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Some pharmacodynamic properties of carrageenin in the rat

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Summary

1. Carrageenin oedema is suppressed by pre-treating the rats with cellulose sulphate, a kininogen depleting agent. This inhibition is closely related to the dose of cellulose sulphate and to the time course of kininogen depletion.

2. Oedema induced by egg white or by dextran, in which the mediators are histamine and 5-hydroxytryptamine, is quite unaffected by cellulose sulphate treatment.

3. Carrageenin injected intravenously lowers the arterial blood pressure of rats. This hypotensive effect is unaffected by histamine antagonists and is abolished by protease inhibitors and thus seems to be due to kinin release from plasma substrates.

4. Like cellulose sulphate, carrageenin enhances the esterolytic activity of the blood from treated rats when incubated with benzoyl-arginine ethyl ester.

5. The ability of carrageenin to activate the kinin-forming system could account for both its inflammatory and hypotensive effects.

Introduction

The use of carrageenin, a sulphated polysaccharide derived from *Chondrus crispus*, to induce foot oedema in the rat's paw was introduced by Winter, Risley & Nuss (1962) and is now a routine test commonly used in evaluating activity of anti-inflammatory agents.

Preliminary results (Di Rosa & Sorrentino, 1968) indicated that aprotinin, the well known protease inhibitor (Trautschold, Werle & Zickgraf-Rudel, 1967), depressed the carrageenin oedema and showed the ability of carrageenin to release kinin-like substance(s) from plasma substrates.

Recent observations (Rothschild, 1968) have shown that cellulose sulphate may be used as a plasma-kininogen depleting agent in the rat.

In the present investigation, inflammatory effects of carrageenin in kininogen depleted rats were studied to test the hypothesis that carrageenin causes the release of kinins. Further, the selective interference of cellulose sulphate with such a releasing process was investigated by eliciting oedema by egg white or by dextran in kininogen depleted rats. These inflammatory agents were used because they act via mediators such as histamine and 5-hydroxytryptamine (5-HT) (Parrat & West, 1958 ; Ankier & Starr, 1967).

Changes in arterial blood pressure and in esterolytic activity of the blood of rats treated with carrageenin were also studied.

Methods

Cellulose sulphate was prepared from Whatman ashless cellulose powder according to Astrup, Galsmar & Volkert (1944).

Kininogen in plasma was determined according to Diniz & Carvahlo (1963) employing the following procedures. All syringes, tubes and pipettes employed in collecting and in transferring blood or plasma samples were of polyethylene. Trypsin hydrolysate was dried under reduced pressure in a rotary vacuum evaporator at 35° C. Contractions of the guinea-pig ileum were recorded on a smoked drum by means of an isotonic microdynamometer manufactured by U. Basile (Milan, Italy). Synthetic bradykinin was used as standard.

To induce plasma-kininogen depletion, cellulose sulphate was administered intravenously three time for each dose employed, 10 min elapsing between successive injections (Rothschild, 1968). Inflammatory responses were elicited after different intervals as specified in the following paragraph.

Male Wistar-Morini rats (150–250 g) were used. Oedema of the hind paw was produced by injecting 0·1 ml. of 1% carrageenin in 0·9% NaCl solution into the plantar surface. The volume of the paw was determined immediately after this injection, as reported by Winter, Risley & Nuss (1963) and using a differential volume measuring instrument manufactured by U. Basile. Subsequent readings for the same paw were carried out every 30 min until 180 min and compared with the initial one.

The same procedure was followed to produce and to evaluate the oedema by undiluted egg white or by 6% dextran.

Other drugs were injected through the superficial veins of the penis or the polyethylene-cannulated right external jugular vein.

Esterolytic activity on benzoyl-arginine ethyl ester (BAEE) was determined by the colorimetric method of Brown (1960). Blood samples were collected according to Rothschild (1968) in order to prevent spontaneous inactivation of the enzyme.

Blood pressure of rats under pentobarbitone anaesthesia (50 mg/kg) was recorded from left carotid artery using a Condon mercury manometer.

