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Pharmacology of Bradykinin and Related Kinins*

D. REGOLI† AND J. BARABÉ‡

Department of Physiology and Pharmacology, Medical School, University of Sherbrooke, Sherbrooke, Quebec, Canada

I. Introduction

THE history of kinins began in 1909 when two French surgeons, Abelous and Bardier (2), observed a transient fall of blood pressure in man after the intravenous injection of fractions extracted from human urine. In 1928, Frey and Kraut (157) attributed the hypotensive effect of urines to the presence of a thermolabile, nondialysable substance. They called it "Kreislaufhormon," on the assumption that it might be a circulating hormone originating outside the kidney and excreted in the urine. The search for the gland of origin resulted in the discovery of a similar hypotensive principle in the pancreas and the substance was given the name "kallikrein" (231). Kallikrein has been partially purified (159) and its biological unit (the Frey unit) was defined as the quantity of powder that produces the same hypotensive effect in dogs as 5 ml of urine from healthy humans (159). Soon the literature was filled with reports on the hypotensive effects of kallikrein in various species (159, 447) and on the identification of kallikreins in the salivary glands (477) and elsewhere (475, 476); kallikrein was even employed by Frey in patients with arterial occlusion of the legs, but patients complained of pain (472). Frey added a local anaesthetic and continued the tests: the results were inconclusive. The new agent did not appear to be of much utility in therapeutics; its effect in vivo was transient because blood and tissues contain principles that are able to inactivate kallikrein rapidly and efficiently (476). In the meantime, Werle et al. (474) found that kallikrein is practically inactive on the guinea-pig isolated intestine and soon discovered that incubation of kallikrein with blood brings about a considerable increase of activity. Kallikrein is an enzyme that reacts with a plasma component to release a small molecule that Werle first named "Darmkontrahierender Stoff" and later "kal-

* This review is dedicated to the memory of W. K. Park, in recognition of his substantial contribution to the synthesis of the peptides utilised in the present study. This study was completed in December 1979 and covers most of the literature that appeared before November 1979. The rules of IUPAC-IUB Commission on Biochemical Nomenclature (214) have been followed for the designation of synthetic peptides.

† Career Investigator of the Medical Research Council of Canada.

‡ Scholar of the Medical Research Council of Canada.

lidin" (473). This substance is a potent stimulant of the rat isolated uterus (128); its actions are therefore not limited to the cardiovascular system.

A few years later, Rocha e Silva et al. (377) found that trypsin, incubated with blood, releases an agent that contracts the guinea-pig ileum; the response of this tissue develops slowly, compared with its response to histamine, so the authors named the new agent "bradykinin." In the years that followed, Rocha e Silva (373) determined that bradykinin is a peptide and obtained it in pure form (9). The peptide was again isolated by Elliott et al. (113), but the identification of the sequence was not an easy task (111). However, thanks to the systematic work of a group of Swiss chemists, the correct sequence of bradykinin was identified and the nonapeptide was synthesised (35).

Early findings, obtained with crude preparations of bradykinin, were consolidated by using the synthetic compound [e.g. the pain-producing effect (12, 13)]; new biological actions were discovered [e.g. in inflammation (244, 421)] and attempts were made to define the pharmacological effects of bradykinin by means of sensitive and specific bioassays (143, 447). In 1967, the inactivation of bradykinin by the lung was described by Ferreira and Vane (144); from this moment the early suggestion that kinins might be circulating hormones was seriously challenged.

The work performed on kallikreins and kinins during the four decades from 1928 to 1968 was summarised and critically analysed in a superb publication edited by E.G. Erdös in 1970, the 25th volume of the *Handbook of Experimental Pharmacology* (116). In addition to their hypotensive effects in vivo, kinins appear to exert numerous other physiological and pathological actions: they cause pain (12), mediate reactive hyperaemia in exocrine glands (207), participate in vascular and cellular events that accompany the inflammatory processes (13, 200, 244), appear to be involved in the control of blood pressure (227), and may act as protective agents against hypertension (227, 289). In pathological states, kinins are thought to be implicated in bronchoconstriction [asthma (60, 61)], inflammatory diseases [rheumatoid arthritis (108), other forms of arthritis (285)], vascular changes occurring in migraine (408), myocardial infarction (446), cardiovascular failure accompanying vasovagal syncope (66), carcinoid and postgastrectomy dumping syndromes (66), hyperbradykininism syndrome (428), haemorrhagic and endotoxic shock (225, 316), and in other pathological conditions (see 225). In 1970, Rocha e Silva (374) suggested that kinins should be considered "as a class of tissue or local hormones in the same sense given to them by Feldberg and Schilf (136) of a group of active materials which are locally released from diffuse stores in the body, not having specialised glands of secretion and being rapidly inactivated at the site of release."

In the last 10 years, consistent progress has been accomplished in the isolation and purification of the components of the kallikrein-kinin system (148, 169) and in the definition of the complex role that they may play in blood coagulation and fibrinolysis (300). New inhibitors of kallikreins have been discovered (463). Radioimmunoassays (437, 187), which improve constantly, have been developed and extensively used (436) in normal and pathological states. The renal kallikreins have been identified; their site of production and their functional roles as well as their protective action in hypertensive disease are currently under investigation (280, 322, 290). The discovery of a bradykinin-potentiating factor (BPF) by Ferreira (137) resulted in the development of potent and fairly specific inhibitors of the converting enzyme that enhance the biological effect of kinins (16, 378). The degradation of kinins has been extensively investigated and the role of various proteolytic enzymes in the metabolism of kinin peptides has been definitely established (121). Other investigators have turned their attention to the study of the mechanism of action of kinins at the cellular level [see review by Odya and Goodfriend (326)] and to the identification of receptors for these peptides (358, 18). It has been suggested that various actions of kinins on the vessels (441) and on other organs (308) may be mediated by the release of prostaglandins obtained by enhancing the availability of arachidonic acid consequent to the activation of phospholipase A_2 (233, 280). Work is in progress to evaluate the relations between kinins and the newly discovered thromboxanes and prostacyclin (299a). Components of the system have been identified in the brain [see review by Clark (58)] and it has been suggested that bradykinin may act on the central nervous system (67). New biological actions of kinins have been identified, such as the control of sperm motility (161) and the stimulation of cell proliferation (368, 369). Other effects [e.g. the stimulation of catecholamine release from the adrenal medulla (420, 438) and the release of histamine (421, 501)] have received much less attention than in the 1960s. Structure-activity studies and the synthesis of analogues of kinins have been actively pursued in various laboratories (150, 336, 366) and a specific and competitive antagonist for some of the pharmacological effects of kinins has been reported (358). Various kinin-like substances have been identified in tissues of mammals (347) or of other species (26, 347). These and other recent findings in the field have been summarised in the supplement to volume 25 of the *Handbook of Experimental Pharmacology* edited by E.G. Erdös (120).

Important new developments concern both kallikreins and kinins. In the present paper, we will restrict our coverage to kinins, since the biochemical, biological, and functional features of kallikreins have been recently reviewed by Pisano (346), Movat (300), and, in this journal, by Schachter (394).

Of the numerous kinins isolated from various sources, we will analyse the mammalian kinins, namely bradykinin (BK), Lys-BK, Met-Lys-BK, and some of their metabolites. Kinins of nonmammalian origin have been reviewed by Bertaccini (26); other kinins to be found in

mammals [e.g. leukokinins (189), colostrokinin (195), neurokinin (54), and several others mentioned by Schachter (390)], have either not yet been obtained in pure form or are the subject of recent reviews [e.g. leukokinins (190)]; they will not be considered here.

II. The Kallikrein-Kinin System

Before describing and discussing the pharmacology of kinins, we shall briefly summarise present knowledge of their formation and breakdown.

Kinins are formed in biological fluids by the activation of naturally occurring hormogens (kininogens), similar to angiotensins (374). In this respect, kinins and angiotensins differ from the numerous other endogenous agents (hormones, neurotransmitters, autacoids) that are synthesised and stored in specialised cells. The activation of hormogens is brought about by enzymes (kallikreins, renin) produced by the liver (plasma kallikreins) and by exocrine glands (glandular kallikreins) (159, 346, 348), particularly the kidney (kallikreins and renin). While most of the circulating renin is released in active form from the kidney (199), kallikreins are contained as inactive precursors (prekallikreins) in the liver (475), the pancreas (148), and the intestine (405). Kallikreins in active form have been found in homogenates of kidneys and salivary glands (346), but it is still premature to affirm that the precursors are absent in these organs, since activation may have occurred during the isolation (346). Even if some organs produce active kallikreins, their activity is modulated by various small proteins (108, 201, 463) contained in blood and tissues and that are able to inhibit a number of proteinases, including trypsin, plasmin, and kallikreins. As early as 1926, Frey observed that samples of urine contaminated with blood are inactive (159).

Plasma prekallikreins generated in the liver (475) are proteins whose molecular weight (M.W.) averages 130,000 (490, 496) and are precursors of the smaller (M.W. 95,000 to 100,000 (65, 388)) plasma kallikreins. Glandular kallikreins are acidic glycoproteins (M.W. between 27,000 and 43,000) (346, 392, 432), showing immunological, physicochemical (molecular size and charge), biochemical (rate of reaction with synthetic substrates), and pharmacological (inhibition by antagonists) properties different from species to species and from plasma kallikreins within the same species (159, 391, 475). Prekallikrein in plasma is activated by the Hageman factor when blood comes into contact with negatively charged surfaces, inert powders such as kaolin, or other negatively charged particles (265, 317). As pointed out by Colman (63): "The work of these enzymes illustrates the various control mechanisms possible in proteolysis, including activation of inactive precursors, positive feedback, stochiometric inhibition, multistep amplification and enzymatic degradation of active products." In fact the activation of the Hageman factor brings about the stimulation of a number of proteolytic enzymes that circulate as inactive precursors and a succession of biochemical amplifications leading to intricate reactive mechanisms of which the most prominent are: blood clotting, fibrinolysis, plasma kinin formation, and reactions involving complement (108). In this context, the kallikrein-kinin system has been considered one of several interlocking networks that helps to mediate cellular and vascular responses necessary for defense and repair (108, 472).

Kallikreins activated in blood and tissues in this way or released in active form in the extracellular fluid of producing organs react with kininogens, the precursors of kinins. Kininogens exist in at least two forms: a) a low molecular weight kininogen (LMWK) [M.W., 48,000 (228, 342, 343) or 70,000 (343), depending on the species], which presumably crosses the capillary filter and may be utilised by tissue and urinary kallikreins (52), and b) the high molecular weight kininogens (HMWK), which are large proteins (M.W. 100,000/200,000) (198, 344), presumably confined to the blood stream to provide substrates for plasma kallikreins (344). Recent findings (257) suggest that plasma prekallikrein may be coupled to kininogen to form a large complex (M.W. 300,000 and more) that is ready to release kinins at the site where activation of prekallikrein may occur. Kinins formed in plasma or diffusing through the capillary wall or via the lymph from the tissues are rapidly inactivated by several enzymes (kininase I, kininase II, etc.) contained in plasma and in tissues. The fraction of kinins that escapes this inactivation and kinin metabolites reach the peripheral tissues, where kinins combine with receptors and with proteases presumably located on the surface of the cells. By analogy with other peptide hormones [e.g. angiotensin (362), insulin (76) and others (87, 100, 160)], it is assumed that receptors for kinins are different from the enzymes of degradation and that the two types of molecules (receptors and enzymes) compete for the kinins, since they show similar affinities for these peptides. In fact, it is well known that inhibition of kininases enhances the potencies of kinins not only in vivo (191, 378, 412), but also in numerous isolated organs suspended in vitro (55, 117). The hypothesis that metabolic enzymes and receptors are different entities is supported by the existence of pharmacological preparations whose response to kinins is not affected by inhibitors of kininase II (22). This topic will be discussed further in section VI.

III. Inactivation of Kinins

The primary structures of Lys-BK and of BK, as well as the enzymes involved in their activation or inactivation, are shown in figure 1. Met-Lys-BK has not been included, since this kinin is released only after exposure of biological fluids to acid conditions (112), which seldom occur in vivo (391). Several proteolytic enzymes implicated in the metabolism of kinins are considered (fig. 1), although a distinction has to be made between the enzymes contained in blood or located at the surface of endothelial or other cells (139, 384) and those found in

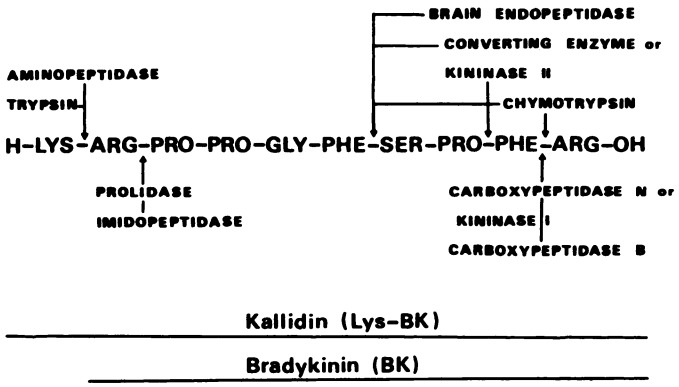

FIG. 1. Primary structure of kallidin and of bradykinin. Site of action of proteolytic enzymes.

cell homogenates. In fact, highly charged peptides such as Lys-BK and BK are unlikely to cross the cell membranes and therefore should have no access to intracellular proteases. By using radiolabeled bradykinin, Ryan et al. (383) were able to show that rat isolated lungs perfused in vitro do not retain any radioactivity.

Among the enzymes active at the amino end, only the aminopeptidase is present in blood (124, 325, 470). This enzyme has been isolated and purified by Guimarães et al. (193) from human serum as a protein with a molecular weight of 95,000. The enzyme cleaves Lys from Lys-BK, Met and Lys from Met-Lys-BK, but is inactive on the Arg^1-Pro^2 bond of BK; acidic residues are not cleaved; therefore the enzyme is almost inactive against angiotensins (193) and it is different from leucine-aminopeptidase (193). Trypsin is present in blood in inactive form (406, 409), except for some pathological conditions (e.g. acute pancreatitis) (409). Imidopeptidases and prolidases have been found only in homogenates of erythrocytes (7) and of renal cortex (125). Ryan et al. (381), however, have provided some indirect evidence that an imidopeptidase may take part in the breakdown of kinins in the isolated lung of the rat.

The functions of the proteolytic enzymes that act at the N-terminus of kinins can therefore be summarised as follows: aminopeptidases can convert Lys-BK to BK. The same is true for trypsin, although this enzyme is active only in special conditions and converts only a small fraction (5% to 13%) of Lys-BK (470). Since these two enzymes are inactive against BK, and the other two (imidopeptidase, prolidase) are located intracellularly, it is assumed that enzymes acting at the N-terminus have no substantial role in the inactivation of kinins.

The most important enzymes involved in the degradation of kinins are those acting at the C-terminal end. The relative contribution of kininase I and kininase II, two metalloproteins, has been indirectly evaluated by Girey et al. (181) by blocking their activities with 1,10-phenanthroline and other metal-chelating inhibitors (127). In the presence of these inhibitors, the formation of kinins stimulated by kaolin in plasma proceeds without appreciable loss over 10 min. In the absence of inhibitors, the peak concentration of kinins observed after 1 min of incubation is rapidly (in 3 to 4 min) reduced by half. Kininase I and II have different sites of action and different locations. Kininase I (also called carboxypeptidase N) is an exopeptidase that removes the C-terminal Arg from BK and from Lys-BK at approximately the same rate (127, 332, 333), while kininase II is a carboxydipeptidase that cleaves the dipeptide Phe^8-Arg^9 (497) and thus inactivates BK, since des-Arg^9, des-Phe^8-BK is practically inactive in vivo and in vitro (121; see also table 12). The best substrate of kininase II is BK, Lys-BK and especially Met-Lys-BK being less susceptible to the degradation by kininase II than BK (95, 96, 365, 376). Kininase I is present in blood, while most kininase II is located on the surface of the lung endothelial cells and in various other vascular beds and organs (126, 127, 144, 382, 454).

Kininase I is responsible for 90% of the kinin destruction in plasma (435, 500), the other 10% being accounted for by kininase II, which is thought to be identical with the enzyme that converts angiotensin I to angiotensin II (117, 123). Kininase II is definitely the major contributor to the degradation of kinins in the lung (381, 383).

The efficiency of the two enzymes and their relative contribution to the overall degradation of kinins can be evaluated by comparing the half-life of BK in circulating blood and the fraction of BK that is inactivated in a single passage through the lung. Both processes appear to be very rapid. With the use of an extracorporeal circulation, McCarthy et al. (277) estimated that the half-life of BK in dog blood is about 0.27 min. Similar values were obtained by Ferreira and Vane (144) in the cat by the use of the blood-bathed organs technique (453). Lys-BK (kallidin) is inactivated at approximately the same rate as BK [half-life of Lys-BK, 0.32 min (277)]; this suggests that the process of inactivation at the C-terminus is probably more rapid than the activation of Lys-BK to BK by the aminopeptidases. As a matter of fact, Lys-BK injected in vivo is about twice as potent as BK (365, 20) and it is therefore unlikely that much conversion of Lys-BK to BK occurs before it reaches the receptor sites. The degradation of kinins in the lung is even more rapid, since 80% to 95% of the biological activity of BK is eliminated during the few seconds required for passing through the pulmonary vascular bed. This important phenomenon, observed by Kroneberg and Stoepel in 1963 (232), was carefully investigated by Ferreira and Vane in 1967 (143) and has since been intensively studied and confirmed in vivo and in isolated lung preparations by several workers (125, 127, 30, 381, 382). For further details the reader is referred to review articles by Erdös (117, 118, 127).

Biochemical studies have established that kininase I is a large protein (M.W. 280,000) active against kinins (332), C3a anaphylatoxin (36), and other peptides containing Lys or Arg at their carboxyl ends (123, 333). Kininase I has been isolated and purified from human plasma by

Oshima et al. (332, 333), who demonstrated that it is different from carboxypeptidase B (M.W. 24,000), the pancreatic enzyme that cleaves C-terminal Arg and Lys of peptides and proteins (156). Under physiological conditions, however, carboxypeptidase B appears to be present in blood at concentrations (179) too low to account for any important carboxypeptidase activity. Therefore the cleavage of the C-terminal Arg of kinins in plasma is carried out principally by kininase I. This enzyme releases des-Arg^9-BK and des-Arg^{10}-kallidin, two potentially biologically active metabolites of the kinins (see section VII).

The enzyme that converts angiotensin I (AT_I) to angiotensin II (AT_{II}) was discovered over two decades ago by Skeggs et al. (239, 411) in horse plasma. Ten years later Ng and Vane (314, 315) found that the conversion of AT_I to AT_{II} in plasma was too slow to account for the generation of AT_{II} in vivo, and discovered the conversion function of the lung. It is now well established that the angiotensin I converting enzyme is the same as the enzyme that inactivates kinins (kininase II) (55, 119, 121). This enzyme is bound to cell membranes and it is widely distributed in a variety of vascular beds (5, 221, 456), in the pituitary gland (499), the brain (351, 499), the choroid plexus (211), the kidney (468), and the testicles (78). Kininase II is a glycoprotein (M.W. 129,000 to 480,000 (81, 94, 121, 303) with a variable (between 8% and 26%) carbohydrate component, which presumably accounts for the large variation in molecular weight (121). The enzyme cleaves C-terminal dipeptides provided that the last residue has a free carboxyl [except for Glu (110)], and that the adjacent residue is not Pro (498). BK is among the best natural substrates for kininase II, since its K_m is lower than that of AT_I (117) and of other synthetic or natural substrates (16, 238). Kininase II is efficiently inhibited in vivo and in vitro by a large number of natural and synthetic compounds, which will be further discussed in section VII E.

Inactivation of kinins is brought about by two other enzymes, chymotrypsin and an endopeptidase from rabbit brain (43). Chymotrypsin splits the C-terminal residue more rapidly than the Phe^5-Ser^6 bond (111, 395) thus releasing des-Arg^9-BK and des-Arg^{10}-kallidin, two potentially active metabolites (see below). However the relative contribution of chymotrypsin to the overall metabolism of kinins has not been established. The Phe^5-Ser^6 bond is also a site of cleavage by the endopeptidase isolated by Camargo et al. (43) from rabbit brain.

In conclusion: kinins exist in circulating blood in very low concentrations (see also the following section), their low levels being the result of the balance between simultaneous processes of production and inactivation. As shown above, the enzymes responsible for the inactivation of kinins are multiple, very potent, and distributed in blood and in tissues such that the half-life of kinins is very short. BK appears to be inactivated more rapidly than Lys-BK and other naturally occurring kinins (120). Under normal physiological conditions, the lung and tissue inactivating systems (kininase II) appear to be prominent (454), both in terms of efficiency and of the quantity of kinins inactivated per unit of time. This system is very efficient since, by removing the C-terminal dipeptide Phe^8-Arg^9, it eliminates completely the biological activities of both BK and Lys-BK. When kininase II is inhibited, e.g. by the administration of recently discovered inhibitors (79, 329, 330), the inactivation of kinins may proceed more slowly and be less efficient, since the remaining inactivating system (plasma kininase I) cleaves only the C-terminal Arg^9 and releases fragments that are biologically active in some circumstances (see further discussion in section VII).

IV. Components of the Kallikrein-Kinin System in Biological Fluids. Critical Analysis of Assay Methods

Because of the rapid inactivation by kininases, free kinins are not easily detected in the circulation. Moreover, the limitation of measuring the blood levels of what are probably, if anything, local hormones, should be kept in mind at the beginning of an analysis directed to evaluate the concentrations or activities of the various components of the kallikrein-kinin system in biological fluids. Such an analysis is of fundamental importance to the pharmacologist, since, by knowing the concentration of circulating kinins, he can discriminate between the effects of physiological interest and those that have to be considered as pharmacological or toxic manifestations. In the absence of satisfactory and specific antagonists for most of the biological effects of kinins, the measurement of kinin levels in blood is probably the only valuable way to get information about the significance of kinins in physiological and pathological processes, as well as about the relevance of the pharmacological effects. For this reason, assays have been developed to measure the content of kininogen, the activity of kallikreins, and the concentration of kinins in plasma and urine. A short survey of these assays is essential to appreciate problems and progress in the field. The assays can be divided into three categories: a) bioassays to measure the biologically active products, the kinins; b) enzymatic assays (esterolytic) to measure the activities of the kallikreins; and c) radioimmunoassays to measure either the enzymes or the active products.

Several workers (50, 134, 446, 460, 462, 490) have attempted to evaluate the activity of the kallikrein-kinin system by measuring changes of plasma kininogen in various experimental or pathological conditions. The amount of kininogen is evaluated by activating the kallikreins and by measuring the released kinins by bioassays. The major criticism of such an approach has been that blood contains such an enormous quantity of kininogen [in the order of 4 to 12 μg/ml, when expressed by the capacity of the plasma to generate kinins in presence of trypsin (50, 134)], that only a tiny fraction of kininogen

needs to be consumed to provide an important increase of circulating kinins (which are usually found in concentrations of nanograms/milliliter). Therefore, changes of kininogen may be observed only in extreme circumstances and the sensitivity of the assay is probably very low.

Plasma kallikreins can be measured by radioimmunoassays. A new method has been developed recently by Saito (388). The assay appears to be very sensitive since it measures prekallikrein concentrations down to 1.5 to 2.0 μg/ml of human plasma (0.3% of the concentration that is found in the plasma of healthy subjects). A good correlation ($r = .71$) was found between titers of prekallikrein measured by the Fletcher factor clotting assay and by the radioimmunoassay in 40 normal subjects. The availability of an antibody against human prekallikrein permits the direct measurement of the proenzyme in the plasma, thus avoiding the complex and risky procedures commonly used in other similar assays for activating kallikreins or for extracting and purifying kinins. The assay described by Saito is indeed a promising one, but the specificity of the antibody remains to be proved definitely.

