Arg-OH
XXXII	7	Gly^{7}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-*Gly*-Phe-Arg-OH
XXXIII	7	His^{7}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-*His*-Phe-Arg-OH
XXXIV	8	Ala^{8}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-*Ala*-Arg-OH
XXXV	8	D-Phe^{8}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-D-*Phe*-Arg-OH
XXXVI	8	Phe(F)^{8}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-^{F}*Phe*-Arg-OH
XXXVII	8	D-Phe(F)^{8}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-D-^{F}*Phe*-Arg-OH
XXXVIII	2+4+6+8	Phe^{2}-Ser^{4}-Gly^{6}-Pro^{8}-Bradykinin «Retrobradykinin»	H-Arg-*Phe*-Pro-*Ser*-Phe-*Gly*-Pro-*Pro*-Arg-OH
XXXIX	2–8	Arg-heptagly-Arg	H-Arg-*Gly-Gly-Gly-Gly-Gly-Gly-Gly*-Arg-OH
XL	5+6+8	Leu^{5}-Thr^{6}-Leu^{8}-Bradykinin	H-Arg-Pro-Pro-Gly-*Leu-Thr*-Pro-*Leu*-Arg-OH
XLI	6+8	Thr^{6}-Leu^{8}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-*Thr*-Pro-*Leu*-Arg-OH
XLII	9	Ala^{9}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Ala*-OH
XLIII	9	Cit^{9}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Cit*-OH
XLIV	9	Gly^{9}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Gly*-OH
XLV	9	His^{9}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*His*-OH
XLVI	9	Lys^{9}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Lys*-OH
XLVII	9	Orn^{9}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Orn*-OH
XLVIII	1+9	Cit^{1}-Cit^{9}-Bradykinin	H-*Cit*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Cit*-OH
XLIX	1+9	Dab^{1}-Dab^{9}-Bradykinin	H-*Dab*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Dab*-OH
L	1+9	Lys^{1}-Lys^{9}-Bradykinin	H-*Lys*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Lys*-OH
LI	1+9	Lys^{1}-Lys(BOC)^{9}-Bradykinin	H-*Lys*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-^{BOC}*Lys*-OH
LII	7+8+9	Phe^{7}-Arg^{8}-Pro^{9}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-*Phe-Arg-Pro*-OH
			(γ) Bradykinin analogues by shortening the peptide chain
LIII	1	Des-(Arg)^{1}-Bradykinin	H-[ ]-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
LIV	3	Des-(Pro)^{3}-Bradykinin	H-Arg-Pro-[ ]-Gly-Phe-Ser-Pro-Phe-Arg-OH
LV	7	Des-(Pro)^{7}-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-[ ]-Phe-Arg-OH
LVI	3+4+7	Des-(Pro^{7})-Gly^{3}-Pro^{4}-Bradykinin	H-Arg-Pro-*Gly-Pro*-Phe-Ser-[ ]-Phe-Arg-OH
LVII	4+5+7	Des-(Pro^{7})-Phe^{4}-Gly^{5}-Bradykinin	H-Arg-Pro-Pro-*Phe-Gly*-Ser-[ ]-Phe-Arg-OH
LVIII	3+7	Des-(Pro^{3}-Pro^{7})-Bradykinin	H-Arg-Pro-[ ]-Gly-Phe-Ser-[ ]-Phe-Arg-OH

