those compounds having a relative activity greater than 0.4 when compared to lidocaine are included in the table. Of the compounds tested, only **36**, **38**, and **72** have an activity (on a molar basis) approaching that of lidocaine. However, **36** and **38** are extremely irritating which rules out their use as local anesthetics. Compound **72** appears to be almost as effective as lidocaine on a molar basis but it is more irritating slightly more toxic, and has a shorter duration of action. It is of interest that **38**, the only secondary amine in the series with significant activity, is also the most irritating.

Acknowledgment.—The authors are indebted to the Service Central de Microanalyse, Paris, France, for performing the microanalyses.

6-Glycine-8-phenyllactic Acid Bradykinin. Its Synthesis, Biological Activity, and Splitting by Kininase (Carboxypeptidase N)

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The synthesis of a new depsipeptide analog of bradykinin is described. The biological activity of the analog has been studied. The vasodepressive effects in the rat and rabbit and the effects on isolated rat uterus and capillary permeability in rabbit skin of the depsipeptide were evaluated as 4, 2, 0.5, and 0.04 times, respectively, the potency of bradykinin. In the rat the analog has three phases of action: primary hypotension, partial restoration of the arterial blood pressure level, and secondary hypotension. The analog has no antibradykinin action. The rate of human and rabbit sera kininase induced degradation of the analog *in vitro* is about 20–35% slower than that of bradykinin; the enhanced and prolonged vasodepressive action of the depsipeptide may be explained on this basis.

In recent years considerable information has accumulated on the relation between the activity of biologically active peptides and the nature, configuration, sequence, and number of their amino acid residues. However, there is practically no knowledge as to the part played by the characteristic structural element of the polypeptide chain, the amide group, in particular, the necessity of its presence for the compounds to manifest their specific biological properties.

In 1964–1965 the depsipeptide analogs of a number of biologically active peptides, namely ophthalmic acid,[1] glutathione,[2] and bradykinin[2,3] in which one or more of the amide groups are replaced by an ester group, were synthesized and it was shown[2-5] that in a number of tests 6-glycolic acid bradykinin practically does not differ from bradykinin,[6] whereas the activity of 4-glycolic acid bradykinin is three to four orders of magnitude lower.

It was thus found that the replacement of an amide group by the spatially and electronically similar ester group does not abolish biological activity, although the degree of its retention does depend on the position of the replaced amide group in the peptide chain.

(1) L. A. Shchukina, A. L. Zhuze, E. P. Senkin, and S. N. Krasnova, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 685 (1964).

(2) L. A. Shchukina, G. A. Ravdel, M. P. Filatova, and A. L. Zhuze, *Acta Chim. Acad. Sci. Hung.*, **44**, 205 (1965).

(3) L. A. Shchukina, G. A. Ravdel, and M. P. Filatova, *Khim. Prirodn. Soedin.* 265 (1966).

(4) M. S. Surovikina, T. P. Egorova, and T. S. Paskhina, *Farmakol. Toksikol.*, in press.

(5) M. M. Shemyakin, L. A. Shchukina, E. I. Vinogradova, G. A. Ravdel, and Yu. A. Ovchinnikov, *Experientia*, **22**, 535 (1966).

(6) Since the replacement of serine by glycine caused no marked change in activity of bradykinin [M. Bodanszky, J. T. Sheehan, M. A. Ondetti, and S. Lande, *J. Am. Chem. Soc.*, **85**, 991 (1963); E. Schröder, *Ann.*, **673**, 185 (1964)] the more readily available 6-glycolic acid bradykinin was used for comparison.

In a continuation of our investigations into the depsipeptide analogs of bradykinin we have undertaken the synthesis of 6-glycine-8-phenyllactic acid bradykinin (I).[7]

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

The biological investigation of this analog was of particular interest because the replacement of phenylalanine by other amino acids is known to cause the greatest change in the biological activity of bradykinin.[8] On the other hand, Erdos has shown[9] that the main path of the inactivation of bradykinin in an organism is the splitting of the C-terminal Phe–Arg bond by blood plasma kininase (carboxypeptidase N). It could be expected that modification of the molecule in the direct proximity of the grouping undergoing attack would markedly affect its behavior toward this enzyme.

