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Action of rare earth metal complexes on neurogenic as well as on bradykinin-induced inflammation

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Both neurogenically evoked inflammation and that induced by bradykinin are not satisfactorily inhibited by the usual anti-inflammatory agents. It has been found that anticoagulant rare earth metal compounds inhibit these types of inflammation. In rats, neurogenic inflammation induced either by antidromic electrical stimulation of the saphenous nerve, or by orthodromic stimulation of sensory nerve endings with capsaicin could almost totally be prevented by a neodymium complex of pyrocatechol sodium disulphonate. In rats and rabbits a dose-dependent inhibition of the permeability increase was observed at the site of the injection of bradykinin, kallikrein or bothrops venom. Similarly, there was marked inhibition of "thermic oedema." The results are in accord with the hypothesis that the blood clotting system plays an important role in the mechanism of inflammation.

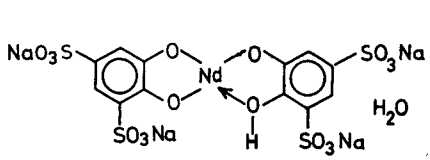
Jancsó & Jancsó-Gábor (1960) and Jancsó (1961) put forward the hypothesis that the blood clotting system plays an important role in the mechanism of inflammation, and the inhibition of blood coagulation prevents or greatly reduces the inflammatory responses. This hypothesis was recently confirmed by Barnhart (1968) and supported by several other authors. For instance Fontaine, Grand & others (1967) demonstrated with many coumarin and indandione derivatives that the anti-inflammatory and anticoagulant actions are inseparable. Wiseman & Yi-Han Chang (1968) reported a marked inhibition of the carrageenan oedema by heparin. In addition, several authors emphasize the inhibitory role of fibrinolysis and that of decrease of the fibrinogen level in the development of inflammation (Aschheim, Tsuluca & Copley, 1963; Gryglewski, 1966; Glenn, 1969).

In view of these considerations it was tempting to investigate whether anticoagulant agents are effective also in those types of inflammation which could scarcely be influenced by the commonly used anti-inflammatory drugs. Previous experiments revealed that rare earth metal complexes with anticoagulant action are effective antiphlogostic agents in many types of inflammation (Jancsó & Jancsó-Gábor, 1960; Jancsó, 1961; Oyvin, Baluda & others, 1964; Oyvin, Uklonskaya & Romanovskaya, 1966; Oyvin, Volodin & others, 1966). In the present experiments the effect of these compounds was tested on neurogenic and bradykinin-induced inflammation.

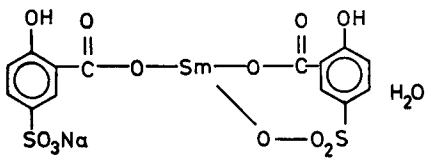
EXPERIMENTAL

Materials

The neodymium complex of pyrocatechol sodium disulphonate (I) synthesized by Jancsó (1961) was used for intravenous injections in form of a 5% solution (Phlogodym). For local treatment an ointment containing 3% samarium sulphosalicylate (II) (Phlogosam, Gedeon Richter) was used.



I



II

Other drugs used were: indomethacin, flufenamic acid, sodium salicylate, amidopyrine, phenylbutazone, cyproheptadine, bradykinin (Parke, Davies), *Bothrops jararaca* venom (Light), kallikrein (Depot Padutin, Bayer), azovan blue, capsaicin. Capsaicin was dissolved by means of ethanol and polysorbate 80 as described earlier (Jancsó, Jancsó-Gábor & Szolcsányi, 1967). Indomethacin was used as a suspension, the other drugs were dissolved in isotonic saline.

Methods

Neurogenic inflammation. In rats under pentobarbitone anaesthesia (40 mg/kg) an inflammatory reaction was induced in the skin area supplied by the saphenous nerve by antidromic electrical stimulation of the nerve for 10 min using rectangular pulses of 8 V, 8 ms at 25/s (Jancsó & others, 1967).

Neurogenic inflammation was also induced in the eye of the anaesthetized rat by instilling a drop of a 1% solution of capsaicin. In both experiments, 10 min before the stimulus, 50 mg/kg azovan blue dye was injected into the tail vein. The animals were killed by bleeding after the completion of nerve stimulation or 10 min after the instillation of capsaicin into the eye. The saphenous skin area or the eye lids and conjunctivae were excised and the amount of the exuded dye quantitatively determined by the suramin extraction method (Jancsó-Gábor, Szolcsányi & Jancsó, 1967).

