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BIOLOGICAL EFFECTS OF DEGRADATION PRODUCTS OF COLLAGEN BY BACTERIAL COLLAGENASE

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1 Collagen degradation products (CDP) resulting from bacterial collagenase digestion were fractionated by gel filtration and their biological activities in rats were estimated.

2 CDP induced the following kinin-like effects: increase in permeability of skin blood vessels, contraction of the isolated intestine of the rat, depression of locomotor activity and of motor coordination.

3 The most active CDP fraction was CDP III containing peptides of mol. wt. < 1000 D with a high percentage of hydroxyproline.

4 As compared with bradykinin, CDP III was less active in the skin permeability test and was 15,000 to 20,000 fold less effective in induction of isolated intestine contraction.

5 Depression of the CNS induced by 30 μg of CDP III administered into the brain ventricle was similar to that observed after 4 μg of bradykinin given by the same route.

6 CDP III prolonged the duration of sleep evoked by thiopentone and enhanced the threshold of convulsion induced by pentazol.

7 The activity of CDP in comparison to other low molecular weight peptides is discussed.

Introduction

The list of biologically active peptides resulting from the lysis of various proteins in mammalian organisms has been growing rapidly in recent years. Besides kinins, other peptides cleaved from blood plasma proteins increase the permeability of capillary vessels and induce changes in the arterial pressure, blood flow and heart rate (Buluk & Małofiejew, 1969; Sobaniec, 1974; Wiśniewski, Tarasiewicz, Maćkowiak, Buczko & Moniuszko-Jakoniuk, 1974; Buczko, Franco, Bianchetti, Donati, de Gaetano & Garattini, 1976; Buczko, 1977).

Low molecular weight products of proteolysis of serum albumin, globulins and of fibrinogen change the distribution and pharmacological effects of some drugs and influence the function of the central nervous system in rats (Sobaniec, 1974; Buczko & Moniuszko-Jakoniuk, 1975; Wiśniewski, Buczko and Moniuszko-Jakoniuk, 1975; Tarasiewicz, Maćkowiak, Moniuszko-Jakoniuk & Michalak, 1977; Zwoliński & Buczko, 1977).

The aim of the present work was to investigate the effects of products of the digestion of collagen by bacterial collagenase, on the permeability of skin capillaries, on smooth muscle and on the activity of the CNS.

Methods

Male Wistar rats, weighing approx. 160 g were used for experiments.

Calf skin acid-soluble collagen was purified according to Kang, Nagai & Piez (1966). The hydroxyproline content in the final preparation was 13%. Before use, collagen was dissolved in 0.05% acetic acid and dialysed exhaustively against 0.05 M Tris-HCl buffer, pH 7.5, containing 0.005 M $CaCl_2$.

Collagen degradation products (CDP) were prepared as follows: samples of collagen were digested with collagenase from *Clostridium histolyticum* (Sigma, type I) at an enzyme: substrate weight ratio of 1:100 and at 37°C. Proteolysis was interrupted by acidification to pH 3.0 with 1 M HCl. CDP were fractionated by gel filtration on Sephadex G-25 columns (2 × 60 cm) using distilled water for elution. Calibration of the columns was performed under the same

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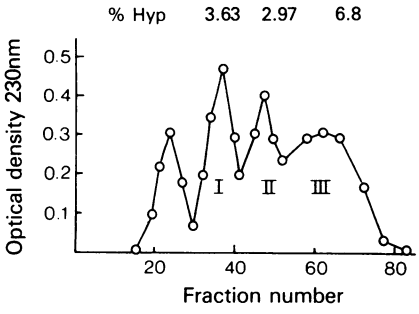

Figure 1 Elution from Sephadex G-25 column of the collagen degradation products (CDP) released from collagen by *Clostridium histolyticum* collagenase. Each fraction was 3 ml.