Chemistry.—The synthesis of the nonadepsipeptide I was carried out according to Scheme I, the protected intermediate pentadepsipeptide X being prepared in two ways, a and b. The initial compound for both ways was *t*-butyl benzyloxycarbonyl-L-prolyl-L-phenyllactate (II), obtained by condensation of benzyloxycarbonyl-L-proline with *t*-butyl phenyllactate[10] with the aid of benzenesulfonyl chloride.

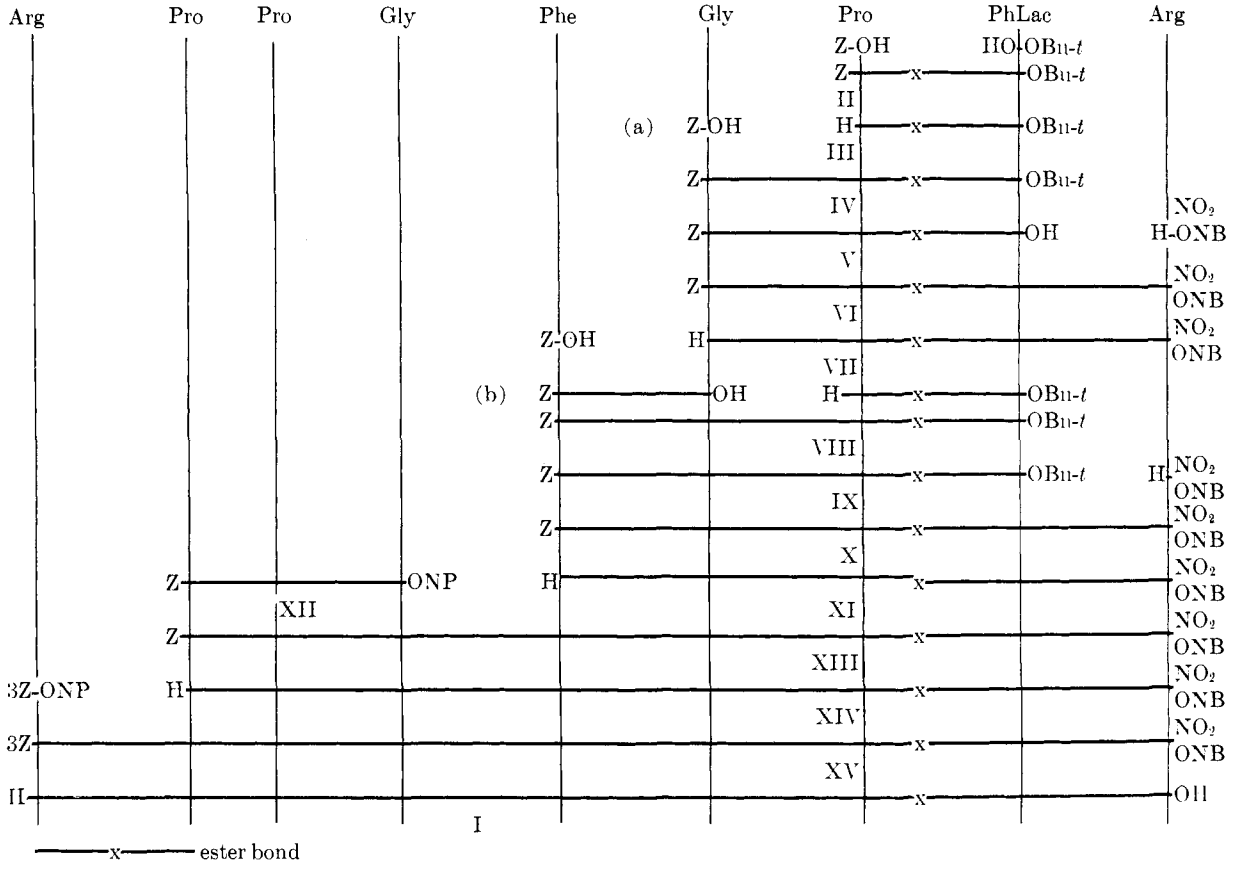
(7) The abbreviations of the amino acids and their derivatives are those adopted by the 5th European Peptide Symposium, Oxford, Sept 1962. Additional abbreviations are: PhLac = L-3-phenyllactic acid, Glyc = glycolic acid.