Moreover, it is still uncertain if the measurement of prekallikrein or kallikrein in plasma or in other biological fluids reflects the actual activity of the kallikrein-kinin system, which is obviously expressed in terms of concentration of active kinins. In several instances, the plasma kallikrein activity does not vary in accord with the concentration of kinins. Recent studies by Vinci et al. (460, 462) show that the observed increase of bradykinin in plasma of patients affected by Barrter's syndrome or of normal subjects submitted to sodium restriction was not associated with changes of prekallikrein or kallikrein levels. Since this appears to be a general finding (187, 436, 461), it is our opinion that assays for kallikreins might not be sufficiently precise to detect certain changes in the enzyme activity of the plasma system such as those which may occur in physiological conditions and in some pathological states. Direct radioimmunoassays for human and rat urinary kallikreins have also been described (49, 259). The major problem with these assays is that they measure not only kallikreins but also prekallikreins and inhibitor-complexed kallikreins (259).

In the majority of the studies in which kallikreins have been measured in plasma and urine, prekallikrein was activated in vitro by kaolin, or by contact with a glass surface, or by other means to give active kallikreins, which are tested for their ability to split *p*-toluene-sulphonyl-L-arginine-methyl ester (TAME) (147, 178, 212, 242, 268, 291, 404), or some new promising tripeptide substrates, such as Bz-Pro-Phe-Arg-*p*-nitroanilide (180) and Bz-Phe-Val-Arg-*p*-nitroanilide (203), or to release kinins on incubation with natural substrates (343). Assays of urinary kallikreins were extensively reviewed by Obika in 1978 (324) and by Levinsky in 1979 (240). Both authors have emphasised the lack of specificity of the esterolytic assays as well as their advantages and limitations. Esterolytic assays have the advantage of eliminating the need to purify kininogen, to inactivate kininases, and to extract the kinins. However, these assays appear to be more adequate for evaluating the activities of kallikreins than to measure their concentrations, since, in most instances, there is no way to determine whether the activation of prekallikrein is partial or complete.

Concentrations of kinins in biological fluids have been measured by radioimmuno- or bioassays. Radioimmunoassays are being used increasingly frequently (436); the advantages of radioimmuno- over bioassays are the smaller sample of blood required for each estimation, the convenience afforded by the simultaneous processing of a large number of samples, and possibly the specificity. In recent years, radioimmunoassays have therefore replaced progressively the bioassays, particularly for bradykinin. However, an important question remains unanswered: whether this constitutes actual progress. Although the radioimmunoassays are improving rapidly, problems and limitations encountered with kinins appear to be more numerous and complex than in similar methods employed to study a variety of other endogenous agents (e.g. polypeptide hormones) (187). Limitations and pitfalls of radioimmunoassays for kinins have been analysed in two excellent recent review articles by Goodfriend and Odya (187) and by Talamo and Goodfriend (436). These two papers, like others, direct the attention of the reader to the weak immunogenicity of kinins, to the unavailability of labelled kinins with sufficient specific activity, to the crossreactivity of bradykinin antibodies with kininogen (51), and to the numerous difficulties related to collecting biological samples, while avoiding the formation or the inactivation of kinins. Talamo and Goodfriend (436) conclude that results of radioimmunoassays for kinins are not necessarily indicative of the in vivo state, because of the large number of factors that might affect kinin levels in vivo during the collection of the blood samples and during the assay in vitro. In particular, the preparation of samples for the bradykinin immunoassays involves addition of various agents [see (187) for a review] to block formation and degradation of kinins. How these agents affect components of plasma that might accompany kinins through extraction of purification steps, or how they can affect the antigen-antibody reaction or even the free kinins remains to be established (187).

Kinins have also been measured by bioassays. For almost two decades, after the discovery of bradykinin by Rocha e Silva et al. (377), kinins were tested for their hypotensive effects in living animals and for their stimulant actions on isolated intestines [the guinea-pig ileum (372, 377), the cat ileum (129)] or the rat uterus (128). The rat uterus assay, introduced by Erspamer in 1948 (128) was extensively used by Schröder (400), Schröder and Lübke (401), and Stewart et al. (422, 423, 426) for studying the structure-activity relations of synthetic kin-

ins, because of its high sensitivity and despite its lack of selectivity (167). The same applies to the guinea-pig ileum used by other investigators (366, 431). Lack of selectivity did not represent a major problem for work with synthetic peptides but was a serious limitation for the bioassay of kinins in biological fluids. This was realised by Ferreira and Vane (143, 144) when they tried to estimate the release and fate of kinins in the circulation of the dog. In fact, both the rat uterus and the guinea-pig ileum contract not only with bradykinin, but also with 5-hydroxytryptamine, acetylcholine, angiotensin, and prostaglandins, four substances that are present in blood in active concentrations under the same conditions in which bradykinin is released. Ferreira and Vane (144) found that the cat isolated ileum was more selective for kinins than the other two preparations and were able to check the presence of other agents in blood by using a cascade of four organs; the rat stomach strip for serotonin, the chick rectum for catecholamines, the rat colon for angiotensin, and the cat ileum for bradykinin. In addition, they treated the animals and the tissues with a bradykinin-potentiating factor that blocks the inactivation of bradykinin in vivo and increases the sensitivity of some smooth muscles to this peptide. The approach of Ferreira and Vane soon revealed some limitations when several other peptides were found to be released in blood and to have pharmacological properties similar to kinins. Some of these peptides have been recently classified into four major groups by Erspamer et al. (130) and Bertaccini (26). Although the majority of these new peptides were found in the skin of amphibia, at least one member of each group has since been demonstrated to occur in mammals. These are substance P (a tachykinin), cholecystokinin (a caerulein-like peptide), and bombesin. Substance P is present in mammalian blood (318); cholecystokinin and bombesin have been identified as immunoreactive material in central and peripheral neurons (364, 466) as well as in other tissues [human foetal lung (480) and rat brain (349), among others]. The pharmacological properties of these peptides, analysed in table I, show similarities with those of the kinins.

Among other features, it is worth mentioning that the cat ileum appears to be less selective for kinins than previously thought, since it contracts also in response to histamine (144), caerulein, and bombesin. While the action of histamine can be blocked by specific antagonists, such compounds are not yet available for the peptides. The bradykinin-potentiating agents developed by Ondetti et al. (330) cannot be used to make the cat ileum more selective for kinins, since they are unable to potentiate the effect of bradykinin in this preparation (22). The data summarised in table 3 also suggest that it is unlikely that isolated vessels or other preparations would provide bioassays selective for kinins, since substance P and caerulein are hypotensive in vivo, relax the arterial smooth muscles, and stimulate the veins, similar to kinins. Even if substance P and cholecystokinin are secreted in small amounts from the producing organs, they are likely to remain in the blood longer than kinins, because they are not inactivated by the lung (34, 71). The ability of kinins to stimulate the release of prostaglandins cannot be used as a specific test, because of the complexity of

TABLE 1

Comparison of properties of "kinins" subdivided into four groups according to Erspamer et al. (130) and Bertaccini (26)

Test	Bradykinin	Substance P	Caerulein	Bombesin
Blood pressure (anaesthetized rat)*	Decrease (209)	Decrease (339)	Decrease (27)	Increase (131)
Peripheral blood flow (human forearm)	Increase (86)	Increase (109)	Increase (26)	Not tested
Isolated artery (dog's carotid)	Relaxation (71)	Relaxation (71)	Relaxation (high doses) (22)	Relaxation (high doses) (22)
Vascular permeability	Increase (504)	Increase (504)	Increase (27)	No change (22)
Isolated vein (dog's saphenous)	Contraction (184)	Contraction (22)	No effect (22)	Not tested
Isolated intestine (cat ileum)	Contraction (26)	No change (26)	Contraction (26)	Contraction (26)
Isolated uterus (rat uterus)	Contraction (26)	Contraction (31)	Contraction (high doses) (26)	Contraction (26)
Isolated bladder (rat urinary)	Contraction (26)	Contraction (132)	Contraction (high doses) (26)	Contraction (26)
Release of histamine (rat mast cells)	Stimulation (220)	Stimulation (220)	Stimulation (220)	Not tested
Salivary secretion (rat submandibular)	No change (130)	Increase (130)	No change (130)	No change (130)
Release of prostaglandins (evaluated from the effect of indomethacin in dog's saphenous vein)	Yes (184)	No (22)	Not tested	Not tested
Pulmonary inactivation (evaluated from the effects of the i.v. and i.a. administration in rats)*	Yes (454)	No (70)	Not tested	No (22)

* Blood pressure in anaesthetized rat decreased (22) and no pulmonary inactivation (22) occurred with *C*-terminal octapeptide of cholecystokinin.

the prostaglandin system (see section V). The analysis and the examples of the peptides discussed above show that isolated smooth muscle preparations are unlikely to provide selective and specific bioassays for measuring naturally occurring kinins in biological fluids until specific antagonists for kinins are discovered.

This critical analysis of the assays currently used for measuring kinins may help to explain the wide range of values of plasma kinins reported by various authors and summarised in table 2. Thus, concentrations of BK immunoreactive material in plasma or blood of healthy humans vary between 5.0 (462) and 0.07 ng/ml [a factor of 71) (274)]. Changes due to posture or dietary electrolytes cannot account for such a large difference, since upright position or sodium restriction increases plasma concentrations of BK only by a factor of 1.5 to 3.0 (462, 493). Changes in the rate of metabolic degradation can also be excluded, since the administration of potent inhibitors of the converting enzyme in concentrations sufficient to block the activation of AT_I to AT_{II} had no (286, 461) or little effect (433, 485) on plasma kinins; moreover, in hyperbradykininism (428), where the activity of kininases is significantly reduced, plasma BK increased only by a factor of 4.0.

When applied in patients affected by various diseases, radioimmunoassays of BK have given consistent results only in pathological states associated with an increase of plasma BK [postgastrectomy (66, 492) or carcinoid (66, 436) dumping syndromes, and Barrter's disease (460)]. Variable and inconclusive results were obtained in cases of sepsis (276, 325), dengue fever (105), and acute febrile illness (105). In pathological states associated with a presumed decrease of plasma BK [liver cirrhosis (491), Williams (64) and Fitzgerald trait (387), or in Fletcher factor deficiency (93)] the radioimmunoassay is useless (436) because it is not sensitive enough. Another weak feature of immunoassays for bradykinin is represented by the antibodies that crossreact to various extents with kininogen (187) and do not discriminate between bradykinin and larger kinins (210, 274, 328, 460), or some of their metabolic fragments [e.g. des-Arg^9-BK (152)]. However, recent reports (210, 328, 355, 437) have shown that antibodies raised against kininogen (437) or bradykinin coupled to ovalbumin (210, 355) are quite specific for the C-terminal Arg and can therefore discriminate between the kinins and major metabolites derived from their breakdown by kininase I and II. This represents definite progress with respect to early studies, in which the cross-reactivity of BK antibodies with fragments (415) or analogues (413, 414) of BK was found to be fairly high. Specificity of antisera is another area of progress; for example, while previous studies (186) indicated that the specificity of a BK antiserum changed with the radioactive antigen, recent reports (187, 328) show that specificity is a function only of the antiserum (187). Still the experts (187) emphasise that the choice of the carrier or the method of coupling BK to the carrier can markedly influence the final results of the immunoassay. Finally, a variable fraction of kinins is generally lost during the purification procedures required to isolate the endogenous kinins before reacting with the antiserum or testing on isolated organs. [^{3}H]Bradykinin is the most used compound for evaluating recovery, which has been found to vary from 100% (274) to 39% (210).

Table 2 also summarises some of the studies performed in the sixties, when kinins were measured by bioassay. Estimates of concentration of bradykinin-like material in plasma of normal subjects fall in the range of 1 to 79 ng/ml and show enormous variations in the various studies (40, 323, 428, 502). Bradykinin-like activity was measured in the rat uterus (323, 428, 502) and the peptidic nature of the material was established by incubating the plasma samples in the presence of chymotrypsin (323, 502) or carboxypeptidase B (428). However, while some authors (428) used atropine and methysergide to avoid the interference of acetylcholine and serotonin on the uterus, and measured the lower values of plasma kinins, others (323, 502) did not use antagonists. The sensitivity of the bioassays utilised in these studies varies from 0.1 to 1.0 ng of BK/ml of plasma and is similar to the sensitivity of the immunoassays (187).

Kinins have also been measured in biological fluids other than plasma, as shown in table 2. Urinary kallikreins and kinins have been studied in relation to human and experimental hypertension. They have been found to be decreased (407) or unchanged in hypertensive patients and animals. For further reading on this topic, the reader is referred to the comprehensive reviews by Ward and Margolius (469) and Mills (290). Kinins appear to be increased in nasal secretion of allergic patients (1, 91), compared to patients suffering from nonallergic infections of the upper respiratory tract. A consistent increase of kinins has been found in synovial fluid taken from patients affected by rheumatoid arthritis (285) and other forms of inflammatory disease of the joints (see table 2).

From the foregoing brief critical analysis of problems related to bradykinin immunoassays, it emerges that much more work is needed before these assays can be used for adequately evaluating changes of kinin concentrations in blood of normal subjects and of patients affected by various diseases in which kinins are expected to intervene as pathological factors. The low levels of circulating bradykinin detected with the most sensitive methods (see table 2), as well as recent experiments with inhibitors of the converting enzyme (SQ 20881, SQ 14225) in humans, raise the question as to whether circulating bradykinin is of any importance in normal conditions. In fact, in spite of the extreme potency of converting enzyme inhibitors in blocking the kinin inactivating system (29, 115) the administration of these inhibitors to normal subjects failed to induce an increase in circulating bradykinin (433, 461, 485) while blocking efficiently the conversion of angiotensin I. We favour the interpretation that either there is no continuous production of kinins in

TABLE 2

Concentration of bradykinin(BK)-like material [measured by radioimmunoassay (R) or by bioassay (B)] in plasma and in biological fluids of normal subjects and of patients affected) by various diseases

Condition	No.	BK (ng/ml)	Assay	Ref.
Kinins (Bradykinin-like) in Plasma				
Normal	7	0.07 ± 0.03*	R	(274)
Normal	19	5.0 ± 2.0	R	(490)
Normal	32	0.04–0.46*	R	(210)
Normal	15	0.2 ± 0.04	R	(273)
Normal				
Hand arterial blood	6	0.15–0.9	B	(271)
Hand venous blood		0.1–0.5		
Effect of heating 33°C	3	0.13		
Hand (venous) 45°C		0.19		
Hand (venous) 17°C	1	0.21		
Reactive hyperaemia after ischaemia	2	0.23		
Effect of cooling, hand venous	1	0.35		
Normal (N)	14	1.38 ± 0.45	R	(493)
N Na^+ depleted	7	3.56 ± 1.00		
N Na^+ loaded	4	0.54 ± 0.01		
After 120 min, upright		0.96 ± 0.07		
Normal (N)	11	2.2	R	(286)
N Na^+ depleted	5	2.8 ± 0.8		
After SQ 20881		4.5 ± 0.9		
N recumbent/upright	29	3.3 ± 0.3/4.5 ± 0.5	R	(462)
N Na^+ depleted recumbent/upright	17	5.4 ± 0.7/8.3 ± 0.7		
N Na^+ loaded recumbent/upright	8	2.9 ± 0.3/4.0 ± 0.6		
Pregnancy	56	<2.0	B	(284)
Umbilical artery at delivery		2.0–15		
Umbilical vein at delivery		2.0–90		
Open heart surgery				
Before	58	0.17 ± 0.04		
1 min after		0.19 ± 0.04		
20 min after		2.51 ± 0.46		
At the end		2.60 ± 0.9		
Normal	9	3.2 ± 0.7	R	(485)
After SQ 20881		4.0		
Hypertension	6	5.5 ± 1.3		
After SQ 20881		7.4 ± 2.6		
Hypertension	14	3.2 ± 0.7	R	(433)
After SQ 20881		4.5 ± 1.3		
Hypertension	6	1.1 ± 0.2		
After SQ 20881		1.0 ± 0.4		
Hypertension (H)	13	Approx. 7.0	R	(461)
After SQ 20881		Approx. 7.0		
H Na^+ depleted	9	Approx. 9.0		
After SQ 20881		Approx. 7.0		
Hypertensive	10	7.0 ± 1.8	R	(461)
After SQ 20881 5 min		7.0 ± 1.6		
After SQ 20881 60 min		5.1 ± 1.1		
Normal	7	1.4–2.2	R	(437)
Normal	7	<1.25		
Hereditary angioedema	13	1.5–35.0		
Normal (N)	19	2.95 ± 1.75	R	(460)
After indomethacin		2.7 ± 0.6		
Barrter's syndrome	7	13.2 ± 4.2		
After indomethacin	6	3.9 ± 0.9		
N Na^+ depleted	14	6.0 ± 0.9		
Liver cirrhosis	20	<0.25	R	(491)
Sepsis + hypotension	11	↑ in 7	R	(325)
		= in 4		
Normal	18	1.52 ± 1.42	R	(105)
Fever	8	2.39 ± 3.55		
Dengue fever	7	1.52 ± 0.68		
Dengue shock	11	1.06 ± 0.7		
Normal (N)	16	2.8 ± 0.4	B	(40)
N + vasovagal fainting	2	30		
Dumping syndrome (D)	4	0.7–7.0		
D + glucose		14–140		
Postgastrectomy				
Without dumping	5	<3.0	R	(492)
With dumping	5	3.0–15		
Dumping syndrome	6	0–110	B	(77)
Normal (N)	4	1.7–3.7	B	(502)
N + glucose		2.2–3.0		
Dumping syndrome (D)	4	1.5–6.8		
D + placebo		1.7–8.8		
D + glucose		15.3–2165		
Carcinoid flush	4	24–53		
Normal (N)	13	<1.0	B	(323)
N + noradrenaline		<1.0		
Carcinoid (C)	5	<1.0–8.0		
C + noradrenaline		2.0–12		
Normal	9	0.2 ± 0.6	B	(1)
Bronchial asthma	19	2.9 ± 2.7		
Normal	11	0.96 ± 0.11	B	(428)
Hyperbradykininism	5	4.24 ± 0.60		
Kinins (Bradykinin-like) in Other Fluids				
Urines				
Normal	8	3.0–60 ng/ml	R	(272)
		5.0–80 ng/ml	B	
Normal (N)	17	12.5 ± 1.3	R	(462)
N Na^+ depleted	29	13.4 ± 0.9		
N Na^+ loaded	8	14.7 ± 2.1		
Normal	10	37.9 ± 3.9	R	(407)
Renal failure	3	9.0 ± 5.0		
Essential hypertension	12	24.2 ± 5.2		
Nasal Secretion				
Allergic	12	1.0–5.0 ng/ml	B	(91)
Infectious	6	0–<1.0 ng/ml		
Synovial Fluid				
Arthritis, gouty	7	1.6–4.3 ng/ml	B	(285)
Psoriatic	2	10–14 ng/ml		
Unknown	2	4.9–12 ng/ml		
Rheumatoid	6	2.9–44 ng/ml		
Spontaneous attack	1	58 ng/ml		
Induced attack	1	43 ng/ml		

* Measured in blood.

the body or that inactivating systems other than the kininase II are sufficiently active to compensate for its absence. Based on these considerations, we will view with caution any effect produced by kinins at a site distant from their release (hormonal effects) when such effect is obtained with concentrations of exogenous kinins in the microgram range.

Assays for plasma prekallikrein and urinary kallikreins

and kinins are rapidly improving and it is hoped that they will soon permit an adequate measurement of the concentrations of these endogenous agents in biological fluids. Despite the limitations of the presently available methods, a large body of information has already been obtained to indicate that kallikreins and kinins may play an important role in health and disease. For instance in the normal kidney they appear to be essential regulatory factors (52) primarily involved in the distribution of renal blood flow (290). As far as renal pathology is concerned, the recent survey by Carretero et al. (52) summarises the most relevant data favouring a possible involvement of kinins in the pathogenesis of hypertension, kidney diseases, and disorders of fluid and electrolytes.

V. Biological Actions of Kinins

A. Cardiovascular System

From the very beginning, kallikreins and kinins have been associated with the regulation of the cardiovascular system (202, 374). Numerous investigators have shown that the haemodynamic and vascular effects of kinins (particularly bradykinin) will vary depending upon the species studied, the organ under observation, the dose of kinin applied, and the interference by other endogenous vasoactive substances. When injected intravenously into mammals, bradykinin produces a rapidly reversible fall of blood pressure that is due to arteriolar vasodilation and to the resulting decrease of peripheral resistance. Threshold hypotensive doses of bradykinin vary from 0.02 to 4.0 μg/kg depending on the species; the rabbit (20, 337, 429) and the dog (277) are among the most sensitive. The hypotensive effect increases gradually with the dose up to a certain level; with high doses, hypotension is prolonged (385). Intravenous infusions of bradykinin fail to maintain a stable hypotension in animals (20, 385) and in man (385). Prolonged hypotension can be produced only by gradually increasing the rate of infusion (146). The rapid reversibility of the hypotensive effect of kinins has been attributed to the reflex increase of heart rate, cardiac output, myocardial contractility (202, 205, 304), and to the redistribution of blood flow to the various organs, which follow the initial rapid decrease of blood pressure. In the cat (234), the rat (74, 237), the dog (338), and the rabbit (237, 287, 458), bradykinin has a biphasic effect (a fall of blood pressure followed by an increase over preinjection levels), particularly after high doses (234), repeated injections (237, 338), and in animals with low initial pressure levels (74), or in absence of anaesthesia (338, 438). A rise in blood pressure has also been observed when bradykinin is injected into the carotid artery of cats (234) and dogs (197). Numerous recent studies have shown that bradykinin has a variety of effects on nervous structures. For details, see the recent review by Clark (58). In the context of the cardiovascular actions, we will mention only that bradykinin applied centrally is hypertensive. When injected into the ventricles in the rat (67), this peptide produces a marked increase of the arterial pressure, while substance P and des-Arg^9-BK are inactive. These findings suggest that, in addition to its peripheral vasodilator effect, bradykinin may exert peripheral and central nervous effects that tend to increase blood pressure and that could be involved in the biphasic or pressor responses described above. Early interpretation (237, 338) of these unexpected responses suggested that they are mediated via activation of the peripheral sympathoadrenal system, after the demonstration by several workers (135, 420) that bradykinin releases catecholamines from the adrenal medulla and stimulates the sympathetic ganglia (245). Such an interpretation was challenged by Staszewska-Barczak and Vane (420), who measured the release of catecholamines induced by kinins in the dog and in the cat with the blood-bathed organ technique (453). The effect of BK on the adrenal medulla was found to be unpredictable and to require concentrations of peptide much higher than those producing significant hypotensive effects. In the same study (420), it was demonstrated that BK acts directly on the adrenal medulla, since its effect resists ganglion blocking agents and is abolished by adrenalectomy. Release of catecholamines as well as stimulation of the sympathetic ganglia (245) evoked by BK when applied by close arterial injection of 0.5 to 10 μg are likely to be pharmacological actions; their physiological importance is not established (135, 420). Their contribution to the pressor effect of kinins is not certain; other mechanisms may be involved in this effect (see below).