Carrageenin (Viscarin 402, lot No. 532218) was generously supplied by Marine Colloids Inc. (Springfield, U.S.A.). Synthetic bradykinin was obtained from Sandoz, aprotinin (Trasylol) from Bayer. Iniprol, the polypeptide extracted by Kunitz & Northrop (1936) from beef pancreas, was kindly supplied by Choay. Dextran, molecular weight 80,000, was supplied by Pharmacia.

All other chemicals were analytical grade preparations obtained from usual commercial sources.

Results

Table 1 shows the results of experiments in which oedema formation by carrageenin, egg white and dextran was induced in rats previously treated with cellulose sulphate.

Cellulose sulphate reduced the swelling induced in the rat's paw by carrageenin. This inhibitory effect was observed on each of the six occasions that it was studied and was closely related to the dose administered (Fig. 1).

On the other hand, oedema induced by egg white or by dextran was not significantly reduced by cellulose sulphate treatment.

TABLE 1. *Effect of cellulose sulphate (CS) on oedema induced by* 0·1 *ml. of carrageenin* (1% *soln in* 0·9% *saline*), 0·1 *ml. of undiluted egg white or* 0·1 *ml. of dextran* (6% *solution in* 0·9% *saline*)

Dose of CS* (mg/kg)	30 min	60 min	90 min	120 min	150 min	180 min
			Carrageenin oedema‡			
Controls†	0·65	0·81	0·93	1·07	1·12	1·25
0·5	0·63	0·72	0·74	0·78	0·92	0·96
1·0	0·61	0·69	0·63§	0·56§	0·55§	0·61§
2·0	0·49	0·52§	0·51§	0·41§	0·35§	0·28§
			Egg white oedema‡			
Controls†	2·43	2·65	2·37	2·07	1·85	1·58
0·5	2·55	2·62	2·43	2·13	1·88	1·55
1·0	2·18	2·47	2·30	1·92	1·72	1·47
2·0	2·03	2·35	2·00	1·67	1·47	1·35
			Dextran oedema‡			
Controls†	1·52	2·15	2·32	2·30	2·35	2·35
0·5	1·48	2·07	2·13	2·07	2·15	2·18
1·0	1·28	1·92	2·08	2·02	2·05	2·10
2·0	1·22	1·83	2·01	1·97	2·00	1·98

* Each dose was administered intravenously (in a volume of 0·25 ml./kg) three times at 10 min intervals, the last was given 10 min before carrageenin injection.
† Controls were injected with 0·9% NaCl.
‡ Each value is expressed in ml. and represents the mean of the determinations carried out on six animals.
§ The observed effect was significantly different ($P < 0.05$) from controls at corresponding time (Student's t test).

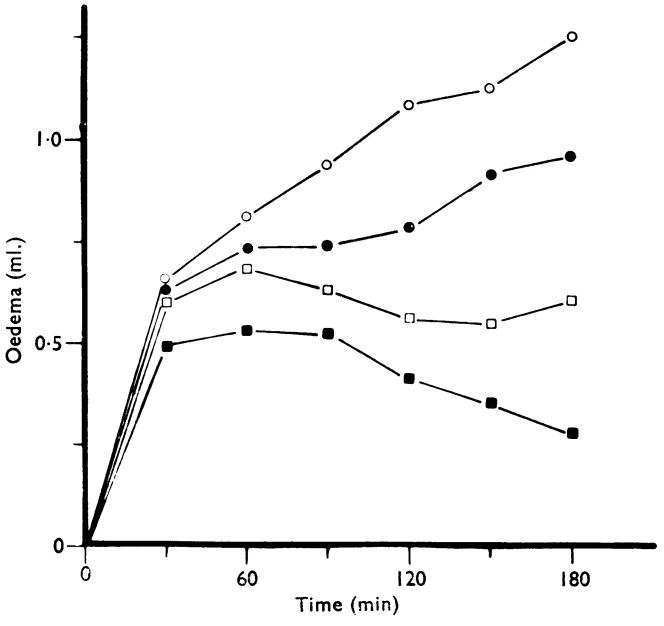

FIG. 1. Effect of cellulose sulphate on the mean carrageenin oedema induced in groups of six rats over a 180 min period. ○, Response to 0·9% NaCl; ●, response to cellulose sulphate 0·5 mg/kg; □, response to cellulose sulphate 1·0 mg/kg; ■, response to cellulose sulphate 2·0 mg/kg. Each dose was administered intravenously (in a volume of 0·25 ml./kg) three times at 10 min interval, the last given 10 min before carrageenin injection.