Biological activities					References
Rat uterus (isolated)	Guinea-pig ileum (isolated)	Rabbit blood pressure	Guinea-pig blood-pressure	Guinea-pig broncho-constriction	
1/80	1/100	< 1/1000	—	—	SCHRÖDER [104, 110]
—	—	—	1/1000	0	NICOLAIDES et al. [103]
1/100	—	—	—	—	BODANSZKY et al. [102, 118]
—	< 1/1500	~ 1/1000	—	—	SCHRÖDER et al. [115]
~ 1/1500	~ 1/1000	~ 1/1000	—	—	SCHRÖDER [104,110]
—	—	—	1/4	1/60	NICOLAIDES et al. [103]
—	—	—	1/4	1/20	NICOLAIDES et al. [119]
—	—	—	1,5	1,4	NICOLAIDES et al. [103]
—	—	—	1,5	1,4	NICOLAIDES et al. [119]
—	—	—	1/3	1/100	NICOLAIDES et al. [119]
inactive up to 10^{-5}	—	—	—	—	VOGLER et al. [120]
inactive up to $8 \cdot 10^{-5}$	—	—	—	—	LANDE [121]
< 1/5000	—	—	—	—	BODANSZKY et al. [102]
0	—	—	—	—	BODANSZKY et al. [102, 118]
slight activity	—	—	—	—	STEWART [122]
Bradykinin-like activity, in small doses anti	—	—	—	—	STEWART [122]
1/500	< 1/800	< 1/1000	—	—	SCHRÖDER [104, 110]
1/400	—	—	—	—	BODANSZKY et al. [57, 102]
1/400	—	—	⩽ 1/100 (rat)	—	ONDETTI [112]
—	—	—	1/20	1/1000	NICOLAIDES et al. [103] DEWALD and NICOLAIDES [123]
< 1/1500	< 1/1500	~ 1/1000	—	—	SCHRÖDER [104]
—	—	—	1/20	1/2000	NICOLAIDES et al. [103]
—	—	—	1/20	< 1/2000	DEWALD and NICOLAIDES [123]
—	~ 1/500	~ 1/1000	—	—	SCHRÖDER (unpublished)
1/500	1/5	1/10–1/30	—	—	SCHRÖDER [104, 114]
—	1/100	1/400	—	—	SCHRÖDER et al. [115]
< 1/5000	—	—	—	—	BODANSZKY et al. [57, 102]
< 1/5000	—	—	< 1/100 (rat)	—	ONDETTI [112]
inactive up to 10^{-4}	—	—	—	—	VOGLER et al. [124]
< 1/1500	< 1/1500	1/500	—	—	SCHRÖDER [104, 114]
< 1/1500	< 1/1500	< 1/1000	—	—	SCHRÖDER [104, 114]
< 1/300	1/150	< 1/1000	—	—	SCHRÖDER [104]
—	—	—	1/80	< 1/2000	NICOLAIDES et al. [103, 113]
1/20	1/100	—	—	—	GUTTMANN and BOISSONNAS [15]
inactive up to 10^{-5}	inactive up to 10^{-5}	—	—	—	BOISSONNAS et al. [14] SCHWYZER et al. [125] NICOLAIDES et al. [126]
inactive up to 10^{-5}	inactive up to 10^{-5}	—	—	—	GUTTMANN and BOISSONNAS [15]
inactive up to 10^{-5}	inactive up to 10^{-5}	—	—	—	GUTTMANN and BOISSONNAS [15]
—	inactive up to $2{,}6 \cdot 10^{-6}$	—	—	—	GUTTMANN and BOISSONNAS [15]

Nr.	Variation in position	Substance	Amino acid sequences
LIX	9	Des-(Arg^{9})-Bradykinin	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-[ ]-OH
LX	1+9	Des-(Arg^{1}-Arg^{9})-Bradykinin	H-[ ]-Pro-Pro-Gly-Phe-Ser-Pro-Phe-[ ]-OH
LXI	1+5+6+8	Des-(Arg^{1})-Leu^{5}-Thr^{6}-Leu^{8}-Bradykinin	H-[ ]-Pro-Pro-Gly-*Leu-Thr*-Pro-*Leu*-Arg-OH
			(δ) *Bradykinin analogues by lengthening the peptide chain* (Kallidin and Kallidin analogues)
		Kallidin	H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH 1 2 3 4 5 6 7 8 9 10
LXII	1	Arg^{1}-Kallidin	H-*Arg*-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
LXIII	1	Gly^{1}-Kallidin	H-*Gly*-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
LXIV	1	Orn^{1}-Kallidin	H-*Orn*-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
LXV	1	Phe^{1}-Kallidin	H-*Phe*-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
LXVI	2	Lys^{2}-Kallidin	H-Lys-*Lys*-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
LXVII	7	Gly^{7}-Kallidin	H-Lys-Arg-Pro-Pro-Gly-Phe-*Gly*-Pro-Phe-Arg-OH
LXVIII	9	Phe(F)^{9}-Kallidin	H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-*Phe*-Arg-OH (F)
LXIX	10	Lys^{10}-Kallidin	H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-*Lys*-OH
LXX		(Met-Lys-Arg)^{1}-Bradykinin	H-Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH

