provide evidence to support the hypothesis that triamterene acts in the distal tubule to reduce Na reabsorption. While this drug acts in the same area of the tubule as aldosterone and causes effects on sodium reabsorption opposite to those attributed to aldosterone(9), competitive inhibition cannot be inferred. The fact that triamterene causes a natriuresis in adrenalectomized rats and dogs indicates that the drug acts by other mechanisms than aldosterone antagonism.

There were no consistent changes in urine flow rate, free flow urine Na concentration, and proximal urine Na concentration in 4 experiments where stop flow studies were done before and after triamterene. While proximal tubular events are not as easily assessed by the stop flow method(6), there is no evidence to support the idea of a proximal tubular action of the drug.

Summary. The pteridine compound triamterene was studied in 11 dogs using the stop flow method. These data support the hypothesis that the site of action of triamterene is the distal tubule of the kidney and that triamterene produces its natriuretic effect by reducing Na reabsorption in this area. No evidence was obtained for a proximal renal tubular effect.

The authors wish to thank Dr. W. S. Wilde for helpful criticism on stop flow and for the use of laboratory facilities and grant support in his absence.

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Received April 4, 1963. P.S.E.B.M., 1963, v113.

Effects on Smooth Muscle and Blood Vessels of Polypeptides Related to Bradykinin and to Kallidin. (28357)

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The physiological and pharmacological actions in experimental animals of synthetic or of naturally occurring bradykinin have been reviewed recently(1,2,3,4). Among the actions of bradykinin are transient vasodilatation of peripheral and coronary arteries, transient hypotension, increased capillary permeability, induction of edema, migration of leukocytes, production of pain after topical application to the base of a blister or after intra-arterial injection, bronchoconstriction in certain animal species, and stimulation or relaxation of excised gastrointestinal or uterine muscles.

Four of these actions (transient hypotension, coronary dilatation, increased capillary permeability, stimulation of excised uteri) provided the basis for comparing the actions of 9 synthetic analogues of bradykinin and one of kallidin.

Materials. Bradykinin and the 10 analogues were synthesized by members of our Organic Chemistry Section. Preliminary reports on the syntheses of these peptides as well as on their stimulant activities on excised rat uteri have appeared recently(5-9). The 10 peptides related to bradykinin or to kallidin were 1-citrulline bradykinin(5,6,8),

9-citrulline bradykinin, 1, 9-biscitrulline bradykinin(5,8), 6-glycine bradykinin, arginyl-heptaglycylarginine, 7-glycine bradykinin(5, 9), bisnitroarginine bradykinin(5,6), retro-bradykinin (reverse sequence of bradykinin) (5,7), 7-glycine kallidin(9), and fully protected bradykinin(6). All of these peptides were nonapeptides with the exception of 7-glycine kallidin, a decapeptide. The fully protected nonapeptide derivative corresponding to bradykinin was Nα-benzyloxycarbonyl-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenyl-alanyl-nitro-L-arginine methyl ester.

Methods. In vitro, *rat's uterus.* Virgin female albino rats were pretreated with estradiol benzoate (2 γ i.m./day for 3 days). Each excised uterine horn was mounted in an isolated tissue bath of 10 ml capacity and perfused with a modified Tyrode's solution, maintained at 27-28°C and aerated with 95% O_2-5% CO_2. These uterine strips were typically devoid of spontaneous contractions in the resting state.

Each peptide was solubilized in perfusion fluid; fully protected bradykinin, however, required solution in N, N-dimethylformamide before dilution with perfusion fluid. Each peptide solution (0.05 ml) was added to the bath for a 5-minute period, before washing with peptide-free perfusion fluid. During this 5-minute period, the amplitude and frequency of contractions, and the changes in tone were recorded. The amplitude of the initial contraction was used in each instance as the criterion for evaluation of stimulant activity relative to bradykinin; graphical estimates of activity were made on a log concentration-response basis. Estimates of relative activity were also obtained for each peptide by analyzing graphically overall changes in tone or in frequency of contractions.

Log concentration-response curves were obtained for the effects of bradykinin and each test peptide on each tissue. Bradykinin was tested at 0.00125, 0.005, and 0.02 γ/ml. Each peptide was tested first in the same concentration range as bradykinin, and, if relatively inactive, was also tested at 4 increasing concentrations, 0.08, 0.32, 1.28, and 5.12 γ/ml. One inactive peptide, retrobradykinin, was studied also at 20.5 and 82 γ/ml.

Isotonic contractions were recorded kymographically with a frontal-writing lever or isometrically (Grass FT .03 force transducer) on an Offner type R Dynograph.

Each test peptide was also examined for *in vitro* anti-bradykinin activity. Uterine strips were pretreated for 5 minutes with 1/100 and 1/10 of the minimal stimulant concentration of each peptide or of the highest non-stimulant concentration employed. This was followed by a stimulant concentration, 0.02 γ/ml, of synthetic bradykinin left in the bath for 5 minutes.