(8) E. Schröder and R. Hempel, *Experientia*, **20**, 529 (1964).

(9) E. Y. Erdos and E. M. Sloane, *Biochem. Pharmacol.*, **11**, 585 (1962).

(10) L. A. Shchukina, G. F. Gromova, and G. A. Ravdel, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 519 (1966).

Scheme I



Removal of the aminoacylhydroxy acid II protection by hydrogenation in methanol at room temperature was accompanied by complete splitting of the resultant *t*-butyl L-prolyl-L-phenyllactate (III) to L-proline and *t*-butyl L-phenyllactate. The hydrogenation was therefore carried out in the presence of tartaric acid, the crystalline salt of the *t*-butyl ester III then being transformed into the base at −8° which was condensed at that temperature with benzyloxycarbonylglycine (route a) or with benzyloxycarbonyl-L-phenylalanylglycine[11] (route b).

On preparing the protected pentadepsipeptide X from benzyloxycarbonyl-L-phenylalanine and *p*-nitrobenzyl glycyl-L-prolyl-L-phenyllactyl-nitro-L-argininate (VII) (route a) it was observed that the latter is stable only in the form of the hydrobromide, whereas the base, both in nonaqueous and aqueous solutions, is completely split at −5 to 0° into two neutral compounds, of which one was identified as glycyl-L-prolyldiketopiperazine, and the second as apparently *p*-nitrobenzyl L-phenyllactyl-nitro-L-argininate. Although breakdown could not be avoided, still the protected pentadepsipeptide X could be obtained in reasonably high yield by liberating the base VII from the hydrobromide in the presence of benzyloxycarbonyl-L-phenylalanine and using the carbodiimide method of condensation. The product was purified from the accompanying degradation products by chromatography (on an alumina column), which entailed considerable loss. For this reason route b was found to be more convenient.

(11) D. W. Clayton, J. A. Farrington, G. W. Kenner, and J. M. Turner, *J. Chem. Soc.*, 1398 (1957).

Preparation of the protected nonadepsipeptide XV was carried out by the successive addition to the peptide XI of the tripeptide benzyloxycarbonyl-L-prolyl-L-prolylglycine and tribenzyloxycarbonyl-L-arginine by the *p*-nitrophenyl ester method. All of the protective groups were simultaneously removed by hydrogenation in a mixture of methanol and acetic acid and, after freeze drying, Gly[6]-PhLac[8] bradykinin acetate was isolated, the individuality of which was confirmed by paper chromatography and electrophoresis.

Experimental Section

***t*-Butyl L-Prolyl-L-phenyllactate (III).**—To a stirred solution of 1 g of benzyloxycarbonyl-L-proline in 5 ml of pyridine, cooled to −5°, 0.5 ml of benzenesulfonyl chloride was added. After 10 min a solution of 0.8 g of *t*-butyl L-phenyllactate in 1 ml of pyridine was added, the mixture was stirred 2 hr at 0–5° and 1 hr at room temperature and then poured into ice–water. The oil was extracted with ethyl acetate, this extract was washed with 1 *N* HCl, saturated $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated *in vacuo*. The oily residue (1.5 g) was hydrogenated at room temperature and ordinary pressure in 10 ml of methanol, containing 0.75 g of tartaric acid, in the presence of Pd black for 3 hr, adding the catalyst twice more (0.2 g total). The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The solid was triturated with ether and after the crystallization from a mixture of absolute ethanol–ether (1:1) the *t*-butyl ester (III) tartrate was obtained; yield 1.2 g (71%), mp 146°, $[\alpha]^{18}D$ −20° (*c* 1.0, H_2O).

Anal. Calcd for $C_{18}H_{25}NO_4 \cdot C_4H_6O_6$: C, 56.31; H, 6.74; N, 3.10. Found: C, 56.28; H, 6.66; N, 2.98.