It appears that kinins have no consistent direct action on either the parasympathetic or the sympathetic nerve terminals. Early work by Khairallah and Page (226) in the isolated guinea-pig ileum showed that while the contractions induced by angiotensin II were reduced in the presence of atropine and morphine those provoked by BK were not influenced by these drugs and presumably result from a direct action of the kinin on the muscle. Little if any evidence has since been provided in favour of a direct action of BK on the parasympathetic nerve terminal or the intramural ganglia. No mention of kinins is made by Westfall (478) or by Langer (235) in their extensive reviews of all natural agents and drugs involved in the local regulation of adrenergic neurotransmission. Recent findings from our laboratory (22, 251) indicate that, while angiotensin II and substance P are able to potentiate the contractions of the isolated rat vas deferens by acting predominantly at the presynaptic (sympathetic nerve ending) level, kinins act exclusively on the muscle. In strips of pulmonary artery and in isolated hearts of rabbits stimulated electrically, bradykinin was found to inhibit the contraction of the muscle and the associated release of noradrenaline (312, 416) not through a direct action but mainly by the intermediation of prostaglandins. Similar findings have been described recently in the rabbit isolated kidney (253).

Two other effects of kinins need to be considered in relation to their haemodynamic actions; namely, the nociceptive effect and the ability of kinins to promote the production and/or the release of prostaglandins. Kinins are potent algesic agents, since they cause pain when applied to the blister base in man (224). In animals, bradykinin causes nociception when injected i.p. in rats and mice (458), and provokes pseudoaffective responses when given by close intraarterial injection in the spleen or in other organs of dogs and cats (138, 140, 141), and when applied either to the knee joint, according to Moncada et al. (294), or on the surface of the heart (419) in the dog. Among the pseudoaffective responses, the increase of heart rate, blood pressure, and renal vascular resistance are the most prominent. These responses derive from the ability of kinins to stimulate pain receptors and to increase the discharge of afferent sympathetic fibres (450). Pseudoaffective responses are of a reflex nature, integrated either at the spinal (255) or supraspinal level. The dose of bradykinin required to elicit these responses is fairly small (419) and therefore this phenomenon may be of physiological importance in the overall cardiovascular action of kinins in living animals, particularly the biphasic response observed in nonanaesthetised animals (202, 438).

While the nociceptive action can be evoked to explain the biphasic or pressor effects of kinins, the release of prostaglandins is actually involved in the vasodilation. Among the vasoactive peptides tested as prostaglandin releasers, bradykinin and, in some systems, angiotensin appear to be the most active (311, 360); substance P (360) and eledoisin (281), among others, are inactive. Bradykinin has been shown to increase the production of prostaglandins in vivo from the rabbit lung (458), the dog kidney (281, 306), spleen (141), and uterus (442), and in vitro from a variety of organs, including the guinea-pig lung (345), the dog spleen (293) and kidney (441), and the rabbit heart (32, 310, 311, 494). Other indirect evidence in favour of such an effect has been obtained in studies performed in vivo and in vitro (see 308 and 441 for reviews) with antiinflammatory drugs (indomethacin, aspirin, meclofenamate) that have been shown to block the synthesis of prostaglandins (455); these drugs reduce several biological actions of kinins. Data obtained in pharmacological preparations suspended in vitro and/or perfused with physiological solutions have to be treated with caution, because the release of prostaglandins is activated by almost all the manipulations currently used to prepare the organs for biological experiments (279) or the samples of body fluids for biological assays. The simultaneous use of several organs with the blood-bathed organ technique eliminates some of the artifacts and has provided consistent evidence that kinins induce prostaglandin formation and release. It must also be considered that circulating prostaglandins of the E and F_α (PGE, PGF) series are inactivated by the lung (145) and are therefore unlikely to exert physiological actions unless they are released from the lung into the arterial blood. This is precisely what has been found by Vane and Ferreira (458) in the rabbit and by other workers in the dog (297) and in the rat (22). The hypotensive effect produced by high doses of BK given i.v. is prolonged with respect to that of the same dose given intraarterially and its duration is reduced by indomethacin. At least in some species, the hypotensive action of bradykinin might be in part mediated by prostaglandins released from the lung. Work by Ferreira et al. (138, 140, 142), McGiff et al. (280–282), and Needleman et al. (311, 312) over the years has produced convincing evidence that prostaglandins intervene as mediators or modulators of several local actions of kinins, such as the production of pain, the modification of renal excretory functions, and coronary vasodilation. With regard to the type of prostaglandin liberated by kinins, it appears that products with chemical, chromatographic, and biological properties similar to PGE_2 and $PGF_{2\alpha}$ are released from the arteries and the veins respectively (32, 246, 439, 444, 494). However, because of the recent discovery of prostacyclin (192, 295), an unstable intermediate of arachidonic acid metabolism and an extremely potent vasodilator (299), most of the roles and functions attributed to PGE_2 and $PGF_{2\alpha}$ need to be reevaluated.

Recent work on prostacyclin (PGI_2) is summarised in review papers (298, 299) and in a symposium (279). It has been demonstrated that PGI_2 is generated by the vascular wall and is continually released by the lung into the blood stream, so that this prostaglandin could act as a circulating hormone, in contrast with PGE_2 and $PGF_{2\alpha}$, which are inactivated in the lung (145). PGI_2 is the major metabolite of arachidonic acid in arteries and veins of several species, including man (296, 298), and is a potent vasodilator of several vascular beds, such as those of the heart, kidney, mesentery, and skeletal muscle (37, 102, 153, 310). In the recent review paper by Terragno and Terragno (441) no mention is made as to whether kinins promote the formation of PGI_2 or not. However, Needleman et al. (310, 410) have found that bradykinin and arachidonic acid, when injected into the coronary circulation of rabbit isolated hearts, promote the release of a prostaglandin that relaxes isolated strips of bovine coronary artery and is presumably PGI_2 (410). In fact, when the phospholipid pool of the heart is labelled with [^{14}C]arachidonic acid, bradykinin releases mostly [^{14}C]6-keto-$PGF_{1\alpha}$, the stable product of prostacyclin hydrolysis. Since the release of PGI_2 from rabbit isolated hearts is blocked by indomethacin (410), it is legitimate to deduce that the partial inhibition (360) or the total block (312) of the bradykinin-induced coronary vasodilation by indomethacin is due to a reduced production of prostacyclin. This is an example of how data and findings obtained before the discovery of PGI_2 need to be reinterpreted. Another example is provided by the reinterpretation of the renal effects of kinins, proposed by McGiff (278, 282). It appears that PGI_2 takes part in the haemodynamic

actions of kinins and in their possible role in modulating renin release, while renomedullary PGE_2 or $PGF_{2\alpha}$ may be involved only in the regulation of water and perhaps sodium excretion by kinins. Recently it has been shown that bradykinin promotes the release of PGI_2 from the dog kidney (297). Reports from McGiff's laboratory (305, 309) suggest also that kinins increase the production of prostaglandin E-like substances in the rabbit and dog kidney.

Another example of how kinins and prostaglandins interact is provided by the arterial tissues involved in the circulatory changes of the newborn. Firstly, it has been shown that the kinin concentration in plasma increases approximately 6-fold during delivery (284); secondly, it has been found that kinins constrict certain vessels (umbilical artery and vein, ductus arteriosus) (44, 284) and relax others (pulmonary artery) (44), as required for the transition of foetal to neonatal circulation (284). Moreover, Terragno et al. (441) have reported that human umbilical vessels synthesise two or three times more prostaglandin than other vascular tissues (e.g. mesenteric artery) (439). Bradykinin is a potent releaser of prostaglandins from both the umbilical artery and vein (440). However, while PGI_2 is the major prostaglandin produced by the foetal blood vessels (440) and possibly released by bradykinin in the foetus (441), presumably to relax the pulmonary artery, the umbilical vessels only and exclusively synthesise PGE_2 (the artery) and $PGF_{2\alpha}$ (the vein) (439, 440). These prostaglandins contract the respective producing vessels. In this way two natural systems (kinins and prostaglandins), one of which is contained in blood and may be activated during labour and the other, located in the vessels to determine tissue responsiveness, may cooperate to mediate the circulatory changes required to convert the foetal circulation to that of the adult.

The fall of systemic blood pressure induced by kinins is certainly the result of decreased vascular resistance in various organs, among others the heart, kidney, intestine, skeletal muscle, and the liver (202). The effects of kinins on segmental circulations have been studied in various species. Work performed before 1970 has been reviewed by Haddy et al. (202) and the most recent findings by Johnson (219). Various approaches have been utilised for evaluating the actions of kinins (generally bradykinin has been tested) in the peripheral vessels of several animals.

Thus, very small amounts (20 to 25 ng/min) of bradykinin given intraarterially in the forearm of normal subjects (85, 250) produce vasodilation and increase peripheral blood flow, either by opening the arteriovenous shunts (85) or by dilating the arteriolar vessels and increasing blood flow to the capillaries (250). In anaesthetised animals, when changes of peripheral blood flow were evaluated with electromagnetic flowmetry, rotametry, or with photomicrography, the majority of investigators (25, 41, 183, 304) found that i.v. administration of bradykinin dilates various peripheral vessels and increases regional blood flow. The intraarterial application of minute doses of the same peptide increased markedly blood flows of coronary (304), femoral (283, 306), brachial, and renal vascular beds (275, 283) and decreased the peripheral resistance in the respective organs without changing heart rate, mean systemic blood pressure, and myocardial contractile force (283, 304, 306). In some of these studies, the observed increase of mean pulmonary and of left atrial pressure was probably due to the enhanced venous return caused by bradykinin (202, 304). In other experiments, changes of flow and of organ function produced by bradykinin were studied in explanted organs perfused in vitro with blood or with physiological media (281, 312, 360). In some instances, the flow was inceased (82, 88), while in others a definite fall of venous outflow was observed (196). As pointed out by Ward and Margolius (469), data obtained in such experiments have to be regarded with caution, since the kinin or the prostaglandin systems may be strongly activated under the artificial conditions required for maintaining the explanted organs. Moreover, in all experiments analysed above, exogenous kinins are given on the assumption that they can reproduce the effects evoked by the activation of the endogenous system; this is unlikely to be true, both in terms of concentration achieved at the site of action, and of localisation of the effect. Definite conclusions about the role of kinins in the regulation of regional blood flow still await the availability of competitive and specific inhibitors.

Finally, the contractile or relaxing effects of kinins have been studied in segments or strips of isolated arteries and veins of humans and animals (6, 86, 172, 443, 448, 449). Table 3 summarises a large number of published results. In several studies, bradykinin has been tested only for its contractile action (86, 172, 230, 430, 448); in others (174, 175, 418, 444), the isolated vessels have also been made to contract with prostaglandins (PG), potassium chloride (K^+), histamine (H), or with noradrenaline (NA) in order to enable the measurement of the relaxing effect of kinins. In humans, bradykinin is inhibitory in all vessels studied except the umbilical artery and vein. In dogs, the coronary, carotid, renal, mesenteric, and iliac arteries are inhibited by kinins and show high sensitivity, while most of the other vessels respond to kinins with a contraction. The dog's veins contract without exception. Vessels of cats, rabbits, guinea pigs, rats, sheep, and pigs, both arterial and venous, are stimulated by kinins when applied in concentrations varying from 10^{-9} to 10^{-5} M. However, in some instances (6, 172, 448, 449), the eventual relaxing effect was not tested. The utility of these preparations for pharmacological studies will be discussed in section VI.

The most prominent actions of kinins are on the peripheral vessels, where these peptides have been shown to increase vascular permeability (202). Intraarterial or topical administration of kinins and the direct microscopic examination of precapillary, capillary, and post-

TABLE 3

Responsiveness of isolated blood vessels of various species to bradykinin

Species	Response*	Dose† (M)	Ref.
Human arteries			
Cerebral	R (K^+, PG)	2.10^{-10}	(444)
Basilar	R (K^+, PG)	2.10^{-10}	(444)
Internal carotid	R (K^+, PG)	2.10^{-10}	(444)
Umbilical	C	10^{-8}	(6, 114, 182)
Human veins			
Umbilical	C	10^{-9}	(6)
Saphenous	R (PG)	10^{-7}	(241)
Dog arteries			
Aorta	C	10^{-8}	(17, 174, 444)
Cerebral	C	2.10^{-10}	(444)
Basilar	C	2.10^{-10}	(444)
Internal carotid	C	2.10^{-10}	(444)
External carotid	C	2.10^{-10}	(444)
Common carotid	R (NA, K^+)	2.10^{-10}	(17, 71, 444)
Pulmonary	C	10^{-7}	(17, 174)
Coronary	R (K^+, PG)	2.10^{-10}	(229, 444)
Mesenteric	R (K^+)	2.10^{-10}	(174, 444)
Renal	R (K^+)	2.10^{-10}	(71, 444)
Hepatic	C	10^{-8}	(174)
Femoral	C/R (K^+, NA)	$10^{-7}/10^{-10}$	(71, 444)
Iliac	R (NA)	10^{-7}	(71)
Subclavian	C	10^{-8}	(174)
Skin arterioles	C	10^{-3}	(489)
Mesenteric arterioles	C	10^{-3}	(489)
Dog veins			
Inferior cava	C	10^{-8}	(448)
Superior cava	C	10^{-8}	(448)
Pulmonary	C	10^{-8}	(448)
Hepatic	C	10^{-8}	(448)
Splenic	C	10^{-8}	(448)
Portal	C	10^{-8}	(448)
Renal	C	10^{-5}	(448)
Mesenteric	C	10^{-6}	(86)
Jugular	O	10^{-5}	(448)
Femoral	C/O	10^{-6}	(86, 448)
Saphenous	C/O	$10^{-9}/10^{-6}$	(22, 184, 185, 444)
Cephalic	O	10^{-6}	(22)
Azygos	C	10^{-5}	(448)
Cat arteries			
Arota	C	10^{-6} no R (NA)	(17)
Common carotid	C	10^{-6} no R (NA)	(17)
Basilar	O/R (K^+)	10^{-5} no R (NA)	(17, 444)
Pulmonary	R/C	$10^{-6}/10^{-5}$ no R (NA)	(17, 174)
Renal	O	10^{-5} no R (NA)	(17)
Subclavian	C	10^{-7} no R (NA)	(174)
Cat veins			
Inferior cava	C	10^{-8}	(174, 449)
Superior cava	C	10^{-8}	(174)
Pulmonary	C	10^{-8}	(174)
Jugular	C	10^{-8}	(174)
Mesenteric	C	10^{-7}	(174)
Renal	C	10^{-8}	(174)
Sheep vessels			
Coronary artery	C	10^{-9}	(229)
Umbilical artery	C/O	10^{-6}	(103, 243)
Foetal ductus arteriosus	C	10^{-7}	(230)
Monkey vessels			
Umbilical vein	R	10^{-8}	(104)
Rabbit arteries			
Ear	R (Hz)	10^{-6}	(418)
Aorta	C	10^{-7}	(17, 172, 358)
Carotid	R (Hz)/C	$10^{-6}/10^{-7}$	(17, 418)
Pulmonary	C	10^{-7}	(17, 418)
Renal	C	10^{-7}	(17)
Mesenteric	R (Hz)	10^{-6}	(418)
Femoral	R (Hz)	10^{-6}	(418)
Cerebral	R (H)	2.10^{-10}	(444)
Brachial	R (Hz)	10^{-6}	(418)
Subclavian	C	10^{-9}	(174)
Mesenteric arterioles	O	10^{-3}	(489)
Skin arterioles	O	10^{-3}	(489)
Rabbit veins			
Anterior cava	C	10^{-9}	(217, 361)
Posterior cava	C	10^{-5}	(194, 430)
Jugular	C	10^{-5}	(217, 361, 430)
Mesenteric	C	10^{-8}	(319, 361)
Portal	O	10^{-5}	(53)
Saphenous	C	10^{-8}	(213)
Guinea-pig arteries			
Aorta	C	10^{-7}	(17)
Carotid	C	10^{-7}	(418)
Pulmonary	C	10^{-7}	(174, 418)
Mesenteric	C	10^{-7}	(418)
Brachial	C	10^{-7}	(418)
Subclavian	C	10^{-9}	(174)
Femoral	C	10^{-7}	(418)
Ear	C	10^{-7}	(418)
Guinea-pig veins			
Inferior cava	O		(174)
Superior cava	O		(174)
Jugular	C	10^{-9}	(174)
Mesenteric	C	10^{-7}	(174, 320)
Portal	C	10^{-7}	(53)
Rat arteries			
Aorta	O		(17)
Carotid	R (Hz)	10^{-6}	(418)
Femoral	R (Hz)	10^{-6}	(418)
Pulmonary	C	10^{-6}	(418)
Brachial	R (Hz)	10^{-6}	(418)
Mesenteric	C/R (Hz)	10^{-6}	(319, 320, 418)
Rat veins			
Inferior cava	O		(174)
Superior cava	C	10^{-8}	(174)
Mesenteric	C	10^{-6}	(174, 319, 320)
Portal	C	10^{-7}	(53)
Pig vessels			
Coronary artery	C	10^{-9}	(174)
Inferior cava	C	10^{-7}	(217)
Superior cava	O	10^{-6}	(217)
Jugular vein	C	10^{-7}	(217)
Saphenous	C	10^{-6}	(217)
Femoral	C	10^{-6}	(217)
Bovine vessels			
Coronary artery	R (H, 5-HT)	10^{-6}	(229)

* R, relaxation of vessels contracted with potassium chloride (K^+), prostaglandin E_2 (PG), histamine (H), 5-hydroxytryptamine (5-HT), noradrenaline (NA), or electrical stimulation (Hz); C, contraction; O, no effect.

† Threshold or average effective concentration of bradykinin.

capillary vessels, has been used in a large number of studies performed before 1970 and extensively reviewed by Haddy et al. (202). These authors summarise the major peripheral vascular events evoked by kinins as follows: "There is an immediate dilatation of the arterioles, which will raise the pressure in both capillaries and venules. At the same time, the endothelial cells of the venules respond by contraction; since they contract in a vessel under high pressure, their filling does not result in a decrease in diameter of the lumen, but in a partial detachment of the intercellular connections. If the larger veins contract, this may help to raise the intravenular pressure, as does the arteriolar dilatation." In the years since, brilliant studies by Gabbiani et al. (164, 165) with electron microscopy have established that the postcapillary venules are indeed the vessels responsible for major alterations of vascular permeability; endothelial cell contraction and widening of intercellular junctions have also been documented (166, 252) after the application of noxious stimuli and of various agents, including bradykinin. Pharmacological studies on the veins of several species have also shown that the predominant effect of the kinins, both in vivo and in vitro, is a contractile one (table 2). Therefore, the interpretation proposed by Haddy et al. (202) has been substantially validated.

However, several questions remain to be answered: a) whether kinins act directly or through prostaglandins, particularly prostacyclin; and b) whether the efflux of solutes from the blood is a passive phenomenon or if kinins not only enlarge capillary pores but also promote the active transport of macromolecules (proteins) and by which mechanism. For further discussion of this topic the reader is referred to the reviews by Haddy et al. (202) and by Johnson (219).

In conclusion, kallikreins and kinins were first described in terms of their hypotensive effect. Indeed, recurrent hypotension is observed in some pathological states (e.g. postgastrectomy and carcinoid syndromes, hyperbradykininism syndrome, pancreatitis) associated with hyperproduction or reduced degradation of kinins. In spite of the fact that kinins can produce either dilation or constriction of arterial vessels, dilation occurs in the majority of the vascular beds and predominates in vivo. Dilation of arteriolar vessels may result from a direct inhibitory effect of kinins on the vascular smooth muscle or from the release of vasodilator prostaglandins (prostacyclin?); constriction of veins is presumably produced by the stimulation of the venous smooth muscles or by the release of venoconstrictor prostaglandins ($PGF_{2\alpha}$) or from the conversion of PGE_2 to $PGF_{2\alpha}$ by PGE 9-ketoreductase (246).

By reducing arteriolar resistance, kinins bring about an increase of pressure and flow in the capillary bed, thus favouring the efflux of fluid from the blood to the tissues. The efflux may be further facilitated by a) the increase of capillary permeability resulting from the formation of large pores or gaps in the capillary endothelium, contracted by kinins, and b) the increase of venous pressure, consequent to the constriction of the veins. No wonder that in these conditions water and solutes (including proteins) pass from the blood to the extracellular fluid, lymph flow increases, and oedema may result. We entirely agree with Haddy et al. (202) that these peripheral vascular events may represent the most consistent actions of kinins in physiological and pathological states. These considerations favour therefore the interpretation that kinins are local hormones primarily involved in the regulation of peripheral blood flow to various organs and in the physiological defense reactions of tissues against noxious stimuli.

Pressor effects occasionally observed in animals appear to be due to the reflex activation of the sympathetic nervous system after the stimulation of paravascular nociceptive structures and of other sensitive nerves by kinins; it is unlikely that kinins exert a direct chemical activation of sympathetic ganglia or sympathetic nerve terminals or that they promote the release of catecholamines from the adrenal medulla under physiological conditions. It is premature to assume that circulating kinins have a central action, since they may not cross the blood-brain barrier. A local kallikrein-kinin system seems to be present in the brain [see review article by Clark (58)]. With an indirect immunofluorescence method, Correa et al. (68) have recently described a neuronal system containing bradykinin-like immunoreactivity in the rat brain. The bradykinin-like material is localised in hypothalamic cells that make connections with various parts of the brain. The physiological significance of this system is still unknown.

B. Inflammation

The above description explains the contribution of kinins to the vascular events occurring in the inflammatory process, studied by various authors (47, 254, 365) and reviewed extensively by Wilhelm (482), Willoughby (488), and Garcia Leme (169) among others. Kallikreins and kinins have been shown to reproduce the basic symptoms of inflammation in several animal species (254, 288, 484). Indeed, local production of kinins is definitely increased in inflammatory lesions produced by carrageenin (487), urate crystals (285), heat (417), and other noxious manipulations (169). Activation of kallikreins occurs in inflamed tissues and degradation of kinins may be reduced by the decreased pH of the oedematous fluid (169, 244). Because kallikreins are inactivated much more slowly than the kinins, it has been suggested (244) that the production of kinins may be prolonged in such a way that these peptides could participate not only in the initial but also in the intermediate and late phases of the inflammatory process. It is still not established whether kinins promote cell migration from blood to the tissues since the observations by Graham et al. (188) were not confirmed by other authors (467). Once located in the tissues, white blood cells may perpetuate the production

of kinins, since they contain kallikreins and possibly kinins (190, 301, 501). In spite of the numerous data accumulated in the past in favour of a possible role of kinins in the inflammatory reaction, inflammation remains a very complex phenomenon in which several endogenous systems intervene and interact with one other. It is therefore premature to conclude that kinins play a definite role in the inflammatory process. This is underlined by Wilhelm (482, 483) and Garcia Leme (169) in their critical analyses of this subject.