In vitro, *Langendorff preparation, coronary perfusion of excised cat heart*(10). Excised hearts from male or female cats weighing 2 to 3 kg were mounted in the Anderson-Craver perfusion apparatus(11) and perfused with Chenoweth-Koelle's solution(12) at 37°C under 40 cm of water pressure. Isometric recordings of force of contraction were obtained from the right ventricle with a Statham GI 8-350 force transducer. Coronary perfusion rate was monitored by a Statham P23BB pressure transducer which detected changes in line pressure each time the coronary flowmeter (10 ml) emptied. An Offner Dynograph yielded continuous records of contractile force, ventricular rate, and coronary perfusion rate.

Each peptide was dissolved in perfusion medium and injected in doses of 1, 10, 100, or 1000 γ; at least 3 dose levels were employed for each peptide. Each injection of test peptide was alternated with an injection of bradykinin. Actual volumes of peptide solutions injected were 0.5 to 1.0 ml, followed by a "flush" of 1.5 ml with perfusion medium during consecutive periods of 30 and 45 seconds, respectively. Evaluation of each peptide's activity in relation to bradykinin was based only on changes in coronary perfusion rate, since, in agreement with a report by other investigators(13), positive inotropic or chronotropic changes were absent or minimal.

Increased capillary permeability in albino

rabbits. Male rabbits, ranging in weight from 2 to 3 kg, were employed. Approximately 48 hours prior to test, the hair on the abdomen was clipped and the skin epilated with a commercial depilatory. On the day of test, at least 17 squares (2.5 cm × 2.5 cm) were marked off on the abdomen of each rabbit. One intradermal injection of 0.2 ml of test material, dissolved in physiological saline, was made within each square with a ½ inch, 27-gauge needle and a glass syringe. The order of injections was randomized so that a site received one of the following: 0.001, 0.01, 0.10, 1.0, 10.0 or 100 γ of each test peptide alone or combined with 10 γ of bradykinin or of 0.01, 0.10, 1.0, or 10 γ of bradykinin alone. Three sites received injections of saline alone. The combinations of test peptide and bradykinin measured any antibradykinin action. Immediately after the last intradermal injection into each rabbit, 10 ml of a 1% aqueous solution of trypan blue was infused into an ear vein during a 60-second period. The blueness and diameter of each wheal were determined at 15, 30, 60 and 120 minutes after injection of dye. Maximal blueness occurred in 15 or 30 minutes. The degree of blueing was given one of the 5 following scores: 0, none; 1, trace; 2, light; 3, medium; or 4, dark. The diameters were measured with a millimeter ruler to ± 1 mm. Graphical analysis of data from experiments with bradykinin suggested that blueing scores and diameters were roughly correlated (Table I). Graphical evaluation of results with each test peptide *vs* bradykinin gave the same estimate of relative activity for each parameter.

Hypotensive activity in anesthetized rats. Male, Sprague-Dawley rats, ranging in weight from 250 to 400 g were anesthetized with urethane injected subcutaneously and then given heparin sodium i.v. according to the procedures described by Dekanski(14). Atropine sulfate, 1.5 mg/kg, i.v., was also administered. Injections were made into a jugular vein cannulated with PE50 polyethylene tubing; mean blood pressure was recorded continuously on an Offner Dynograph through a Statham P23AA pressure transducer at-

TABLE I. Dose or Concentration Effects of Bradykinin.

Animal	No. of animals	Test preparation	Test response	Bradykinin dose or concentration — Avg response				
Rat	10	Excised rat's uterus	Oxytocic; amplitude of contraction	Conc, γ/ml	.00125		.005	.02
				mm	15.0		25.2	34.8
"	11	Anesthetized, atropinized; i.v. injections	Hypotension	γ/kg, i.v.	.31	1.0	3.1	10.0	31.0
				mm Hg decr.	1.2	7.0	13.2	16.2	19.8
Rabbit	9	Intradermal injections followed by trypan blue, i.v.	Increased capillary permeability	γ/0.2 ml	.01	.10	1.0	10.0
			Wheal blueing	Score	1.5	1.3	2.8	3.6
			Wheal diameter	mm diam.	8.8	12.4	16.8	21.4
Cat	6	Excised cat's heart, coronary perfusion	Increased coronary perfusion rate	γ	1.0		10.0	100.0
				% incr.	30.6		64.8	79.2

TABLE II. Approximate Relative Activities of Peptides Related to Bradykinin.