Benzyloxycarbonylglycyl-L-prolyl-L-phenyllactic Acid (V).—The tartrate of III (0.4 g) was dissolved at −8° in a mixture of 10 ml of 0.5 *N* $NaHCO_3$ and 20 ml of ether, and the organic layer was separated, dried for 10 min ($MgSO_4$) at 0–5°, and concentrated at this temperature. To the residue 0.2 g of benzyloxy-

TABLE I
BIOLOGICAL ACTIVITY OF BRADYKININ AND Gly⁶- PhLac⁸ BRADYKININ

	Vasodepression, g/kg		Isolated rat uterus, g/ml	Capillary permeability g/ml
	in rat	in rabbit		
Bradykinin	1.16×10^{-6}	0.58×10^{-7}	2×10^{-11}	5.6×10^{-9}
Gly⁶- PhLac⁸ bradykinin	0.26×10^{-6}	0.33×10^{-7}	4×10^{-11}	1.3×10^{-9}

carbonylglycine and 0.2 g of dicyclohexylcarbodiimide was added at 0°. After 20 hr at 20° the urea derivative was filtered off, the filtrate was washed with 1 *N* HCl, 0.5 *N* $NaHCO_3$, and water, dried, and then evaporated *in vacuo*. The thoroughly dried oil was treated with 2 ml of CF_3COOH for 30 min, the resulting solution was concentrated at 20°, the residue was dissolved in 20 ml of ethyl acetate, and the product was extracted with saturated $NaHCO_3$. The water solution was acidified with 5 *N* HCl and the product (V) was filtered off; yield 0.25 g (65%), $[\alpha]^{20}D$ −30° (c 1.0, ethanol).

Anal. Calcd for $C_{24}H_{26}N_2O_7 \cdot 0.5H_2O$: C, 62.19; H, 6.08; N, 6.04; H_2O, 1.80. Found: C, 62.35; H, 5.98; N, 5.80; H_2O, 1.88.

p-Nitrobenzyl Benzyloxycarbonylglycyl-L-prolyl-L-phenyllactylnitro-L-argininate (VI).—The protected tridepsipeptide V (0.42 g) was dissolved in 2 ml of $SOCl_2$. After 40 min the excess $SOCl_2$ was distilled *in vacuo*, and dry benzene was added to the residue and evaporated *in vacuo*. The resultant acid chloride was dissolved in 3 ml of dry benzene and added to the solution of *p*-nitrobenzyl nitro-L-argininate (from 0.53 g of hydrobromide[12] and 0.18 g of triethylamine) and 0.15 g of triethylamine in 4 ml of dimethylformamide (DMF) at 20°. The mixture was kept for 1 hr at 0–5° and 3 hr at room temperature; the solution was diluted with 100 ml of ethyl acetate, washed with 1 *N* HCl and 0.5 *N* $NaHCO_3$, dried ($MgSO_4$), and evaporated *in vacuo*. The solid residue of VI weighed 0.6 g (83%), mp 77–81°, $[\alpha]^{18}D$ −32° (*c* 1.0, DMF).

Anal. Calcd for $C_{47}H_{42}N_8O_{12}$: C, 56.20; H, 5.36; N, 14.17. Found: C, 56.19; H, 5.51; N, 14.37.

Benzyloxycarbonyl-L-phenylalanylglycyl-L-propyl-L-phenyllactic Acid (IX).—Aminoacylhydroxy acid ester III, obtained from 5 g of the tartrate, was kept in 13 ml of acetonitrile with 0.4 g of benzyloxycarbonyl-L-phenylalanylglycine[11] and 0.24 g of dicyclohexylcarbodiimide. After 20 hr at 20° the mixture was processed as described in the preparation of V, yield 0.41 g (59%), mp 95°, $[\alpha]^{20}D$ −56° (*c* 1.0, ethanol).

