Tab. I. Vergleich der Agglutinin- und Giftwirkung von Bohnenfraktionen

Fraktion	Agglutinierung von Mäuseblut	Dosis/kg Maus mg	Anzahl der gestorbenen Tiere/injizierte Tiere
A	1:64000	100	10/10
		75	10/10
		60	10/10
		50	19/20
		40	5/20
B	1:16000	250	9/20
		200	1/10
C	1:16000	200	14/20
		150	12/20
		125	5/10
E	1:8000	500	9/10
		250	10/10
		200	5/20
A (an DEAE-Cellulose chromatographiert)	1:16000	60	0/5
		100	3/7

Tab. II. Toxizität der Bohneneiweissfraktion A in Mäusen verschiedener Herkunft

Verwendete Mäuse	Dosis/kg Maus mg	Anzahl der gestorbenen Tiere/injizierte Tiere	Agglutinierung der Erythrocyten der Mäuse
Weisse Mäuse (MPI)	150	1/5	1:64000
	300	11/11	
Braune Mäuse (Heston)	100	2/9	1:64000
	125	3/7	
Weisse Mäuse (MPI) mit Papillomen	100	0/10	1:64000
	200	0/12	
	400	8/12	

Ricin und 1/40 der von Diphterietoxin[7]. In den von ihm hergestellten Bohnenfraktionen fand PIERKARSKI[8] die entsprechenden toxischen Dosen zwischen 25 mg und 500 mg/kg Maus.

Bei einem Aufenthalt in München hatten wir Gelegenheit, einige weitere Versuche an Mäusen von 2 verschiedenen Stämmen sowie an Mäusen mit Papillomen zu machen. Wie aus den Daten der Tabelle II hervorgeht, lag die toxische Dosis in diesen Fällen höher als in der ersten Versuchsreihe, was zeigt, dass ein Vergleich zwischen Daten verschiedener Provenienz nicht unbedingt möglich ist. Am höchsten lag die Dosis bei den Versuchen mit Mäusen, in denen durch Behandlung mit Crotonölfraktionen Papillome erzeugt worden waren. Obwohl die Unterschiede gegenüber den Tieren des gleichen Stammes bei der geringen Zahl nicht unbedingt als gesichert angesehen werden können, erschien die Beobachtung erwähnenswert, da NUNGESTER und VON HALSEMA[9] in haemagglutinierenden Bohnenextrakten eine inaktivierende Wirkung auf Flexner-Sobling Karzinomazellen bei Ratten beobachtet haben, was mit der Bindung von Phytagglutinin an Zellrezeptoren der Krebszellen in Zusammenhang gebracht wird.

Einen Unterschied in der Agglutinierbarkeit der Blutzellen der verschiedenen untersuchten Mäusestämme konnten wir nicht feststellen.

Über die Wirkungsweise der Bohnentoxine können wir noch keine Aussagen machen. Lediglich haben wir als Erklärung der beobachteten Wachstumshemmung nach oraler Applikation eine Reduktion der Darmabsorption zeigen können[10,11]. Wir vermuten, dass die parenterale Toxizität mit einer Reaktion der untersuchten Phytotoxine mit Zellrezeptoren ähnlich denen der Erythrocyten zusammenhängt. Über die anatomisch-pathologischen Befunde soll später berichtet werden.

Unsere Ergebnisse – zusammen mit denen von LIENER über das toxische Soyabohnenagglutinin[12] – lassen weitere toxikologische Untersuchungen an andern bisher als ungiftig angesehenen Phytagglutininen wünschenswert erscheinen[13].

Summary. Soluble proteins from black beans (*Phaseolus vulgaris*) have been separated into 4 fractions, one soluble in 1% NaCl-solution and 3 water soluble. The latter were separated by ammonium sulfate fractionation. The haemagglution of these fractions is compared with their toxicity in mice when applied by intraperitoneal injection. The LD_{50} of the most active fraction was about 50 mg/kg. When used in mice of different strains, the toxicity was not the same while the blood cells were agglutinated by the same final concentration. Croton-oil-induced papilloma-bearing mice were slightly more resistant than normal animals.

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[13] Wir danken dem Consejo del Desarollo Cientifico y Humanistico der Venezolanischen Zentraluniversität sowie der Fundación Creole, Caracas, für die finanzielle Unterstützung dieser Arbeit. Herrn Dr. E. HAECKER und Frl. I. BRACHMANN, Max-Planck-Institut für Biochemie, München, danken wir für die freundliche Überlassung der im 2. Versuch verwendeten Mäuse.

