It would appear that the anti-rat hemagglutinins detectable in sera of burned guinea pigs, rabbits and humans were elicited by the autologous tissue antigens altered as a result of the injury. An alternative possibility should also be considered that these antibodies were engendered by bacterial infection complicating the wound healing. This interpretation, however, requires a rather unlikely assumption that all the animals suffered from an infection by microorganisms sharing antigens with rat erythrocytes. The final answer to this point was sought in experiments on germ-free animals. Thus far, however, the burned germ-free animals could not be maintained alive long enough to conclude the experiments. Formation of anti-gamma globulin factors might have been stimulated by denatured autologous gamma globulin in a similar fashion as was the case in the above quoted studies(11,12).

Summary. Anti-rat hemagglutinins and anti-human gamma globulin factors were demonstrated in sera of guinea pigs and rabbits exposed to repeated thermal injury. Similar factors were also found in sera of some patients with severe skin burns.

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Received January 16, 1967. P.S.E.B.M., 1967, v125.

Effect of Some Analogues of Bradykinin Upon Vascular Permeability.* (32034)

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Increased vascular permeability is probably an important and early response of the microcirculation to injury in an inflammatory response(1). Bradykinin has been shown to induce several changes associated with experimental inflammatory responses including vasodilitation(2-4), increased vascular permeability(5), and margination of leukocytes (6). It was therefore of interest to assess some of the synthetic analogues of bradykinin in regard to their effect upon vascular permeability, and to compare this action with some other pharmacologic properties of these compounds.

Materials and methods. The synthetic bradykinin and analogues listed in Table I were synthesized by Nicolaides and his associates(7). Permeability properties were measured in the skin of living, unanaesthetized guinea pigs by observing changes in permeability induced by intradermal injection of these compounds suitably diluted in buffer. This technique has been described by Miles

* This work was supported by grants HE-08865 and HE-01661 from Nat. Inst. Health, USPHS, and by grants from Am. Heart Assn. and the Fulton, Paulding, Williams, Henry and Defiance County Branches, Northwest Ohio Heart Assn.

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TABLE I

Compound	Diameter of lesion, 10^{-7} M (mm)	Relative effectiveness in assays for: Vascular permeability	Bronchoconstriction(7)	Hypotension(7)
Bradykinin (Bk)	8.0	1	1	1
1-D-arginine Bk	2.8	1/500	Not tested	Not tested
1-lysine Bk	3.0	1/100	1/3	2/3
1-ornithine Bk	2.0	1/100	1/1000	1/50
BK methyl ester	2.5	1/100	Not tested	Not tested
8-p-fluoro-D-phenylalanine Bk	3.1	1/50–1/100	1.4	1.5
8-D-phenylalanine Bk	3.0	1/100	1/60	1/4
4-homoglycine Bk	2.8	1/100	Not tested	Not tested
9-D-arginine Bk	2.1	1/50	"	"
5-tyrosine Bk	2.4	1/100	"	"
5-D-phenylalanine Bk	4.0	1/10	"	"
5-aminovaleric acid Bk	3.3	1/1000	"	"
6-threonine Bk	7.4	1/2	"	"
6-O-carbamylserine Bk	2.0	1/100	1/2000	1/10–1/100
6-D-serine Bk	5.5	1/10	1/40	1/2.5
Glycyl Bk	8.0	1	Not tested	Not tested
Lysyl Bk (Kallidin I)	8.6	10	1/500	1/20
9-p-fluoro-D-phenylalanine kallidin	2.4	1/100	1/3	2/3

Effect of each compound listed upon vascular permeability is given in terms of the size of blue lesions induced by injections of 0.1 ml of 10^{-7} M concentrations of each. Injections of buffer induced lesions of 2.5 mm or less. Kallidin (lysyl bradykinin) in concentrations of 10^{-8} induced a lesion 7.9 mm in diameter, which is approximately equal to the size of lesion induced by 10^{-7} M bradykinin. Concentrations of 5-amino valeric acid bradykinin as high as 10^{-5} M induced a lesion 5.4 mm in diameter, which was approximately equal to that induced by 10^{-8} M bradykinin. The data included relating to the bronchoconstrictor and hypotensive effects of these compounds have been published(7).