Anal. Calcd for $C_{33}H_{35}N_3O_8$: C, 65.87; H, 5.86; N, 6.98; neut equiv, 601. Found: C, 65.63; H, 6.25; N, 6.89; neut equiv, 600.

p-Nitrobenzyl Benzyloxycarbonyl-L-phenylalanylglycyl-L-prolyl-L-phenyllactylnitro-L-argininate (X). A.—A solution of 4 *N* HBr in acetic acid (3 ml) was added to a solution 0.42 g of the protected tetradepsipeptide VI in acetic acid (3 ml). The solution was kept for 40 min at room temperature and evaporated to dryness. The semisolid mass was repeatedly triturated with ether, until solid mass (0.38 g, 81%) was obtained.

Anal. Calcd for $C_{24}H_{36}N_8O_{16} \cdot 2.5HBr$: Br, 23.29. Found: Br, 23.0.

The hydrobromide thus obtained and 0.14 g of benzyloxycarbonyl-L-phenylalanine were dissolved in 5 ml of DMF. Triethylamine (0.15 ml) and 2 ml of ethyl acetate containing 0.1 g of dicyclohexylcarbodiimide were added at 0°. After 48 hr at 20° the solid was filtered off and the solution was diluted with 30 ml of ethyl acetate, washed with water, 1 *N* HCl, and 0.5 *N* $NaHCO_3$, dried, and evaporated to dryness. The residue (0.35 g, 90%) in portions of 100 mg was chromatographed on a neutral Al_2O_3 column, using a mixture of ethyl acetate–benzene (7:3) and methanol with a gradual increase in concentration of the latter from 1 to 13%. Total yield of protected pentadepsipeptide X was 0.34 g (56%), mp 87–90°, $[\alpha]^{20}D$ −37° (*c* 1.0 ethanol).

Anal. Calcd for $C_{46}H_{51}N_9O_{13} \cdot H_2O$: C, 57.79; H, 5.38; N, 13.19; H_2O, 1.88. Found: C, 57.96; H, 5.55; N, 13.23; H_2O, 1.62.

B.—The protected tetradepsipeptide IX (0.3 g) was dissolved in 1 ml of $SOCl_2$ and after 40 min at 20° the solution was evaporated *in vacuo*; the dry benzene was added to residue and again distilled *in vacuo*. The resulting acid chloride was dissolved in dry benzene and the solution was added at 0° to the solution 0.22 g of *p*-nitrobenzyl nitro-L-argininate hydrobromide[12] and 0.075 ml of triethylamine in 2 ml of DMF, and then 0.07 ml of triethylamine was added to the resulting mixture. The mixture was kept for 1 hr at 0–5° and 3 hr at room temperature and diluted with 30 ml of ethyl acetate, and the solution was washed with 1 *N* HCl, 0.5 *N* $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated, giving 0.3 g (70%) of the protected pentadepsipeptide X.

p-Nitrobenzyl Benzyloxycarbonyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenyllactylnitro-L-argininate (XIII). To a solution of 0.6 g of X in 3 ml of glacial acetic acid was added 3 ml of HBr–4 *N* acetic acid and the mixture was kept for 40 min at 20°. The solution was evaporated *in vacuo* and a resulting oil was triturated with dry ether until it solidified, yielding 0.6 g (94%) of hydrobromide.

To a solution of this hydrobromide in 5 ml of DMF was added at 0° 0.077 ml of triethylamine, 0.3 g of *p*-nitrophenyl L-prolyl-L-prolylglycinate,[13] and 0.03 g of imidazole. After 48 hr at 20° the mixture was diluted with 30 ml of ethyl acetate, washed with water, 1 *N* HCl, again with water, dried ($MgSO_4$), and evaporated. The residue was triturated with ether and recrystallized twice from $CHCl_3$–ether (1:1). The yield of purified XII was 0.53 g (70%), mp 111–114°, $[\alpha]^{20}D$ −40° (*c* 1.0, ethanol).

