Digital Vascular Responses to Intra-Arterial Injections of Bradykinin, Kallidin, and Eledoisin in Man

By N. P. DePasquale, M.D., and G. E. Burch, M.D.

BRADYKININ, KALLIDIN, AND ELEDOSIN are vasoactive polypeptides recently synthesized in pure form.\(^1\)\(^-\)\(^3\) Bradykinin and kallidin exist in man, whereas eledoisin has been found only in the posterior salivary glands of Eledone. Although the three polypeptides have potent vasodilating and hypotensive properties, only bradykinin and kallidin are closely related structurally (fig. 1).

The nonapeptide structure of bradykinin was established by Boissonas and associates\(^4\) in 1960. Pierce and Webster\(^5\) and Werle and associates\(^6\) concluded independently that kallidin is a decapeptide and suggested that it is the lysine-homologue of bradykinin, a fact later confirmed by Nicolaides and his co-workers.\(^2\) Bradykinin and kallidin are both released from the alpha-2-globulin in man.\(^7\) Eledoisin was first isolated from the posterior salivary glands of two molluscan species, Eledone moschata and Eledone aldrovandi, by Erspamer and Anastasi\(^8\) in 1961 and synthesized by Boissonas and Sandrin\(^9\) in 1962. Eledoisin is an endecapeptide with an amino acid sequence markedly different from that of bradykinin and kallidin (fig. 1).

Interest in the vasoactive polypeptides has been aroused because of the role that they may play in local vascular control; however, relatively few studies of the influence of bradykinin, kallidin, and eledoisin on the circulation have been performed on man. In 1958, Fox and Hilton\(^10\) showed that bradykinin was released in the upper extremity when the trunk and lower extremity were heated. They suggested that bradykinin was responsible for functional hyperemia of the sweat glands during heating. Fox and associates\(^10\) found that intra-arterial injection of bradykinin resulted in an increase in blood flow to the forearm and hand. DeFreitas and associates\(^11\) found that the intravenous infusion of bradykinin decreased systemic arterial pressure and peripheral vascular resistance and increased cardiac output in man. Similar observations were made by Kontos and co-workers\(^12\) during intravenous infusions of eledoisin into man. They also observed an increase in hand and forearm blood flow during intravenous injection of eledoisin. Kontos and colleagues concluded that the general circulatory effects of eledoisin resemble those of bradykinin, but eledoisin is a much more potent vasodilator in man.

In a previous report from this laboratory,\(^13\) the digital vascular responses to the ipsilateral intra-arterial (brachial) injection of bradykinin in man were described as follows: (1) flushing of the digit, (2) increase in skin temperature, (3) increase in the magnitude of the volume pulse wave, (4) decrease in digital inflow volume of blood, and (5) increase in the total volume of the digit. These responses are similar to those observed during reactive hyperemia and are best explained by selective constriction of the A-V shunts with associated dilatation of the arterioles, capillaries, venules and veins.

\[\text{BRADYKININ: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg}\]
\[\text{KALLIDIN: Lys-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg}\]
\[\text{ELEDOSIN: Pyr-Pro-Ser-Lys-Asp-Phe-Ileu-Gly-Leu-Met}\]

**Figure 1**

Amino acid sequence of bradykinin, kallidin, and eledoisin.
The present study was undertaken to compare the digital vascular responses to intra-arterial injections of bradykinin, kallidin, and edeloisin. As far as we have been able to determine, the influence of kallidin on the circulation of man has not been studied previously.

**Methods**

The digital rheoplethysmogram (RPG) was recorded in 10 subjects ranging in age from 31 to 66 years (mean 53 years). The second (index) right finger tip (2RF) was enclosed in a plethysmographic cup, and a pneumatic occluding (collecting) cuff was placed just proximal to the cup. The volume inflow \( (I_V) \) for a single pulse cycle was obtained by sudden inflation of the cuff to 60 mm Hg pressure. The third (middle) right finger tip (3RF) was also placed in a plethysmographic cup in order to record the volume pulse \( (D_V) \) during digital occlusion. By means of difference circuits the volume outflow \( (O_V) \) of blood from the digit was also obtained, that is, \( O_V = I_V - D_V \). By means of differentiating circuits the rate of inflow \( (I_R) \), the difference between the rates of inflow and outflow \( (D_R) \) and the rate of outflow \( (O_R) \) were recorded. Details of the rheoplethysmographic technique used in this laboratory have been presented previously.\(^{14,15}\)

Skin temperature was recorded by means of fine wire (copper-constantan) thermocouples placed on 2RF just proximal to the occluding cuff.

The subjects rested quietly in a hospital-type bed in a climatic room maintained at 22°C and 46% relative humidity. The digits were supported comfortably at heart level. A Cournand arterial needle was placed in the right brachial artery and connected by means of a three-way stopcock and polyethylene tubing to a strain gauge and amplifier in order to record arterial blood pressure directly. The three-way stopcock made it possible to inject the drugs intra-arterially without interrupting the blood pressure recording for more than a few seconds.