The values given by SCHRÖDER, resp. SCHRÖDER et al., for guinea-pig ileum and rat uterus have been obtained by a comparison of the threshold sults (half-logarithm scale) within the range of a 10%–35% decline.

also Gly^{7}-bradykinin (XXXII)]. Contrary to Ala^{5}-bradykinin (XIX) the blood pressure of rabbits shows only a loss of activity to 1/300 in the case of Tyr^{5}-bradykinin (XX). Following a *p*-substitution of the Phe^{8}-residue by a fluor atom [Phe(F)^{8}-bradykinin (XXXVI)] an increased activity can be observed on the blood pressure of guinea-pigs and the bronchoconstriction. Even after a reversal of the configuration [D-Phe^{8}-bradykinin (XXXV)] and also after additional *p*-substitution [D-Phe(F)^{8}-bradykinin (XXXVII)] a high activity of 1/4 or 1/3 is maintained at the guinea-pig ileum. The bronchoconstrictory activity is somewhat lower in this case. The aromatic side chain in position 8 must, therefore, subsist if biological activity is to be maintained. A *p*-substitution does not turn out to be disadvantageous. However, it is not yet possible to judge if this also refers to substituents other than the one examined so far. Similar results concerning the importance of the aromatic side chain apply also to position 5.

Besides replacing the N- and C-terminal arginin residues by neutral amino acids, the influence of acid, or of other basic amino acids, and of citrullin, structurally resembling the arginin, have been examined in these positions. Lys^{1}-bradykinin (VII) has 1/10 of the bradykinin activity on the blood pressure of guinea-pigs and 1/250 on the blood pressure of rabbits. Lys^{9}-bradykinin (XLVI) is considerably more active with 1/10–1/30 of the bradykinin activity on the blood pressure of rabbits. The corresponding Orn-analogues (VIII or XLVII) are less active. Citrullin analogues showed interesting findings. While Cit^{1}-bradykinin (IV) has 1/250 of the bradykinin activity on the blood pressure of guinea-pigs and thus has a lower activity than the Lys^{1}-compound, a value of 1/20 is obtained for Cit^{9}-bradykinin (XLIII). Cit^{1}- and Cit^{9}-bradykinin with a value of $<1/100$ demonstrate equal activity on the blood pressure of rats.

From the higher activity of the bradykinin analogues which are modified in position 9 as compared with those in position 1, it can be concluded that the Arg^{1}-residue is of great importance. The activities of Des-(Arg)^{1}-bradykinin (LIII) and of Des-(Arg)^{9}-bradykinin (LIX), with 1/80 and $<1/2000$ respectively of the bradykinin activity, on the blood pressure of guinea-pigs are, however, contrary to these observations. The high activity of the Cit-compounds as compared with the Ala- and Gly-derivatives shows a distinct influence

Biological activities					References
Rat uterus (isolated)	Guinea-pig ileum (isolated)	Rabbit blood pressure	Guinea-pig blood pressure	Guinea-pig broncho-constriction	
—	—	—	< 1/2000	< 1/2000	NICOLAIDES et al.[103] DEWALD and NICOLAIDES[123]
—	—	—	< 1/2000	< 1/2000	NICOLAIDES et al.[103]
inactive	—	—	—	—	STEWART[122]
ca. 1/2–2/3	1/3 ± 1/10	1,9 ± 0,4	1/2–2/3 (cat)	—	PLESS et al.[99]
ca. 1/2–2/3	1/3 ± 1/10	1,9 ± 0,4	3,3 ± 0,8 (rat)	—	STÜRMER and BERDE[92]
—	—	—	2/3	1/3	NICOLAIDES et al.[100]
1/10	1	2	—	—	SCHRÖDER and GIBIAN[101]
1	1/10	1/3–1/4	—	—	SCHRÖDER et al. (unpublished)
1–2	1	1/2–1/3	—	—	SCHRÖDER (unpublished)
1/10	1/3	1/5–1/10	—	—	SCHRÖDER and GIBIAN[101] SCHRÖDER[104]
1	1/2	1/5	—	—	SCHRÖDER[104]
—	—	—	1/20	1/500	NICOLAIDES et al.[103]
1,4–1	—	—	—	—	BODANSZKY et al.[118]
~ 1/10	~ 1/10	2–3	—	—	SCHRÖDER (unpublished)
—	—	—	2/3	1/3	NICOLAIDES et al.[103]
1/500	1/100	1,5	—	—	SCHRÖDER[104]
1/4	1/4	—	—	1/4	ELLIOTT et al.[19]
1/3	1/3	2–3	—	—	SCHRÖDER[95]

doses with synthetic bradykinin. The blood-pressure values in rabbits were ascertained from a graph by these authors of the dose-response re-

of the structural factors in these positions besides the importance of the basicity. The relatively high activity of a Glu[1]-bradykinin (V) on the blood pressure of guinea-pigs (1/300 that of bradykinin) is somewhat surprising.