Anal. Calcd for $C_{58}H_{68}N_{12}O_{15} \cdot H_2O$: C, 57.70; H, 5.68; N, 13.92; H_2O, 1.49. Found: C, 57.60; H, 5.92; N, 14.07; H_2O, 1.62.

p-Nitrobenzyl Tribenzyloxycarbonyl-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenyllactylnitro-L-argininate (XV).—The benzyloxycarbonyl group of the protected octadepsipeptide XIII (0.3 g) was removed as described above for the preparation of XIII and 0.13 g of *p*-nitrophenyl tribenzyloxycarbonyl-L-argininate[14] was coupled to the resulting hydrobromide. The resulting product was crystallized three times from a mixture ethyl acetate–ether (1:3); yield 0.26 g (70%), mp 101–105°, $[\alpha]^{20}D$ −41° (*c* 1.0, ethanol).

Anal. Calcd for $C_{80}H_{92}N_{16}O_{21} \cdot 2H_2O$: C, 58.23; H, 5.80; N, 13.59; H_2O, 2.18. Found: C, 58.17; H, 5.87; N, 13.65; H_2O, 2.5.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-phenyllactyl-L-arginine (I).—The protected nonadepsipeptide *p*-nitrobenzyl ester XV (0.1 g) was hydrogenated for 20 hr in a mixture of methanol (3 ml) and acetic acid (3 ml) in the presence of Pd black. After filtering off the catalyst, the filtrate was evaporated *in vacuo*. The residue was repeatedly triturated with ether, dissolved in water, and freeze dried, giving 80 mg (96%) of nonadepsipeptide (I), mp 179–181°, $[\alpha]^{19}D$ −75° (*c* 0.5, water).

Anal. Calcd for $C_{45}H_{70}N_{14}O_{11} \cdot 5H_2O \cdot 1.5CH_3COOH$: C, 51.57; H, 7.16; N, 16.19; H_2O, 7.51; CH_3CO, 5.82. Found: C, 51.20; H, 7.21; N, 16.50; H_2O, 8.02; CH_3CO, 5.95.

This product was shown to be homogeneous by paper chromatography (butanol–acetic acid–water, 4:1:5, R_f 0.40; and isoamyl alcohol–pyridine–water, 35:35:30, R_f 0.27) using ninhydrin and Sakaguchi reagent for detection. It was also shown to be homogeneous by paper electrophoresis (Veronal buffer, pH 8.6, 450 v) using the same reagents. The quantitative amino acid analysis gave the ratio of amino acids: arginine: proline: glycine: phenylalanine, 1.96:2.77:1.83:1.0.

Biological Activity of Gly⁶-PhLac⁸ Bradykinin and Its Kininase-Catalyzed Degradation. Methods.—The bradykinin-like activity of depsipeptide I was assayed by measuring its ability to decrease blood pressure in rats and rabbits, to stimulate rat uterus, and to increase capillary permeability in skin of rabbit. Results of these assays were compared with the activity of bradykinin in corresponding biological sytems. The arterial blood pressure (AP) was recorded from the carotid artery of a rat anesthetized by urethan.[15] The substances were injected in the femoral vein; the amount causing a drop in AP values by 5–7 mm was assumed to be a threshold dose.

(12) R. A. Boissonnas, St. Guttmann, and P.-A. Jaquenoud, *Helv. Chim. Acta*, **43**, 1349 (1960).

(13) L. A. Shchukina, G. A. Ravdel, M. P. Filatova, E. P. Senkin, and S. N. Krasnova, *Khim. Prirodn. Soedin.*, 124 (1966).

(14) E. D. Nicolaides and H. A. DeWald, *Nature*, **187**, 775 (1960).

(15) J. Dekanski, *Brit. J. Pharmacol.*, **7**, 567 (1952).

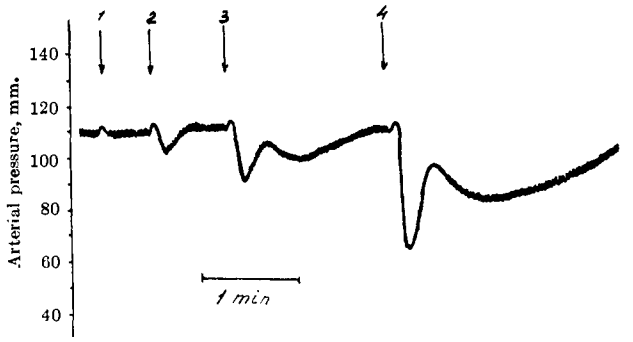


Figure 1.– Decrease in arterial pressure of a rat (208 g) after intravenous injections of bradykinin and gly⁶-PhLac⁸ bradykinin. Arrows indicate the time of injections of the preparations: 1, 0.85% NaCl, 0.2 ml; 2, bradykinin, 1.5×10^{-6} g/kg; 3, Gly⁶-PhLac⁸ bradykinin, 0.5×10^{-6} g/kg; 4, same as 3, 2.5×10^{-6} g/kg.

