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Assay of Kinins by Their Effects on Canine Femoral Blood Flow[1] (39063)

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The involvement of the kallikrein–kinin system as a mediator of physiological and pathophysiological processes has been suggested (1). Development in this area, however, has been impaired, to a certain extent, by difficulties inherent with the accurate and specific quantification of the small amounts of kinins which usually occur in biological fluids. The most commonly used kinin bioassay procedures involve the utilization of smooth muscle tissues as assay organs (1). Although these techniques are sensitive and easy to perform, they are susceptible to a variety of factors such as oxygen tension, calcium concentration, ionic strength, and pH of the bathing fluid, and to seasonal variations in the spontaneous contractility of some assay tissues (1). Assay procedures based on the hypotensive (2) and vasodilator properties of kinins (3) overcome the inconveniencies associated with isolated smooth muscle preparations but are less sensitive. In this paper we describe a method that permits the assay of as little as 0.1 ng of kinins by their effect on canine femoral blood flow. The procedure is easy to perform and permits the assay of large numbers of samples in a single preparation.

Methods. Male mongrel dogs, weighing 20–30 kg, were fasted overnight but allowed free access to water. Sodium pentobarbital was administered (30 mg/kg, iv), the trachea was cannulated and the lungs were ventilated mechanically (Model 607A, Harvard Apparatus Company, Inc.). Mean blood pressure was measured by a strain-gauge transducer (P23Db, Statham Instruments, Inc.) via a catheter placed in the left brachial artery. The femoral arteries were exposed below the inguinal ligaments and their blood flows were measured with noncannulating electromagnetic flowmeters (Model M-4001, Statham Instruments, Inc.). Blood pressure and blood flows were recorded with a multichannel direct writer (Model 7720, Hewlett-Packard). The femoral arteries were punctured, 2 cm distal to the flow probes, with a 26-gauge needle and a polyethylene catheter (PE-50) was placed 4 cm inside the lumen of the vessel without obstructing the flow. Bradykinin and other vasoactive drugs were rapidly injected (2 sec) through the femoral catheters in a volume of 0.5 ml. The dead space was rinsed with 0.5 ml of 0.9% NaCl. The dose-vasodilator response relationship of each of the following agents was examined: bradykinin, kallidin, kinins generated by incubation of kininogen with either trypsin or urinary kallikrein, histamine (free base), substance P, acetylcholine chloride and prostaglandin E_2. The effect of DFP treated carboxypeptidase B (Sigma) on the vasoactive properties of the aforementioned agents was investigated by comparing the effect of the agonists on femoral blood flow before and after incubation of 1 ml of each test solution with 1 unit of the enzyme at 37° and pH 7 for 15 min. In addition we also studied the effect of the kininase II inhibitor and bradykinin potentiating peptide ($BPP_{9\alpha}$) Pyr-Trp–Pro–Arg–Pro–Gln–Ile–Pro–Pro (SQ-20881, Squibb) on the femoral blood flow responses elicited by bradykinin, kallidin and enzymatically generated kinins. In those experiments, the kininase II inhibitor $BPP_{9\alpha}$ was dissolved in saline and given intravenously (forelimb vein) as a bolus (300 μg/kg) followed by a continuous infusion at a rate of 10 μg kg^{-1} min^{-1} in 0.5 ml of 0.9% NaCl until the experiments were terminated.

Bradykinin kallidin, histamine substance P and acetylcholine chloride were purchased

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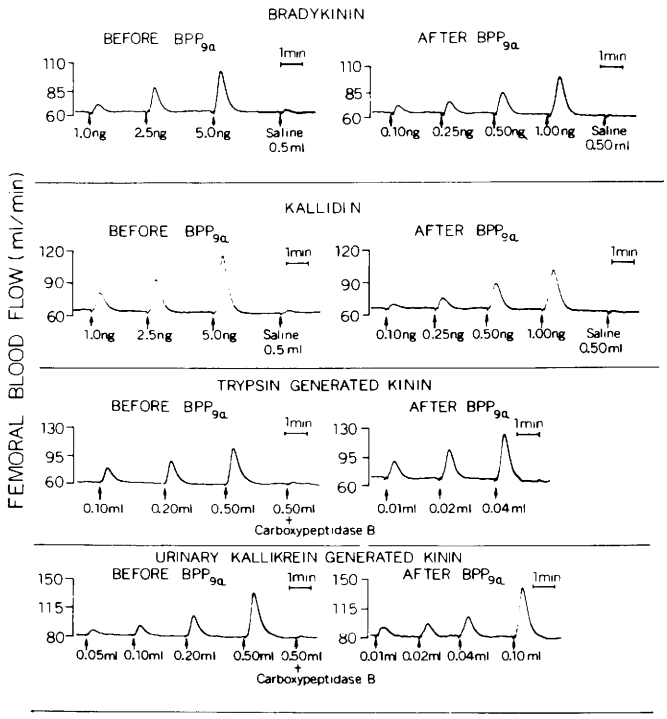