C. Exocrine Glands

Prekallikreins and kallikreins have been found in exocrine glands such as the pancreas, kidney, intestine, salivary, and sweat glands. These enzymes are released by the stimulation of the autonomic nervous system, by drugs, and other stimuli (see 28, for an extensive review) into the secretory fluid of the individual organs, either in active form or as precursors that may undergo rapid activation. Kallikreins have been found to release kinins in the kidney of various species (49, 158, 346) and in the submandibular gland of the dog (142). The secretory fluids of other organs (e.g. pancreas, intestine, salivary glands) appear to contain the enzymes, while the active products are absent or have not been measured (28). Controversial opinions have been expressed as to whether a fraction of the released kallikreins enters the blood stream or remains confined in the secretory fluid. Recently, Nustad et al. (321) have been able to detect an antigen immunologically identical to salivary kallikrein in rat plasma; several studies have shown that kallikreins can be found in the venous effluent of salivary glands (28, 207), kidney (49), and the pancreas (28), but only when the organs are perfused with physiological media (207, 370). De Bono and Mills (84) have observed simultaneous increases in kallikrein in renal lymph and urine during saline infusion in the dog. If kallikreins or active kinins diffuse from the organ to the blood, they may participate in the regulation of blood flow and possibly sustain the changes of vascular resistance and permeability that are generally produced by the same stimuli that bring about the release of kallikreins. This is a very controversial point, particularly for the salivary glands, and the opposite point of view has been expressed by Hilton (207), the author of the vasodilation theory for glandular kallikreins, and by Schachter (392, 393), who maintains that the glandular kallikrein-kinin systems may play a minor role in the regulation of glandular blood flow. Without entering into this argument, we simply wish to point out that, if kallikreins or kinins enter the blood from the tissues, they may act on capillaries to increase the filter permeability and on the venules to increase the intracapillary pressure, two phenomena that may together facilitate the efflux of fluid from the blood to the organ and activate the secretory process. There is perhaps no need for functional arterial dilatation, which constitutes the major point of the controversy. As pointed out by Wilhelm (482), vasodilation and increased permeability may occur independently.

Other functional roles or actions of glandular kallikreins have been considered. These actions may be exerted directly by the kallikreins or by kallidin. Thus, several workers have suggested that kallikreins or kallidin participate in the regulation of salivary and pancreatic secretions (24, 173, 200). Nustad et al. (322) have recently reviewed the evidence for the participation of glandular kallikrein-kinin systems in membrane transport of electrolytes, glucose, and amino acids. Some of the proposed actions occur in the producing organ [e.g. the kidney (52) or the salivary glands (399)], and they may have a functional role (e.g. in the excretion of potassium); others may be exerted outside the glands, for instance in the gastrointestinal tract, which receives the secretions from the pancreas, the intestinal, and the salivary glands. Like other serine proteases, kallikreins may take part in digestion (313). Since kinins have such striking effects on smooth muscle, they may modulate the tone of pancreatic or salivary ducts, as well as the motility of the intestine or of the urinary tract.

D. Other Actions (Kallikreins)

It is also worth mentioning that kallikreins are located in the β-cells of the pancreas and may be involved in the physiological activation of proinsulin since, in concert with carboxypeptidase B, they convert bovine proinsulin to insulin (292). Other workers have suggested that renal kallikreins may convert prorenin to renin in the kidney and in plasma (402, 403). These interesting new effects have been confirmed in other systems [see (394) for a recent survey], thus indicating that kallikreins exert another very important role: that of promoting the conversion of prohormones to active hormones, similar to other proteases (313). Work on urinary kallikreins has been extremely active in recent years and has been adequately reviewed in recent reports dealing with the formation and functions of urinary kallikreins and kinins (240, 346, 469), as well as with the possible role of these agents in the regulation of blood pressure (289, 290) and of kidney excretory functions (289, 290, 346) and in the pathogenesis of hypertensive disease (52, 227, 267). The relationships between the kallikrein-kinin system, the renin-angiotensin-aldosterone system (278, 305), and the renal prostaglandin system (308) have also been recently reviewed. In summary, renal kallikreins are formed in the distal nephron (322, 324), where they appear to control water and electrolyte excretion (52, 346). Kallikreins may diffuse to the adjacent juxtaglomerular cells to promote the conversion of prorenin to renin or into the blood, where they release the vasodilator kinins, which, either directly or through the activation of the prostaglandin system, may affect the distribution of renal blood flow (52, 308). Numerous studies in humans and animals indicate that the excretion of urinary kallikreins is decreased in most types of hypertension (52, 289, 290) with

the exception of that caused by mineralocorticoids (266, 267). Whether the reduced kallikrein excretion in hypertension reflects a pathogenetic mechanism or an alteration in the kidney not related to hypertension remains to be established.

Kallikreins and kinins have also been implicated in various other processes such as the regulation of glucose uptake in working and hypoxic skeletal muscle (89, 90), the regulation of smooth muscle tone (in the vessels, the bronchi, the gastrointestinal tract, and the uterus), and the control of sperm motility and cell proliferation. The analysis of these effects falls beyond the scope of the present paper and the reader is therefore referred to recent comprehensive reviews (219, 346, 394). We will focus our discussion on the actions of kinins on smooth muscles in relation to the utilisation of these tissues in pharmacological assays.

VI. General Pharmacology of Kinins

Basic peptides such as kinins presumably act on the cell membrane, where they may interact with numerous negatively charged sites. Some of the resulting complexes between kinins and their membranous counterparts are able to evoke biological responses. These active membranous sites, currently termed receptors, not only bind the kinins, but also undergo a functional change that triggers the sequence of biophysical and biochemical events leading to the effect. By analogy with other systems, analysed by Ariëns (10), the kinin-receptor interaction is likely to involve two phases: a) the binding of the kinin to the receptor (occupation); and b) the functional change of the receptor molecule (activation). Occupation and activation of receptors are not necessarily mediated by the same chemical group of the peptide molecule. Such groups appear to be distinct, for example, in the octapeptide angiotensin II (362). The assumption that a similar distinction between binding and active sites exists for the kinins is supported by the results obtained in our laboratory (358, 359) on the B_1 receptor system (see section VII, A and B for details) and constitutes the basis of the pharmacological analysis presented in this review. The ability of kinins and their analogues to occupy the receptors will be evaluated in terms of affinity and the ability to activate the same receptors in terms of intrinsic activity, according to Ariëns (10). The various compounds will therefore be classified into three groups: full agonists, partial agonists, and antagonists. Results of all pharmacological experiments will be interpreted according to the occupation theory, as elaborated by Ariëns (10), accepting, however, that this theory is, in the most favourable case, an oversimplification of reality. The occupation theory, as well as the "one-receptor-site concept" (11), together represent the simplest model for interpreting drug-receptor interactions. As pointed out by Ariëns et al. (11), "A disadvantage of the more complex models—and there is no limit to the complexity—is that, because of the large number of variables, rate constants, etc., involved, they usually will give a variety of answers in the interpretation of certain biological phenomena and thus do not show discrimination." Peptides are larger and more complex molecules than the biologically active amines. The effects of peptides are antagonised specifically and competitively by analogues whose structures are very similar to those of the native hormones [e.g. angiotensin (362), des-Arg^9-bradykinin (358), vasopressin (260, 261), oxytocin (260)]. There is as yet no indication that competitive antagonists for peptides also bind to accessory receptor sites, as is the case for biologically active amines (11). Thus, the affinities of the most potent antagonists for peptides are practically identical to those of the native hormones and the kinetics of the antagonist-receptor interactions are very close to those inferred for the respective agonists (260, 358, 362) even for kinins (359). We therefore do not consider the adoption of one of the complex receptor models reviewed recently by Ariëns et al. (11) to be preferable to the simpler "one-receptor-site concept" for several small peptides and specifically for kinins.

A. Pharmacological Preparations

Among the numerous biological actions of kinins, analysed in section V, the stimulation or inhibition of isolated smooth muscles has been widely used for pharmacological experiments, particularly in the various structure-activity studies of kinins (336, 400, 401, 422, 423, 431). Practically any kind of pharmacological test can be utilised with some success in this type of study, although it is preferable that the preparation complies with the following criteria:

1. It should respond to threshold concentrations of kinins of 10^{-8} M or less.

2. It should be an isolated tissue rather than a perfused organ or a living animal, in order to permit the recording of the maximal response.

3. Kinins should exert a direct action on the preparation, because, even in the favourable situation, in which the intermediate endogenous substance (e.g. prostaglandins in the specific case of kinins) contributes to the pharmacological effect (e.g. the rat uterus, in fig. 2B), the production or the release of such a substance may depend on or vary with the time, the dose of peptide, or with other unknown factors. In other preparations (e.g. the dog saphenous vein, fig. 2A), bradykinin appears to be almost inactive when applied in the absence of indomethacin; the kinin is, however, fairly active in tissues treated with the inhibitor of the prostaglandin synthesis (455), which suggests that bradykinin is a potent stimulant of this vein, but its effect is reduced by the simultaneous release of an inhibitory prostaglandin.

4. The response of the preparation should derive from the activation of receptors specific for the kinins. These peptides are stimulants or inhibitors of a large variety of smooth muscles, which also respond to other hormones, neurotransmitters, and autacoids, for which specific an-

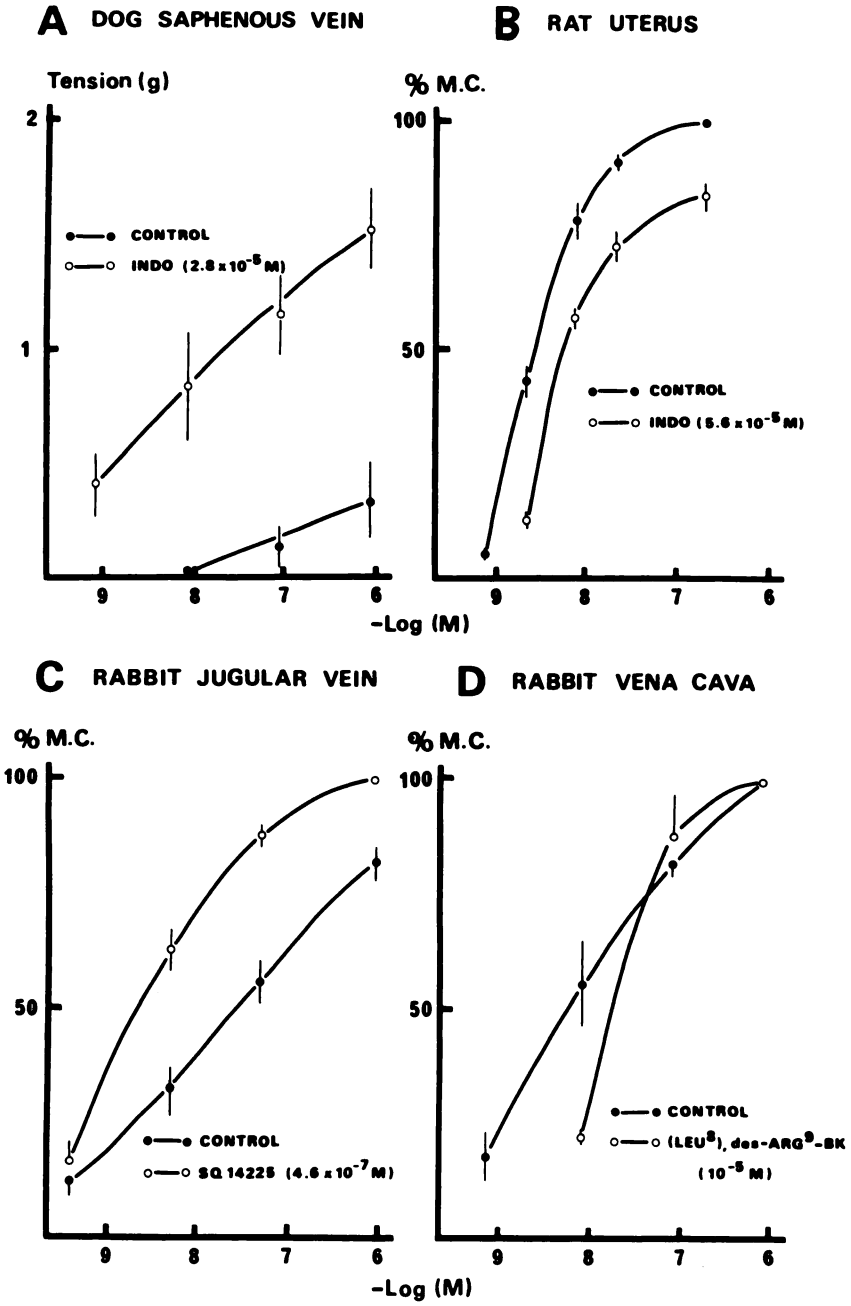

FIG. 2. A, B, and C, concentration-response curves of bradykinin as measured. A and B, in absence and in presence of indomethacin (INDO). C, in absence and in presence of a kininase II inhibitor, SQ 14225. D, concentration-response curves of kallidin as measured in absence and in presence of a B_1 receptor inhibitor, [Leu^8],des-Arg^9-BK. Abscissa, concentration of agonist. Ordinate, in A, contractions of the dog saphenous vein are expressed in grams (g) of tension; in B, C, and D, the contractile effects of bradykinin (BK) and of Lys-BK are expressed in percent (%) of the maximal contraction. Points indicate the mean and vertical bars the standard error of the means of 8 to 12 determinations. Experimental details are in references 18 and 20.

tagonists are available. These antagonists (e.g. anticholinergic, antiadrenergic, antihistaminic, antiserotonin, and antiangiotensin agents, etc.) should be tested in order to find out if they modify the response of the preparation to the kinins. If the response to kinins is not affected, the assumption that these peptides may act on specific receptors is legitimate.

5. The recorded concentration-response curve should fall along the theoretical curve given by Clark's equation (57). When the slope of the experimental concentration-response curve is the same as that of the theoretical curve (which is based on the mass-action law), the type of reaction between drug and receptor is a bimolecular one and the ratio between the doses producing 16% and 84% of the maximal contraction corresponds to 1:27 (one drug molecule interacts with one receptor). If two molecules of drug unite with one receptor or if one molecule of drug unites with two receptors, the ratio to be expected, according to Clark (57) is 1:5.2 and 1:730 respectively. Deviation from the theoretical curve may be found

in a nonlinear distribution of the agonist concentration between the fluid in the bath and the biophase or the receptor compartment. Another factor that can affect the slope of the dose-response curve is the tissue metabolism. This is important for kinins since it is known that some tissues contain very active kininases that can interfere with the concentration of the agonist in the receptor compartment.

6. The presence of intramural degradation of kinins should be considered in the choice of a preparation to be used in structure-activity studies of these peptides. In fact, the affinities of analogues and fragments susceptible to metabolism will obviously be underestimated with respect to the affinities of compounds resistant to degradation. The existence of kinin degradation can be demonstrated in isolated smooth muscles by using inhibitors of kininases. Among the numerous compounds described in the literature and recently reviewed by Stewart (424), SQ 14225 appears to be a potent and specific inhibitor of kininase II (79). Recent findings in our laboratory show that SQ 14225 does not interfere with the action of BK either on the B_1 receptor of the rabbit aorta or on the B_2 receptors of the other three preparations: the rat uterus, the cat ileum, and the dog carotid artery [(22, 175) and see table 4]. The increased affinity of BK observed in the guinea-pig ileum and the rabbit jugular vein probably results from the inhibition of kininase II by SQ 14225, because it occurs with BK and [Ala^6]-BK but not with [D-Phe^8]-BK (22). The first two compounds are much less potent hypotensive when administered intravenously in anaesthetised rats (relative potency approximately 10%), than when given intraarterially, while [D-Phe^8]-BK is equally active by the two routes (336).

Concentration-response curves of BK were also measured on the rabbit jugular vein in the absence and the presence of SQ 14603 and SQ 24798, which inhibit respectively carboxypeptidase A and carboxypeptidase B (329). These two compounds were found to be totally inactive. It is also worth mentioning that SQ 24798, when used at the same concentrations as those applied on the rabbit jugular vein (10 μg/ml), was able to inhibit the conversion of BK into des-Arg^9-BK by human plasma (263); this conversion is presumably mediated by kininase I. Concentration-response curves to BK were therefore measured on the six preparations analysed in table 4 both in the absence and presence of SQ 14225. The concentration-response curves of BK were found to be displaced to the left without change of the maximum response in two of the six preparations, namely the guinea-pig ileum and the rabbit jugular vein. As illustrated in figure 2C, the inhibition of the tissue kininase II by SQ 14225 in the rabbit jugular vein increases the apparent affinity of BK and changes the Clark ratio from a value of 1:2000 (measured in the absence of kininase II inhibitor), to 1:83 (see fig. 2C). From the above results, we conclude that the guinea-pig ileum and the rabbit jugular vein contain active kininase II, which reduces the affinity of BK for its receptors, probably by decreasing the fraction of peptide in the receptor compartment. In the other four preparations, the enzyme might be present, but does not interfere with the measurement of the biological activity of BK and presumably of other kinins.

7. When challenged with kinins, isolated smooth muscles respond in two ways: *a*: with contractions or relaxations that remain stable as long as the agent is kept in contact with the organ; and *b*: with a rapidly reversible change of tension that fades off despite the presence of the stimulant. Preparations showing response *a* are to be preferred, since stable plateaux of response, obtained at all levels of agonist concentration, suggest that, when the drug has diffused into the biophase and a steady state is reached, each drug-receptor complex contributes a quantum of activity during its full existence. Preparations of the *a* type are adequate for measuring cumulative concentration-response curves and for testing antagonists during the steady change of tension produced by the agonist. The other type of response (*b* type) is less satisfactory because, in the majority of the systems so far studied, it is not known whether the fading depends on a) the kinetics of the drug-receptor interaction, b) receptor inactivation after contact with the drug, c) drug metabolism, or d) functional changes in the intracellular machinery that mediates contraction or relaxation of smooth muscle. Preparations of the *b* type may, however, be useful for desensitisation experiments, a use that will be discussed later.

8. Finally the type of receptor for kinins that is present in the preparation constitutes another important criterion of choice. After the demonstration of the existence of at least two receptor types for kinins [the B_1 receptor in the rabbit aorta (358) and the B_2 receptor in the cat terminal ileum and the rat uterus (18)], various isolated organs have been found to contain both B_1 and B_2 receptors. In such preparations (see example of the rabbit vena cava in fig. 2D) the contribution of the B_1 receptor to the overall concentration-response curve of Lys-BK can be evaluated by using a specific and competitive inhibitor of this receptor, namely [Leu^8],des-Arg^9-BK. When the B_1 receptors are blocked, the slope of the curve is modified and the apparent affinity of Lys-BK is significantly reduced. Lys-BK was used instead of BK in this experiment because this kinin has a fairly good affinity for both receptor types, while BK is a very weak stimulant of the B_1 receptor.

In table 4, we analyse six preparations that have been used in structure-activity studies of kinins. This analysis is intended to determine whether these preparations fulfill the criteria discussed above.

In spite of its high sensitivity to kinins, the rat uterus in oestrus is a preparation of the *b* category that is, however, unsuitable for desensitisation experiments, because it responds to kinins with a rapidly reversible contraction followed by a series of smaller and irregular increases of tension (21). According to Malone and Trot-

TABLE 4

Do preparations used in structure-activity studies of kinins respond according to the eight criteria discussed on pages 16 to 18?

Criterion	Rat Uterus	Guinea-Pig Ileum	Cat Ileum	Rabbit Aorta	Rabbit Jugular Vein	Dog Carotid Artery
1. Sensitivity to bradykinin (BK)	++++*	+++	+++	(+)	+++	++++
Sensitivity to des-Arg^9-BK	(+)	(+)	(+)	++	(+)	(+)
2. Possibility of recording maximal response	+†	+	+	+	+	+
3. Specificity of response (direct or indirect action)	(?)	+	+	+	+	+
4. Specificity of action (presence of specific receptors)	+	+	+	+	+	+
5. Shape of the concentration-response curve (Clark's ratio)‡	+ (1:19)	+ (1:40)	+ (1:28)	+ (1:19)	+ (1:83)	+ (1:167)
6. Sustained effect (S.E.) or fade (F)	F	F	S.E. after small or average doses	S.E.	F	F
7. Absence of metabolism by kininase II	+	−	+	+	−	+
8. Receptor type	B_2	B_2	B_2	B_1	B_2	B_2

* Sensitivity has been quantified in terms of threshold concentration of BK: ++++ 10^{-10} M; +++ 10^{-9} M; ++ $<10^{-8}$ M; (+) $<10^{-6}$ M.

† +, criterion is fulfilled; − or (?), criterion is not or not entirely fulfilled.

‡ Clark's ratio in case of bimolecular interaction is 1:27.

tier (256), the contractions of the rat isolated uterus in response to bradykinin are partially mediated by prostaglandins because they are reduced in the presence of indomethacin. In confirming this observation, we (21) have found that indomethacin not only displaces the concentration-response curve of bradykinin to the right, but also reduces the maximum response of the uterus (fig. 2B). More recently, Whalley (479) has shown that the response of the whole uterus to bradykinin is significantly reduced in the presence of indomethacin and of polyphloretin phosphate, while the response of the myometrium is not altered. Since the endometrium has been shown to be a source of prostaglandins (486), which stimulate or sensitise the myometrium to the action of other stimulants (75), it is suggested that the contractile response of the whole isolated uterus to bradykinin is the result of a) a direct stimulation of the myometrium, and b) an indirect action via the release of prostaglandins from the endometrium (479). As reported in table 4, the myotropic effect of BK in the rat uterus is not modified in the presence of SQ 14225, indicating that kininase II, even if it is present, does not interfere with the biological effect of BK.

The guinea-pig ileum belongs also to the *b* category and presents the major disadvantage of containing active kininase II. Like the uterus, the ileum responds to numerous endogenous agents and is not selective for kinins. Unlike the uterus, the fading in the presence of kinins is practically complete and therefore this preparation has been found useful for desensitisation experiments (356). Moreover the guinea-pig ileum appears to contain large amounts of active kininase II, since the effect of bradykinin is potentiated by the inhibitors of the angiotensin converting enzyme (379, 380, 22).

The cat ileum is as sensitive to kinins as the guinea-pig ileum, but it is more selective (143). The major advantage of the cat ileum is that it appears to be devoid of active kininase II, since the effect of BK is not modified either by SQ 20881 or by SQ 14225 (22), two potent antagonists of the converting enzyme. Ferreira and Vane (144) preferred this preparation to the rat duodenum because it responds to kinins with well-maintained contractions in the experimental conditions required by the blood-bathed organ technique, while the duodenum fades. We have confirmed these findings in tissues superfused in a cascade system with physiological media. It is to be noted, however, that contractions evoked by maximal concentrations (10^{-6} M) of BK are not fully maintained. The preparation is therefore not to be recommended for testing antagonists in tissues contracted by the agonists. In the early description of the cat ileum by Erspamer et al. (129), it was recommended to keep the tissue at 4°C for several hours before experimenting, in order to increase its sensitivity to kinins. We also used this manipulation in our early studies (21). More recently, however, we have observed that the longitudinal strip of the cat ileum is fully sensitive to kinins as soon as it is taken from the animal and the sensitivity remains stable during at least 8 hr of incubation in vitro (22). The cat ileum has been used in our laboratory to compare the effects of the kinins, as well as of their fragments and analogues, on a nonvascular receptor of the B_2 type, which appears to be very similar to the receptors of the rat uterus and the guinea-pig ileum (for more details, see section VII B).

The other three preparations analysed in table 4 are isolated vessels. The choice of such preparations for testing kinins has been determined by three major considerations: a) the kinins have definite actions on the cardiovascular system and, therefore, we thought it would be appropriate to look for vascular preparations in order to measure the effect of kinins directly on vascular receptors; b) isolated vessels cut into strips provide excellent pharmacological preparations for studying vasoactive agents [one of the most eloquent examples is the rabbit aorta strip developed by Furchgott and Bhadrakom (163)]; c) recent work from our laboratory suggests that some of the isolated vessels of the rabbit and other

species contain a new type of receptor for kinins, the B_1 receptor. This receptor was first identified in the rabbit aorta (358) and it has since been found in several other vascular and nonvascular tissues (see table 8) and in living animals (263).