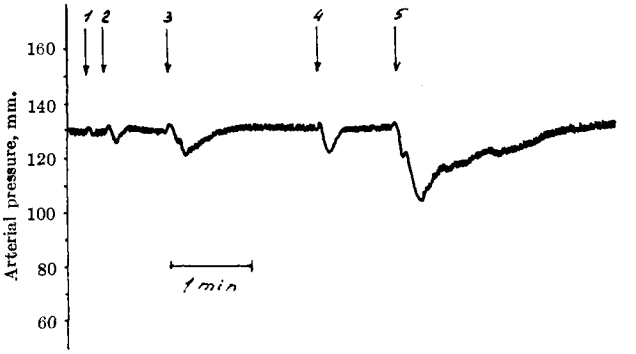


Figure 2.—Decrease in arterial pressure of a rabbit (2.96 kg) after intravenous injections of the same doses of bradykinin and Gly⁶-PhLac⁸ bradykinin. Arrows indicate the time of injection of the preparations: 1, 0.85% NaCl, 0.3 ml; 2, bradykinin, 0.33×10^{-7} g/kg; 3, Gly⁶-PhLac⁸ bradykinin, 0.33×10^{-7} g/kg; 4, bradykinin, 0.66×10^{-7} g/kg; 5, Gly⁶-PhLac⁸ bradykinin, 0.66×10^{-7} g/kg.

The tests on rat uteri were carried out in a conventional way.[16] Virgin rats weighing 150–200 g were injected subcutaneously with stilbestrol (1–2 μg/g) 24 hr before use; a segment of the uterus horn was suspended in a de Jalon solution at 29–30°.

The effects on capillary permeability were examined in rabbit skin by means of a modified Menkin's method[17] with 0.5% Evans blue; the amount of substance causing appearance within 10–15 min, after intracutaneous injection, of a blue spot about 7–10 mm in diameter was considered as a threshold dose. The rates of kininase (carboxypeptidase N)-catalyzed degradation of bradykinin and of the analog were estimated by measuring the amount of arginine produced by 1 mg of enzymatically active protein during 1 min at 37°. Arginine content was estimated by means of a quantitative ninhydrin method in eluates from paper chromatograms.[18]

Results and Discussion

As may be seen in Table I the vasodepressor activity of Gly⁶-PhLac⁸ bradykinin in rat and rabbit is four and two times greater, respectively, than that of bradykinin. However, tests on the rat uterus (contracting effect) or rabbit skin (effect on capillary permeability) showed that the activity of the analog is $^1/_2$ and $^1/_{25}$ times, respectively, as great as that of bradykinin.

The biological effect of both bradykinin and depsipeptide analog on the smooth muscle and capillary permeability is rather similar, but the action of the latter on the blood vessels exhibits some peculiar patterns. Gly⁶-PhLac⁸ bradykinin like Gly⁶ bradykinin has three phases of action: (1) primary decrease in AP (the phase of primary hypotension); (2) partial (60–70%) restoration of AP as compared with the level reached during first phase; (3) second (35–40%) drop in AP as compared with the level reached in the second phase (the phase of secondary hypotension). The three phases are demonstrated in Figure 1.

The vasodepressor effect in rats and rabbits of depsipeptide I lasts two or three times longer than that of bradykinin. For example, the restoration of initial AP values in rats after the intravenous injection of some (2–3) threshold doses of depsipeptide I takes place during 2.5–3 min, after the injection of 10–100 doses during 6–10 min. Under these conditions the action of bradykinin lasts only 20–30 sec or 1–2 min, respectively. The same observation was made in rabbits.

