STUDIES ON THE VASCULAR ACTION OF BRADYKININ

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Bradykinin (BK) is widely regarded as a potent vasodilator capable of increasing blood flow, increasing vascular permeability, and lowering systemic blood pressure through microcirculatory dilatation.

On the other hand, reports have appeared in the literature which indicate that BK may constrict some vessels.

Lecomte and Trouquet (1), Gersmyer and Spitzbarth (2) and Klupp and Konzett (3) demonstrated the constriction of the lung vessel. Guth et al. (4) and Rowley (5) reported that BK caused venoconstriction of the rabbit ear and rat skin, respectively. Shimamoto et al. (6) also reported the constriction of the rabbit saphenous vein by BK.

The present experiments were undertaken to clarify the BK action on the various portions of blood vessel using several methods. Furthermore some tests were tried to study on the mechanism of venoconstrictive action of BK.

METHODS

Experiments in the rabbit in situ

1. Injection into the central artery of the ear

Rabbits weighing about 2.0 kg were anesthetized with urethan (1 g/kg subcutaneously). One carotid artery and ear central artery of the different side were cannulated and connected with the polyethylene tube to carry the autoperfusion. Plethysmographic method was used to observe the change of vessel diameter. The sheared ear was glued to the glass stage of the projector (Olympus SP-150) and projected on the screen by light through heat filter. CdS photo cells were affixed onto the figures of artery and vein on the screen of the projector and connected to the bridge circuit and electronic amplifier (Nihonkohden ADH-2). The blood pressure of the carotid artery was measured by the electronic manometer (Nihonkohden MP-3A). Blood flow of the marginal vein was measured using the modified crossed double thermocouple method (7, 8). A wire type electrode was affixed to the marginal vein and connected to Shincorder (Shin-ei CTE-201). Four parameters were recorded simultaneously on the ink writing oscillograph (Nihonkohden WI-260 M) as shown in Fig. 1. Furthermore, the change of vessels including smaller parts of artery and vein, were observed microscopically.

BK used in the experiments is the product of Sandoz Pharmaceuticals or of the Insti-
2. Local administration

The topical application of BK was undertaken. The small portion of the ear skin was exfoliated and vessels there were observed microscopically. The drug solution was dropped down topically.

Experiments with the isolated rabbit ear and isolated guinea pig and rat hindquarters

Isolated rabbit ear and guinea pig hindquarters were perfused with Ringer's solution or rabbit's blood diluted with the same volume of Ringer's solution. Rat hindquarters were also perfused with Ringer's solution. Perfusion pressure was 30-50 cm H₂O for Ringer's solution and 50-100 cm H₂O for blood. Furthermore, separate perfusions were undertaken in the isolated rabbit ear. Isolated ear was cut into the two parts which contain mainly the central artery and the marginal vein respectively as shown in Fig. 2. Each vessel preparation was perfused with Ringer's solution at a pressure of 10-30 cm H₂O or with blood containing the same volume of Ringer's solution at a pressure of about 50 cm H₂O. Outflow was recorded using the drop-counter (Miyano) and ink writing oscillograph. Experiments were done at a temperature of about 20°C. In other animals reserpinization was undertaken. Rabbits were administered reserpine of 1 mg/kg intraperitoneally, daily for 2 days before the experiment. Perfusion of Ringer's solution was applied to the isolated whole ear and ear vein preparations from these animals. Guinea pigs were administered reserpine of 3 mg/kg intraperitoneally, daily for 2 days before the experiment. Perfusion of Ringer's solution was applied to the isolated hindquarters from these animals.
Fro. 2. Separate perfusion of the isolated rabbit ear vessels. Perfusion of the artery (A) and vein (B).

Fig. 2. Separate perfusion of the isolated rabbit ear vessels. Perfusion of the artery (A) and vein (B).

Experiments with the rabbit external jugular vein strips

Rabbit external jugular vein was cut to rings 3-5 mm wide, two of which were tied together to form a chain. The tension of the preparation was measured by means of mechano-electric transducer (Nihonkohden SB-1T) and electronic recorder (Toa ERR-2T) in the bath containing Ringer's solution at 32°C.

Phentolamine was used to observe if there was a participation of catecholamine in the action of BK.