and Wilhelm(8) and consists of the following maneuvers. Guinea pigs were depilated and given injections of a mixture of 1% Pontamine Sky Blue 6x (E. I. DuPont de Nemours and Co., Wilmington, Del.) in amounts of 1.2 ml per kilogram of body weight in 0.075 M sodium chloride mixed with an antihistaminic agent, triprolidine (Actidyl, Burroughs Wellcome Co., London, 0.1 mg per kilogram) intravenously. Dilutions of test compounds in barbital-saline buffer, pH 7.4, ionic strength 0.15, were injected intradermally with disposable 1.0 ml polystyrene syringes and 27 gauge needles (Monoject, Roehr Products Co., Deland, Fla.) into each of 4 animals used in each experiment. The increase in permeability was estimated by averaging the largest diameter of extravasated blue dye and its perpendicular in each animal measured 15 minutes after the last intradermal injection. The average of the 4 mean values was expressed as a numerical value, (Tables I and II) which was a function of vascular permeability. Logarithmic differences in concentrations of peptides from 10^{-5} M to 10^{-8} M were tested. Control injections of barbital-saline buffer were performed in each experiment.

Results. Fourteen nonapeptides, 2 decapeptides resembling bradykinin, and bradykinin methyl ester were tested for permeability properties. All but glycyl-bradykinin and lysyl bradykinin (Kallidin I) were less active than bradykinin (Table I). Of these, 5-aminovaleric acid bradykinin was least active in increasing vascular permeability. While glycyl bradykinin was as effective as bradykinin, lysyl bradykinin (Kallidin I) increased vascular permeability even more. These were the only two decapeptides tested in which the bradykinin sequence of the molecule was unaltered. When the phenylalanine in the 8-position of lysyl bradykinin (*i.e.*, the 9-position of Kallidin I) was substitued with p-fluoro-D-phenylalanine the decapeptide was less active than bradykinin. Although 8-p-fluoro-D-phenylalanine bradykinin has been shown to have greater bronchoconstrictor and hypotensive actions(7) than bradykinin, it was less effective in increasing vascular permeability (Table I).

TABLE II

Injection #1	Injection #2: Diameters of lesions, mm:		
	Buffer	Bradykinin, 10^{-7} M	Lysyl bradykinin, 10^{-7} M
Buffer	2.8	6.9	6.9
Bradykinin, 10^{-6} M	2.9	6.0	5.7
" , 10^{-7} M	2.4	5.8	6.5
Lysyl bradykinin, 10^{-6} M	2.4	4.8	6.0
" " , 10^{-7} M	2.4	4.8	6.0

Samples of substances given as injection #1 were given intradermally in 0.1 ml amounts 30 to 50 min before Pontamine Sky Blue dye was given intravenously. Substances given as injection #2 were injected into the same sites as #1 immediately after injection of dye.

It was possible that an inactive analogue might inhibit the action of bradykinin. Therefore, the least active compound, 5-amino valeric acid bradykinin, was injected intradermally before the intravenous injection of dye into animals, and bradykinin injected into the same site 30 minutes after the dye was given. The prior injection of this inactive analogue did not inhibit the action of the bradykinin. Failure of a number of less active analogues to block the action of bradykinin in other assays has been reported(7,9). When bradykinin was injected *before* the dye and the same, or a lower concentration of either bradykinin or lysyl bradykinin reinjected into the same site, a somewhat smaller increase in permeability was observed than if the pre-dye injection consisted of buffer alone (Table II). In addition, when lysyl bradykinin (Kallidin I) was injected intradermally before administration of dye, the effect of a later injection of bradykinin or lysyl bradykinin into the same site was also decreased. The potency of lysyl bradykinin in this permeability assay decreased during its storage in a solution of 0.15 M sodium chloride at −65°C and repeated thawing. Bradykinin in solution seemed to deteriorate less readily.