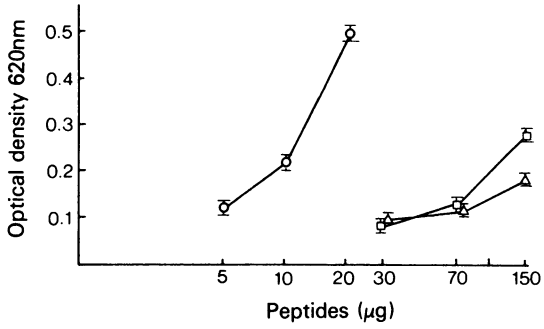

Figure 2 Enhanced skin capillary permeability to Evans blue induced in rat by intradermal injection of collagen degradation products fractions (CDP): CDP I (△); CDP II (□); CDP III (○). Optical density of eluted spots was measured at 620 nm. Eluate from the site injected with saline 0.1 ml was used as a blank. Each point represents the mean value of at least six experiments; vertical lines show s.d.

conditions with bradykinin (mol. wt. 1240), trasylol (mol. wt. 6500) and cytochrome C (mol. wt. 12,600) as standards. Fractions of 3.0 ml were collected. Material eluted in individual peaks of absorbancy at 230 nm (Kang, Bornstein & Piez, 1967) was pooled and concentrated under vacuum to about 1/10 of the initial volume. Concentrated fractions were desalted on Sephadex G-10 columns (2 × 50 cm). Before biological testing, CDP were diluted with saline and the pH adjusted to 7.5 with 0.2 M Tris. The contribution of fractions to the total material subjected to gel filtration was determined by measuring the areas under absorption peaks.

Hydroxyproline was determined by the method of Stegman & Stalder (1967).

Permeability of rat skin capillaries was examined according to Udaka, Takeuchi & Movat (1970) after intradermal injection of 0.1 ml samples of CDP. Results are expressed as absorbancy of extracted Evans blue at 620 nm.

Contraction of the smooth muscle of distal segments of rat jejunum was measured as described by Hori, Masumura, Mizuta & Kondo (1969).

Locomotor activity was evaluated by Lat's (1965) test. Individual rats were carefully observed and periods of walking, washing and numbers of standing up reactions were recorded during a 10 min period. Rats of similar initial locomotor activity were selected for this assay on the basis of 3 day observations.

Motor coordination was tested by the method of Denker Christensen (1973). A rat was placed on a rotating cone (18 turns/min) and pushed forward by an automatically moving spiral wall. Motor coordination was determined by the distance which the rat travelled before it fell into one of ten numbered boxes placed under the apparatus.

CDP or bradykinin (Bk) was introduced into a lateral brain ventricle as described by Herman (1970). CDP, Bk or buffered saline were administered in a volume of 20 μl, 30 min before starting Lat's test or the motor coordination assay.

The duration of thiopentone (30 mg/kg, i.p.) sleep was measured from the loss of postural reflexes to the recovery of the standing postion.

The time of onset of convulsions was recorded after subcutaneous injection of pentazol (75 mg/kg s.c.). CDP or saline were administered 30 min before thiopentone or pentazol.

Statistical evaluation of results was by Student's *t* test.

Results

The whole digest of collagen by *Clostridium histolyticum* collagenase increased the permeability of rat skin capillaries when applied in a volume of 0.1 ml. The potency increased with time of digestion (up to 2 h).

CDP formed after 2 h of incubation were fractioned on Sephadex G-25 columns (Fig. 1). The first peak eluted at the void volume of the column was discarded. It contained macromolecular material including acid-denatured enzyme and amounted to 19.3% of the total absorbancy of the eluate. Of the three following peaks (CDP I, II and III), CDP III was the richest in hydroxyproline (Hyp) and greatly increased the permeability of skin capillaries (Figure 2). CDP III comprised 44.5% of the total peptide material subjected to gel filtration (Figure 1) and contained peptides of mol wt. up to 1000 daltons. CDP II and CDP I were much less active. CDP III was less active than bradykinin in enhancing skin permeability (Figure 3).

On the isolated distal jejunun of the rat CDP III

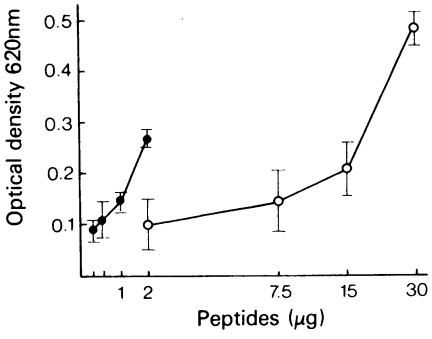

Figure 3 Skin capillary permeability after bradykinin (●) and CDP III (○). Each point represents the mean of at least ten experiments; vertical lines show s.d.