As shown in table 4, the rabbit aorta strip fulfills all the criteria discussed above. It is the only organ, among the six, that contains the B_1 type of receptor for kinins. Like other tissues that have B_1 receptors, the sensitivity of the aorta to BK is rather low at the beginning of the incubation in vitro. This represents a special feature that will be discussed in section VI C. The action of kinins on the aorta is direct and their degradation, if any, is slow and does not interfere with the development and maintenance of the biological response.

The rabbit jugular vein contains a B_2 receptor type. The preparation has the same sensitivity to BK as the cat ileum and has been used to establish the similarity of vascular with intestinal B_2 receptors. The degradation of bradykinin by the converting enzyme appears to be fairly active and interferes with measurement of the biological activity (fig. 2C). The effect of kinins in the rabbit jugular vein fades almost completely in a few minutes; therefore this preparation could be useful for desensitisation experiments.

The last preparation, the dog carotid artery, is of particular interest because it responds to kinins with a relaxation, similar to various isolated human and canine arteries (see table 3). The preparation can be made extremely sensitive to kinins by increasing its tone with noradrenaline. This has to be done in the presence of indomethacin, in order to block the noradrenaline-induced production of prostaglandins and their relaxing effects, which interfere with that of the kinins (71). Recent results from our laboratory (71) indicate that the relaxing effect of bradykinin is not modified by SQ 14225. The testing of kininase inhibitors in this preparation (which has to be contracted with noradrenaline and treated with indomethacin), however, is not as easy as in the other organs.

In conclusion, of the six preparations analysed in table 4, the rabbit aorta has been used for studying the effects of kinins on the B_1 receptor system and three others have been chosen for evaluating the affinities and activities of kinins, their fragments, and analogues in a structure-activity study of B_2 receptors (see section VII C). These preparations are the cat ileum, the rabbit jugular vein, and the dog carotid artery. As shown in table 4, the four preparations fulfill satisfactorily most of the criteria discussed at the beginning of this section. The interference by kininase II in the rabbit jugular vein, but not the fading (22), has been eliminated by treating this tissue with SQ 14225. In this way it has been possible to compare the affinities and potencies of kinins and their analogues on an intestinal excitatory (cat ileum), a vascular excitatory (rabbit jugular vein), and a vascular inhibitory (dog carotid artery) B_2 receptor system.

B. Receptors for Kinins

The receptor hypothesis was proposed at the beginning of this century from the observation that trypanosomes, when made insensitive to arsenicals, may be killed by other drugs (107). The hypothesis became a theory when substances such as atropine and ergotoxine were found to block the actions of acetylcholine and adrenaline without affecting those of other agents (57). It was proposed that hormones and drugs produce their biological effects by activation of specific structures or sites in the target organs (57). The isolation of some receptors has substantially contributed to the definition of the modern receptor concept, which implies the existence, in the cells, of macromolecules (generally proteins) having high affinity for their agonist counterparts. Kinins are no exception to this rule. They are very potent agonists in numerous organs and practically all their biological actions are thought to be mediated by specific receptors. Examples are provided in table 5, by showing that all preparations currently used in pharmacological assays of kinins respond to these agents with contractions or relaxations that are not affected by inhibitors of other hormones or neurotransmitters.

Recent studies in our laboratory have established the specificity of kinin action in the rat uterus (18), the rabbit aorta (358), the guinea-pig ileum (22), the cat jejunum and ileum (21), the rabbit jugular vein (20), and the dog carotid artery (71). The reader is therefore referred to these publications for details about the doses of agonists and antagonists and the experimental protocols used in each preparation. The dose-response relations for the action of kinins can be fitted by the simple mass-action equations of Clark (57). This has been established in the cat ileum (21), the rabbit aorta (358), and the rabbit jugular vein (357). Such an analysis is not yet available for the other preparations.

Practical criteria for the characterisation of receptors have been elaborated and successfully used in the field of catecholamines and histamine by Schild (398) and applied to angiotensin (356). These criteria are: a) the comparison of the order of potency of agonists; b) the estimation of the affinity of competitive antagonists; and c) classification by densensitisation. These criteria will be used here in the characterisation of receptors for kinins.

Figure 3 shows the dose-response curves of bradykinin, one of its fragments, des-Arg^9-BK, and one of its analogues, [Tyr(Me)^8]-BK, on the three preparations used for studying structure-activity relations of kinins on vascular receptors. The order of potency of agonists is: des-Arg^9-BK>BK>[Tyr(Me)^8]-BK in the rabbit aorta and [Tyr(Me)^8]-BK≥BK>des-Arg^9-BK in the other two preparations. This suggests that two different receptor types for kinins may exist, by analogy with the work of Ahlquist (3) on catecholamines.

Results of a large number of experiments performed in

TABLE 5

*Specificity of receptors mediating the contractile or relaxing effects of kinins in six isolated smooth muscle preparations**

Antagonist	Rat Uterus		Guinea-Pig Ileum		Cat Ileum		Rabbit aorta		Rabbit Jugular Vein		Dog Carotid Artery	
Atropine	Ach	+	Ach	+	Ach	+			BK	−	Ach	+
	BK	−	BK	−	BK	−					BK	−
Morphine	BK	−	BK	−	BK	−						
					Ach	−						
Methysergide	5-HT	+	5-HT	+			5-Ht	+				
	BK	−	BK	−	BK	−	BK	−	BK	−	BK	−
Diphenhydramine	BK	−	Hist	+	Hist	+	Hist	+	Hist	+	Hist	+
			BK	−	BK	−	BK	−	BK	−	BK	−
Burimamide or cimetidine	BK	−			BK	−	Hist	−			BK	−
							BK	−			Hist	+
Phentolamine	BK	−	BK	−	BK	−	NA	+	BK	−	BK	−
							BK	−				
Propranolol	BK	−	BK	−	BK	−	BK	−	BK	−	BK	−
											Iso	+
[Leu^8]-AT$_{II}$	AT$_{II}$	+	BK	−	BK	−	AT$_{II}$	+	AT$_{II}$	+	BK	−
	BK	−					BK	−	BK	−	AT$_{II}$	+
Indomethacin	BK	+	BK	+	BK	−	BK	−	BK	−	BK	−
			Subst. P	+							Subst. P	−

* +, the antagonist reduces or blocks the effect of the corresponding agonist; − the antagonist is ineffective; Ach, acetylcholine; BK, bradykinin; 5-HT 5-hydroxytryptamine; Hist, histamine; Iso, isopropyl noradrenaline; AT$_{II}$, angiotensin II; Subst. P, substance P.

order to characterise kinin receptors in various preparations are summarized in table 6. It is worth noting that the aortic receptor is sensitive not only to the fragment des-Arg^9-BK, but also to a naturally occurring kinin, Lys-BK. This finding suggests that increased affinity for the aortic receptor (denoted receptor B_1) can be achieved either by removing a positive charge (Arg^9) at the carboxyl-end (as in des-Arg^9-BK) or by adding a similar charge at the amino end (as in Lys-BK). The combination of the two chemical changes has provided a way of finding potent agonists and antagonists of the B_1 receptor (see table 9 and section VII A).

Structure-activity studies on the octapeptide des-Arg^9-BK have resulted in the discovery of specific and competitive inhibitors of the effects of kinins on B_1 receptor. One of these compounds has been used in order to apply the second classification criterion. It has been found that the octapeptide antagonist [Leu^8],des-Arg^9-BK inhibits the actions of des-Arg^9-BK, BK, Lys-BK and several analogues of bradykinin on the rabbit aorta, while it is completely inactive on other preparations containing B_2 receptors. These results strongly suggest the existence of at least two different receptor types for kinins.

In section VII we will review the antagonists for kinins. At this point it is important to mention, however, that no competitive and specific inhibitors of the action of kinins in preparations containing receptors other than the B_1 type are yet available. It is therefore obvious that we cannot apply the second criterion for proving or disproving that receptors for kinins in the rat uterus, the guinea-pig ileum, and other preparations are a single entity. We will use the term B_2 to indicate receptors for kinins that are not inhibited by the octapeptide antagonists active on B_1 receptors knowing that it is possible that more than one type of B_2 receptor may be present in the body or even in the six preparations analysed in table 4. In table 6, we show results obtained with two compounds that had been described as competitive antagonists of kinins in the guinea-pig ileum (170, 177). These compounds are noncompetitive and nonspecific (cf. reference 18 and section VII) and they cannot be used for receptor classification. It should be noted, however, that they reduce the effects of kinins in preparations containing B_2 receptors, while they are totally inactive on the rabbit aorta.

The classification by desensitisation has seldom been used for kinins. The utilisation of this criterion is limited for the following reasons: a) desensitisation is practically inapplicable to preparations containing B_1 receptors, because they show a continuous increase of sensitivity that appears to be due to the de novo formation of receptors (see section VI C); b) it can be used in preparations containing B_2 receptors, preferably in those that show complete fading (e.g. the guina-pig ileum, the cat ileum, and the rabbit jugular vein); however, the classical protocol suggested by Schild (398) has to be modified when applied to peptides and particularly to kinins (fig. 4). The modification consists in leaving the desensitising concentration of the peptide in contact with the organ during the whole duration of the test for desensitisation, because kinins dissociate very rapidly from B_2 receptors. We have recently discussed this topic (356) and we have shown some examples of desensitisation experiments with kinins and angiotensin (99, 356). The test has been found of some utility to demonstrate that Lys-BK acts on the same receptor as BK in the cat ileum (99) and that receptors for bradykinin are different from those for histamine (fig. 4). Moreover, receptors for BK in the guinea-pig ileum are different from those of angiotensin and substance P (356). These examples indicate that

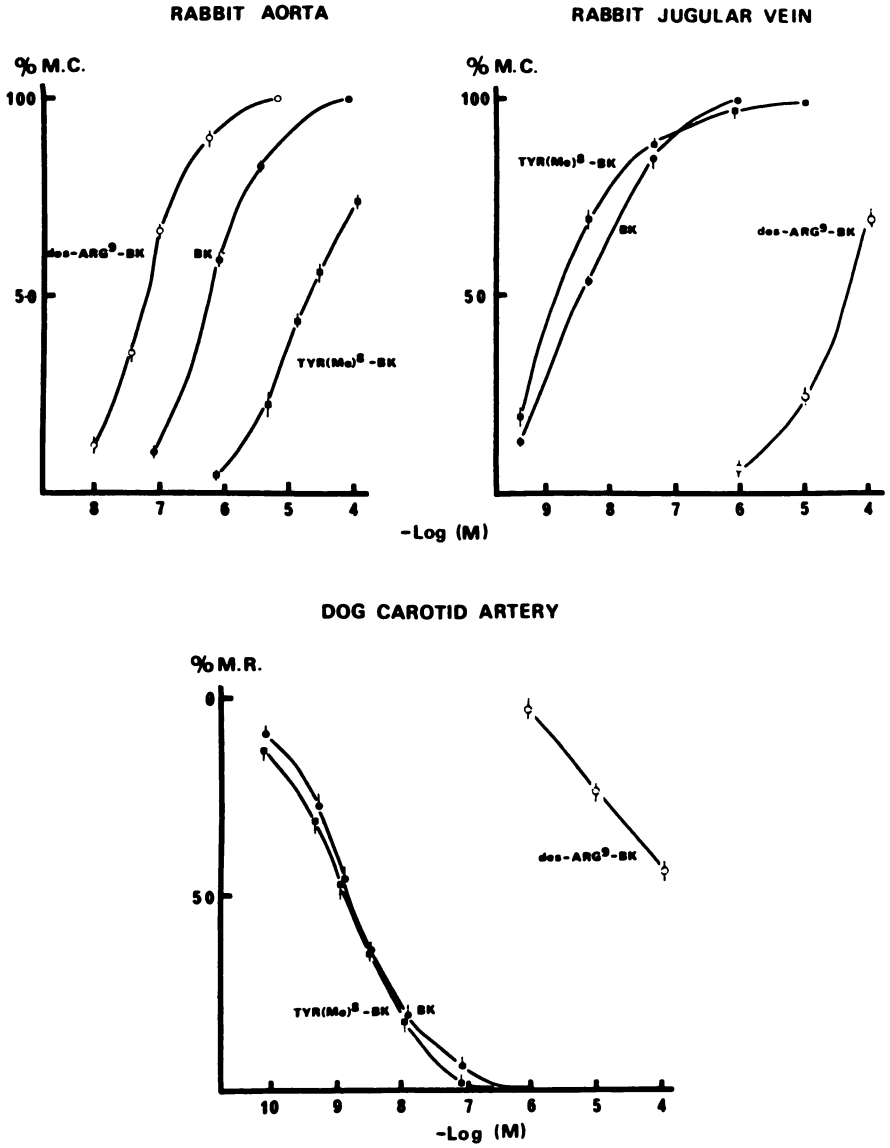

FIG. 3. Concentration-response curves of bradykinin (BK), des-Arg^9-BK, and [Tyr(Me)^8]-BK in three vascular preparations containing receptor B_1 (the rabbit aorta) and receptor B_2 (the rabbit jugular vein and the dog carotid artery). Abscissa, concentration of peptide. Ordinate, contractile (rabbit aorta and jugular vein) and relaxing (dog carotid artery) effect expressed in percent (%) of the maximal contraction or maximal relaxation. Points indicate the mean and vertical bars the standard error of the mean of 12 to 50 determinations. Experimental details are in references 18, 20, and 358.

TABLE 6

Relative potency of bradykinin (BK), analogues, and fragments in four pharmacological preparations and effects of antagonists

	Rabbit Aorta	Cat Ileum	Rabbit Jugular Vein	Dog Carotid Artery
Agonists				
BK	8.50	100.00	100.00	100.00
Lys-BK	95.00	89.00	150.00	64.00
des-Arg^9-BK	100.00	0.16	<0.01	<0.01
des-Arg^{10}-kallidin	2100.00	18.00	0.06	3.80
[Tyr(Me)^8]-BK	0.24	105.00	140.00	100.00
Antagonists*				
[Leu^8], des-Arg^9-BK	+C	–	–	–
Dihydrochlorprothixene	–	+NC	+NC	+NC
β-Phe-hexyl ester	–	+NC	+NC	+NC

* +, effects of kinins are blocked by the antagonist, which is competitive (C) or noncompetitive (NC); –, antagonist is inactive.

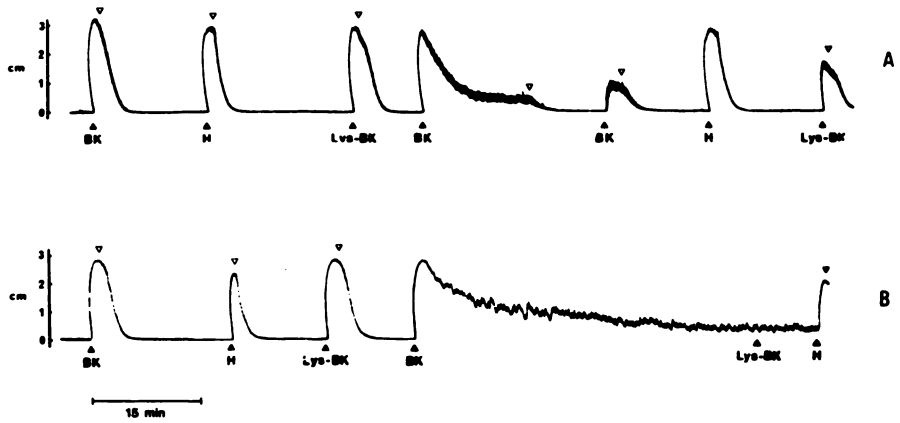

FIG. 4. Contractions of the cat ileum strip to bradykinin (BK) (8.1×10^{-6} M), histamine (H) (9.1×10^{-6} M), and Lys-BK (7.0×10^{-6} M) before and after desensitisation with BK (8.1×10^{-5} M). Tracing A shows the Schild protocol in which the desensitising agent (BK) is washed out before testing the agonists. Tracing B shows the alternative protocol in which BK is kept in contact with the tissues during the testing of Lys-BK and H. Abscissa, time in minutes; ordinate, contraction in centimetres. [Reproduced with the permission of the editors from J.-N. Drouin, S. St-Pierre, and D. Regoli, Can. J. Physiol. Pharmacol. 57: 375–379, 1978 (99).]

desensitisation experiments may provide indications of receptor specificity, and be of some utility for receptor classification.

Receptors for kinins have been classified into P (mediating pain) and S (mediating swelling) by Rocha e Silva (374). Receptors of the S type were found to be present in the guinea-pig ileum and the veins of several species. S receptors appear also to be mediating the hypotensive effect of BK (374). The distinction of P and S receptors is not based on any of the criteria recommended by Schild (398) and remains hypothetical.

Furthermore, Collier and Shorley (62) have proposed the existence of two different receptors for kinins after the observation that antiinflammatory drugs antagonise only some of the biological actions of BK. This classification has to be reconsidered, however, in the light of the observations by Vane (455) and numerous other workers that antiinflammatory drugs are potent inhibitors of prostaglandin synthesis. Therefore, these drugs might be useful tools to eliminate any prostaglandin effects that complicate the interaction of BK with its receptors, but cannot be used for classification of kinin receptors. For further discussion on this point, see section VII D.

C. De Novo Formation of B_1 Receptors and Possible Role in Physiopathology

A peculiar feature of preparations containing B_1 receptors is that their sensitivity to kinins increases progressively during the incubation of the isolated organ in vitro. The phenomenon has been extensively studied in the anterior mesenteric vein of the rabbit (361). Similar studies have been performed for comparison in the rabbit jugular vein, which contains receptors of the B_2 type. Results are presented in table 7. Thus the sensitivity of the mesenteric vein to des-Arg^9-BK, as well as to other kinins, increases significantly after 3 hr and even more after 6 hr of incubation, while the sensitivities to SP and NA are stable. No changes of sensitivity to BK, SP, and NA are observed in the jugular vein.

The application of medium containing high concentrations of des-Arg^9-BK or BK at the beginning of the incubation (priming) has no effect, contrary to what has been observed with other peptides [e.g. LHRH (106)]. The changes of sensitivity are not due to indirect effects mediated by catecholamines or prostaglandins, since the continuous application of phentolamine and indomethacin are ineffective. Cycloheximide and actinomycin D completely block the sensitisation, but only when applied from the beginning of the incubation.

These findings have been interpreted as indicating that receptors of the B_1 type are progressively synthesised and become functionally active during the incubation of the anterior mesenteric vein in the artificial in vitro conditions currently used for pharmacological experiments (361). Receptors for SP and NA in the anterior mesenteric vein and those for BK, SP, and NA in the jugular vein of the rabbit appear to be stable cellular components whose number or function do not change during the incubation of the organs in vitro and are not affected by cycloheximide. All preparations so far studied that contain receptors of the B_1 type show the same phenomenon. These preparations are listed in table 8.

The de novo formation of B_1 receptors in the rabbit isolated mesenteric vein has been demonstrated by using a radioligand receptor binding assay (19). The specific binding of [H^3]-des-Arg^9-BK per unit of wet weight of tissue increases in parallel with the biological activity when the isolated rabbit vein is incubated in vitro under the experimental conditions currently used for the biological assay. The sensitisation, as well as the increase of specific binding, proceeds from initial low levels, which suggests that receptors of the B_1 type may be scarce in vivo and that they are formed de novo after the trauma and exposure of the tissues to artificial conditions. B_1 receptors can be induced in vivo by noxious stimuli. For

TABLE 7

De novo formation of receptor B_1 in the rabbit anterior mesenteric vein; data obtained in the rabbit jugular vein, which contains a receptor of the B_2 type, are shown for comparison

Treatments	Anterior Mesenteric Vein Response*			Jugular Vein Response*		
	Time	des-Arg^9-BK (1.3×10^{-6} M)	Substance P (7.4×10^{-8} M)	Time	Bradykinin (1.1×10^{-6} M)	Substance P (7.4×10^{-8} M)
Control	1 hr	0.06 ± 0.03	1.11 ± 0.23	1 hr	0.40 ± 0.06	0.11 ± 0.02
	6 hr	1.15 ± 0.10	0.90 ± 0.18	6 hr	0.39 ± 0.03	0.10 ± 0.02
Without priming	1 hr					
	6 hr	0.91 ± 0.09				
Indomethacin† (1.1×10^{-6} M)	1 hr	0.09 ± 0.06				
	6 hr	0.82 ± 0.12				
Phentolamine (3.5×10^{-6} M) and propranolol (7.7×10^{-6} M)†	1 hr	0.06 ± 0.04				
	6 hr	1.14 ± 0.02				
Cycloheximide (7.2×10^{-5} M) applied at 0 hr	1 hr	0	0.96 ± 0.18	1 hr	0.40 ± 0.06	0.16 ± 0.03
	6 hr	0.03 ± 0.02‡	0.97 ± 0.19	6 hr	0.42 ± 0.04	0.13 ± 0.02
Cycloheximide (7.2×10^{-5} M) applied at 5 hr 45	1 hr					
	6 hr	1.21 ± 0.40				
Actinomycin D (2.0×10^{-6} M) applied at 0 hr	1 hr	0.01 ± 0.01	0.74 ± 0.31	1 hr	0.36 ± 0.05	0.11 ± 0.02
	6 hr	0.02 ± 0.02‡	0.73 ± 0.21	6 hr	0.33 ± 0.03	0.10 ± 0.02
Actinomycin D (2.0×10^{-6} M) applied at 5 hr 45	1 hr					
	6 hr	0.87 ± 0.26				

* The responses are contractions expressed in grams of tension.
† The drugs were applied after recording the 1-hr response.
‡ $P < .001$.

TABLE 8

Preparations containing the receptors

Type B_1
Rabbit: Aorta, renal artery, pulmonary artery, carotid artery, anterior mesenteric vein, portal vein, jejunum
Cat: Aorta, carotid artery
Types B_1 and B_2
Rabbit: Posterior vena cava, renal vein, trachea
Rat: Anterior mesenteric vein, stomach, ileum, urinary bladder
Dog: Jugular vein, mesenteric vein, superior vena cava, subclavian artery
Man: Circular muscle of the colon

example, injection of Triton X-100 into the urinary bladder through the urethra in ether-anaesthetised rats induces the appearance of B_1 receptors at the site of injury in a few hours (262). This sensitisation has been demonstrated by recording the changes of bladder pressure in vivo or in inflamed bladders explanted and suspended in vitro (262). The i.v. injection of a pyrogenic lipopolysaccharide (LPS, *Escherichia coli*, Sigma L-3129) into rabbits induces the generation of B_1 receptors in the cardiovascular system in 5 hr. The treated rabbits respond to the i.v. injection of des-Arg^9-BK (a compound that is practically inactive in normal animals) with a significant fall of blood pressure that is blocked by the specific B_1 receptor antagonists [Leu^8],des-Arg-BK and [Leu^9],des-Arg^{10}-kallidin (264). Moreover, arteries (aorta, pulmonary, renal) and veins (anterior mesenteric) taken from treated animals are sensitive to des-Arg^9-BK from the beginning of the incubation in vitro, while the same tissues of the normal animals are practically insensitive. These results indicate that the activation of B_1 receptors in the cardiovascular system results in hypotension and are in accord with previous observations that the coronary vasodilator effect of BK is partially inhibited by [Leu^8],des-Arg^9-BK (20). The findings may be representative of a mechanism of receptor regulation involved in the response of tissues to injury, particularly in fever, inflammatory, or allergic reactions, whereby the formation of B_1 receptors could mediate some of the effects of kinins released locally by the noxious stimulus or by the accumulating white blood cells.

