Pharmacological Characteristics of Bradykinin B

The chemical isolation and some pharmacological properties of bradykinin resulting from the interaction of ox plasma and *Bothrops jararaca* venom have recently been described in a short communication from our laboratories[1]. Although chemical and pharmacological analyses have so far revealed no differences between this bradykinin and that obtained from the interaction of ox plasma and trypsin, we should like to designate the former as bradykinin B (*Bothrops*), as opposed to the latter, bradykinin T (trypsin).

Very soon in the course of the purification procedure outlined in our previous communication[1], it became evident that several test methods would have to be used in order to characterise bradykinin B and to differentiate it more accurately from other polypeptides acting on smooth muscle (isolated gut and uterus; blood-pressure response, etc.).

The following is a short account of the pharmacological properties of the purest bradykinin B[2] preparation available (CIBA 27 994-Ba).

Methods. Tests were performed on the following isolated organs:

A) 1. Terminal guinea-pig ileum suspended in Mg-free Tyrode; bath volume: 20 ml;

2. Rat colon suspended in de Jalòn's fluid; bath volume: 20 ml; and

3. Rat uterus suspended in de Jalòn's fluid; bath volume: 20 ml.

In addition, the preparation was tested *in vivo* as follows:

B) 1. Blood-pressure (bl' pr') of the cat anaesthetised with Dial-urethane (Dial-CIBA®, 30 mg/kg i. p. and 30 mg/kg s. c.);

2. Bl' pr' of the rabbit anaesthetised with urethane (1,5 g/kg, administered intravenously); and

3. Bl' pr' of the cock anaesthetised with phenobarbital-sodium = Luminal®-Na(180–200 mg/kg, administered into the pectoral muscle).

C) Bradykinin B was also injected intracutaneously in varying local concentrations (0·001–0·3 μg) into the dorsal skin of male albino guinea-pigs which had been injected beforehand intravenously with 'Pontamine'®, Sky Blue 6 BX (5% in saline; 0·1 ml/100 g body weight). Dye extravasation was measured in flaps of reflected skin from animals sacrificed 30 min after intracutaneous administration of bradykinin B.

Results. A: 1. The isolated terminal guinea-pig ileum contracted upon addition of bradykinin B in final concentrations as low as 1 to 3×10^{-12} (g/ml), i.e. 1 to 3 ng/ml.

2. The isolated rat colon proved slightly less sensitive to bradykinin B (bB) than the guinea-pig ileum inasmuch as concentrations of $5–10^{-12}$ to 1×10^{-11} caused contractions of the rat organ which were quantitatively about equal to the contractions elicited by synthetic angiotensin[3,4] in concentrations of $1–3 \times 10^{-8}$.

3. The isolated rat uterus responded to concentrations of bB even lower—roughly 10 times lower—than those causing contractions of the isolated guinea-pig ileum or the rat colon.

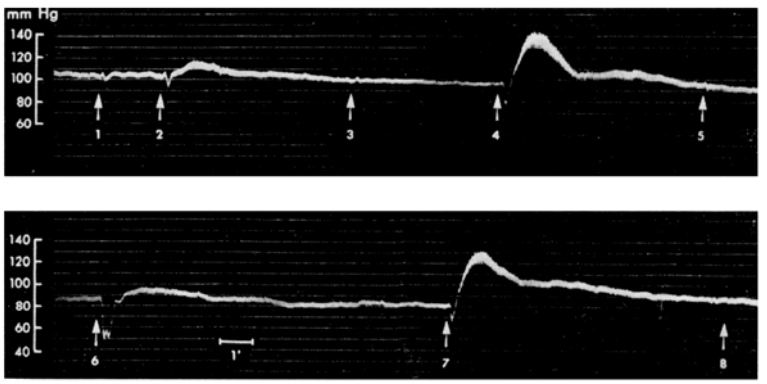
In all the experiments mentioned above in § 1 to 3, bradykinin B caused contractions of the respective test-organ which differed from those produced by fast-acting substances such as histamine or 5-hydroxytryptamine (serotonin) in that they started with a short latency of 5 to 15 sec and reached their maximum effect only after the substance had been in contact with the respective test-organ for 30–120 sec. On the whole, no noticeable tachyphylaxis or hyporeactivity were observed when bradykinin B was administered every 8th min and washed out after the contraction had reached its maximum. In addition, it was noted that neither atropine sulphate nor mepyramine in final concentrations of 1×10^{-8} caused any appreciable reduction in the contractile response of the guinea-pig ileum. Bradykinin B thus displayed an activity which was described in 1938 by FELDBERG and KELLAWAY as typical of so-called 'slow-reacting substances' = 'SRS'[5].