Peptide	Excised rat's uterus; stimulation	I.V. rat; vasodepression	Intradermal, rabbit; incr. capillary permeability	Excised cat's heart; coronary dilatation
B (=bradykinin)	[1]	[1]	[1]	[1]
6-glyc. B.	1	1 to 2	1	1
7-glyc. kallidin	1/4 to 1	1/3 to 1	1/10 to 1	1/10
7-glyc. B.	1/90	1/300	1/100	<1/100*
9-citr. B.	1/400	<1/100 1/140	1/10 to 1 1/100 to 1/10	1/10 to 1 1/1000 to 1/100
1-citr. B.	1/800 to 1/400	<1/100	1/1000	<1/100
Bisnitro B.	1/1000 to 1/100	<1/100	1/100 to 1/10	<1/100
Arginylheptaglycylarginine	<1/4000	<1/100	1/1000 to 1/100	<1/1000
1,9-biscitr. B.	<1/4000	<1/100	1/1000 to 1/100	1/100 to 1/10†
Fully protect. B.	<1/4000	<1/100	—	—
Retro B.	<1/80,000	<1/300	1/1000 to 1/300	<1/1000

* Slight coronary constriction.
† Secondary slight constriction, following transient dilatation.
< = Inactive at highest dose or concentration tested.

tached to PE50 polyethylene tubing in a carotid artery. Each peptide, dissolved in physiological saline, was injected i.v. in a 0.05-0.10 ml volume and then flushed in with 0.50 ml of saline during a 10-second period; injections were made at 5- to 20-minute intervals. At least 3 doses of each peptide, alternated with doses of bradykinin, were given. The doses of synthetic bradykinin were 0.31, 1.0, 3.1, 10 or 31 γ/kg and of test peptide 1.0, 3.1, 10, 31 or as high as 1000 γ/kg.

Results. The average effects of synthetic bradykinin in each of the 4 types of test preparations are shown in Table I. The initial contractions produced by bradykinin on the excised rat's uterus occurred within the first minute. The nadir of the hypotensive response in the anesthetized rat to bradykinin occurred within the first or second minute; this response lasted no more than 2 minutes. The maximal effects of bradykinin on capillary permeability of rabbit skin occurred within 15 to 30 minutes after injection of the dye. The peak increases in coronary perfusion rate of the excised cat's heart occurred one to 2 minutes after injection of bradykinin; these effects declined to control levels during the next 2 to 4 minutes.

The estimates of activity for each test peptide, relative to bradykinin, are shown in Table II. None of the peptides produced effects which lasted longer than those produced by bradykinin.

The moderate to marked decreases in vasodepressor activity of 9-citrulline bradykinin were consistent in 2 tests in rats; however, as shown in Table II, the effects of this peptide on rabbit's skin and on excised cat's heart were extremely variable.

Fully protected bradykinin was tested only for effects on the rat's uterus and on the rat's blood pressure; N,N-dimethylformamide solvent controls were devoid of activity in both preparations.

None of the peptides antagonized either the stimulant action of bradykinin on the excised rat's uterus or the increased capillary permeability in the rabbit.

Discussion. Bradykinin is a nonapeptide of the following composition: H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH (15,16). Kallidin has been reported to be a decapeptide with lysine preceding the first arginine of bradykinin, *i.e.*, H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH (17,18,19,20).

In the 4 test systems employed, the following structure-activity relationships were discerned among the 10 peptides related to bradykinin or to kallidin. The peptide with glycine substituted for 6-serine in bradykinin retained full activity. Substitution of glycine

for 7-serine in kallidin diminished activity slightly; kallidin has been reported to be more potent than bradykinin as a vasodepressor agent in the rat but less potent than bradykinin as a uterine or ileal stimulant *in vitro*(1,22) or as a bronchoconstrictor *in vivo* (1,21).

The replacement of 7-proline in bradykinin by glycine decreased biological activity moderately. Substitution of citrulline for either 1-arginine or for 9-arginine or for both terminal arginines in bradykinin resulted in moderate to marked decreases in activity. Blocking both terminal arginines of bradykinin with nitro groups also decreased activity moderately. Fully protected bradykinin exhibited even less activity in the 2 types of tests employed. Arginylheptaglycylarginine (glycine substituted in positions 2, 3, 5, 6, 7, and 8 of bradykinin) showed very low activity. Retrobradykinin, in which all of the amino acids of bradykinin are present in reversed order, also showed very low activity in each of the 4 test procedures.

The activities of these 10 peptides related to bradykinin or to kallidin suggest a high specificity of structural requirement for biological activity. Furthermore, within the limits of the approximate estimates of relative activities, no gross dissociation of one type of biological activity from any other could be detected in any of the peptide analogues.

In the 2 types of tests in which the test peptides were examined for anti-bradykinin activity, none was active.

Summary. Ten synthetic peptides, structurally related to bradykinin or to kallidin, were compared with bradykinin for (a) stimulant activity on the excised rat's uterus, (b) i.v. vasodepressor activity in the anesthetized rat, (c) increased capillary permeability effects in rabbit's skin, and (d) coronary dilator activity in the excised cat's heart.

The decreasing order of activity of these peptides was as follows: (1) bradykinin and 6-glycine bradykinin, (2) 7-glycine kallidin, (3) 7-glycine bradykinin, (4) 9-citrulline bradykinin; 1-citrulline bradykinin; bisnitrobradykinin, (5) arginylheptaglycylarginine; 1,9-biscitrulline bradykinin; fully protected bradykinin; and retrobradykinin. None of the bradykinin analogues manifested anti-bradykinin activity on the excised rat's uterus or in rabbit's skin.

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Received April 5, 1963. P.S.E.B.M., 1963, v113.