The early observation that bradykinin given by aerosol is bronchoconstrictive in asthmatic patients while inactive in normal subjects (206) is consistent with the interpretation that the sensitivity of damaged tissues to kinins may be increased due to the de novo formation of B_1 receptors.

As far as B_2 receptors are concerned, changes of sensitivity to kinins have been described in isolated organs both after treatment of the donor animal and after application of several agents in vitro. A well-known example of the first category is the increased sensitivity to kinins of the rat isolated uterus in oestrus induced by steroids (128). The ileum of female guinea pigs is more sensitive to BK than that of the male (128); the sensitivity can be increased by castration and reduced again by treating the castrated male with testosterone (471). The responses of smooth muscle preparations suspended in vitro to kinins can be enhanced by chymotrypsin and trypsin [guinea-pig ileum (464)], by the reduction of pH in the physiological medium to 6.0 [guinea-pig ileum (464)], and by thiol compounds [guinea-pig ileum (56) and rat uterus (258)]; it is reduced in the presence of or after contact with urea [rat stomach (154)], by increasing the pH of the physiological medium to 9.0 [guinea-pig ileum (464)], by treatment with neuraminidase [rat uterus, rat stomach (495)], and by contact with high concentrations of

thiol compounds [guinea-pig ileum (15)]. As pointed out by Odya and Goodfriend (326), potentiation or inhibition of tissue sensitivity to kinins by thiols has been attributed to: "Kininase inhibition (341), alteration of excitation-contraction coupling (15), facilitation of acetylcholine release (352), and increased permeability to bradykinin (443). Since thio compounds are known to inhibit kininases, potentiation could not be interpreted as an effect on the bradykinin receptor unless it could be demonstrated that kininase activity was not limiting." The example of the thiol compounds indicates the difficulty in interpreting data obtained in whole organs in terms of receptor function when using drugs whose mechanism of action is not entirely understood or may be complex; it also shows again that the metabolic inactivation of kinins interferes with their biological activities.

VII. Relationships between Chemical Structure and Biological Activities

This section will be divided into four parts in order to analyse separately structure-activity relationships of kinins in the two receptor systems described above and their respective antagonists. A short paragraph has been added on compounds that potentiate some of the pharmacological effects of bradykinin and on radioligand assays.

A. Structure-Activity Relationship of des-Arg^9-Bradykinin and Its Derivatives on the B_1 Receptor System

After the observation that des-Arg^9-BK is much more potent than BK itself on the B_1 receptor of the rabbit aorta (358), several synthetic analogues of des-Arg^9-BK were prepared and tested in order to perform a systematic structure-activity study directed to identify the chemical groups responsible for binding to and/or activation of the B_1 receptor. The final purpose of this study was 3-fold: a) to evaluate the activities of naturally occurring kinins and some of their major metabolites on B_1 receptors; b) to find compounds more active than des-Arg^9-BK; and c) to develop specific and competitive antagonists.

The compounds were synthesised by the solid-phase method: they were checked for purity with several chemical techniques (336, 427) and were tested on the rabbit aorta strip (B_1 system) and the cat ileum (B_2 system) for comparison. Some of them were also injected into anaesthetised rats to measure their effect on blood pressure.

The first series of compounds are analysed in table 9. They include: a) the natural kinins and some of their metabolites; and b) 26 analogues of des-Arg^9-BK, in which the natural residues were replaced one by one with L-Ala (compounds no. 8 to 15), the C-terminal carboxyl was esterified or amidated (compounds no. 16 to 17), or the phenylalanines in positions 5 and 8 were replaced with other aromatic or aliphatic residues (compounds no. 18 to 33).

The results of the biological assays, performed on rabbit aorta strips and on the cat ileum and shown in table 9, suggest the following. 1) The octapeptide fragment des-Arg^9-BK is 10 times more active than the naturally occurring nonapeptide, while the heptapeptide fragment des-Phe^8, des-Arg^9-BK is inactive. 2) The other two natural kinins, Lys-BK and Met-Lys-BK, are approximately 10 and 76 times more active than BK. It appears that the affinity of kinins for the B_1 receptor system can be improved either by eliminating a positive residue (Arg^9) at the carboxyl end, or by adding an isosteric and isofunctional residue (Lys) at the amino end. The activity can be further improved by extending the peptide chain at the amino end with a Met. 3) When the first two chemical features are combined, as in des-Arg^{10}-kallidin, the increase of affinity is greater than additive, while the extension at the amino end is less effective in the absence of Arg^{11} (see compound no. 7).

Compounds no. 1 to 7 stimulate the rabbit aorta by the activation of the same B_1 receptor (99) and they are all full agonists, except no. 3. This would be compatible with the observed changes of activity being due to differences in affinity, although alterations in efficacy [in Stephenson's sense (421a)] have not been ruled out. Increased affinity is presumably due to a prolonged duration of the peptide-receptor interaction (98), at least for compounds no. 4, 5, and 6. The unexpected reduction of activity of compound no. 7 remains unexplained.

In an attempt to evaluate the individual role of each residue in the binding and the stimulation of B_1 receptors by kinins, a series of L-Ala derivatives of des-Arg^9-BK was prepared (compounds no. 8 to 15). Des-Arg^9-BK was chosen instead of kallidin or the more potent des-Arg^{10}-kallidin as the basic structure and reference agonist for analogues designed to study the chemical features required to activate B_1 receptors, because the octapeptide des-Arg^9-BK is easier to synthesise and is more selective for B_1 receptors than the other two larger peptides. In fact, kallidin and des-Arg^{10}-kallidin maintain 89% and 18% of the activity of BK on the B_2 receptor system (see table 9), while des-Arg^9-BK is practically inactive ($P < .01$). As shown in table 9, L-Ala is perfectly tolerated only when used to replace Ser^6. In all the other positions, the substitution of the natural residue with L-Ala is accompanied by a drastic reduction of apparent affinity to values below 1% (compounds no. 10, 11, 15) or to 10% (compounds no. 8, 9, 12, 14) of des-Arg^9-BK. For some analogues (compounds no. 10, 11, 15) the maximal response of the aorta could not be recorded, even using concentrations of peptides in the order of 10^{-4} M. However, the slope of the dose-response curves and their parallelism with that of des-Arg^9-BK suggest that compounds no. 10 and 11 are full agonists, while the other two (compounds no. 14 and 15) are partial agonists. The L-Ala analogues of des-Arg^9-BK are practically inactive on the B_2 receptor system of the intestine (cat ileum).

These results suggest that: 1) in order to interact with B_1 receptors des-Arg^9-BK needs a critical conformation

TABLE 9

Primary structure and biological activities of kinins, des-arg^9-BK and its analogues

No.	Sub.*	Name	Primary Structure	Rabbit Aorta			Cat Ileum
				α^E	pD_2	RA†	RA
			1 2 3 4 5 6 7 8 9				
1		Bradykinin (BK)	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Phe- Arg-OH	1.0	6.22	8.50	100.00
2		des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Phe- OH	1.0	7.29	100.00	0.16
3	8, 9	des-Phe^8, des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro-OH			0	<0.01
			1 2 3 4 5 6 7 8 9 10				
4		Lys-BK (kallidin)	H- Lys- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Phe- Arg-OH	1.0	7.27	95.00	89.00
5	10	des-Arg^{10}-kallidin	H- Lys- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Phe- OH	1.0	8.61	2100.00	18.00
			1 2 3 4 5 6 7 8 9 10 11				
6		Met-Lys-BK	H- Met-Lys- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Phe- Arg-OH	1.0	8.17	760.00	7.60
7	11	des-Arg^{11}-Met-Lys BK	H- Met-Lys- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Phe- OH	1.0	7.28	98.00	68.00
8	1	[Ala^1], des-Arg^9-BK	H- Ala- Pro- Pro- Gly- Phe- Ser- Pro- Phe- OH	1.0	6.09	6.30	<0.01
9	2	[Ala^2], des-Arg^9-BK	H- Arg- Ala- Pro- Gly- Phe- Ser- Pro- Phe- OH	1.0	5.86	3.70	<0.01
10	3	[Ala^3], des-Arg^9-BK	H- Arg- Pro- Ala- Gly- Phe- Ser- Pro- Phe- OH		4.74	0.28	<0.01
11	4	[Ala^4], des-Arg^9-BK	H- Arg- Pro- Pro- Ala- Phe- Ser- Pro- Phe- OH		4.45	0.14	<0.01
12	5	[Ala^5], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Ala- Ser- Pro- Phe- OH	1.0	6.03	5.50	0.01
13	6	[Ala^6], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ala- Pro- Phe- OH	1.0	7.29	100.00	0.44
14	7	[Ala^7], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Ala- Phe- OH	0.61	5.64	2.20‡	0.05
15	8	[Ala^8], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Ala- OH		4.78	0.31‡§	<0.01
16	8, 9	[Phe-OMe^8], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Phe-OMe	1.0	6.69	25.00	0.25
17	8, 9	[Phe-NH$_2^8$], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Phe-NH$_2$	0.88	6.59	16.80‡	<0.01
18	5, 9	[Tyr^5], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Tyr- Ser- Pro- Phe-OH	1.0	6.32	11.00	0.03
19	5, 9	[Tyr(Me)^5], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Tyr(Me)- Ser- Pro- Phe-OH	1.0	5.27	0.95	0
20	5, 9	[Cha^5], des-Arg^9-BK†	H- Arg- Pro- Pro- Gly- Cha- Ser- Pro- Phe-OH	1.0	7.21	83.00	0.01
21	5, 9	[D-Phe^5], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- D-Phe Ser- Pro- Phe-OH			<0.01‡	<0.01
22	5, 9	[Car^5], des-Arg^9-BK†	H- Arg- Pro- Pro- Gly- Car- Ser- Pro- Phe-OH	0.76	6.32	11.00‡	
23	5, 9	[Leu^5], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Leu- Ser- Pro- Phe-OH	1.0	5.81	3.10	<0.01
24	8, 9	[Tyr^8], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Tyr-OH	1.0	4.39	0.10	0.02
25	8, 9	[Tyr(Me)^8], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Tyr(Me)-OH		4.28	0.10	<0.01
26	8, 9	[Cha^8], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Cha-OH	0		—§	0.09
27	8, 9	[D-Phe^8], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- D-Phe-OH	1.0	7.89	400.00	0.04
28	8, 9	[Car^8], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Car-OH			<0.10	
29	8, 9	[Leu^8], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Phe- Ser- Pro- Leu-OH	0		—§	<0.01
30	5, 8, 9	[Tyr(Me)^{5,8}], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Tyr(Me)- Ser- Pro- Tyr(Me)-OH			<0.10	<0.01
31	5, 8, 9	[Cha^{5,8}], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Cha- Ser- Pro- Cha-OH	0		—§	0.02
32	5, 8, 9	[D-Phe^{5,8}], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- D-Phe- Ser- Pro- D-Phe-OH			<0.10‡	0.31
33	5, 8, 9	[Car^{5,8}], des-Arg^9-BK	H- Arg- Pro- Pro- Gly- Car- Ser- Pro- Car-OH			<0.10	

* Sub., residues that have been removed or replaced.

† α^E, intrinsic activity; pD_2, -log of the molar concentration of peptide producing 50% of maximal response; RA, relative affinity in percent of that of des-Arg^9-BK (rabbit aorta strip) or of that of BK (cat ileum); Cha, cyclohexylalanine; Car, carboranylalanine.

‡, compound is a partial agonist; §, compound is an antagonist.

that is conferred by Pro^3 and Gly^4, since the replacement of these residues with L-Ala brings about the most important decrease of apparent affinity; b) the first six residues of des-Arg^9-BK appear to play a role in the binding of the peptide to B_1 receptors, because the corresponding L-Ala substitutes maintain full intrinsic activity; c) the two C-terminal residues, Pro^7 and Phe^8, appear to be involved in stimulation of receptors, because the corresponding L-Ala substitutes are partial agonists. Therefore, the stimulating group of des-Arg^9-BK is located at the carboxyl end. This assumption has been confirmed by testing the compounds no. 14 and 15 for antagonism and by the finding that, at very high doses, they reduce the stimulating effect of des-Arg^9-BK on the rabbit aorta (97). Moreover, [Gly^7], des-Arg^9-BK has been found to be a weak antagonist (22).

The C-terminal carboxyl group appears to be involved in the binding of des-Arg^9-BK to the receptors, because its methylation or amidation reduces affinity with little or no reduction in the intrinsic activity (see results with compounds no. 16 and 17).

Results obtained with compounds no. 18 to 33 to assess the role of Phe^5 and Phe^8 in the interaction between des-Arg^9-BK and the B_1 receptor allow the following conclusions: a) Phe^5 appears to be involved primarily in the process of binding, because its replacement with aromatic [Tyr, Tyr(Me)], or aliphatic (Leu, Cha) residues is accompanied by a decreased affinity without changes in the intrinsic activity; b) the aromatic nucleus of Phe^8 is the stimulating group, because Phe^8 can be replaced with aromatic residues [Tyr, Tyr(Me), D-Phe] without changing the intrinsic activity, while replacement with aliphatic residues (Cha, Leu) results in analogues that are potent antagonists of the effect of kinins on the B_1 receptor system.

Des-Arg^9-BK does not tolerate the double substitu-

tions in 5 and 8, as indicated by the negative results obtained with compounds no. 30 to 33. When tested for antagonism, only [Cha^{5,8}], des-Arg^9-BK has given positive results, but it is a weak antagonist. Finally, the use of D-Phe and of carboranylalanine (Car) in position 5 and 8 has provided interesting results. The expected modification of conformation produced by D-Phe in position 5 is not tolerated, while in position 8 it produces an increase of affinity by a factor of 4 in the rabbit aorta and yields a compound that is much more potent than des-Arg^9-BK in the rat blood pressure assay (22). On the other hand, the utilisation of Car, an amino acid with a rigid ring containing 10 atoms of boron in a cage that has the volume of a phenyl nucleus rotating about its C(1)–C(4) axis and that is more lipophilic than phenylalanine, gave the opposite results. When used in position 5, this residue increases the duration of binding and therefore prolongs the duration of action of compound no. 22 on the B_1 receptor system, while, when placed in position 8, it reduces the affinity and does not confer to compound no. 28 any antagonistic properties. These results suggest that the structural requirements for optimal attachment into the active site of the B_1 receptor are limited to the benzyl ring, while the binding site that normally interacts with Phe^5 accepts nonaromatic (Cha), more rigid (Car) and larger nuclei (Car, Tyr) than Phe. The residue in position 8 must be aromatic and have the size of a phenyl nucleus in order to enable activation of the aortic receptor, while aromaticity appears not to be essential for the binding contribution by Phe^5.

B. Antagonists of Kinins on the B_1 Receptor System

Structure-activity relationships of antagonists that inhibit the effect of kinins on the B_1 receptor system are presented in table 10. All compounds were tested by administering the antagonist a few minutes before or during the stable contraction evoked by des-Arg^9-BK in the rabbit aorta (98, 358, 359). pA_2 and pA_{10} were measured, according to Schild (397), in order to demonstrate the competitivity of the antagonism. For some compounds [e.g. [Leu-OMe^8],(des-Arg^9-BK)], the plot of log (DR-1) against − log [B] was calculated according to Arunlakshana and Schild (14) and the relationship was found to be linear with a slope close to unity (22, 358). This demonstrates that the antagonist is competitive. In table 10 we have given pA_2 values of 19 antagonists as measured in the rabbit aorta strip against des-Arg^9-BK. Some of the compounds were also tested against BK. The values of the difference pA_2–pA_{10} measured with each antagonist against des-Arg^9-BK are also presented in table 10.

Compounds no. 1 to 8 are all analogues of des-Arg^9-BK, monosubstituted in position 8. All substitutions have been made with aliphatic residues containing side chains of various lengths from Ala to Nle. The replacement of Phe^8 by L-Ala gives a very weak antagonist; affinity increases by about two log units with Cha and it reaches a maximum with Leu. [Leu^8],des-Arg^9-BK has approximately the same affinity as des-Arg^9-BK for the B_1 receptor. Amidation or esterification of the C-terminal

TABLE 10

Primary structure and pharmacological parameters of antagonists that block the effects of kinins on the B_1 receptor system of the rabbit aorta

No.	Name	Primary Structure	pA_2		$pA_2 - pA_{10}$
			des-Arg^9-BK	BK*	des-Arg^9-BK
		1 2 3 4 5 6 7 8			
1	[Ala^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Ala-OH	4.40	3.95	
2	[Cha^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Cha-OH	6.42	5.67	1.05
3	[Leu^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-OH	7.27	6.75	1.00
4	[Leu-NH$_2^8$], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-NH$_2$	7.04	6.36	0.92
5	[Leu-OMe^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-OMe	6.70	6.66	0.92
6	[D-Leu^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-D-Leu-OH	6.44	6.22	1.06
7	[Ile^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Ile-OH	6.40	6.25	1.02
8	[Nle^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Nle-OH	6.35	6.31	0.83
9	[Cha^5, Cha^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Cha-Ser-Pro-Cha-OH	6.47		0.99
10	[Car^5, Leu^8], des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Car-Ser-Pro-Leu-OH	7.84		0.93
11	[Car^5, D-Leu^8]-des-Arg^9-BK	H-Arg-Pro-Pro-Gly-Car-Ser-Pro-D-Leu-OH	6.31		0.74
12	[Car^5, Leu-NH$_2^8$], des-Arg^9-BK	H-Arg-Pro Pro-Gly-Car-Ser-Pro-Leu-NH$_2$	7.36		1.03
		1 2 3 4 5 6 7 8 9			
13	[Leu^9], des-Arg^{10}-Kall.	H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-OH	8.37		1.10
14	[Leu-NH$_2^9$], des-Arg^{10}-Kall.	H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-NH$_2$	8.07		1.03
15	[D-Leu^9], des-Arg^{10}-Kall.	H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-D-Leu-OH	7.50		1.04
16	[Car^6, Leu^9], des-Arg^{10}-Kall	H-Lys-Arg-Pro-Pro-Gly-Car-Ser-Pro-Leu-OH	7.69		1.16
17	[Car^6, D-Leu^9], des-Arg^{10}-Kall.	H-Lys-Arg-Pro-Pro-Gly-Car-Ser-Pro-D-Leu-OH	6.61		0.93
		1 2 3 4 5 6 7 8 9 10			
18	[Leu^{10}], des-Arg^{11}-Met-Lys-BK	H-Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-OH	8.22		1.05
19	[Leu-NH$_2^{10}$], des-Arg^{11}-Met-Lys-BK	H-Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-NH$_2$	8.17		1.03

* pA_2 was measured by using des-Arg^9-BK or BK as agonists. pA_2, -log of the concentration of antagonist that reduces the effect of a double dose of agonist to that of a single dose (396).

carboxyl, as well as the use of D-Leu, to prevent metabolism by carboxypeptidases, present no advantage since compounds no. 4, 5, and 6 show less affinity than [Leu^8], des-Arg^9-BK. The same effect is observed with a beta-branching side chain (with Ile) or a straight chain (with Nle). The affinity of these same antagonists is slightly lower when measured against BK. A possible explanation for this difference is that some test tissues (rabbit aortae) may contain a small number of receptors of the B_2 type that are not blocked by the antagonist and could contribute to an overestimation of BK activity. Compounds no. 2 to 8 appear to be competitive, according to the pA_2–pA_{10} difference, which varies from 0.83 to 1.06.

The second series of compounds (no. 9 to 12) are double-substituted and three of them contain Car in position 5. This modification was used in order to increase the duration of action of the antagonists. The most active compound is [Car^5, Leu^8],des-Arg^9-BK, which shows a pA_2 of 7.84, much higher than that of the monosubstituted [Leu^8],des-Arg^9-BK. The duration of action of this antagonist is significantly prolonged (69, 97). Compound no. 9 does not present any advantage with respect to the monosubstituted congeners, while compounds no. 10, 11, and 12 are long-acting and competitive (see value pA_2–pA_{10} in table 10).

The third group (compounds no. 13 to 17) includes 5 octapeptides extended at the amino end by a Lys, in order to improve the strength of binding with the aortic receptor. Indeed, pA_2 values of [Leu^9],des-Arg^{10}-kallidin average 8.37, and they are similar to the pD_2 value of the corresponding agonist (see compound 5 of table 9). Again, the highest antagonist affinity is conferred by the replacement of Phe by Leu at the C-terminal end; all other substitutions are much less favourable than that of compound 13. Finally, the peptide chain was extended at the amino end by the addition of Met-Lys (compounds no. 18 and 19). Although very potent, these antagonists are slightly less active than [Leu^9],des-Arg^{10}-kallidin and they are competitive.

The specificity of the antagonism for kinins was studied on the rabbit aorta by testing the effect of [Leu-OMe^8],des-Arg^9-BK against several stimulants of this vascular smooth muscle. The results summarised in table 11 indicate that [Leu-OMe^8]-des-Arg^9-BK and presumably all the other antagonists inhibit only the kinins (des-Arg^9-BK) and are therefore specific for these peptides. Recent results obtained with [Leu^8],des-Arg^9-BK support this assumption (22).

C. Structure-Activity Relationships of Bradykinin, Its Homologues, Fragments, and Analogues on the B_2 Receptor System

More than 300 analogues of bradykinin have been studied over the last 20 years. The compounds reported before 1970 have been reviewed by Schröder (400) and those of the last decade by Stewart (424). The synthetic peptides have been tested in several different bioassays, of which the most commonly used were the tests in vivo (the rat and rabbit blood pressure assays) and in two in vitro preparations, the rat uterus and guinea-pig ileum (400, 424).

The limitations of in vivo assays for studying drug action at the receptor level have been outlined by Furchgott (162) and by Ahlquist (4). In the specific case of kinins, the currently used measurement of the hypotensive effect after i.v. administration of these peptides into anaesthetised rats and rabbits, appears to be a poor test for three reasons; firstly, because a variable fraction of each kinin is inactivated in the lung before reaching the peripheral vessels; secondly, because the overall hypotensive effect of kinins is the result of several interfering components analysed in section V A, which may be influenced in different ways by the various peptides; and thirdly, because bradykinin has been shown to promote

TABLE 11

*Specificity of the inhibitory action of [Leu-OMe^8], des-Arg^9-BK on rabbit aortae**

Agonists (M)	[Leu-OMe^8], des-Arg^9-Bradykinin (10^{-5} M)	Contraction† (mm)	
		Without Antagonist	With Antagonist
des-Arg^9-Bradykinin (9.8×10^{-8})	0.11	33.6 ± 2.6	17.0 ± 1.9‡
	1.08	32.9 ± 1.8	4.1 ± 0.7‡
Bradykinin (BK), (2.0×10^{-6})	0.11	38.0 ± 2.5	25.2 ± 3.1§
	1.08	37.1 ± 1.4	8.0 ± 1.4‡
Noradrenaline (1.4×10^{-8})	0.11	34.2 ± 1.5	34.0 ± 1.4
	1.08	36.4 ± 1.5	37.2 ± 1.5
5-Hydroxytryptamine (1.1×10^{-7})	0.11	39.3 ± 1.6	38.5 ± 5.1
	1.08	40.0 ± 1.4	38.6 ± 1.1
Histamine (2.2×10^{-6})	0.11	31.9 ± 2.6	31.1 ± 2.4
	1.08	32.6 ± 2.1	32.2 ± 1.8
Angiotensin$_{II}$ (2.3×10^{-9})	0.11	35.0 ± 1.6	34.6 ± 2.2
	1.08	31.1 ± 2.1	28.5 ± 1.1

* Modified with the permission of the editors from D. Regoli, J. Barabé, and W. K. Park, Can. J. Physiol. Pharmacol. 55:855–867, 1977 (358).
† Results are given by the means ± S.E. of at least six determinations.
‡ $P < .001$.
§ $P < .005$.

the release of a vasodilator substance from the lung of both the rat (22) and rabbit (458), and its hypotensive effect is thus partly indirect. The in vivo test can be improved by injecting kinins intraarterially or by treating the animals with inhibitors of kininase II. Under these conditions the potency of bradykinin and its analogues undergoing metabolic degradation by kininase II is substantially increased (22, 371, 425). When the kinins are tested both by the i.v. and the intraarterial route, the difference of potency provides a useful indication of the pulmonary inactivation. Several analogues of BK have been tested in this way by Roblero et al. (371), by other workers [see review article by Stewart (424)], and by us (336). The interference by prostaglandins released from the lung can be avoided by treating the animals with indomethacin (22, 458). Thus, despite the inadequacy of the in vivo assays for a precise evaluation of kinin action at the receptor level, these assays are important to measure the overall hypotensive effect of each kinin and should be used in parallel with the assays in vitro.

