EFFECT OF BRADYKININ AND HISTAMINE ON CAPILLARY PERMEABILITY

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The concentration of bradykinin disturbing permeability in 50% of cases (ED_{50}) is $1 \cdot 10^{-11}$ g/ml for rabbits, $7 \cdot 10^{-12}$ g/ml for guinea pigs, and $4 \cdot 10^{-8}$ g/ml for rats. The cutaneous capillaries of the rabbit are 131,600 times, those of the guinea pigs 289,000 times, and those of the rat 526 times more sensitive to bradykinin than to histamine (calculated on the basis of molar proportions).

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In 1958, Lewis [16] and later other workers [2, 7, 20] suggested that kinins may take part in tissue reactions, especially in inflammation. Bradykinin causes the cardinal signs of inflammation: vasodilatation [12], an increase in capillary permeability [8, 9, 11], pain [4], accumulation of leukocytes at the site of injection [17], and stimulation of phagocytosis [18]. Several investigations have been made on the effect of bradykinin on vascular permeability [5, 9, 13-21]. However, the sensitivity of the skin capillaries of various species of animals to bradykinin, the kininase activity of the skin, and interaction between kinins and histamine and other mediators still remain incompletely studied.

EXPERIMENTAL METHOD

Experiments were carried out on three species of animals: 30 male Wistar rats weighing 350-420 g, 40 guinea pigs of both sexes weighing 430-520 g, and 40 male chinchilla rabbits aged 6 months and weighing 2.7-3.3 kg or aged 3 months and weighing 1.8-2.1 kg. The effect of bradykinin and histamine on capillary permeability was studied by means of the dye Evans' blue. Five minutes after injection of a solution of the dye, various doses of bradykinin or histamine, made up in 0.1 ml physiological saline, were injected intradermally. The index of a disturbance of capillary permeability was the appearance of a blue coloration of a papule not less than 7 mm in diameter in the course of 10 min. The duration of action of bradykinin or histamine on vascular permeability was determined by Menkin's method [19].

The results obtained were analyzed by the probit method as described by Litchfield and Wilcoxon and modified by Roth [1]. Synthetic bradykinin (Sandoz, Switzerland), histamine hydrochloride (Riga Pharmaceutical Chemical Factory), and Evans' blue (Reanal, Hungary) were used in the investigation. The histamine was identical in activity with the preparation manufactured by the firm of G. Lawson (England).

EXPERIMENTAL RESULTS

It was shown experimentally that small concentrations of bradykinin ($1 \cdot 10^{-11}$-$1 \cdot 10^{-10}$ g/ml for rabbits and guinea pigs and $1 \cdot 10^{-7}$ g/ml for rats) produced diffuse coloration of the papule without sharp borders. Higher concentrations of bradykinin ($1 \cdot 10^{-7}$-$5 \cdot 10^{-7}$ g/ml for rabbits and guinea pigs and $2 \cdot 10^{-6}$ g/ml for rats) produced appreciable vasodilatation. The blue coloration spread uniformly to all parts of the skin and the papule had well-defined borders. With a further increase in concentration ($1 \cdot 10^{-5}$ g/ml for rabbits and $1 \cdot 10^{-7}$ g/ml for guinea pigs, a papule with a white ischemic center and a blue border was formed. The vasoconstrictor action of the sensitivity of the skin capillaries to bradykinin chosen in the investigation was the concentration (calculated from the graph in Fig. 1) causing a disturbance of permeability in 50% of cases (ED_{50}). ED_{50} of bradykinin for rabbits was found to be $1 \cdot 10^{-11}$ g/ml ($3 \cdot 10^{-12}$-$4 \cdot 10^{-11}$ g/ml), for guinea pigs $7.3 \cdot 10^{-12}$ g/ml ($3.2 \cdot 10^{-12}$-$16.8 \cdot 10^{-12}$ g/ml), and for rats $4 \cdot 10^{-8}$ g/ml (1.9

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TABLE 1. Time of Appearance (in sec) of Blue Coloration (passage of Evans' Blue into the skin) at Sites of Intradermal Injections of Bradykinin (M ± m)