RESULTS

Experiments in the rabbit in situ
1. Injection into the central artery of the ear

It was observed by plethysmography that an injection of 0.1 ml of $10^{-6} \text{g/ml}$ BK into the central artery of the ear, caused a dilatation of the artery and vein and an increase of the blood flow of the marginal vein in many cases (Fig. 3). In fewer cases, a constriction of the vein and a decrease of the blood flow were observed. Even in the latter cases, the artery was dilated. There was no change or slight decrease in the systemic blood pressure.

Changes of vessels including smaller artery or vein than those observed plethysmographically, were also observed microscopically. From Fig. 4 it is able to see that intra-
arterial injection of BK in the same doses, caused a dilatation of smaller arteries and a constriction of smaller veins markedly.

2. Local administration

One-tenth ml of $10^{-5}$ to $10^{-7}$ g/ml BK in saline applied around vessels, caused arterial dilatation and venous constriction in all cases.

Experiments with the isolated rabbit ear, and guinea pig and cat hindquarters

Effects of 0.1 ml of $10^{-7}$ to $10^{-5}$ g/ml BK on the isolated tissues perfused with Ringer's solution and blood containing Ringer's solution, were summarized in Table 1.
**Fig. 4.** Micrograph of the rabbit ear vessels before (left) and after (right) bradykinin. A: artery. V: vein.

**Table 1.** Effects of bradykinin on the isolated organ vessels.

| Perfusion | Ringer Bradykinin | Blood Bradykinin |
|-----------|-------------------|------------------|
| Organs    | Outflow           | 10⁻¹ | 10⁻² | 10⁻³ | 10⁻⁴ | 10⁻⁵ | 10⁻⁶ |
| Rabbit whole ear | No change | ....   | .... | ....   | .... | .... | .... |
| Rabbit ear artery | ↑ | 10⁻¹ | 10⁻² | 10⁻³ | 10⁻⁴ | 10⁻⁵ | 10⁻⁶ |
| Rabbit ear vein | ↓ | 10⁻¹ | 10⁻² | 10⁻³ | 10⁻⁴ | 10⁻⁵ | 10⁻⁶ |
| Guinea pig hindquarters | ↑ | 10⁻¹ | 10⁻² | 10⁻³ | 10⁻⁴ | 10⁻⁵ | 10⁻⁶ |
| Rat hindquarters | ↑ | 10⁻¹ | 10⁻² | 10⁻³ | 10⁻⁴ | 10⁻⁵ | 10⁻⁶ |
Perfusing with Ringer’s solution, BK caused no change or a decrease of the outflow from the rabbit whole ear and guinea pig and rat hindquarters. In the separate perfusions of the rabbit ear vessels, BK caused no change in the outflow from the artery and a marked decrease in that from the vein. Therefore, a decrease in the outflow of the Ringer’s solution from the whole ear was considered to be due to the constriction of the vein.

On the other hand, perfusing with blood containing Ringer’s solution, BK caused an increase in the outflow from the whole ear and guinea pig hindquarters in many cases. In the separate perfusion of the rabbit ear vessels, BK caused an increase in the perfusate from the artery and, therefore, an increase in the whole ear was probably due to the dilatation of the artery. In the separate perfusion of the vein, the outflow did not increase but decreased.

Reserpinization did not affect the response to BK in the outflow from the whole ear and ear vein of the rabbit and from the hindquarters of the guinea pig, as shown in Table 2.

**Table 2. Effects of bradykinin on the isolated organ vessels from reserpinized animals.**

| Isolated organs          | Drug | Bradykinin |
|--------------------------|------|------------|
|                          |      | 10⁻⁴       | 10⁻⁵       | 10⁻⁶       |
| Rabbit whole ear         | Increase | ↑     | ⋯⋯⋯⋯      | ⋯⋯⋯⋯      |
|                          | No change | ⋯⋯⋯⋯      | ⋯⋯⋯⋯      |
|                          | Decrease | ↓     | ⋯⋯⋯⋯      | ⋯⋯⋯⋯      |
| Rabbit ear vein          | ↑     | ⋯⋯⋯⋯      |
|                          | ↓     | ⋯⋯⋯⋯      |
| Guinea pig hindquarters  | ↑     | ⋯⋯⋯⋯      |

Reserpinization did not affect the response to BK in the outflow from the whole ear and ear vein of the rabbit and from the hindquarters of the guinea pig, as shown in Table 2.