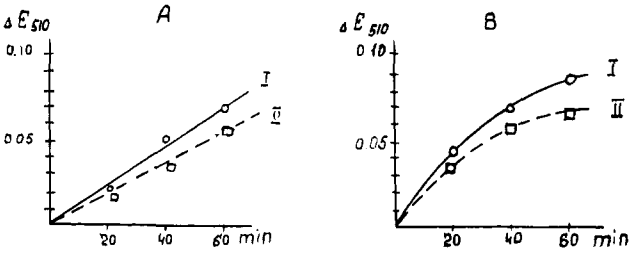


Figure 3.—Rates of liberation of arginine from bradykinin (I) and Gly⁶-PhLac⁸ bradykinin (II) by human and rabbit kininases. Concentrations of the substrates, 0.8×10^{-3} *M*; enzymes, 0.07 mg/ml; 0.01 *M* phosphate buffer, pH 7, 8, containing 10^{-4} *M* $CoCl_2$. Abscissa, time; ordinate, increase in optical density of eluates at 510 mμ.

Unlike the phenomena observed in rats, the hypotensive effects of I in rabbits do not exhibit three distinct phases of action (Figure 2).

The results obtained (see Table I) prove that the rabbit blood vessels are more sensitive to the effect of both bradykinin and depsipeptide I than those of the rat. Furthermore, in the rabbit we have observed tachyphylaxis toward bradykinin and especially toward its analog. This phenomenon is observed in case of intravenous injections repeated every 2–5 min, whereas it does not occur if the injections are made at intervals of 6–10 min.

Like the other depsipeptide analogs of bradykinin studied,[4] depsipeptide I does not manifest the properties of an antagonist of this peptide. An additive effect of intravenously injected subthreshold or threshold doses of bradykinin and depsipeptide I was observed in rats and rabbits.

As the intensity and duration of the effect of kinins in organisms depend much on the rate of their inactivation by enzymes in blood and tissuse, we have compared the rates of degradation of bradykinin and Gly⁶-PhLac⁸ bradykinin in the presence of two different samples of kininase, prepared from human and rabbit sera. The results are given Figure 3 and Table II. The rate of hydrolysis of depsipeptide analog I by both preparations of enzyme is about 20–35% lower than that of bradykinin.

(16) D. F. Elliott, E. W. Horton, and G. P. Lewis, *J. Physiol.* (London), **153**, 473 (1960).

(17) T. S. Pashkina, *Dokl. Akad. Nauk SSSR*, **86**, 609 (1952).

(18) T. S. Paskhina in "Sovremennye metody biokhimii," V. N. Orekhovich, Ed., Medicina, Moscow, 1964, p 162.

TABLE II

RELATIVE RATES OF DEGRADATION OF BRADYKININ AND GLY^6-PHLAC^8 BRADYKININ BY THE PARTIALLY PURIFIED KININASES FROM HUMAN AND RABBIT BLOOD (THE MEAN VALUES)

	Arginine, μM/min mg	
	Bradykinin	Gly^6-PhLac^8 bradykinin
Human	0.056	0.045
Rabbit	0.100	0.005

The tendency toward a slower hydrolysis of depsipeptide I was established by us for the dilute human sera and rabbit sera. Replacement of serine in position 6 by glycine may not influence the rate of hydrolysis of that analog by kininase because the rate of degradation of the Gly^6 bradykinin is even higher than that of bradykinin.

The human and rabbit kininases cause liberation of only one amino acid residue, arginine, from either bradykinin or its analog.

Our results suggest that the enhanced and prolonged vasodepressive effect of Gly^6-PhLac^8 bradykinin as compared with the effect of bradykinin may to some extent be accounted for by the relatively low rate of inactivation of the depsipeptide in the blood stream. In tissues containing comparatively low amounts of kininases (for example, rat uterus and rabbit skin) the rate of inactivation does not influence the biological activity of kinins. The relatively low rates of the kininase-induced degradation of Gly^6-PhLac^8 bradykinin as compared with that of bradykinin may be explained by assuming alterations in degree of complementarity between the active site of the enzyme and the C-terminal peptide chain of the depsipeptide molecule.