Inflammation induced by bradykinin, bothrops venom and kallikrein

The inflammatory agents were injected intradermally, in rats in 0·05 ml into the dorsal skin of the hind paws, in rabbits in 0·1 ml into the shaved skin of the back. 10 min before the application of the agents, 50 mg/kg azovan blue was injected intravenously. The degree of the permeability increase was measured as in neurogenic inflammation.

Thermic oedema

Under pentobarbitone anaesthesia one rat hind paw was immersed in water at 46·5° for 30 min, the other paw serving as control. After the experiment the animals were killed, the paws amputated and weighed. The weight increase of the paw immersed at 46·5° was calculated by subtracting the weight of the control paw.

RESULTS AND DISCUSSION

Inhibition of the neurogenic inflammation

Previously it was reported that by antidromic electrical stimulation of a sensory nerve true inflammatory symptoms can be evoked and that these symptoms cannot be inhibited by anticholinergic, adrenergic blocking or ganglionic blocking agents, or by antagonists of histamine and 5-hydroxytryptamine (5-HT) (Jancsó & others, 1967).

The present experiments revealed that non-steroid anti-inflammatory agents and even prednisolone, in non-toxic doses, have no effect on this type of inflammation, or at best exert only slight inhibition. In sharp contrast the rare earth metal complex, Phlogodym, almost abolished the inflammation induced by nerve stimulation.

Table 1. *Effect of different agents on the azovan blue dye accumulation induced in the skin of the paw of rats by antidromic electrical stimulation of the saphenous nerve.* The excess dye values were obtained by subtracting the dye content of control sides from those of stimulated sides. Azovan blue dose: 50 mg/kg.

	No. exp.	Dose mg/kg	Interval min	Dye content (μg) stimulated side	Dye content (μg) control side	Excess dye (μg)	Inhibition %
Phlogodym ..	6	250 i.v.	30	2·5	1·8	0·7	97
	8	100 i.v.	30	4·5	1·8	2·7	88
	6	50 i.v.	30	6·4	1·1	5·3	77
Sodium salicylate	6	250 i.v.	30	16·4	1·3	15·1	35
Amidopyrine ..	6	200 i.v.	30	19·2	1·1	18·1	22
Phenylbutazone ..	6	100 i.v.	30	22·6	1·1	21·5	7
Indomethacin ..	7	10 oral	60	18·4	1·3	17·1	26
Flufenamic acid ..	8	20 i.v.	30	21·7	1·6	20·1	13
Cyproheptadine ..	4	5 i.v.	30	24·7	1·7	23·0	0
Control	33	—	—	24·6	1·5	23·1	—

Table 1 shows the effect of different agents on the permeability increase resulting from antidromic electrical stimulation of the saphenous nerve. In untreated rats the mean value of the exuded dye was 23·1 μg. This inflammatory response was slightly inhibited by non-steroid anti-inflammatory agents, but the histamine- and 5-HT-antagonist, cyproheptadine was ineffective. Phlogodym exerted a marked, dose-dependent inhibition which was very long-lasting: the dose of 100 mg/kg exerted 70% inhibition even 6 h after the injection. In rats pretreated with prednisolone (2 × 10 mg/kg) 24 and 1 h before the nerve stimulation, no inhibition was seen.

Phlogodym also prevented the inflammation evoked by orthodromic stimulation of sensory nerve endings with chemical irritants.

Table 2 shows marked inhibition of the inflammatory effect of the neurogenically acting capsaicin (Jancsó & others, 1967) on the eye of rats pretreated with Phlogodym.

Inhibition of the inflammation induced by bradykinin, bothrops venom and kallikrein

Some of the non-steroid anti-inflammatory agents are able to counteract the actions of bradykinin on musculature of the guinea-pig lung (Collier & Shorley, 1960, 1963), or on the vascular smooth muscle (Starr & West, 1966; Northover, 1967a). But the findings concerning its permeability increasing effect are contradictory, and in most cases inhibition could only be achieved with very high doses of anti-inflammatory agents (Collier & Shorley, 1960; Walters & Willoughby, 1965; Greaves & Shuster, 1967; Giordano & Scapagnini, 1967; Martelli, 1967; Starr & West, 1967; Northover, 1967b).