FIG. 1. Effects of bradykinin, kallidin and enzymatically generated kinins on canine femoral blood flow and the influence of $BPP_{9\alpha}$ on these effects. All kinins were injected into a femoral artery. $BPP_{9\alpha}$ was given intravenously (300 μg/kg).

from Schwartz Mann, prepared as 1.0 mg/ml stock solutions in saline and stored at −20°. Prostaglandin E_2 (The Upjohn Co.) was kept at −20° as 1.0 mg/ml stock solution in 95% ethanol. On the day of the experiment, all the stock solutions were diluted with 0.9% NaCl sufficient to inject a constant volume (0.5 ml).

Kinins generated by the action of trypsin and urinary kallikrein upon kininogen were obtained as follows: Canine plasma was heated at 61° for 2 hr followed by acidification (pH 2.8) with 5 *N* HCl and incubation at 4° for 15 min. After adjusting the pH to 7.5, the plasma was centrifuged and kept at −20° until used. The treated plasma (0.10 ml) was diluted to 10 ml with 0.01 *M* sodium phosphate buffer (pH 7.5) containing 0.15 *M* NaCl and 0.003 *M* disodium EDTA and incubated for 30 min at 37° with either trypsin (0.5 mg) or dialyzed dog urine (0.2 ml). The enzymatic reaction was terminated by heating in boiling water (5 min) and the samples were kept at −20° until biological testing. All the results were expressed as mean ± standard error (SE).

Results. Mean arterial blood pressure and mean femoral blood flow were 110 ± 5 mmHg and 75 ± 8 ml/min respectively. Intraarterial injections of bradykinin caused a rapid increase in femoral blood flow, which reached its high point in about 22 sec and lasted approximately 80 sec (Fig. 1). The responses to bradykinin were reproducible and the sensitivity of the preparation permitted the detection of at least 1 ng of peptide. Some preparations were more sensitive and responded to 0.5 ng of bradykinin (Fig. 2). Increases in femoral blood flow produced by bradykinin were related to dosage between 0.5 and 10 ng and the steepest segment of the log dose-response curve was between 2.5 and 10 ng (Fig. 2). The responses to kallidin were qualitatively similar to those elicited by bradykinin, although its potency was 1.5 times greater than that of the nonapeptide (Figs. 1 and 2). Femoral blood flow responses to kinins released by incubation of

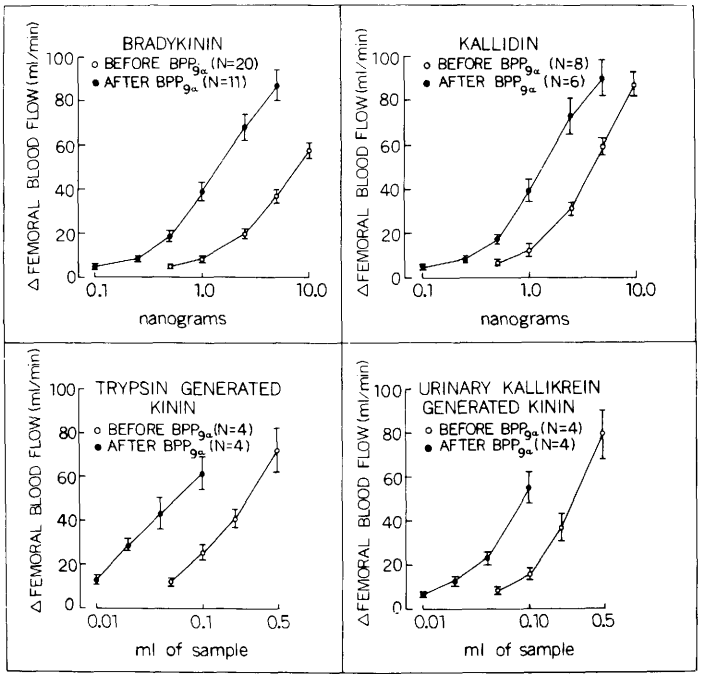

FIG. 2. Effect of $BPP_{9\alpha}$ (300 μg/kg, iv) on the femoral vasodilator responses of the dog to femoral arterial injections of bradykinin, kallidin and enzymatically generated kinins. Numbers between parentheses indicate the number of experiments. Vertical bars represent the SE.