Animals	Concentration (in g/ml)							
	$1 \cdot 10^{-11}$	$1 \cdot 10^{-10}$	$1 \cdot 10^{-9}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-7}$	$5 \cdot 10^{-7}$	$1 \cdot 10^{-6}$	$5 \cdot 10^{-6}$
Rabbits	214,7 ± 13,35 (9)	179,4* ± 10,46 (13)	140,3* ± 9,48 (10)	105,7* ± 8,25 (13)	89,6* ± 4,30 (16)	72,0* ± 2,26 (10)	69,0 ± 6,66 (10)	75,9 ± 2,21 (11)
Guinea pigs	172,9 ± 4,56 (12)	148,8* ± 20,78 (16)	102,2* ± 5,35 (16)	87,6* ± 6,49 (15)	89,6 ± 3,17 (15)	69,5* ± 3,90 (10)	75,0 ± 1,64 (11)	78,5 ± 7,15 (10)

Note. Number of animals given in parentheses; an asterisk denotes values differing significantly ($P < 0.05$) from those obtained during the action of an earlier, lower concentration.

Fig. 1. Action of bradykinin on permeability of skin capillaries of a guinea pig (1), rabbits (2), and rat (3). Here and in Fig. 2, abscissa: negative log of concentration; ordinate: probits.

Fig. 2. Action of histamine on permeability of skin capillaries of a guinea pig (1), rabbits (2), and rat (3).

$\times 10^{-8}$-$7.9 \cdot 10^{-8}$ g/ml). ED_{50} of histamine for rabbits was $1.6 \cdot 10^{-7}$ g/ml ($8 \cdot 10^{-8}$-$3.2 \cdot 10^{-7}$ g/ml), for guinea pigs $2.3 \cdot 10^{-7}$ g/ml ($1.3 \cdot 10^{-7}$-$4.1 \cdot 10^{-7}$ g/ml), and for rats $2.2 \cdot 10^{-6}$ g/ml ($1.6 \cdot 10^{-6}$-$3.1 \cdot 10^{-6}$ g/ml; Fig. 2).

As Figs. 1 and 2 show, the dose–effect curves for histamine and bradykinin were parallel for all species of animals (the parallel nature of the curves was demonstrated by the probit analysis method), so that it was possible to compare the sensitivity of the skin capillaries to bradykinin and histamine. ED_{50} of histamine for rabbits was 14,000 times greater than ED_{50} of bradykinin, i.e., the skin capillaries of the rabbit were 14,000 times more sensitive to bradykinin than to histamine. ED_{50} of histamine for guinea pigs was 30,900 times greater than ED_{50} of bradykinin. When converted to molar proportions, the sensitivity of the skin capillaries of rabbits was 131,600 times, of guinea pigs 289,000 times, and of rats 526 times greater to bradykinin than to histamine.

The intensity of coloration was assessed by a four-point system. With an increase in the concentration of bradykinin the intensity of coloration increased proportionally only up to a certain limit, amounting in rabbits to a concentration of $1.1 \cdot 10^{-6}$ g/ml. In rats the intensity of coloration rose proportionally to the increase in concentration within the range of concentrations tested up to $5 \cdot 10^{-5}$ g/ml.

The speed with which disturbances of permeability arose after injection of the various doses of bradykinin was determined from the time of appearance of coloration of the papule after injection of the mediator. For rabbits and guinea pigs, with an increase in bradykinin concentration from $1 \cdot 10^{-11}$ to $5 \cdot 10^{-7}$ g/ml the time of appearance of coloration of the papule fell significantly with an increase in concentration (Table 1). For rats, within the range of concentrations tested up to $5 \cdot 10^{-6}$ g/ml, the speed of disturbance of permeability rose proportionally to the increase in dose.