During preliminary experiments the intra-arterial injection of 0.2 \( \mu \)g of bradykinin consistently produced a digital vascular response which was maximal between 22 and 76 seconds after injection. The digital vascular response was elicited without significant change in arterial blood pressure or cardiac rate. Thus, any changes in the digital circulation could be attributed to a direct effect of the drug on the circulation to the upper extremity. Therefore, in the present studies 0.2 \( \mu \)g of synthetic bradykinin, kallidin, and edeloisin were injected intra-arterially into each subject. At least 20 minutes elapsed between each injection.

**Results**

**General Responses**

The intra-arterial injections of 0.2 \( \mu \)g of bradykinin, kallidin and edeloisin were associated with pain in the right elbow or hand or both in two of the 10 subjects. Another subject experienced burning pain in the finger tips of the right hand following injection of kallidin but not following injection of bradykinin or edeloisin. Skin temperature of the digit increased in all subjects following injections of each of the polypeptides. No subject developed headache, generalized flushing, syncope, or abdominal distress.

**Figure 2**

Summary of the digital vascular responses to the intra-arterial injections of bradykinin, kallidin, and edeloisin in 10 subjects. Basal inflow rate represents the rate of digital blood flow at the end of diastole whereas the maximal inflow rate represents the rate of inflow which is superimposed upon the basal component as a result of systolic ejection.

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Arterial Blood Pressure
There was no significant difference between the control arterial blood pressure and the arterial blood pressure at the time measurements of digital blood flow were made, that is, 22 to 76 seconds after the injection of bradykinin, kallidin, and eleodoisin (fig. 2).

Pulse Cycle Duration
The relative durations of systole and diastole at the time that measurements of digital blood flow were obtained did not vary significantly from the control.

Time Course of Digital Pulse Volume (D_v)
Although there was a tendency for bradykinin, kallidin, and eleodoisin to increase the height of the digital volume pulse wave, the differences from the control were not statistically significant (fig. 2). The contour of the digital pulse wave, however, changed definitely in all but two subjects. The change in contour consisted of a slow ascent of the anacrotic limb of the pulse wave and a rounding of the descending limb associated with complete or partial loss of the dicrotic notch (figs. 3 and 4). When changes in the contour of the digital volume pulse wave developed, they were most obvious with eleodoisin (fig. 4b), next most obvious with bradykinin (fig. 3b) and least obvious with kallidin (fig. 4a).

**Figure 3**
Control (a) and digital vascular responses to intra-arterial injections of 0.2 μg of bradykinin (b). In this and in the following illustrations, the original digital rheoplethysmographic (RPG) recording is shown at the top. The simultaneous time course curves of the volumes and rates of inflow, outflow, and difference between inflow and outflow for a single pulse cycle are shown below. $I_v$ = time course of digital inflow volume; $O_v$ = time course of digital outflow volume; $D_v$ = time course of the difference between inflow and outflow (the volume pulse wave); $I_R$ = time course of the rate of digital inflow; $O_R$ = time course of the rate of digital outflow; $D_R$ = time course of the difference between the rates of inflow and outflow. 2RF refers to the second right (index) finger and 3RF refers to the third right (middle) finger.
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Figure 4

Digital vascular responses to intra-arterial injections of 0.2 µg of kallidin (a) and eledoisin (b). See figure 3 for control.

Time Course of Digital Inflow Volume (Iv)

Digital inflow volume decreased in six subjects (figs. 3 to 6) and increased in four subjects. The direction of the response in a particular subject was the same for each of the three polypeptides, that is, when one polypeptide resulted in a decrease or increase in Iv the other two polypeptides produced a similar response. However, the magnitude of the responses was greater with eledoisin than with bradykinin or kallidin. Thus, in six subjects bradykinin and kallidin resulted in a 36% and 32% decrease in Iv, respectively, whereas eledoisin produced a 43% decrease in Iv. In four subjects bradykinin and kallidin produced a 26% and 43% increase in Iv, respectively, whereas eledoisin produced a 40% increase. Because of the variability in the response of Iv to intra-arterial injections of the polypeptides, the differences between the means and the control were not statistically significant for any polypeptide.

Time Course of Digital Outflow Volume (Ov)

In five of the six patients in whom there was a decrease in digital inflow volume (Iv) following injection of a polypeptide, the simultaneous curves of inflow volume and outflow volume were widely separated temporally, particularly during the early portion of the pulse cycle (figs. 3b and 4). This separation was due to a "lag" in the outflow curve. A lag in the curve of outflow volume was not observed in any of the four subjects in whom Iv increased after injection of the polypeptides.

Time Course of Basal and Pulsatile Flow Rate

The changes in the rates of basal and maximal pulsatile flow14, 15 reflected the changes in Iv described above. Thus, basal and maximal pulsatile flow rates decreased in six subjects and increased in four subjects.

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Figure 5
Control (a) and digital vascular responses to intra-arterial injection of 0.2 μg of bradykinin (b). Note the abrupt increase in total digital volume following the injection of bradykinin indicated by the rise in the I curve in the slow portion of the original RPG record prior to venous occlusion.