A simultaneous replacement of the Arg[1]- and Arg[9]-residue, as in Cit[1]-Cit[9]-bradykinin (XLVIII), Dab[1]-Dab[9]-bradykinin (XLIX) and Lys[1]-Lys[9]-bradykinin (L), leads to practically ineffective derivatives, of which the Lys[1]-Lys[9]-compound with 1/500 of the activity of bradykinin is still the relatively most active.

By replacing the amino acids in the positions 2, 3, 4, and 6 by alanin, the activity of bradykinin is maintained to a more or less considerable degree. An analogue could be found in Ala[3]-bradykinin (XII) which possesses the full activity of bradykinin both on isolated smooth muscles and on the blood pressure of rabbits. An Ala[2]-bradykinin (IX) with 1/200 has a distinctly lower activity. The activities of Val[2]-bradykinin (X) and Val[3]-bradykinin (XIV) are lower than those of the corresponding Ala-derivatives. A comparison of the activities of Ala[2]-, Ala[3]-, and Ala[7]-bradykinin allows an estimation of the importance of the Pro-residues in positions 2, 3, and 7 of the original bradykinin. The Pro[7]-residue is more important than that in position 2 and ultimately more than that in position 3.

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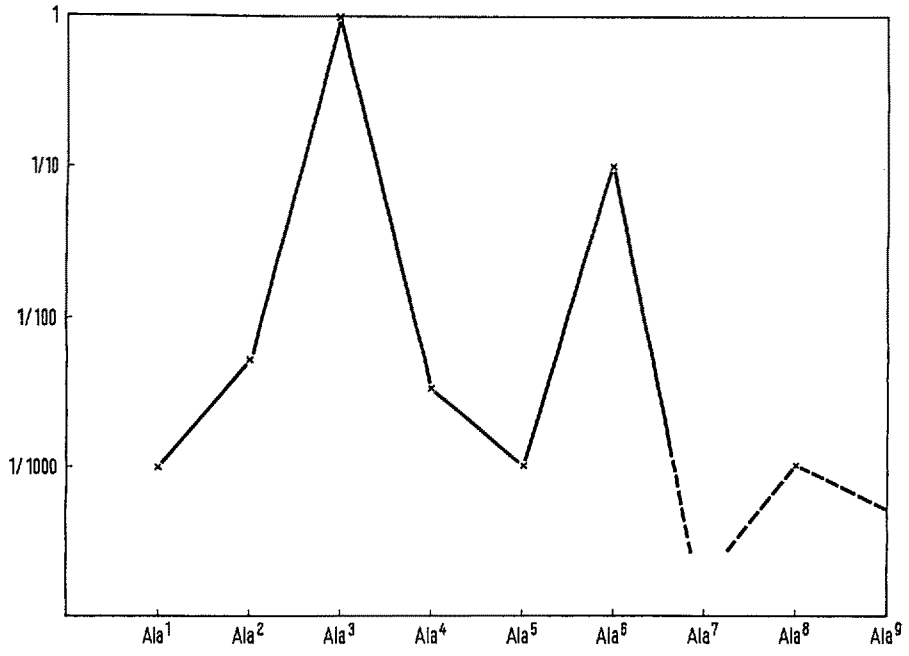

Fig. 9. Activities of the ala-analogues of bradykinin (bradykinin = 1)

These findings are in conformity with those values found for Des-(Pro)^3-bradykinin (LIV) and Des-(Pro)^7-bradykinin (LV). While 1/20 and 1/100 respectively of the bradykinin activity was still found for the first compound on the rat uterus and the guinea-pig ileum, the Des-(Pro)^7 compound is inactive up to 10^{-5} g/ml.