The pharmacological data obtained by various authors (400, 401, 422–424, 431) in vitro and in vivo with analogues and fragments of kinins describe only the relative activities (assays in vitro) and the relative potencies (assays in vivo) of each compound. One of the most interesting purposes of structure-activity studies is the identification of chemical groups involved in binding the peptide to its receptors as well as of the group mediating the biological effect. Such an identification is possible by measuring complete dose-response curves and the maximum effect of each compound (see section VI).

The analysis of the structure-activity relations of kinins on several B_2 receptor systems presented below is primarily intended to eliminate some of the deficiencies of previous pharmacological studies by giving the actual values of the intrinsic activities of at least a limited number of compounds. This analysis required the reassay and, in some instances, the resynthesis of previously published compounds, which were then tested in three preparations selected according to the criteria discussed in section VI A. Two of the preparations (the cat ileum and the dog carotid artery) fulfill these criteria, while the rabbit jugular vein has the major disadvantage of containing active kininase II. This metabolic interference was eliminated by testing all the peptides in the presence of a high concentration of SQ 14225 (4.6×10^{-7} M), which inhibits kininase II (22) (see section VI). Direct stimulating actions of the kinins, mediated by specific B_2 receptors in the intestine (cat ileum) and in the venous smooth muscles (rabbit jugular vein), as well as the inhibitory vascular action (dog carotid artery), are analysed together and compared with the results obtained previously on the rat uterus and the rat blood pressure, in order to provide an extensive and adequate pharmacological characterisation of each compound and to identify possible receptor differences between the various preparations. The results are presented in table 12.

The three natural kinins are full agonists, whose apparent affinities for the intestinal, uterine, and arterial smooth muscle systems decrease in parallel with the extension of the peptide chain at the amino end, while, in the venous system, Lys-BK is definitely more potent than BK and Met-Lys-BK. Recent results from our laboratory confirm these observations in the anterior mesenteric vein of the guinea pig (176). This difference may be of physiological interest, since Lys-BK is the principal product of glandular kallikreins (353). As discussed in section V C, a stimulant effect of Lys-BK on the capillary endothelium and on the venules might explain the potent action of this peptide on vascular permeability (365).

The removal of the C-terminal Arg reduces the activity of BK to a greater extent than that of Lys-BK. The effect of eliminating an Arg at the C-terminal end is partially compensated for by the addition of Lys at the amino end in two of the five preparations, namely the cat ileum and the dog carotid artery. Elimination of the C-terminal dipeptide Phe-Arg leads to a drastic decrease of activity, however, more pronounced for des-Phe^8, des-Arg^9-BK. These findings suggest that the inactivation of kinins by carboxypeptidases (e.g. kininase I) is less efficient than that by kininase II, also considering the fact that des-Arg^9-BK and des-Arg^{10} kallidin are potent stimulants of B_1 receptors (see section VII A). Arg^1 appears to be very important for the binding of BK to the B_2 receptors of all preparations and it is a full agonist in the arterial smooth muscle. Previous reports from our laboratory (18) showing that this compound is a partial agonist on the cat ileum have not been confirmed (22). The low activity of this compound suggests that if the degradation of BK from the amino end occurs in blood and tissues, it would be an efficient mechanism. The drastic reduction of activity observed with compounds 4 to 8 confirm the important role assigned to the Arg residues by Stewart (424) and indicate that the length of the peptide chain is critical for the biological activity (424). This is in contrast with the findings obtained with other peptides such as substance P (42, 70) or neurotensin (48) for which the N-terminal part of the molecule appears to be much less important.

In contrast with other systems [e.g. angiotensin (362), des-Arg^9-BK (section VII A)], the replacement of the natural residues of bradykinin one by one with L-Ala has not provided definite indications either of the location of groups responsible for binding and activation of receptors or whether these groups may be distinct. In fact, a variable decrease of affinity is observed with all L-Ala analogues of BK. Some compounds (e.g. no. 19, 12, 13, 16, and 17 of table 12) have such low affinities that the maximal response on both the cat ileum and the rabbit jugular vein could not be measured, even with extremely high concentrations (10^{-4} M) of peptide. However, the partial concentration-response curves obtained with these compounds were all parallel to that of BK and their highest values were greater than 50% of the maximal

TABLE 12

Pharmacological parameters of kinins, fragments, and analogues in the B_2 receptor systems of five pharmacological preparations

No.	Compound	Cat Ileum			Rabbit Jugular Vein			Dog Carotid Artery			Rat uterus RA	Rat Blood Pressure RP
		α^{E*}	pD_2	RA	α^{E}	pD_2	RA	α^{E}	pD_2	RA		
1	Bradykinin (BK)	1.0	8.47	100.00	1.0	8.46	100.00	1.0	8.64	100.00	100.00	100.00
2	Lys-BK (kallidin)	1.0	8.42	89.00	1.0	8.63	150.00	1.0	8.44	64.00	50–66	64†
3	Met-Lys-BK	1.0	7.35	7.60	1.0	5.24	0.06	1.0	6.56	0.84	25.00	32†
4	des-Arg^9-BK	1.0	5.67	0.16		4.39	0.01			<0.01	0.96	0.04†
5	des-Phe^8, des-Arg^9-BK			<0.01			0			0	0	0
6	des-Arg^{10}-K	1.0	7.72	18.00		5.33	0.06	1.0	7.22	3.8	0.06	0.02†
7	des-Phe^9, des-Arg^{10}-K		4.58	0.01		4.78	<0.01	1.0	5.32	0.05	0.01	0.01
8	des-Arg^1-BK			<0.01		4.13	<0.01	1.0	4.86	0.02	<0.01	0
9	[Ala^1]-BK		5.27	0.06	1.0	5.07	0.04	1.0	5.97	0.49	0.29	0.1
10	[Ala^2]-BK	1.0	6.80	2.10	1.0	6.10	0.42	1.0	6.33	0.21	0.33	0.5
11	[Ala^3]-BK	1.0	8.37	74.00	1.0	7.19	5.40	1.0	8.34	50.00	100.00	180.00
12	[Ala^4]-BK		5.17	0.04		4.73	0.02	1.0	6.78	4.30	<0.06	0.30
13	[Ala^5]-BK		5.69	0.16	1.0	5.41	0.10		4.40	<0.01	0.10	0.10
14	[Ala^6]-BK	1.0	7.92	27.00	1.0	6.94	3.00	1.0	6.49	0.72	10.00	10.00
15	[Ala^7]-BK	1.0	6.70	1.60	1.0	5.66	0.16	1.0	6.16	0.33	1.20	0.1
16	[Ala^8]-BK			<0.01		4.37	0.01	1.0	5.40	0.06	0.06	2.9†
17	[Ala^9]-BK		5.52	0.11		3.73	<0.01	1.0	5.38	0.06	<0.01	0.1
18	[Ala^{1,9}]-BK			0			<0.01		5.20	0.04	0.09	0
19	[D-Phe^5]-BK	1.0	6.99	3.30	1.0	7.01	3.60	1.0	7.19	3.50	4.6	2.3†
20	[Tyr^5]-BK	1.0	6.84	2.20	1.0	6.21	0.58	1.0	7.55	8.20	0.52	0.64†
21	[Trp^5]-BK	1.0	6.81	2.10	1.0	6.32	0.74	1.0	6.79	1.42	2.2	0.38†
22	[Cha^5]-BK	1.0	8.06	37.00	1.0	8.15	49.00	1.0	7.92	18.00	64.0	43.00
23	[Tyr(Me)^3]-BK	1.0	7.97	30.00	1.0	7.93	30.00	1.0	7.60	9.20	9.6	15.00
24	[Leu^5]-BK	1.0	6.79	2.00	1.0	7.70	17.00	1.0	6.49	0.70	5.0	1.9†
25	[D-Phe^8]-BK	1.0	7.25	5.80	1.0	6.30	0.72	1.0	6.39	0.56	1.70	6.6†
26	[Tyr^8]-BK	1.0	6.74	1.80	1.0	7.34	7.80	1.0	7.15	2.60	20.00	5.0
27	[Trp^8]-BK	1.0	8.36	75.00	1.0	7.83	24.00	1.0	8.07	27.00	120.00	125.00
28	[Cha^8]-BK	1.0	8.47	100.00	1.0	8.15	49.00	1.0	7.82	15.00	100.00	100.00†
29	[Tyr(Me)^8]-BK	1.0	8.51	105.00	1.0	8.59	140.00	1.0	8.64	100.00	165.00	270.00
30	[Leu^8]-BK	1.0	5.65	0.14	1.0	5.77	0.20	1.0	5.66	0.10	0.19	0.23
31	[Tyr(Me)^{5,8}]-BK	1.0	8.24	56.00	1.0	8.07	42.00	1.0	8.44	64.00	33.00	56.00
32	[Cha^{5,8}]-BK	1.0	7.27	6.00	1.0	6.13	0.47	1.0	6.70	1.10	33.00	17.5†
33	BK-methyl ester	1.0	8.26	58.00	1.0	7.69	17.50	1.0	8.13	31.00	21.5	9.2
34	[Lys^9]-BK	1.0	5.96	0.29	1.0	6.78	2.20	1.0	5.26	0.04	0.2	0.44
35	[Nle^9]-BK			<0.01		4.65	0.01	1.0	5.18	0.03	0.14	0.01

* α^E, pD_2, RA as in table 9; RP, relative potency calculated in percent of the potency of BK from effects of intraarterial injections of the peptides.

† Pulmonary inactivation of the compound in the rat is inferior to that of BK.

response produced by BK. We were therefore able to calculate the pD_2 on concentration-response curves whose maxima were obtained by extrapolation. In this way, compounds no. 9 to 17 of table 12 were considered to be full agonists in the intestinal and the venous B_2 receptor systems and therefore no indication was obtained on the location of the stimulating site of BK. The drastic reductions of activity, observed with compounds no. 12 and 15, in which L-Ala was used to replace Gly^4 or Pro^7, suggest that the conformation of the nonapeptide BK is very critical for the peptide-receptor interaction. The aromatic residues in positions 5 and 8 of BK appear to play a major role in affinity, (see compounds no. 13 and 16) and [Ala^5]-BK is very weak on the arterial smooth muscle. The order of potency of the L-Ala analogues in the cat ileum (11 > 14 > 10 > 15 > 13 > 17 > 9 > 12 > 16), the rabbit jugular vein (11 > 14 > 10 > 15 > 13 > 9 > 12 > 16 > 17), and the rat uterus (11 > 14 > 15 > 10 > 9 > 13 > 16 > 12 > 17) are extremely similar, while that of the dog carotid artery (11 > 12 > 14 > 9 > 15 > 10 > 17 = 16 > 13) is different, especially for compounds no. 12 ([Ala^4]-BK) and no. 13 ([Ala^5]-BK). The first is surprisingly active while the other is almost inactive. Another compound (no. 18) containing double substitutions with L-Ala in positions 1 and 9 was found to be inactive.

Considering the fundamental role played by aromatic residues in other peptides [e.g. angiotensin (362)], we tried to evaluate further the relative contribution of Phe^5 and Phe^8 to BK-B_2 receptor interaction by preparing and testing compounds no. 19 to 32. The results, summarised in table 12, can be discussed as follows. 1) Compounds containing aromatic [D-Phe, Tyr, Trp, Tyr(Me)], and aliphatic (Cha, Leu) residues in positions 5 or 8 behave as full agonists. From this it can be concluded that Phe^5 and Phe^8 are primarily involved in the binding of BK to

the B_2 receptor; aromaticity appears not to be essential for stimulation. 2) All analogues substituted in position 5 are less active than BK; this indicates that the receptor site normally interacting with Phe^5 is quite selective for the aromatic nucleus of Phe. On the other hand, the receptor site interacting with Phe^8 accepts a larger and similarly lipophylic (204) group [namely, Tyr(Me)] and binds it more strongly than Phe itself; in fact, the affinity of compound no. 29 is definitely increased with respect to BK. Aromaticity is not required, since [Cha^8]-BK is a full agonist that maintains a good affinity (155). Dunn and Stewart (101) have found that the replacement of Phe^5 and Phe^8 or of both these residues with Thi [β-(2-thienyl)-alanine] gives the most potent agonist ever tested in the rat uterus. 3) Double substitutions in 5 and 8 with Tyr(Me) or with Cha (see compounds no. 31 and 32) do not change intrinsic activity, suggesting that stimulation of the B_2 receptors is probably not the result of combined action by the two aromatic nuclei.

The orders of potency of compounds no. 19 to 24 (substituted in position 5) show minor differences in the four preparations and are practically identical between the cat ileum and the dog carotid artery, and between the rabbit jugular vein and the rat uterus. Of the six residues used for replacing Phe^5, the most favourable is Cha. These results do not reinforce the suggestion derived from results with [Ala^5]-BK that Phe^5 may play a special role on the receptor of the dog carotid artery. The order of potency of compounds no. 25 to 30 is practically the same in the four preparations and the most effective substitute for Phe^8 is Tyr(Me).

A few attempts have been made to explore the function of the C-terminal Arg. Methylation of the free C-terminal carboxyl reduces affinity but does not affect the intrinsic activity. We suggest that the C-terminal carboxyl is not essential for stimulation of B_2 receptors. However, a positive charge seems to be required in position 9 for binding and perhaps for stimulation of the B_2 receptor, since [Lys^9]-BK and [His^9]-BK (336) are full agonists with markedly reduced affinity, while [Nle^9]-BK is very weak.

Most of the compounds described in table 12, particularly the less active, were tested for antagonism against BK in the four isolated organs. A maximal concentration of each compound that had no or very little myotropic effect was applied to the tissue 5 or 15 min before measuring the effect of BK. All compounds were found to be inactive as antagonists.

To summarise, the structure-activity study of the B_2 receptor systems presented above provides a pharmacological characterization of the three natural kinins and their major metabolites as well as of a limited number of analogues. This study also provides new insights into the actions of kinins on vascular B_2 receptors with opposite actions (arterial relaxation and venous contraction). The L-alanine series of analogues, as well as the analogues, in which Phe^5 or Phe^8 were replaced with other residues, have permitted a comparison of the order of potency of kinin peptides in several preparations possessing B_2 receptors. In this way, the first classification criterion recommended by Schild (398) has been extensively applied to show that the four preparations probably contain the same type of B_2 receptor. It is worth mentioning that the same order of potency has been found in the three newly developed preparations (the cat ileum, the rabbit jugular vein, and the dog common carotid artery) and in the rat uterus. Our results, therefore, confirm the previous data of several authors (400, 424) for most of the compounds presented in table 12. This enables a comparison of the data presented in table 12 with those reported by other authors (366, 400, 424, 431) on numerous analogues of BK and other kinins. A complete critical review of these data has been made recently by Stewart (424).

In parallel with the empirical structure-activity approach analysed above, several authors have attempted to determine the conformation of BK in various solvents and to calculate the minimum energy structure of this nonapeptide. Because of the presence of three prolines, BK would be expected to show some degree of ordered structure in solution. The initial studies were therefore focussed on the measurement of optical rotatory dispersion (ORD) and circular dichroism (CD) spectra of BK and some of its analogues. Surprisingly, these studies revealed the absence of rigid geometry in BK, in contrast with secretin (33), and similar spectra were measured with biologically active and inactive analogues of BK (39). It was also found that BK had neither an α-helix nor a β-pleated sheet structure (39). Later on, the CD spectra were reinterpreted by Cann (45) "as being indicative of a secondary structure having some order, most probably due to an internally hydrogen-bonded proline residue." A systematic study of the CD behaviour of BK in water and dioxane resulted in the suggestion that "the secondary structure of bradykinin and its analogues is a time average of two interconverting structures—one disordered and one partially ordered due to a $3 \rightarrow 1$ type hydrogen bond bridging the Pro^7 residue (46)." In a hydrophobic environment, BK adopts a more highly ordered configuration, with the H-bond around Pro^7 while, in water, this bond is weakened and persists in less than 30% of the BK molecules. Charged groups (Arg^1 and Arg^9) and the two aromatic nuclei (Phe^5 and Phe^8) appear not to be involved in ionic bonds (46). Because of the important role played by the arginines and phenylalanines in the biological activity of BK (see section VII), Lintner et al. (247, 248) attempted to evaluate separately the contribution of each aromatic chromophore and of the whole peptide by CD studies of BK in various solvents, at different pH and temperatures, by using a series of analogues of BK modified in position 5 and/or 8. It was found that not only the overall apparent contribution of the two phenylalanyl residues is unequal, but also that each one is influenced differently by changes in the environment of the peptide: Phe^5 appears to have more

rotational freedom than Phe[8] (247). CD spectra of BK, des-Arg[1]-BK, des-Arg[9]-BK, and some analogues (248) confirmed earlier observations (39) suggesting that the conformation of BK is very sensitive to the peptide length. Removal of the N- or C-terminal Arg leads to drastic conformational changes that are compatible with a shift of the conformation towards or away from turns that may be present in the natural peptide.

Recent studies by London et al. (249) by means of ^{13}C nuclear magnetic resonance (NMR) have helped to substantiate or disprove several interpretations and have added important new data. The NMR findings are consistent either with a completely disordered conformation of BK or with an average of an ordered and a disordered conformation in aqueous media. According to London et al. (249) "measurements of the temperature dependence from the amide proton shifts provide no evidence for the involvement of either the Phe[8] NH proton or the Arg[9] amide proton (which could form a 4 → 1 hydrogen bond leading to a β-turn) in an intramolecular hydrogen bond. . . . The ^{13}C shifts of the Pro C_β and C_γ resonances are inconsistent with the exclusive existence of γ-turn configuration . . . no evidence for the existence of a tight ionic bond between the Arg[1] guanido and the Arg[9] carboxylate anion has been found." Thus, the existence of a β-turn in BK or the cyclic model proposed over a period of years by Russian investigators on the basis of physicochemical data (150, 215, 216) and of theoretical calculations (168, 215) has been disproved. London et al. (249) also suggest that the two phenylalanine rings are not folded inward and are thus available to interact with the receptors. Finally an interesting interpretation is presented about the role of the proline residues that constitute one third of bradykinin ". . . a primary function of the prolines may be to prevent the possible intramolecular hydrophobic interactions due to lack of flexibility which they impart to the molecule" (249).

The physicochemical studies on BK summarised above have contributed valuable data on the secondary structure that this peptide assumes in water and in organic solvents. These structures appear to be different, but both may be important since the peptide, generally administered in an aqueous medium, acts on the lipophylic environment of the cell membrane. It is still too early even to attempt an interpretation of biological activities of analogues and fragments in terms of conformational features. We agree with Stewart (424) that "It is very doubtful if the observed conformational features of bradykinin contribute to our knowledge of the conformation of bradykinin when it binds to tissue receptors." This remains a challenge for future work.

D. Antagonists of Kinins on B_2 Receptor Systems

Numerous chemicals have been tested in intact animals and in isolated organs in the hope of finding specific antagonists for kinins. A list of compounds that have been found to reduce the biological effects of these peptides in various pharmacological preparations has been presented by Odya and Goodfriend (326) and includes substances of diverse nature. Here we will attempt to establish the site and possibly the mechanism of action of each antagonist, bearing in mind that: a) kinins are basic peptides that can interact with negatively charged molecules or with other agents before reaching the receptor sites; b) kinins show high affinity for various components of cell membranes, for instance the receptors, some enzymes involved in the release or conversion of prostaglandins, and proteases that break down the peptides (see section VIII); c) kinins increase or diminish smooth muscle tone or produce changes in other cellular functions by initiating a sequence of intracellular events that are common to many other endogenous agents (see section VIII). A block or reduction of a kinin-induced biological effect by a chemical can therefore be the result of: 1) a chemical antagonism (the case contemplated in a); 2) a pharmacological antagonism (the case contemplated in b); and 3) another kind of antagonism (the case contemplated in c) whereby the antagonist interferes with one of the fundamental intracellular processes involved in the expression of the biological effect initiated by the peptide-receptor interaction. Specific antagonists for kinins may be found in groups 1 and 2, but they are unlikely to be found in group 3.

Antagonists of the first category include antibodies against bradykinin produced and tested as antagonists by various authors (83, 269, 270). Antibodies have been found to block the constrictor effect of exogenous bradykinin on the airways of guinea pigs (83) as well as the vasodilator effect of the nonapeptide in autoperfused dog hindlimbs (270).

A similar block of BK-induced hypotension by antibodies was observed in the rat, together with an inhibition of the oxytocic effect of BK (269). Antibodies were also used with the purpose of defining the physiological role of kinins in the regulation of renal function in the rat (268). It was found that antibodies against BK reduce water and sodium excretion.

To the first category belongs also carboxypeptidase B, a potent proteolytic enzyme that is able to cleave Arg[9] from BK (121). This compound has been used by several authors to block kinin effects in peripheral organs [see review by Erdös (121)]. Other compounds, such as butylated hydroxyanisole (BHA), an antioxidant, have been shown to form complexes with bradykinin in solution (92). This food-grade antioxidant shows some specificity for BK [for example, it is inactive against histamine (350)].

Pharmacological inhibitors of kinins remain to be identified. In fact the results reported by Stewart and Woolley (426) with analogues of BK modified in the 5 and 8 positions (e.g. [Tyr-(Me)[5,8]]-BK) have not been confirmed in our laboratory and we have previously discussed the reasons for the discrepancy (18). One possible explanation of the discrepancy is to be found in the

concentration of these compounds used by Stewart and Woolley (426) for testing the antagonism. When such potent stimulants as [Tyr(Me)^{5,8}]-BK are applied at the dose (10^{-6} M) recommended by the authors (426), they would desensitise the tissue in a similar way to BK. The desensitisation persists for some minutes after washing, and after the base-line of the preparation has returned to the control level and is responsible for the partial block of the response of the organ to BK. Our conclusion is based on results obtained in the rat uterus, the cat ileum, and the rabbit jugular vein. This point has recently been discussed by Stewart (424), who concludes that: "The initial demonstration of antibradykinin activity (Stewart and Woolley, 1966) could be done only on rat uterus from a special type of animal, in which the tissue was probably not normal." We have also tested for antagonism against BK, in several pharmacological preparations, a few other compounds that were found to compete with BK in isolated organs. These include: a) dihydrochlorprothixene (DHCP) and cyproheptadine (CH) (170); b) L-phenylalanine-heptyl ester (PHE) (177) and two other similar compounds (18); c) khellin and hesperitin (171); and d) several small peptides with structures similar to that of Chromozym Pk (Bz-Pro-Phe-Arg-pNA) (133).