The relationship between plasma kininogen content and the intensity of carrageenin oedema was investigated by injecting carrageenin into the rat paw at different intervals after the administration of cellulose sulphate. Three hours after carrageenin injection the development of oedema was measured and the plasma kininogen level of the blood was determined. The results show a very close correlation between the time course of plasma kininogen depletion and reduction of oedema (Fig. 2).

Carrageenin administered intravenously (1 mg/kg) caused intense hypotension in the pentobarbitone-anaesthetized rat (Fig. 3). The fall of blood pressure was preceded by a transient rise (60 s), appearing immediately after the injection, and followed by a gradual recovery which was complete within 10 min. A second injection of the same dose elicited a reduced effect on blood pressure; the third injection was completely ineffective. This pattern was typical for all rats, but there was wide variability in the depressor response and in the time of recovery. The

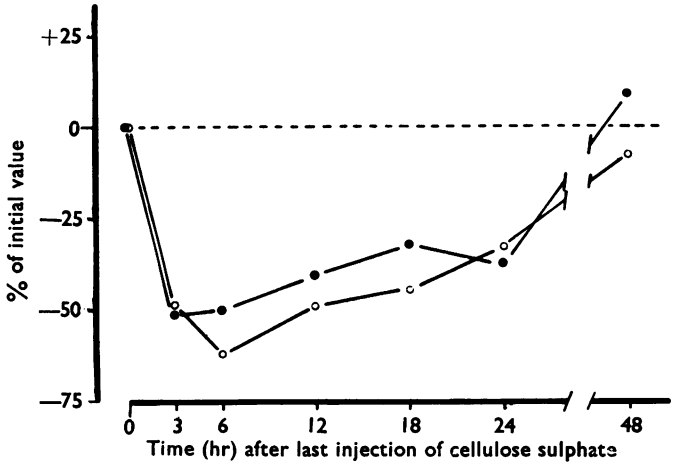

FIG. 2. Relation between % inhibition of carrageenin oedema and % depletion of plasma kininogen, both induced by cellulose sulphate (1 mg/kg) injected intravenously in groups of six rats. Each dose was administered three times at 10 min intervals. ●, Oedema as measured 180 min after carrageenin injection (initial value: 1·35 ± 0·12 ml.); ○, plasma kininogen content as determined immediately after evaluating carrageenin oedema (initial value: equivalent to 5·8 ± 0·65 μg synthetic bradykinin).

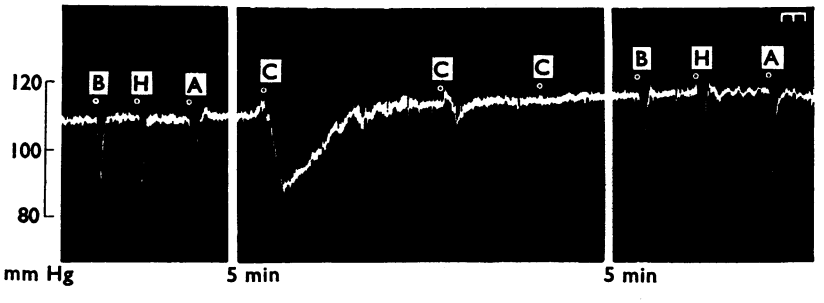

FIG. 3. Record of the arterial blood pressure of a rat anaesthetized with sodium pentobarbitone. Intravenous injections: B, bradykinin 5 μg/kg; H, histamine dihydrochloride 10 μg/kg; A, acetylcholine chloride 10 μg/kg; C, carrageenin 1 mg/kg. Time scale in min.

tachyphylactic effect was not due to loss of sensitivity to effects of bradykinin, histamine or acetylcholine.