Potentiation of Bradykinin Action on Smooth-Muscle by Cysteine

In the course of experiments performed to test purified substrates for bradykinin-releasing enzymes[1], it was necessary to inhibit kininase, a plasma peptidase that rapidly destroys bradykinin. When cysteine[2,3] was used, it was confirmed that it potentiated the smooth-muscle stimulating action of bradykinin, a fact already reported by LEWIS[4]. Such potentiation occurred on the isolated guinea-pig ileum as well as on the rat uterus preparation, two

[1] OLGA B. HENRIQUES, MARIA C. F. OLIVEIRA, and ZULEIKA P. PICARELLI, in press.

[2] E. K. FREY, H. KRAUT, and E. WERLE, *Kallikrein* (Padutin) (F. Enge-Verlag, Stuttgart 1950).

[3] M. ROCHA E SILVA, in *Polypeptides which Stimulate Smooth Muscle* (E. und S. Livingstone Ltd., London 1955), p. 20.

[4] G. P. LEWIS, Physiol. Rev. *40*, 647 (1960).

of the most sensitive structures to bradykinin, although it was much less marked on the rat uterus (Figure 1). It was also observed that the responses of these organs to acetylcholine, histamine and 5-hydroxytryptamine were not affected by cysteine; angiotensin II[5] was weakly potentiated and angiotensin I[5] was rather antagonized by the amino acid.

The experiments here reported were made in order to test the possibility of that potentiation being due to the inhibition of the endogenous kininase present in these pharmacological preparations, since it is well known that kininase occurs in many animal tissues[4].

Segments of terminal ileum removed from guinea pigs weighing 180–250 g were suspended in a 7 ml muscle chamber with aerated Tyrode solution containing atropine (1 μg/ml) and diphenhydramine (1 μg/ml), at 35°C. Contractions were registered with a frontal lever with a load of 1–1.5 g, giving a sixfold magnification.

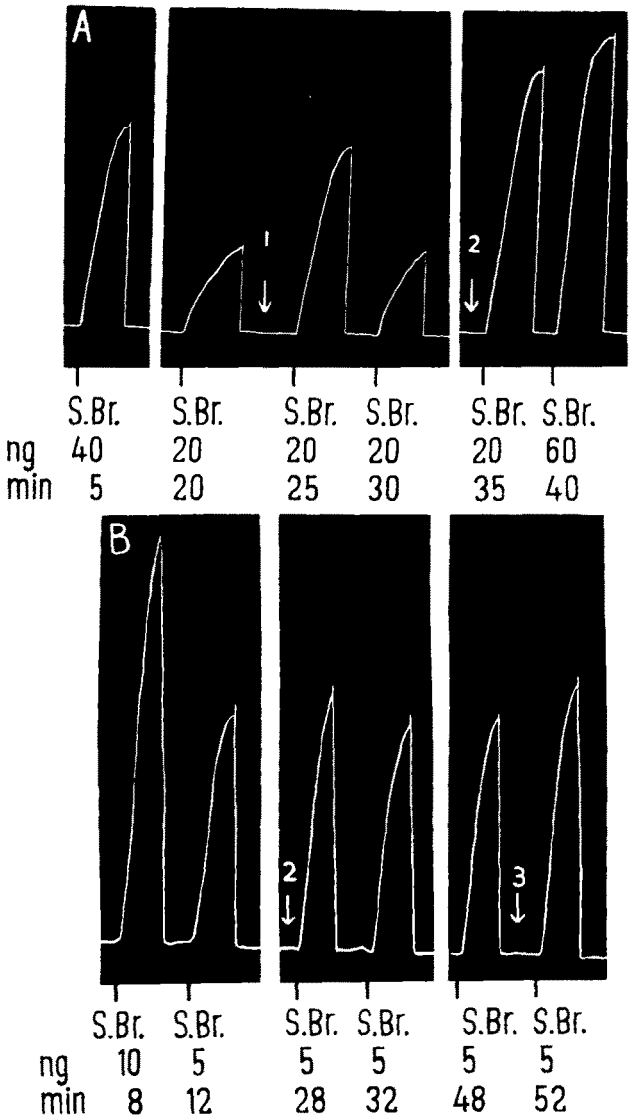

Fig. 1. Effect of cysteine on the response of isolated guinea-pig ileum (A) and rat uterus (B) to bradykinin. – Organs immersed in 7 ml chambers containing respectivelly aerated Tyrode or Jalon's solutions. S.Br = synthetic bradykinin; at 1, 2 and 3 addition of 20, 100 and 500 μg of cysteine. The potentiation of bradykinin on the guinea-pig ileum preparation (A) was already evident with very small doses of cysteine (20 μg); however, on the rat uterus, (B) it was much less marked even with doses as high as 500 μg.