Discussion. In these experiments all of the compounds in which amino acid substitutions were made in the nonapeptide analogues of bradykinin had less ability to increase vascular permeability than the parent molecule. Decapeptides in which the amino acid sequence of the bradykinin molecule was unchanged, but glycine or lysine attached to the N-terminal amino acid, either had the same or greater permeability activity in this assay activity than bradykinin. If the C-terminal arginine was esterified, or if D-arginine was substituted for L-arginine, permeability function was lost. Possibly the free C-terminal L-arginine assumes greater importance in the permeability increasing activity of bradykinin than does the N-terminal arginine. The importance of the carboxyl ends of several peptides in functional assays has been reported by Stewart and Woolley(9). The number of analogues which were tested in which the terminal positions of the bradykinin molecule were altered is too small to permit any conclusions to be drawn as to the nature of specificity endowed at these points which designates permeability function. It is of interest, nonetheless, that the only compounds tested which could induce as much or more of an increase in vascular permeability as bradykinin were decapeptides rather than nonapeptides. Possibly there may be other larger related molecules which can induce permeability changes more effectively than some other pharmacological effects, such as those noted in Table I.

There are many reports of species differences in response to bradykinin and lysyl bradykinin(11,12). The ratios of potency of these two kinins vary when the same pharmacological action is measured in different species (11). Carr and Wilhelm(13) found that a polypeptide kinin varied in its ability to increase vascular permeability upon intradermal injection into different species. The data in Table I represent studies of pharmacologic actions of bradykinin and some related compounds as measured in the guinea pig. The ratios of effectiveness of these compounds differ in these three assays for pharmacologic activity within this species. Bronchocon-

striction and hypotension were induced in the guinea pig most effectively with 8-p-fluoro-D-phenylalanine bradykinin(7), a compound relatively ineffective in increasing vascular permeability in the skin of the same species. Lysyl bradykinin, on the other hand, was highly effective in increasing vascular permeability, but less active as a bronchoconstrictor or hypotensive agent in the guinea pig (Table I)(7). These differences seem to indicate differences in specificity of receptor sites interacting with biologically active polypeptides in the different bioassays.

Stewart and Woolley(9) have reported extensive studies on the ability of synthetic bradykinin analogues to act as bradykinin antagonists. While they found that bradykinin-like function usually correlated with antibradykinin activity, substitution of the phenylalanine in the 5- and 8-position with 0-methyl tyrosine provided a compound which was a potent bradykinin antagonist but had little bradykinin-like activity. None of the compounds tested in the present experiments were doubly substituted but all in which the phenylalanine normally in the 5-position was replaced were less active. When 5-amino valeric was inserted in this position, no permeability activity could be found in concentrations ranging from 10^{-6} to 10^{-8} M, but 10^{-5} M concentrations significantly increased vascular permeability. While a relatively inactive analogue of bradykinin (5-amino valeric acid bradykinin) did not inhibit the effect of a later injection of bradykinin into the same site, both bradykinin and lysyl-bradykinin significantly inhibited the action of a later injection of either compound, as shown in Table II. The bradykinin and lysyl bradykinin appear, then, to act through the same receptor sites. The 5-amino valeric acid bradykinin may bind loosely or transiently to these receptor sites, or it may act upon different receptor sites.

Summary. The decapeptide, lysyl bradykinin (Kallidin I) was more effective in increasing vascular permeability in the skin of guinea pigs than was the nonapeptide, bradykinin, or Kallidin II. Bradykinin has been reported to be more active in some other assays measuring pharmacological effects than lysyl bradykinin. All other analogues of bradykinin tested were less active than bradykinin in the permeability assay with the exception of glycyl bradykinin, another decapeptide, which had an equal effect. While an inactive analogue of bradykinin did not block increased vascular permeability induced by a later injection of bradykinin, two active compounds, lysyl bradykinin and bradykinin inhibited the effect of a subsequent injection of either one into the same site.

Synthetic bradykinin BRS-640 was generously provided by Dr. Harry Baird, Sandoz Pharmaceuticals, Hanover, N. J. Dr. Ernest D. Nicolaides generously provided bradykinin analogues, which were synthesized in the Research Laboratories of Parke Davis & Co., Ann Arbor, Mich. Dr. Miklos Bodanszky Dept. of Chemistry, Western Reserve University, has provided helpful criticisms of this manuscript.

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Received January 11, 1967. P.S.E.B.M., 1967, v125.