The hypotensive effect of carrageenin did not seem to be due to histamine release because it was not affected by pre-treatment with the histamine antagonist mepyramine (Fig. 4).

Recent observations (Di Rosa & Sorrentino, 1968) have shown that aprotinin is able to inhibit the *in vitro* release by carrageenin of kinin-like substance(s) from guinea-pig plasma. The same inhibition seems to be present *in vivo* because the hypotension induced in the rat by carrageenin was suppressed by pre-treating the animal with aprotinin (10,000 units/kg ; Fig. 5).

Soya bean trypsin inhibitor (10 mg/kg) and Iniprol (40,000 units/kg) previously administered were also able to suppress the hypotensive effect of intravenous carrageenin. The doses of protease inhibitors used did not affect the sensitivity of animals to bradykinin, histamine, or acetylcholine.

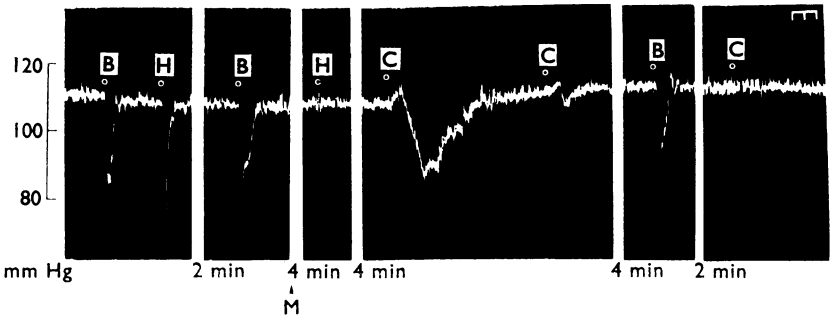

FIG. 4. Record of the arterial blood pressure of a rat anaesthetized with sodium pentobarbitone. Intravenous injections: B, bradykinin 5 μg/kg ; H, histamine dihydrochloride 10 μg/kg ; M, mepyramine 200 μg/kg ; C, carrageenin 1 mg/kg. Time scale in min.

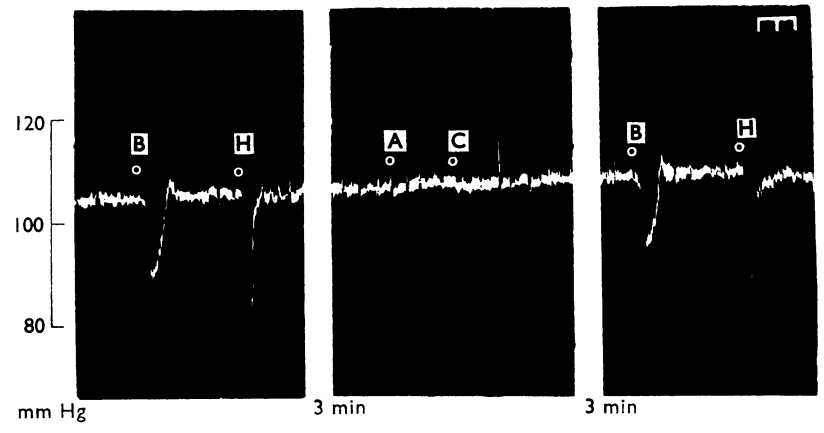

FIG. 5. Record of the arterial blood pressure of a rat anaesthetized with sodium pentobarbitone. Intravenous injections: B, bradykinin 5 μg/kg ; H, histamine dihydrochloride 10 μg/kg ; A, aprotinin 10,000 units/kg ; C, carrageenin 1 mg/kg. Time scale in min.

Figure 6 shows the effects of carrageenin (1 mg/kg) and of cellulose sulphate (3 mg/kg) administered intravenously on the esterolytic activity of rat blood. Both sulphopolysaccharides enhanced the blood esterolytic activity on benzoyl-arginine ethyl ester. This effect was observed within 1–3 min of treatment and disappeared after 12–24 min.