B: On the blood-pressure of the anaesthetised cat, rabbit, and cock, purified bradykinin B displayed the following activities:

1. In both the cat and the rabbit, bradykinin B acted as a depressor substance in dosages starting from 10 to 30 ng/kg; in doses of 30–100 ng/kg depressor reactions of 20–50 mm. Hg were obtained in both species and lasted from 1–5 min.

2. In the cock, dosages of 10 ng–3 μg/kg of bB did not cause any appreciable alteration in the bl' pr', in spite of the fact that in the same animal Pitressin® or angiotensin (synthetic hypertensin, CIBA) caused depressor or pressor responses in dosages of 0·3 I. U. or 1 μg/kg respectively. An example of this behaviour of the cock's bl' pr' in response to i.v. administration of Pitressin®, synthetic angiotensin, and bB is illustrated in the following Figure.

C: Administered intracutaneously in guinea-pigs which had been pre-treated with Pontamine®, bradykinin B caused extravascular leakage of circulating dye when given



Effect of various polypeptides on the arterial blood-pressure of the cock (body weight: 2·4 kg, anaesthetised with Luminal®-Na, 200 mg/kg, i. m.).

At arrows numbered 1–8, the following substances or solutions were injected intravenously: 1) 1·0 ml physiological saline; 2) hypertensin, 0·3 μg/kg; 3) bradykinin B, 1·3 μg/kg; 4) hypertensin, 1·0 μg/kg; 5) bradykinin B, 2·6 μg/kg; 6) vasopressin (Pitressin®), 0·4 I. U./kg; 7) hypertensin, 1·0 μg/kg; 8) bradykinin B, 2·6 μg/kg.

N.B. There is a 5-min interval between the end of the upper tracing and the beginning of the lower tracing.

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[2] The bradykinin B sample under consideration was prepared in our laboratories by Dr. H. ZUBER and Prof. R. SCHWYZER.

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[5] W. FELDBERG and C. H. KELLAWAY, J. Physiol. *94*, 187 (1938).

[6] A. A. MILES and D. L. WILHELM, Brit. J. exp. Path. *36*, 71 (1955); Nature *181*, 96 (1958).

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in local concentrations of 0·03–0·01 μg (contained in 0·1 ml physiological saline). Thus, bradykinin B proved very active in causing increased capillary permeability in the guinea-pig. Lacking any direct comparison on the very same test object between bB and, for example, MILES' permeability factor (PF/Dil)[6], or the mediators described by SPECTOR and WILLOUGHBY[7], no comparison is yet possible between the permeabilising activity of either substance.

The authors are indebted to Miss E. HEITZ and Miss C. ZUBER for their valuable technical assistance during these experiments.

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Pharmazeutische Abteilung der CIBA Aktiengesellschaft, Basel, June 13, 1960.

Zusammenfassung

Die pharmakologischen Effekte (*in vitro* und *in vivo*) eines durch Einwirkung von Schlangengift (*Bothrops jararaca*) auf Rinderplasma erhaltenen, reinen Bradykinins (B) werden beschrieben.

Post Mortem Increase of 5-Hydroxytryptamine in Rat Brain after 5-Hydroxytryptophan Administration

In several animal species injection of 5-hydroxytryptophan (5HTP) causes an increase of 5-hydroxytryptamine (5HT) in the brain which can be enhanced by pretreatment with monoamine oxidase inhibitors, e. g. iproniazid[1–4]. This 5HT accumulation has been attributed to the fact that 5HTP easily penetrates the brain in which decarboxylation occurs. In presence of great amounts of 5HTP the formation of 5HT probably exceeds its catabolism so that the amine accumulates. This is particularly the case when MAO is blocked.