A replacement of the Gly^4-residue by alanin (XV) and even by other amino acids [Prolin (XVI), Sarkosin (XVII), Serin (XVIII)] always weakens the activity which is higher on the blood pressure of rabibts than on isolated smooth muscles.

Remarkable results were obtained by changes in position 6. While a substitution of the hydroxyl group of the serin residue by a carbamyl group (II) on the blood pressure of guinea-pigs only resulted in 1/75 of the activity of bradykinin and reduced the broncho-constrictory activity to $<1/500$, analogues obtained by an exchange of amino acids partly show a very high activity. As an example, an analogue with practically the full activity of bradykinin is at hand in the Gly^6-bradykinin (XXII). D-Ser^6-bradykinin (XXIV) and Thr^6-bradykin (XXV) have half the activity of bradykinin on the blood pressure of guinea-pigs. The Thr^6-compound is somewhat lower with 1/10 on the blood pressure of rabbits, and as active as bradykinin on the rat uterus. The activity of Ala^6-bradykinin (XXI) is comparable with that of the Thr^6-compound. Sar^6-bradykinin (XXIII) represents an exception amongst these highly active derivatives with only 1/1000 of the activity of bradykinin on the blood pressure of rabbits.

An exchange of amino acids in two positions of bradykinin or an interchange of two amino acids leads to differing results. Pro^1-Arg^2-bradykinin (XI) is a practically inactive analogue. The importance of the Arg-residue in the N-terminal position is thus confirmed. A simultaneous changing of positions 3 and 6 by alanin and glycin leads to a very active bradykinin analogue [Ala^3-Gly^6-bradykinin (XXVI)] with 1/6 of the activity of bradykinin on the blood pressure of rabbits, i.e. even in comparison with Gly^6- or Ala^3-bradykinin, which demonstrated the full activity of bradykinin, only a very low loss of the activity occurs. As expected, the activity of Ala^5-Gly^6-bradykinin (XXVIII) and Pro^5-Gly^6-bradykinin (XXIX) is considerably decreased by replacement of the important Phe^5-residue. In contrast to this is the relatively high activity of the Ser^4-Gly^6-bradykinin (XXVII) (guinea-pig ileum and blood pressure of rabbits 1/100), which again points to the less important position of the Gly^4-residue.

An exchange of amino acids in more than two positions (XXXVIII, XXXIX, LII) or a shortening of the peptide chain (LIV, LV, LVIII, LIX, LX) or a shortening with simultaneous replacing of amino acids (LVI, LVII, LXI) leads to inactive derivatives.

Analogues of kallidin have so far only been synthesized to a small extent. An exchange of the N-terminal lysinresidue against neutral or basic amino acids [Gly^1-kallidin (LXIII), Phe^1-kallidin (LXV), Arg^1-kallidin (LXII), Orn^1-kallidin (LXIV)] revealed only a limited loss of activity (to 1/2–1/10) on the blood pressure of rabbits as compared with bradykinin (and correspondingly a greater loss compared with kallidin). A loss of activity to 1/20 follows from an exchange of the Arg^2-residue against lysin [Lys^2-kallidin

Variation in position	1 H—Arg—	2 —Pro—	3 —Pro—	4 —Gly—	5 —Phe—	6 —Ser—	7 —Pro—	8 —Phe—	9 —Arg—OH	Biological activity (blood pressure) bradykinin = 1
Variation only in one position against ...	Lys Orn		Ala			Ala Gly D-Ser Thr		Phe(F) D-Phe D-Phe(F)	Cit His Lys	1–1/50
	Cit Glu	Ala	Val	Ala Sar	Tyr				Orn	1/50–1/500
	Ala Gly		Pip		Ala	Sar	Ala Asp(NH_2) His	Ala	Ala Gly	< 1/500
Variation in two positions against ...			Ala			Gly				1–1/50
	Cit Lys			Ser	Ala	Gly Gly			Cit Lys	1/50–1/500
	Pro	Arg								< 1/500
Variation in more than two positions		Phe		Ser	Leu	Thr Gly	Phe Leu	Arg Pro	Pro	< 1/500

Fig. 10. Amino acid exchange in the bradykinin molecule

(LXVI)]. Corresponding to the Gly[6]-bradykinin, an analogue with practically the full activity of kallidin (blood pressure in rabbits) was also found in Gly[7]-kallidin (LXVII). On the other hand, a Phe(F)[9]-kallidin (LXVIII) is slightly less active than Phe(F)[8]-bradykinin. By replacing the C-terminal arginin residue in kallidin against lysin [Lys[10]-kallidin (LXIX)], a kallidin analogue is formed possessing practically the full activity of kallidin on the blood pressure of rabbits (see Lys[9]-bradykinin). A more remarkable loss of activity occurs on isolated smooth muscles.