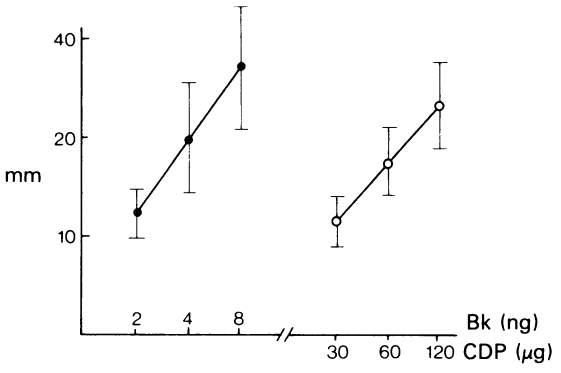

Figure 4 Height of contractions (mm) of rat isolated jejunum in response to bradykinin (Bk, ●) and CDP III (○). Each point represents the mean of seven experiments; vertical lines show s.d.

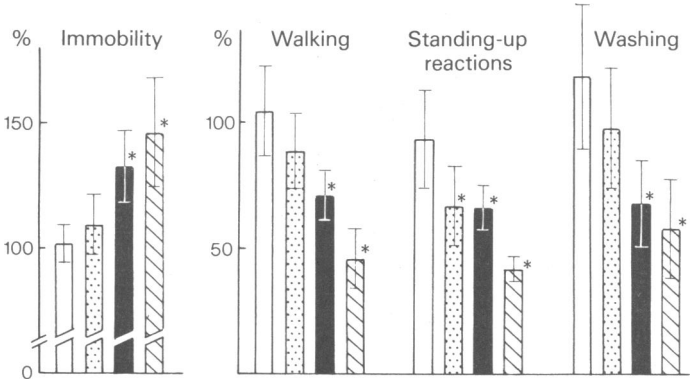

Figure 5 Behaviour of rats (Lat's test) during a 10 min observation period, 30 min after injections (20 μl) of CDP fractions into the right brain ventricle. Results are expressed as percentages of control values obtained in the same rats before CDP injections. Open columns = controls (saline i.c.v.); stippled columns = CDP I (60 μg); solid columns = CDP II (60 μg); hatched columns = CDP III (30 μg). Mean values for 10 rats are shown; vertical lines indicate s.d. * $P < 0.01$.

exhibited only 1/15,000 to 1/20,000 of bradykinin activity (Figure 4).

CDP introduced into the brain ventricle decreased the locomotor activity of rats (Figure 5). Significant prolongation of immobility periods, shortening of periods of walking and washing and decrease in numbers of standing-up reactions was observed after CDP II (60 μg) and III (30 μg); 15 μg of CDP III still induced significant effects but was less potent than bradykinin. CDP III also disturbed the motor co-ordination of rats. Control rats fell into boxes later (mean box no. ± s.e. mean was 5.2 ± 0.9) than treated rats (mean box no. 2.3 ± 1.0 for 15 μg and 2.5 ± 1.3 for 30 μg; both values were significantly different from the control: $P < 0.01$). The dose of 15 μg of CDP III prolonged the duration of sleep provoked by

thiopentone (30 mg/kg i.p.) from 16.3 ± 2.1 min to 35.0 ± 7.7 min; $P < 0.001$. CDP III (30 μg) increased the time of onset of convulsions after subcutaneous injection of pentazol (75 mg/kg) from 5.0 ± 1.0 min to 10.1 ± 2.0 min ($P < 0.001$).

Discussion

Collagen comprises about 33% of the total body proteins in mammals. This protein can be degraded into low molecular weight peptides either under the influence of bacterial collagenases or by sequential action of tissue enzymes (Harris & Krane, 1976; Weiss, 1976). The resulting peptides when tested in rats were found to increase the permeability of skin capillaries, decrease locomotor activity, and to modify pharmacological effects of centrally acting drugs. The CDP III fraction which contained peptides of low molecular weight (⩽1000 D) was the most active. Its high hydroxyproline content indicated that it originated at least in part from sequences of the helical part of collagen.

As mentioned in the introduction, peptides derived from the hydrolysis of fibrinogen, albumin and globulin are similar but less potent in their effects (Buluk & Małofiejew, 1969; Sobaniec, 1974; Wiśniewski *et al.*, 1974; Buczko & Moniuszko-Jakoniuk, 1975). All these peptides may increase the permeability of the blood-brain barrier as well as acting directly on the nervous tissue (Buczko & Moniuszko-Jakoniuk, 1975; Wiśniewski *et al.*, 1975; Zwoliński & Buczko, 1977). Kinin-like effects of CDP may contribute to severe local and general symptoms in clostridial infections such as oedema and shock.

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