plasma with either trypsin or urinary kallikrein were also dose related and undistinguishable from those elicited by synthetic bradykinin and kallidin (Figs. 1 and 2). Intraarterial administration of all aforementioned agents was devoid of effects on contralateral femoral blood flow and mean aortic blood pressure. Intravenous administration of $BPP_{9\alpha}$ did not affect femoral blood flow or systemic blood pressure. However, it potentiated the responses to synthetic bradykinin and trypsin generated kinin by fivefold and those to synthetic kallidin and urinary kallikrein generated kinin by threefold (Figs. 1 and 2). After giving $BPP_{9\alpha}$, the sensitivity of the preparation permitted the detection of as little as 0.10 ng of bradykinin and kallidin and the steepest segment of the log dose–response curves were between 0.5 and 10 ng (Fig. 2). The log dose–response curves of synthetic as well as of enzymatically generated kinins were shifted to the left and paralleled those obtained in the absence of $BPP_{9\alpha}$ (Fig. 2).

Prostaglandin E_2, histamine, substance P and acetylcholine caused dose related increases in femoral blood flow (Fig. 3) which were affected neither by preliminary incubation of the test compounds with carboxypeptidase B nor by intravenous administration of $BPP_{9\alpha}$. Since synthetic as well as enzymatically generated kinins are inactivated by carboxypeptidase B (Figs. 1 and 3) differentiation between responses produced by kinins and those elicited by the aforementioned vasoactive agents is possible.

Discussion. Close arterial injections of synthetic as well as of enzymatically generated kinins produced a dose related increase in femoral flood flow. Relative to bradykinin, the decapapeptide kallidin was 1.5 times more potent (4). After administration of the kininase II inhibitor and bradykinin potentiating peptide $BPP_{9\alpha}$ (5) the femoral blood flow responses to bradykinin and kallidin but not to other vasodilator agents we tested, (two experiments) were augmented by five- and threefold, respectively, and both peptides became equally potent. Responses to enzymatically generated kinins

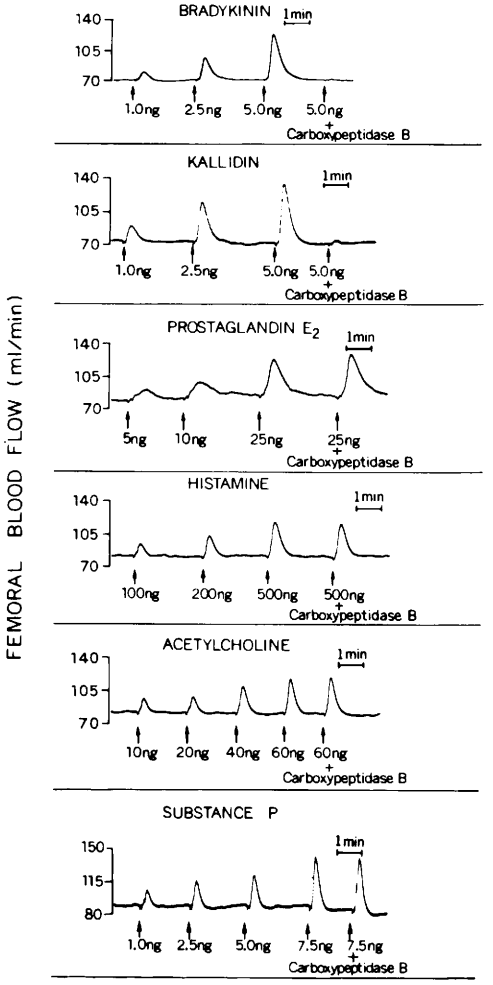

FIG. 3. Effects of bradykinin, kallidin, prostaglandin E_2, substance P, histamine and acetylcholine on canine femoral blood flow and the influence of incubation of the test compounds (1 ml) with carboxypeptidase B (1 unit, pH 7, 37°, 15 min) on these effects. All agonists were injected into a femoral artery.