In the present paper it is shown that 5HTP induced increase of 5HT in brain does not occur *in vivo* only but still continues after decapitation.

Methods. Male Wistar rats fasting for 16 h and weighing 60 to 80 g were decapitated 30 min after i. p. injection of 75 mg/kg DL-5HTP. Part of the animals received 100 mg/kg iproniazid (i.p.) $16^1/_2$ h before 5HTP. The brains were homogenized in 0·1 *n* HCl either immediately after killing (shortest intervall 60 sec) or after the following procedures:

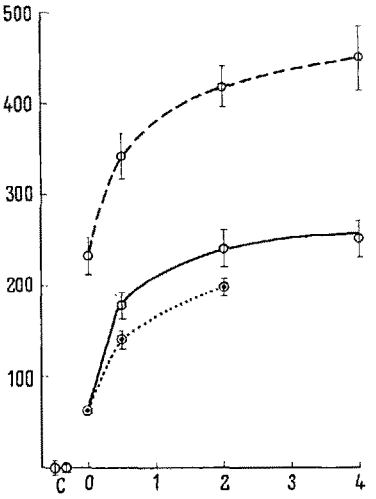
(a) Storage of severed skulls at room temperature and 37°C in closed wet chambers for $^1/_2$ to 4 h.
(b) Storage of whole brains removed immediately after killing in 0·1 *n* HCl at room temperature for 2 h.
(c) Dropping of the skulls into liquid nitrogen immediately after decapitation, removal of the frozen brains in the cold room and homogenization in ice cooled 0·1 *n* HCl.

Animals not treated with 5HTP served as controls. 5HT was determined by a spectrophotofluorometric method[5].

Results. (1) The 5HT content of rat brain was significantly higher after 5HTP injection than in normal animals. The increase of 5HT amounted to 63 ± 5% if the skulls were dropped into liquid nitrogen or to 59 ± 7% in brains taken out at room temperature and homogenized immediately after killing. The difference is not significant ($p > 0{\cdot}05$). Iproniazid pretreatment enhanced this 5HTP induced 5HT increase significantly ($p < 0{\cdot}01$), (Figure, time 0).

(2) If the severed skulls were kept at 37°C before homogenization a further continuous 5HT rise in brain occurred. This was the case in animals with and without iproniazid pretreatment before administration of 5HTP. Storing of the skulls at room temperature had a similar but somewhat less effect (Figure).

(3) Brains of control animals without any treatment kept for 2 h at 37°C showed a significant 5HT decrease during the first half hour as compared with normal brains homogenized immediately after death. In animals only pretreated with iproniazid incubation of the brains for 2 h at 37°C caused no significant change in the 5HT content (Table).



Post mortem increase of 5HT in brain of rats treated with 75 mg/kg 5-hydroxytryptophan i.p. 1/2 h before death.

Abscissa. Time in h after decapitation. 0 time = immediate removal and homogenization after decapitation. *Ordinate.* Percentage 5HT increase as compared with controls (C) not pretreated with 5HTP.

○——○ No treatment with iproniazid. Storage of skulls at 37°C.
○- - -○ i.p. injection of 100 mg/kg iproniazid $16^1/_2$ h before death. Storage of skulls at 37°C.
○·····○ No iproniazid treatment. Storage of intact brains in 0·1 *n* HCl at room temperature.

Each point represents average of 8–10 single values with standard error. 5 HT of 22 control rats (C) amounted to 0.52 ± 0.02 μg/g fresh weight of brain.

Time *post mortem* (h)	0	1/2	2
No pretreatment	100 ± 4	*80 ± 2*	*82 ± 2*
Iproniazid pretreatment	100 ± 4	98 ± 8	103 ± 8

5 HT content in % of normal brains at various intervals after death. Incubation of intact brain at 37°C. 100 mg/kg iproniazid were administered i. p. 16 h before decapitation. Each figure represents the average value of 6 experiments with standard error. Brains removed immediately after decapitation (time 0) served as controls. The underlined values are significantly different from control values ($p < 0{\cdot}01$).

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