Uterus were taken from virgin rats (180–200 g) injected 18–24 h before with diethylstilboestrol (10 μg/100 g body weight). One horn was immersed in aerated atropinized (1 μg/ml) Jalon's solution at 30° C and the contractions recorded in the same way as those of the ileum.

In order to eliminate blood plasma kininase still present in the isolated preparations, rat (anesthetized with nembutal, 8 mg/100 g intraperitoneally) and guinea-pig (anesthetized with ethylether) hindquarters were perfused with the appropriate solution at 40°C and 200 mm Hg for 20–30 min. After this period the presence of blood in rat uterus or guinea-pig ileum was negligible[6]. *Washed* organs were then collected and suspended as usual in the tissue chamber or used for incubation assays.

Cysteine (1.5–60 μg/ml) exhibited its potentiating action on isolated *washed* organs as well as on the usual preparations; here again its effect on the contractions of isolated uterus produced by bradykinin was weaker than on those of guinea-pig intestine.

If synthetic bradykinin[7] was incubated at 35°C with segments of washed ileum or uterus, and samples of these incubates were taken at certain intervals and tested on isolated guinea-pig ileum or rat uterus, it was found that

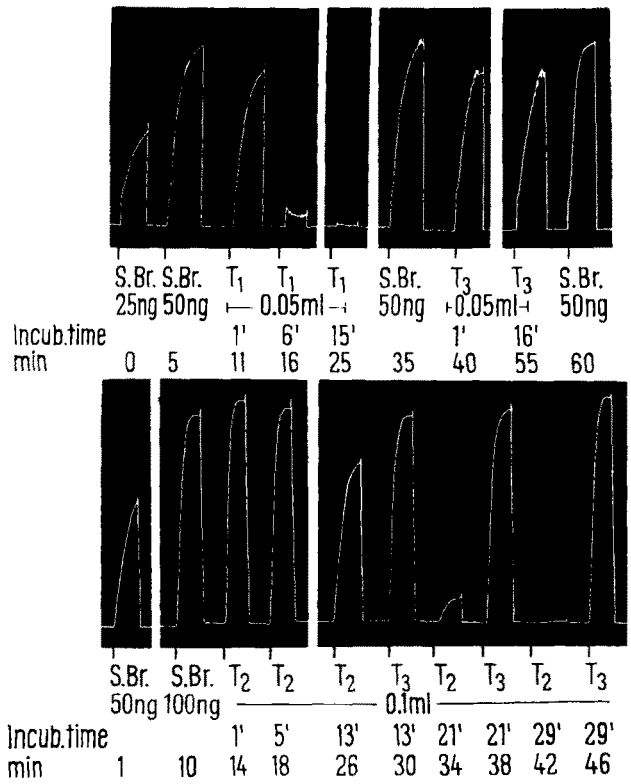

Fig. 2. Presence of kininase in *washed* guinea-pig ileum and rat uterus —Assay on the isolated guinea-pig ileum (same conditions as Figure 1) of samples taken from the following incubates at 35°C: T_1 = Bradykinin (1 μg/ml) + *washed* guinea-pig ileum; T_2 = Bradykinin (1 μg/ml) + *washed* rat uterus and T_3 = Bradykinin (1 μg/ml). S.Br. = synthetic bradykinin. The gradual inactivation of bradykinin by the *washed* gut (upper) was complete after 15 min incubation; with *washed* rat uterus (lower) this was achieved after 29 min at 35°C.

[5] Val[5] angiotensin I, Asp. B-amide and val[5] angiotensin II, Asp. B-amide were obtained through the courtesy of Ciba Ltd., Basel.

[6] E. A. CARLINI, Z. P. PICARELLI, and J. L. PRADO, Bull. Soc. Chim. biol. *40*, 1825 (1958).

[7] The synthetic bradykinin (Sandoz BRS-640) was kindly supplied by Prof. M. ROCHA E SILVA.