were similarly enhanced. Therefore, this potentiation appears to be specific for kinins and may be attributed to reduced inactivation of kinins by kinins II, resulting in enhanced concentration of the agonist at its site of action. The ability of kinins to produce vasodilation in peripheral vasculatures has been used to assay this class of peptides in biological samples (3, 6). The canine hind limb, perfused with a Schuster-Dale pump through the femoral artery, permitted the detection of 5–10 ng of bradykinin (3). A similar technique utilizing the perfused hindquarter of the rabbit allowed the measurement of 0.5–1 ng of bradykinin (6). The bioassay preparation we have described has advantages over the aforementioned procedures. It does not require extracorporeal circulation and its sensitivity permits the detection of 0.5–1 ng of either bradykinin or kallidin. Moreover, treatment with $BPP_{9\alpha}$ augments the sensitivity of the preparation by five to tenfold and enables the detection of as little as 0.1 ng of kinins. This sensitivity and the steepness of the dose-response curves make the procedure suitable for the measurement of kinin concentrations as low as those found in blood. We have used this preparation to assay the kinin content of blood and urine samples (7). The simultaneous utilization of both femoral arteries is possible since ipsilateral arterial injections of kinins do not affect the contralateral femoral blood flow. This, and the maintenance of an adequate sensitivity and response reproducibility for periods of up to 8 hr, permits the assay of large numbers of samples in a single preparation.

A major handicap shared by our assay procedure with most other bioassay techniques is lack of specificity (1). Thus other agents such as prostaglandins of the E and A series, histamine, substance P and acetylcholine have vasoactive properties similar to those of kinins and may interfere with the assay of the latter. Identification of kinins as mediators of the vasodilator activity of unknown samples is considerably improved by demonstrating that preincubation with carboxypeptidase B abolishes their vasodilator effects. Thus, carboxypeptidase B does not affect the vasoactive properties of prostaglandins, substance P, acetylcholine and histamine, but renders kinins inactive by splitting the C-terminal arginine of the peptides (8). The specificity of the measurement of blood and urinary kinins however, will not be appropriate unless an adequate purification of the samples is performed prior to their biological testing.

Summary. In pentobarbital anesthetized dogs, close arterial injections of bradykinin and kallidin elicit a dose related increase in femoral blood flow. Treatment with the

kininase inhibitor $BPP_{9\alpha}$ augments the femoral blood flow responses to bradykinin and kallidin by five and threefold respectively. The sensitivity of the preparation permits the detection of 0.5–1 ng of either bradykinin or kallidin in untreated dogs and as little as 0.1 ng of kinin peptides in animals receiving $BPP_{9\alpha}$. This sensitivity and the steepness of the dose response curves make this procedure suitable for the assay of kinins.

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1. Trautschold, I., *in* "Handbook of Experimental Pharmacology" (E. G. Erdos, ed.) Vol. 25, p. 52. Springer-Verlag, Berlin-Heidelberg-New York (1970).
2. Roblero, J., Ryan, J. W., and Stewart, J. M., Res. Commun. Chem. Pathol. Pharmacol. **6,** 207 (1973).
3. Binia, A., Fasciolo, J. C., and Carretero, O. A., Acta Physiol. Lat. Amer. **13,** 1.1, (1963).
4. McCarthy, D. A., Potter, D. E., and Nicolaides, E. D., J. Pharmacol. Exp. Ther. **148,** 117 (1965).
5. Greene, L. J., Camargo, A. C., Krieger, E. M., Stewart, J. M., and Ferreira, S. H., Circ. Res. **31** (Suppl. II), II, 62 (1972).
6. Abe, K., Mouri, T., Seki, T., Suzuki, M., Takano, R., and Yoshinaga, K., Experientia **24,** 455 (1968).
7. Nasjletti, A., Colina-Chourio, J., and McGiff, J. C., Acta Physiol. Lat. Amer. **24,** 587 (1974).
8. Erdos, E. G., Renfrew, A. G., Slone, E. M., and Wohler, J. R., Ann. N.Y. Acad. Sci. **104,** 222 (1963).

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