Discussion

Carrageenin oedema is depressed by pre-treating rats with cellulose sulphate. Van Arman, Begany, Miller & Pless (1965) observed a suppression of carrageenin oedema by soya bean trypsin inhibitor previously administered in the rat paw and put forward the hypothesis that carrageenin could act through a proteolytic process with consequent formation of a kinin-like substance.

By treating rats with aprotinin, Di Rosa & Sorrentino (1968) showed that anti-protease agents, administered systemically, could prevent carrageenin-induced swelling. These authors demonstrated that carrageenin, when incubated with plasma substrates, is able to release kinin-like substance(s). Further investigations have shown that the susceptibility of the guinea-pig ileum to this kinin-like substance(s) is raised by chymotrypsin (Sorrentino & Di Rosa, unpublished). According to recent observations (Edery & Grunfeld, 1969) this procedure sensitizes smooth muscle to plasma kinins ; therefore the mediator released by carrageenin may be identified as a peptide related to bradykinin.

Cellulose sulphate is a sulphated polysaccharide which induces a plasma kininogen depleted state in the rat (Rothschild, 1968). In rats pre-treated with cellulose sulphate, carrageenin fails to provoke oedema. The depression of paw swelling is closely related to the dose administered and more specifically to the extent and to the time course of circulatory kininogen depletion. Egg white oedema, which results from the release of mediators such as histamine and 5-HT (Parrat & West, 1958), is not inhibited by pretreatment with cellulose sulphate.

This finding supports the conclusion reached by Ankier & Starr (1967) that the kinin system plays no part in dextran oedema.

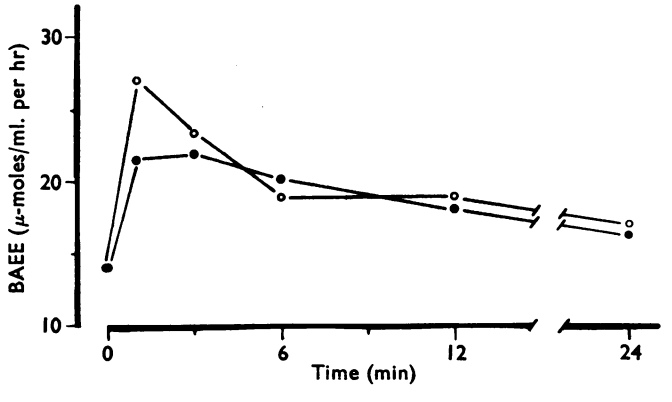

FIG. 6. Esterolytic activity on benzoyl-arginine ethyl ester (BAEE) in the blood of rats injected intravenously with carrageenin 1 mg/kg (●——●) or with cellulose sulphate 3 mg/kg (○——○). Each point represents the mean esterolytic activity of four rats. Samples at zero time (controls) were withdrawn from rats treated with 0·9% NaCl.

From these observations it may be assumed that the inhibitory effect of cellulose sulphate on carrageenin oedema is selective and is due to the depletion of plasma kininogen. The results also support the hypothesis that carrageenin provokes swelling by releasing kinin-like substance(s).

Carrageenin lowers the arterial blood pressure of rats. This hypotensive effect of carrageenin is not affected by the antihistamine agent mepyramine, while it is suppressed by protease inhibitors such as aprotinin, soya-bean trypsin inhibitor, or iniprol. Like the inflammatory response, the hypotensive effect of carrageenin seems to be due to its ability in releasing kinin-like substance(s). Such a hypothesis is also supported by experiments in which an enhancement of blood esterolytic activity in rats treated with carrageenin was observed. This action of carrageenin appears to be the same as that observed for cellulose sulphate as reported by Rothschild (1968).

All these experimental observations support the conclusion that carrageenin activates the kinin-forming system. The kinin release could be brought about by carrageenin acting on the complement system (Willoughby, Coote & Turk, 1969). This would lead to the release of other pharmacologically active substances. Nevertheless, it would seem that the main inflammatory action of carrageenin is via the kinin system, which seems to be a common property of several sulphated polysaccharides (Rothschild & Gascon, 1966).

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