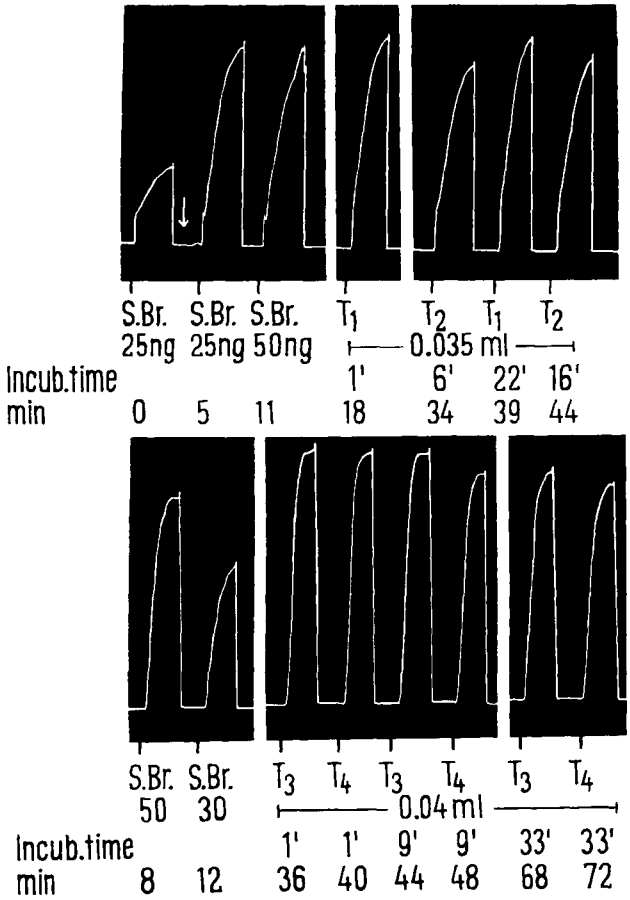

Fig. 3. Inhibition of kininase by cysteine—Assay on the isolated guinea-pig ileum (same conditions as Figure 1) of aliquots of the following incubation mixtures: T_1 = cysteine (2.85 mg/ml) + bradykinin (0.71 μg/ml); T_2 = *washed* guinea-pig ileum + cysteine (2.85 mg/ml) + bradykinin (0.71 μg/ml); T_3 = cysteine (2.48 mg/ml) + bradykinin (0.75 μg/ml); T_4 = *washed* uterus + cysteine (2.48 mg/ml) + bradykinin (0.75 μg/ml). S.Br. = synthetic bradykinin. At the arrow, 100 μg of cysteine were added to the bath. Contact of the *washed* organs with cysteine for 3 min before incubation with bradykinin, prevented the inactivation of the peptide.

the peptide was gradually destroyed, complete inactivation being achieved after 10–30 min (Figure 2). However, if the segments of the *washed* organs were previously left in contact with cysteine for 2–5 min and then incubated with bradykinin, destruction of the peptide was not observed (Figure 3).

These results seem to indicate that the potentiating effect of cysteine on contractions produced by bradykinin on isolated rat uterus or guinea-pig ileum is probably due to inhibition by the amino acid, of kininase present in these tissues. Its smaller effect on the rat uterus could perhaps be related to weaker kininase activity of this organ, which would also contribute to the greater sensitivity of this pharmacological preparation to bradykinin.

Zusammenfassung. Die Wirkung von Bradykinin, aber nicht von Angiotensin I und II auf das isolierte Meerschweinchenileum und den Uterus der Ratte wird durch Cystein verstärkt. Die Sensibilisierung der glatten Muskulatur ist auf die Hemmung der Kininase, die sich in den Geweben jener Organe befindet, durch Cystein zurückzuführen. Fragmente von Uterus oder Ileum von Tieren, deren hintere Extremitäten unter Druck mit Tyrode durchströmt und zur vollkommenen Ausblutung gebracht wurden, inaktivieren das Bradykinin, wenn sie damit inkubiert werden. Diese Inaktivierung kann durch Vorbehandlung der Organe mit Cystein verhindert werden.

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[8] Partially supported by Grant RF 58217 from the Rockefeller Foundation.

Comparison of the Effects of ß-Palmitoyl Lysolecithin and Cardiac Glycosides in two Mammalian Tissues

In 1957 HAJDU et al.[1] reported the isolation of a substance identified as β-palmitoyl lysolecithin from various mammalian tissues. They found that it shared with the cardiac glycosides the properties of: (1) abolishing treppe in the frog's heart, and (2) having positive inotropic effects in the hypodynamic frog heart and the isolated ventricle of the squab. We were interested to see whether these findings could be extended to mammalian tissues. The test systems we used were the human erythrocyte and the isolated guinea pig ventricle. We chose these because of the large amount of data available as to the effects of the cardiac glycosides on them.

The monopalmitoyl-lecithin (β-lysolecithin) used in these studies was prepared as follows: (dipalmitoleyl)-*L*-α-lecithin (containing two unsaturated fatty acids per molecule) was isolated from baker's yeast by the method of HANAHAN and JAYKO[2]. From this material, the corresponding β-lysolecithin, monopalmitoleyl-lecithin, was obtained by enzymatic hydrolysis according to HANAHAN et al.[3], using *Crotalus atrox* venom[4]. This unsaturated lysolecithin was subjected to catalytic hydrogenation[2,3], which resulted in a product with the same melting point (195°) as the monopalmitoyl-lecithin described by HANAHAN et al.[3].

Fresh human red cells were obtained and incubated with $K^{42}Cl$ as previously described[5]. Plasma was separated and counted just before incubation, and at 1, 2 and 3 h.

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