The disturbance of capillary permeability caused by bradykinin did not last long. For rabbits the increase in permeability after injection of bradykinin in a concentration

of $1 \cdot 10^{-7}$ g/ml persisted for 5-6 min. With an increase in bradykinin concentration to $5 \cdot 10^{-6}$ g/ml the duration of the disturbances of permeability increased to 10-11 min for rabbits and 8-9 min for guinea pigs. For rats, bradykinin in a concentration of $5 \cdot 10^{-6}$ g/ml caused a disturbance of permeability which lasted 3-4 min. The short duration of the disturbances of capillary permeability can be explained by the high kininase activity of the skin. Histamine had a more prolonged effect: in a concentration of $1 \cdot 10^{-5}$ g/ml it increased permeability in rabbits for 20 min, while in a concentration of $1 \cdot 10^{-4}$ g/ml the effect lasted 30 min.

Rabbits aged 3 months were less sensitive to the action of the mediators.

The investigations revealed high sensitivity of the skin capillaries of rabbits, guinea pigs, and rats to bradykinin, which disturbed the capillary permeability when injected intradermally. There are reports in the literature describing the relative activities of bradykinin and histamine as mediators of permeability [6, 10, 15]. The quantitative differences between our results and those published in the literature may be explained by the fact that we took as our criterion of action of bradykinin and histamine the minimal doses causing disturbances of capillary permeability in 50% of cases. The disturbances of capillary permeability, which we judged from the speed of development of coloration and the intensity of the color of the papule reached a maximum when the bradykinin concentration rose to $1.1 \cdot 10^{-6}$ g/ml, in agreement with data in the literature [9]. All three species of animals possess high kininase activity. Even when the bradykinin concentration was increased to $5 \cdot 10^{-6}$ g/ml, the inactivation time, judging from the duration of the disturbances of capillary permeability, was only 10 min. The action of bradykinin had the shortest duration in rats. Our results also indicate that the response of young animals to histamine and bradykinin is imperfect.

LITERATURE CITED

1. M. L. Belen'kii, Elements of Quantitative Analysis of a Pharmacological Effect [in Russian], Riga (1959), p. 71.
2. I. A. Oivin and S. M. Shchegel', in: Material on the Pathogenesis of Inflammation and Pathology of the Blood Proteins [in Russian], Dushanbe (1961), p. 167.
3. I. A. Oivin, S. M. Shchegel', and E. G. Yagodkina, Nature, 200, 270 (1963).
4. D. A. Armstrong, J. B. Jepson, C. A. Keele, et al., J. Physiol. (Lond.), 135, 350 (1957).
5. J. Carr and D. L. Wilhelm, Aust. J. Exp. Biol. Med. Sci., 42, 511 (1964).
6. J. Carr and D. L. Wilhelm, Nature, 208, 653 (1965).
7. H. Edery and G. P. Lewis, J. Physiol. (Lond.), 163, 48 (1962).
8. D. F. Elliott, E. W. Horton, and G. P. Lewis, J. Physiol. (Lond.), 153, 473 (1960).
9. M. Frimmer, Arch. Exp. Path. Pharmak., 245, 287 (1963).
10. M. Frimmer, Arch. Exp. Path. Pharmak., 242, 390 (1961).
11. D. J. Holdstock, A. P. Mathias, and M. Schachter, Brit. J. Pharmacol., 12, 149 (1957).
12. F. A. Holton and P. Holton, J. Physiol. (Lond.), 118, 310 (1952).
13. H. Jacquet, Arch. int. Pharmacodyn., 144, 161 (1963).
14. H. Konzett and R. A. Boissinnas, Experientia (Basel), 16, 456 (1960).
15. H. Konzett and E. Stürmer, Brit. J. Pharmacol., 15, 544 (1960).
16. G. P. Lewis, J. Physiol. (Lond.), 140, 285 (1958).
17. G. P. Lewis, Physiol. Rev., 40, 647 (1960).
18. G. Ludany et al., Orv. Hetil., 105, 2026 (1964).
19. V. Menkin, J. Exp. Med., 50, 171 (1929).
20. M. Rocha e Silva and A. Antonio, Med. Exp. (Basel), 3, 371 (1960).
21. D. L. Wilhelm, Pharmacol. Rev., 14, 251 (1962).