None of the bradykinin or kallidin analogues so far described had a prolonged action compared with bradykinin. Apart from Thr[6]-Leu[8]-bradykinin (XLI), without defined dates[122], no antagonistic effects have been reported so far.

Supplementary to the findings obtained from the blood pressure and smooth muscle preparations, some of the bradykinin and kallidin analogues synthesized in our laboratory were examined on the isolated perfused heart of guinea-pigs (Langendorff preparation, Table II). However, while differences of activity up to

Table II. Coronary vasodilation on isolated guinea-pig heart produced by bradykinin and kallidin analogues (bradykinin = 100)

Substance (100 ng)	Coronary vasodilation
Lys^1-Bradykinin	85
Ala^3-Bradykinin	108
Val^3-Bradykinin	89
Ala^6-Bradykinin	82
Gly^6-Bradykinin	97
Thr^6-Bradykinin	91
Lys^9-Bradykinin	105
Lys^1-Lys^9-Bradykinin	75
Kallidin	100
Orn^1-Kallidin	84
Phe^1-Kallidin	93
Lys^{10}-Kallidin	59
Ala^1-Bradykinin	34
Ala^2-Bradykinin	44
Ala^5-Bradykinin	30
Ser^4-Gly^6-Bradykinin	49
Ala^7-Bradykinin	43
Ala^8-Bradykinin	44
Ala^9-Bradykinin	49
His^9-Bradykinin	35

several times the power of ten could be observed on the blood pressure of rabbits following the administration of the different compounds, depending upon the changes of the bradykininmolecule, the isolated heart preparation shows an extremely low differentiation only. A classification of only two groups seems possible: the first group comprises analogues the activity of which is comparable with bradykinin itself (75–100%). In this case, we are concerned with compounds which have also a high activity on the blood pressure of rabbits (except Lys^{10}-kallidin). The second group comprises analogues with $<50\%$ of the bradykinin activity. Apart from Ala^2- and Ser^4-Gly^6-bradykinin, these derivatives were practically inactive, having not more than 1/1000 of the activity of bradykinin on the blood pressure of rabbits. To judge from the coronary vasodilatation, the receptors responding to bradykinin and kallidin are obviously less sensitive regarding modifications of the bradykinin molecule than in the remaining vascular system.

In trying to classify the single positions of the bradykinin molecule corresponding to their influence on the biological activity based on the experimental material available, the following series is obtained:

$$7, 8 > 1 > 9 > 5 > 4 > 2 > 3 > 6$$

i.e. the Pro^7 and Phe^8 residues appear to be most essential for the biological activity (further examples are necessary). The terminal arginin residues can only be replaced by neutral amino acids subject to loss of activity. A replacement by basic amino acids or those amino acids which are structurally similar to arginin (i.e. Citrullin) causes a partial loss of activity only.

The aromatic residue in position 5 is essential (further examples are necessary). An exchange of the Gly^4- and Pro^2-residues causes a medium loss of activity. The Pro^3- and Ser^6-residues can only be replaced subject to minor loss of activity, in part even with complete preservation of the bradykinin activity[127].

Zusammenfassung. Biochemie und Pharmakologie der drei natürlich vorkommenden und in der Struktur bekannten Kinine – Bradykinin, Kallidin und Methionyl-Lysyl-Bradykinin – sowie deren Synthesen werden besprochen. Für die bisher bekannten 70 Analoga des Bradykinins und Kallidins wurden die biologischen Eigenschaften (Rattenuterus, Meerschweinchenileum, Kaninchenblutdruck, Meerschweinchenblutdruck, Rattenblutdruck, Bronchokonstriktoraktivität) tabellarisch zusammengestellt. Die einzelnen Analoga werden untereinander verglichen und Beziehungen zwischen Struktur und Wirksamkeit diskutiert.

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