Figure 6
Digital vascular responses to intra-arterial injections of kallidin (a) and eledoisin (b). Note the abrupt increase in digital volume following injection of eledoisin. See figure 5 for control.

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Change in Total Digital Volume ($\Delta T_v$)

Total digital volume ($T_v$) increased in all subjects after intra-arterial injections of the three polypeptides (figs. 2, 4a, 5b, and 6b). Bradykinin and kallidin increased total digital volume 18% and 15%, respectively, whereas eledoisin increased total digital volume 32%. The increase in total digital volume was statistically significant for the three polypeptides.

Discussion

The most remarkable aspect of these studies was the consistent increase in total digital volume following the intra-arterial injections of bradykinin, kallidin, and eledoisin. In six of the 10 subjects total digital volume increased in spite of the fact that digital inflow volume ($I_v$) decreased. As indicated in previous reports from this laboratory, during reactive hyperemia total digital volume also increases while the volume of digital inflow decreases.\textsuperscript{13, 14} We have interpreted an increase in total digital volume which occurs in spite of a decrease in the volume of digital inflow to be due to closure of the arteriovenous anastomoses. It is well known that the blood supply to the digit is many times that necessary to support the metabolic needs of the digital tissues and that a major fraction of the digital circulation functions in thermal regulation. During rest in a neutral environment the volume of blood passing through the capillary vessels (nutrient or effective blood flow) is relatively small because the needs of the tissues are not great. Sudden closure of the arteriovenous anastomoses decreases the volume of the shunt circulation and increases nutrient blood flow. Under these circumstances the capillaries, venules, and veins become distended with blood and the total volume of the digit increases. Because of increased capillary blood flow, the temperature of the digit increases. On the other hand, because of closure of the A-V anastomoses, the resistance to blood flow through the digital circulation increases, so that total digital blood flow ($I_v$) decreases. Thus, although total digital inflow volume ($I_v$) decreases following injection of the polypeptide, effective digital blood flow increases and a relatively greater fraction of the inflow volume is trapped within the capillaries, venules, and veins than during the resting state. Changes in total digital volume occurred too rapidly to be explained by transudation of fluid from the capillary bed (for example see figures 5b and 6b). Furthermore, transudation of fluid into the tissues would have resulted in a decrease rather than an increase in skin temperature.

Intravenous infusion of bradykinin and eledoisin in man results in an increase in cardiac output, arterial hypotension, a decrease in total peripheral resistance, and in arteriolar dilatation.\textsuperscript{11, 12} Obviously, our conclusions for the human digit are not in agreement with these findings. However, none of the studies on the hemodynamic effects of bradykinin and eledoisin thus far reported distinguish between the vascular responses in muscle and skin. The terminal portion of the digits contain no skeletal muscle, so that the responses described in the present paper apply only to blood flow through the skin. It is well known that the digits are richly supplied with A-V anastomoses, whereas skeletal muscle contains relatively few A-V anastomoses. The intradermal injection of bradykinin on the volar surface of the forearm is associated with a marked increase in the volume of the bleb, but there is little or no cutaneous flare (G. E. Burch and N. P. DePasquale, unpublished observations). If bradykinin dilated the arterioles of the skin, intradermal injection of this substance should produce intense erythema as occurs, for example, following intradermal injection of histamine or hexamethonium. The slight flare which does occur in some subjects following intradermal injection of bradykinin could be due to passive dilatation of the capillaries, venules, and veins secondary to an axon reflex due to the trauma of the injection. In man, therefore, the vascular responses to bradykinin and eledoisin and presumably kallidin apparently differ in the skin from those in skeletal muscle. Although a great deal of emphasis has been placed upon bradykinin as a dilator of smooth
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muscle, it should not be forgotten that the substance constricts the smooth muscle of the intestine and uterus.\(^7\) Furthermore, the vascular responses of the rabbit ear (which contains no skeletal muscle) to bradykinin have also been interpreted to be consistent with closure of the A-V anastomoses.\(^16\) Like the human digit, the rabbit ear is richly supplied with A-V anastomoses.

These studies were not designed to determine dose-response ratios for the various polypeptides. However, at the concentrations used, eleoosin was essentially twice as effective in increasing total digital volume as kallidin and bradykinin.

Bradykinin has been implicated as playing a role in inflammatory reactions.\(^7\) As far as the skin is concerned, closure of the arteriovenous anastomoses in areas of dermal injury would support tissue repair by increasing nutrient blood flow. Teleologically, it would be more economical to increase blood flow to areas of skin injury by closing A-V shunts than to permit the shunts to remain open and dilating the arterioles in addition.

Summary

Intra-arterial injections of bradykinin, kallidin, and eleoosin produce qualitatively similar digital vascular responses. These responses are interpreted as indicating closure of the arteriovenous anastomoses. In these studies eleoosin was twice as effective in producing an increase in total digital volume as either bradykinin or kallidin.

References

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