**Experiments with rabbit external jugular vein strips**

Excised rabbit external jugular vein responded to BK very sensitively and was contracted by BK of concentrations higher than 10⁻⁴ g/ml (final concentration). Dose-response curve is shown in Fig. 5. Dose-response curve of bradykinin on the rabbit vein strips.

![Fig. 5. Dose-response curve of bradykinin on the rabbit vein strips.](image-url)
Fig. 5.
Phentolamine $10^{-5}$ g/ml (final concentration) pretreatment did not change the contractile action of BK on this preparation, while it inhibited completely the action of norepinephrine (Fig. 6).

Fig. 6. Influence of phentolamine (Ph) on the actions of norepinephrine (NA) and bradykinin (Br) on the rabbit jugular vein. Phentolamine was applied 3-5 minutes before norepinephrine or bradykinin.

DISCUSSION

Venoconstrictive action of BK was observed in the following experiments: (a) rabbit ear vein in a topical application around the vessel, (b) isolated rabbit ear vein perfused with blood or Ringer's solution and (c) rabbit external jugular vein strips immersed in Ringer's solution. These results are in accordance with those reported by Guth et al. (4), Bobbin and Guth (9), Sutter (10), Horowitz and Mashford (11) and Sakuma and Ohishi (12).

On the other hand, BK caused a dilatation of the rabbit ear artery in a topical application around the vessel and of the isolated rabbit ear artery perfused with blood. There seems to be definite difference in response to BK between the vein and artery. The reason why artery does not dilate when perfused with Ringer's solution is not always clarified. One of the authors (13) attributed it to the absence of arterial tone.

In the isolated whole rabbit ear and the guinea pig and rat hindquarters perfused with Ringer's solution, the outflow always decreased when BK was administered. These phenomena are probably due to vasoconstriction. In the same preparations perfused with blood, the outflow increased in many cases when BK was administered. Probably the dilatation of the artery was predominant and the vein was also dilated passively by the
pressure through microcirculation from the artery. Similar results were obtained in the rabbit in situ, in which BK administered into the central artery of the ear induced a dilatation of the artery and vein and an increase of blood flow in the marginal vein. However, local venoconstriction was observed microscopically even when the outflow increased.

The possibility might be little under physiological or pathological condition that BK in a considerable quantity circulates in vessels and produces actions in various organs. There could be more possibility that BK originates locally in the tissue and produces pharmacological actions in the comparatively small portions in the organs. If so, some results of the present experiments can not be ignored in which BK was administered topically around the vessels in the rabbit ear and BK was injected into the perfusate of the isolated rabbit ear vein. Rowley (5) and Bobbin and Guth (9) asserted venoconstrictive action by BK which will be the cause of the increase of permeability in the capillary bed.

The experiments were also undertaken to know about participation of catecholamine in the venoconstrictive action of BK. Pretreatment with reserpine did not affect the response to BK of isolated rabbit ear vein and guinea pig hindquarters perfused with Ringer’s solution. Phentolamine, α blocking agent, also did not affect the response of the rabbit jugular vein strips to BK. Therefore, it will be concluded that BK constricts veins directly without releasing catecholamine.

**SUMMARY**

Vascular action of bradykinin (BK) were examined in the rabbit, guinea pig and rat.

BK caused a dilatation of artery and vein and an increase in venous flow by injection into the central artery of the rabbit ear in situ.

In the isolated rabbit ear and the guinea pig and rat hindquarters perfused with Ringer’s solution, BK decreased the outflow from the organs. In the isolated rabbit ear and the guinea pig hindquarters perfused with blood. BK increased the outflow from the organs. In the separate perfusion method, BK caused no change of the outflow from the artery but a decrease of that from the vein using Ringer’s solution as perfusate, and caused an increase of the outflow from the artery and a decrease of that from the vein using blood as perfusate.

BK administered topically around the vessels in the rabbit in situ, caused arteriodilatation and venoconstriction simultaneously.

BK increased the tone of the excised rabbit external jugular vein strips markedly.

BK constricted the veins of the rabbit and guinea pig directly without releasing catecholamine.

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