Most of these compounds have been tested on the cat ileum and on the rabbit aorta strip to determine whether they antagonise the effects of kinins in a B_2 or B_1 receptor system. Several of them were also tested on the rat uterus or the guinea-pig ileum (22) for comparing the effects observed in our laboratory with those previously published or communicated by other authors. All these compounds were found to reduce the stimulating action of bradykinin on the cat ileum or on the rat uterus and the guinea-pig ileum, while they were inactive on the rabbit aorta (18). Moreover, the analysis of the concentration-response curves obtained with bradykinin in the absence and presence of these inhibitors revealed that all compounds were noncompetitive, since they depressed the maximal response. The stimulating effects of other agonists (acetylcholine, histamine, or serotonin) in the same preparations were reduced by these compounds, suggesting that they are not specific for kinins (18).

The fact that all these compounds exert a noncompetitive and nonspecific antagonism against kinins and other stimulants suggest that they do not belong to group no. 2, but rather to group no. 3. Although their mechanism of action was not investigated in great detail, it is assumed that the above substances interfere with membranous or intracellular processes contributing to the pharmacological effects of kinins as well as of other agents. To the same category belongs indomethacin, an agent that blocks the synthesis of prostaglandins, a phenomenon that, in the context of the present discussion, is considered to be an intermediate step between the peptide-receptor interaction and the biological effect.

Finally, it is worth mentioning that some effects of kinins in vivo [e.g. the rat paw oedema (254, 256)] are reduced by antihistaminics or by other types of drugs. Considering the complexity of the phenomena that occur in inflamed tissues and the possibility that kinins may act through the release of endogenous substances (histamine, serotonin, prostaglandins), any inhibitory effect is possibly of interest in therapeutics, but is of limited value for a pharmacological analysis as attempted above.

E. Potentiation of Pharmacological Effects Produced by Kinins

The response of some isolated organs to bradykinin, as well as the hypotensive effects evoked by these peptides when administered i.v. in several animals [e.g. the rat (381), the rabbit (22), the dog (302), etc.] are enhanced by naturally occurring agents (in venoms of various snakes), by synthetic peptides, and by other compounds. In conjunction with the potentiation of bradykinin, these agents have been shown to block the conversion of angiotensin I to angiotensin II (16, 119). The numerous compounds isolated, synthesised, and tested after the discovery of the first bradykinin-potentiating factor in the venom of *Bothrops jararaca* by Ferreira in 1965 (137), have been extensively reviewed by Stewart (424) and have been classified into two major groups: a) bradykinin-potentiating factors of natural origin or synthetic peptides bearing the major structural features of some natural bradykinin-potentiating peptides, in particular BPP5a (<Glu,Lys,Trp,Ala,Pro) and BPP9a (<Glu,Trp,Pro,Arg,Pro,Gln,Ile,Pro,Pro), denoted as SQ 20881, and some fragments of bradykinin; and b) synthetic chelating agents, SQ 14225 being a typical example.

Among the 15 natural peptides whose sizes vary between 5 and 13 amino acids [see review by Stewart (424)], the most potent is BBP9a (424). The basic structures of BBP5a and BPP9a have been taken as models for the synthesis of numerous analogues in order to find more potent compounds (331). These attempts have been successful in the case of BPP9a and some very potent compounds have been obtained by replacing Pro^3, Pro^5, or Pro^9 with dehydroproline (152). Despite their high potency and relatively long duration of action in vivo, the natural and the synthetic peptides have a major limitation, since they are not absorbed by the intestine and have to be administered i.v. This limitation has been overcome with a recent synthesis of chelating agents by Ondetti et al. (330). SQ 14225 is a potent long-acting and orally active inhibitor of kininase II as well as of other proteolytic enzymes (122). The design of this compound was based on a hypothetical model of the active site of the angiotensin-converting enzyme. According to Ondetti et al. (330) "one of the most important interactions of SQ 14225 [(D-3-mercapto-2-methyl-propanoyl)-L-proline] with the angiotensin-converting enzyme is thought to be the binding of its sulphydryl group to the catalytically important zinc ion of this enzyme." This binding is probably responsible for the long duration of action of SQ 14225 in vivo and in vitro (382). In the three years

since its discovery, this compound has been used in a large number of experimental and clinical investigations and has been found to be an active antihypertensive agent (218). Recent experimental and clinical findings with SQ 14225 have been analysed in a symposium (434). We will limit our analysis to the utilisation of SQ 14225 in isolated organ preparations that are currently used in our and other laboratories for pharmacological studies of kinins. In section VI A, we have presented and discussed the experimental evidence supporting the assumption that the potentiation of the biological effects of kinins in the rabbit jugular vein and in other tissues containing active kininase II by SQ 14225 may be due to inhibition of kininase II. Kininase II inhibition is not the only mode of potentiation of BK in intestinal (451, 452) and uterine (445) tissues. Interference by kininase II inhibitors with receptors for BK has been proposed as a possible mechanism for explaining unexpected findings obtained with various types of compounds (445, 451, 452). One controversial preparation is the rat isolated uterus. In fact, Aiken and Vane (5) have shown that the action of angiotensin I on this tissue is partially blocked by competitive inhibitors of kininase II (SQ 20475: <Glu-Lys-Trp-Ala-Pro). Moreover, potentiation of bradykinin on rat uterus has been observed by Kato and Suzuki (222) with the undecapeptide derived from *Agkistrodon halys blomhoffii* and by Tominaga et al. (445) with several synthetic compounds. We could not confirm the results of these authors (222, 386, 445, 451, 452) on rat uterus in oestrus by using SQ 14225. We have therefore classified the uterus among the preparations devoid of active kininase II. A possible interpretation of these contrasting results is that SQ 14225 acts only on kininase II and is therefore more specific than other inhibitors. Another possibility is that, in certain experimental conditions or oestrus phases, the presence of active converting enzyme or even of active kininase II can be demonstrated in the whole rat isolated uterus.

Converting enzyme inhibitors have been used by several authors in physiological studies directed to evaluate the role of the kallikrein-kinin system in the regulation of renal blood flow (305) and of kidney functions (305, 307). Because of the simultaneous actions of the renin-angiotensin and other systems, bradykinin potentiators have major limitations when utilised in physiological studies. In fact, any actual change of function induced by these inhibitors in vivo or in isolated organs perfused with blood can be referred to either an enhancement of kinin or reduction of angiotensin activities. On the other hand, the concomitant use of inhibitors specific for angiotensin, such as saralasin, does not yet provide a definite alternative, since these compounds maintain some angiotensin-like activity in most in vivo assays (236).

F. Radioligand Assays for Bradykinin

A few reports on radioligand assays for BK have been published in recent years (326, 327). The major difficulty with such assays for BK receptors is found in the development of a radioactive ligand with full biological activity and with sufficient specific activity to permit the detection of the limited number of specific receptor sites that may be present in target tissues. Biologically active ^{3}H- and ^{14}C-labelled BK were found to have too little specific activity to be used as radioligands in radioreceptor assays (326). The problem was resolved with the addition of a radioligand at the N-terminal end of the nonapeptide BK. Thus, binding sites with an apparent $K_{assoc.}$ of 3.6×10^{9} M^{-1} for BK were found by Paegelow et al. (334) in plasma membranes isolated from the rat uterus, with [^{3}H]acetyl-(8-erythro-amino-β-phenylbutyric acid)-BK (specific activity 2 Ci/mmol.). Similar results were recently obtained by Odya et al. (327) in bovine uterine membranes, where sites were found showing an apparent $K_{assoc.}$ of 10^{10} M^{-1} for [^{125}I]-Tyr^{1} kallidin (specific activity 1 to 2 Ci/μmol). Binding of BK to bovine uterine membranes was highly specific, since it was inhibited by active analogues of kinins proportionally to their biological activity. Inactive fragments and analogues of BK, as well as oxytocin, angiotensin I and II, and a peptide inhibitor of kininase II (SQ 20881) did not compete with BK even when applied at high concentrations (5 μg/ml). Interference by kininases with the radioreceptor assay was definitely demonstrated by Odya et al. with SQ 20881 (327) and was avoided by Paegelow et al. (334) with an analogue resistant to degradation by the rat uterus membrane.

This analogue increased the binding of [^{125}I]-Tyr^{1}-kallidin to bovine uterine membranes presumably by protecting the peptide from degradation. Other protease inhibitors (ϵ-aminocaproic acid for kininase I and L-1-tosylamide-2-phenylethyl-chloromethyl-ketone for chymotrypsin) were found to be inactive. SQ 20881 was preferred to chelating agents because these agents can allow binding of bradykinin to kininase II even though they may inhibit BK hydrolysis (327). As pointed out by Odya et al. (327): "Proof that the binding studies described above pertained to receptors and not other sites requires knowledge of the cellular events the receptors modulate, or the use of specific inhibitors of their interaction with bradykinin."

VIII. Mechanism of Action of Kinins

The multiple biological effects of kinins discussed in section V are unlikely to be the result of a unique mechanism of cellular action. In fact, kinins mediate opposite effects, such as the contraction of the rabbit jugular vein and the relaxation of the dog carotid artery, through the activation of a similar (probably the same) B_2 receptor system. In these two instances, the biological effects produced by kinins are determined by the intracellular events that follow the activation of the receptors and result in either contraction or relaxation. In other tissues, the biological effects of kinins depend on events occurring at the cell membrane, where kinins may find

at least three different macromolecular entities, fairly specific and ready to interact with them. These entities are: a) specific receptors of the B_1 or the B_2 type; b) two enzymes involved in the formation, release, or conversion of prostaglandins (phospholipase A_2 and PGE 9-ketoreductase); and c) proteases.

Pharmacological studies, analysed in section VI, indicate that kinins are able to exert some of their effects through the activation of specific receptors. The ultimate biological effect in each system is therefore determined by the receptor type on the cell membrane and can be blocked or reduced, in some instances, by specific antagonists, which compete with kinins for the same macromolecular entity (e.g. B_1 receptor). This is the typical case of a direct pharmacological action and the results obtained in such a system can be analysed and interpreted with the concepts and equations of classical receptor theories.

In other tissues [e.g. the rat isolated ileum (72, 73, 465) or the rat duodenum (465)], the contracting (ileum) or relaxing (duodenum) effects of bradykinin result from both a direct and an indirect action, because the concentration-response curves of the nonapeptide are displaced to the right and their maxima are reduced in the presence of indomethacin. Assuming that indomethacin (when used in concentrations of 2.8 μM or higher) completely blocks prostaglandin synthesis, the above results suggest that the contraction of the rat ileum brought about by low concentrations of bradykinin is primarily due to the release of stimulating prostaglandins and is probably the result of the interaction of BK with phospholipase A_2 or with other enzymes involved in the production or release of the stimulating prostaglandins. Higher concentrations of bradykinin appear to interact with a rather insensitive receptor that enables the peptide to contract the ileum in the presence of indomethacin. The analysis of such a complex biological effect, consisting of an indirect and a direct component, requires the use of specific antagonists that enable the study of one component at the time. Antagonists would also permit discrimination between the two alternatives: a) the presence of two separate entities, the enzyme and the receptor; and b) the presence of two receptor populations, acting by different mechanisms. The example described above may not be infrequent in the field of kinins, a group of peptides that stimulate the release of prostaglandins from a variety of tissues (see section VI A).

Activation of membranous phospholipase A_2 is generally followed by the production of arachidonic acid and by its rapid activation to prostaglandins, which may act either in the producing cell or be released in the extracellular fluid for action on neighboring structures. Bradykinin is among the few peptide hormones that have been shown to stimulate phospholipase A_2. As pointed out by Nasjletti and Malik (308), "augmented release of prostaglandins from an organ denotes either enhanced biosynthesis or diminished degradation of prostaglandins, because there is no appreciable storage of prostaglandins in tissues (457). However, the evidence available strongly suggests that the release of prostaglandins evoked by bradykinin is brought about by stimulation of synthesis rather than by inhibition of prostaglandin degradation." In fact, mepacrine, an inhibitor of phospholipase A_2, prevents the release of prostaglandin-like material by bradykinin without affecting the activation of arachidonic acid to prostaglandins in isolated guinea-pig lungs (459) and blocks the bradykinin-induced release of both arachidonic acid and prostaglandins from the rabbit renomedullary interstitial cells (503). Much other evidence in favour of this mechanism of action by bradykinin has been reviewed recently (308) and need not be discussed in detail in this review. The following question, however, remains to be answered before attempting to analyse the above described phenomenon in pharmacological terms. Does bradykinin activate phospholipase A_2 by interacting directly with the enzyme or is the enzyme a second messenger, similar to adenylate cyclase? The same question applies to other similar biochemical actions of bradykinin, such as the proposed activation of PGE 9-ketoreductase and the resulting conversion of PGE_2 to $PGF_{2\alpha}$ in the veins (246, 494) as well as the possible release of other prostaglandins or products of arachidonic acid metabolism (410, 494). The possibility that a hormone could activate an enzyme without the intermediation of a receptor system is a new and fascinating hypothesis that, if definitely proved, would permit the application of basic and theoretical approaches, currently used in biochemistry, to the pharmacology of kinins.

The third group, the proteases, are unlikely to be directly involved in mediating the biological effects of kinins. We are tempted to consider the proteases as functional entities similar to acetylcholinesterases—namely extrinsic proteins (23), responsible for the degradation of kinins. However, the breakdown of kinins by some proteases (e.g. carboxypeptidases) might result in the formation of products that are potent stimulants of certain tissues. This mechanism is supported by the results presented in section VII A and B, as well as from early experiments (80) confirmed recently by Stewart (424), in which des-Arg^9-BK was shown to be a potent releaser of adrenal catecholamines in anaesthetised rats.

The contribution of proteases to the biological effects of kinins is possible only when these enzymes release locally an active fragment that has higher affinity than bradykinin for the organ receptor that mediates the biological action. The rabbit aorta strip provides a good example since it is practically devoid of active kininase II, while it contains active carboxypeptidases (363). In this context it is worth mentioning that the utilisation of potent inhibitors of kininase II in diseases (e.g. in the treatment in hypertension) by eliminating the major tissue pathway of kinin-degradation without influencing kininase I, might create the ideal conditions for a local release of des-Arg^9-BK and of des-Arg^{10}-kallidin, two

potent stimulants of B_1 receptors. Furthermore, if the number of tissue B_1 receptors increases in pathological conditions, as suggested by some preliminary data described in section VI C, the accentuated release of des-Arg^9-BK and des-Arg^{10}-kallidin could become an effective pathogenetic mechanism.

Several studies have been performed in order to elucidate the intracellular events that follow the stimulation of membranous receptors by kinins (335, 367). The analysis of recent literature by Odya and Goodfriend (326) indicates that receptors for kinins behave in a similar way to the receptors of numerous other hormones and neurotransmitters. The great majority of identified biological actions of kinins (see section VI) as well as the effects currently used in pharmacological studies result from contraction or relaxation of smooth muscles. Depending on the organ and on the type of effect (contraction or relaxation), ions and in particular Ca^{++} or cyclic nucleotides (cGMP, cAMP) appear to be the second messengers for kinins. Thus, a) the contraction of the human umbilical artery in response to bradykinin is accompanied by an increase of cGMP, dependent on extracellular Ca^{++} (59), b) the relaxation of the rat duodenum by an increase of cAMP (335), and c) the stimulation of the rat uterus by a decrease of cAMP that presumably leads to release of intracellular Ca^{++} and to contraction (335). Considering the essential role of Ca^{++} in the contraction of smooth muscles (38) and the fact that contraction is associated with either an increase of cGMP (umbilical artery) or a decrease of cAMP (uterus), the interpretation by Andersson (8) of the role of Ca^{++} and cyclic AMP in stimulus-contraction coupling can be extended to explain some of the myotropic effect of kinins. According to Andersson (8): "Cyclic AMP is suggested to be a regulator substance for the contraction-relaxation cycle of smooth muscles. Increase in the tissue level of cyclic AMP would then stimulate an energy-requiring Ca^{++} binding mechanism which, in turn, probably leads to a decreased concentration of myoplasmic Ca^{++} and subsequent relaxation. A reduction of the cyclic AMP level caused by contracting agent either through a stimulation of phosphodiesterase or a reduction of adenyl cyclase activity probably leads to a release of bound Ca^{++} which activates the contracting proteins." This interpretation does not take into account cyclic GMP or other substances that may be present in complex multicellular structures such as strips or segments of smooth muscles. For example, the three organs mentioned above can generate prostaglandins that are released by kinins and may participate in contraction or relaxation by influencing the fluxes of calcium or the intracellular content of cyclic nucleotides. This aspect has not been considered in the quoted studies. Other studies, performed on rather homogeneous populations of isolated cells, such as MC 5-5 fibroblasts (208), suggest that bradykinin stimulates the accumulation of cAMP through the induced release of prostaglandins. In another system, the isolated rat thymocytes (481), bradykinin stimulates the mitotic activity of these cells through accumulation of cAMP, which, in this system, appears to be dependent on Ca^{++} (340), in contrast with smooth muscles. As pointed out by Rasmussen and Goodman (354), "The cyclic nucleotide controls operate within the context of the cell ionic net, and ions are one of the most common and most widespread modulators of protein function. . . . One aspect of this whole concept of an ionic net that has only recently been considered is the possibility of more than one domain of calcium within the cell cytosol and the unique role they may play in intracellular communication."

IX. Conclusions

1. A variety of serine proteases, called kallikreins, are present in blood and in tissues as inactive precursors that can be easily activated for exerting direct actions [see recent review by Schachter (394)] or for releasing kinins, a group of local hormones. The activation of kininogen by kallikreins brings about the release of at least two active peptides (bradykinin and kallidin), which are further broken down to potentially active fragments (des-Arg^9-BK, des-Arg^{10}-kallidin) by carboxypeptidases or to inactive products by carboxydipeptidases. Some of the metabolic and pharmacological features of naturally occurring kinins and their potentially active metabolites are summarised in table 13.

Bradykinin (BK) is the most potent stimulant of B_2 receptors with the exception of those mediating the contraction of venous smooth muscles, which appear to be more sensitive to the product of glandular kallikreins, the decapeptide kallidin. This peptide, as well as Met-Lys-BK, are more resistant than BK to the proteolytic action of the carboxydipeptidase (kininase II) of the lung and of the vascular tissues. However, the higher affinity of kallidin for the venous B_2 receptor cannot be explained in terms of reduced inactivation, because the affinities of the kinins were measured after complete block of kininase II with SQ 14225. Kallidin is also more active than BK on the B_1 receptor, which, however, shows high sensitivity for the N-terminal fragments of BK and kallidin, namely des-Arg^9-BK and des-Arg^{10}-kallidin. Met-Lys-BK is a potent stimulant of B_1 receptors. However, its N-terminal fragment released by carboxypeptidases is less potent than the corresponding fragment of kallidin (97). The possible role of Met-Lys-Bk in oedema and inflammation is still undefined and requires further study.

The different pharmacological features of the three naturally occurring kinins and of some of their metabolic fragments, analysed above, call for sensitive and specific radioimmunoassays, in order to measure the concentration of these products in circulating blood and to define more precisely their physiological and pathological roles.

2. Kinins act on such a variety of smooth muscles that numerous sensitive preparations are available for phar-

TABLE 13

Pharmacological and metabolic features of kinins and of some of their metabolites

Feature	Bradykinin (BK)	Lys-BK (K)	Met-Lys-BK	des-Arg^9-BK	des-Arg^{10}-K
1. Apparent affinity for receptor B_1 (pD_2) (rabbit aorta)	6.22	7.27	8.17	7.29	8.61
2. Apparent affinity for receptor B_2 (pD_2)					
Cat ileum	8.47	8.42	7.35	5.67	7.72
Rabbit jugular vein	8.46	8.63	5.24	4.39	5.33
Dog carotid artery	8.64	8.44	6.56		7.22
3. Potency in vivo (rat, intravenous) (relative potency BK = 100)*	100	450	680	0.31	0.44
Potency in vivo (rat, intraarterial) (relative potency BK = 100)*	100	64.0	32.0	0.04	0.02
4. Pulmonary inactivation (%) (rat)	85–95	72.0	14.0	8.0	20.0
Potentiation in vitro by SQ 14225 (%) (rabbit jugular vein)	38.0	21.0	11.0	0	104.0
5. Vascular permeability (rabbit) (22)	1.0	1.0	0.7	0	
6. Release of catecholamines from the adrenal medulla, in the rat (80) or in the cat (135,420).	1.0	0.5		0.25	

* Similar results have been reported by other authors (365, 375).

macological assays of these peptides. The majority of the preparations respond to kinins with dose-dependent contractions and are mediated by at least two receptor types, denoted B_1 and B_2. In a few preparations, kinins exert inhibitory actions. In addition to producing direct effects mediated by either B_1 or B_2 receptors, kinins may act indirectly by promoting the release of prostaglandins and their effects can be significantly affected by the presence of active kininases in the isolated organs. These interfering factors limit the usefulness of various preparations for pharmacological assays of kinins. Such interference has been avoided by selecting preparations in which kinins do not release prostaglandins, or by the use of inhibitors of kininase II. In this way, it has been possible to interpret the pharmacological effects of kinins according to the concepts of the receptor-occupation theory, as elaborated and applied by Ariëns (10) to biogenic amines and by us to angiotensin (362). The relative contribution of each residue of the kinin molecule to binding and activation of receptor B_1 has been studied with des-Arg^9-BK as the prototype. The identification of the C-terminal Phe as the active group has been followed by the discovery of specific and competitive inhibitors of kinins on the B_1 receptor. Similar studies, directed to the identification of the chemical features of kinins required for binding and stimulation of B_2 receptors, have not provided any definite answer, revealing at the same time the limitations of our structure-activity approach to the study of peptides. The characterisation of the B_2 receptor for kinins remains, therefore, a challenging problem for future research. Its solution will set the pharmacology of kinins on a solid foundation. At the same time it will permit a precise definition of the B_2 receptor as a single entity and will possibly provide fundamental knowledge to be used for other peptides (e.g. substance P). The characterisation of B_2 receptors requires specific and competitive antagonists. Such compounds will be of great utility for pharmacological studies as well as for determining the roles that kinins may well play in a variety of physiological processes and diseases.

3. The great majority of direct pharmacological effects of the kinins can be attributed to excitation or inhibition of smooth muscles (stimulus-contraction, stimulus-relaxation coupling). Kinins appear to be inactive as stimulants of endocrine secretions, except for the release of catecholamines from the adrenal medulla, which however may represent a pharmacological effect rather than a physiological action. The possibility that kinins might inhibit endocrine secretions, in a similar way to somatostatin, has never been tested. Kallikreins and kinins produced by exocrine glands may increase exocrine secretion by acting on the vessels rather than on the secreting cells.

Several indirect pharmacological effects of kinins appear to be due to the ability of these peptides to promote the synthesis and release of prostaglandins. The effect is important both on theoretical grounds, since it raises the possibility that kinins may activate a membranous enzyme rather than a receptor, and from a practical point of view, considering the recent developments in the field of prostaglandins. Further studies on the interrelationships between kinins and prostaglandins are needed in order to determine to what extent these ubiquitous and abundant systems are involved in such fundamental processes as blood coagulation and haemostasis, cell proliferation, tissue defence and repair, and regulation of blood pressure.

4. Kinins exert their biological actions by activating two receptor systems: One of them (the B_2 receptor) is a stable component of the cell membrane that appears to be partially controlled by steroid hormones. The other system (the B_1 receptor) seems to be silent in vivo, but it can be activated in vivo by chemical noxious stimuli and in vitro by the artificial conditions utilised for biological assays. The activation appears to be the result of a de novo formation of receptor proteins. A future task will be to explain how and why noxious and other stimuli induce the formation of receptors specifically for kinins.

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