Table 2. *Azovan blue dye accumulation in the eye lids and conjuctivae of Phlogodym-pretreated and control anaesthetized rats after instillation of a drop of a 1% solution of capsaicin into the eye.* Phlogodym was injected intravenously 30 min before the instillation of the irritant. The excess dye values were obtained by subtracting the dye content of control sides from those of capsaicin-treated sides. Azovan blue dose: 50 mg/kg.

	No. exp.	Capsaicin 1%	Azovan blue (μg)	Excess dye (μg)	Inhibition %
Phlogodym, 250 mg/kg ..	14	+	4	1·7	87
	5	—	2·3		
Control	14	+	15·5	13·1	
	4	—	2·4		

With our quantitative method for measuring the amount of the leaked dye, the permeability increase induced by 0·5 μg of bradykinin could not be inhibited at all with 250 mg/kg of sodium salicylate or 100 mg/kg of phenylbutazone intravenously. In contrast, rare earth metal complexes parenterally or percutaneously greatly inhibited the permeability increase induced by bradykinin. Moreover, the effect of kallikrein and that of bothrops venom which releases bradykinin and potentiates its effect (Rocha e Silva, Beraldo & Rosenfeld, 1949; Ferreira, 1965), were also strongly inhibited by the rare earth complexes.

Fig. 1 shows the log dose-response relation of the mean value of dye exudation induced by 1, 2 and 10 μg bradykinin in the skin of 5 rabbits pretreated with 250 mg/kg Phlogodym and that of 5 controls. The degree of inhibition in the case of different doses of bradykinin was almost the same, between 50 and 63%. Comparable results were achieved if, instead of bradykinin, 1 IU of kallikrein was injected into the skin of the rabbit.

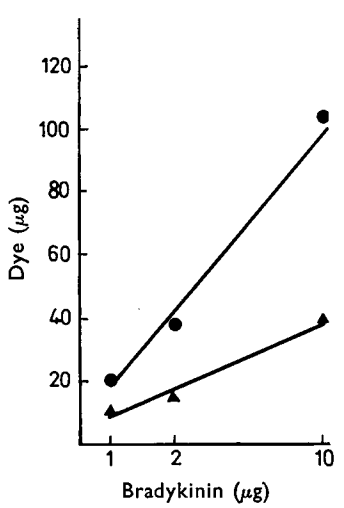


FIG. 1. Dose-response curve of the dye exudation induced by intradermal injection of bradykinin in the rabbit. ▲—▲ = rabbits pretreated with 230 mg/kg Phlogodym 30 min before the experiment; ●—● = controls. Azovan blue dose: 50 mg/kg.

Fig. 2 demonstrates the inhibitory effect of Phlogodym on the local dye exudation induced in the paw of the rat by the intradermal injection of bradykinin, bothrops venom or kallikrein. A marked dose-dependent inhibition (33 to 73%) was observed. Local application of an ointment containing 3% samarium sulphosalicylate (Phlogosam) inhibited (36%) the inflammation induced by bradykinin and (49%) that evoked by bothrops venom.

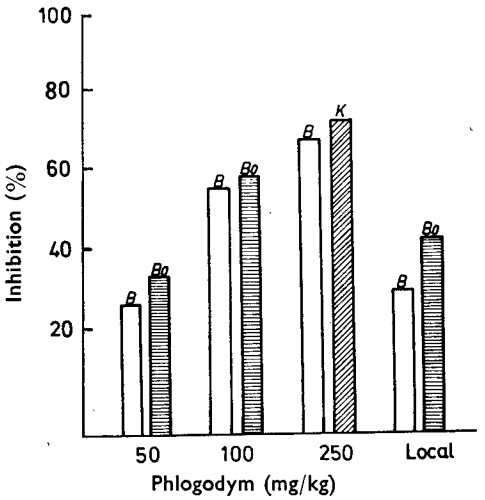


FIG. 2. Effect of 50, 100 and 250 mg/kg Phlogodym intravenously on the local dye exudation induced in the skin of the paw of rats. Phlogodym was given 30 min before the intradermal injection of 0·5 μg bradykinin (B), 2 μg bothrops venom (Bo) and 0·25 IU of kallikrein (K), respectively. The columns show the mean inhibition compared with the control values, on the basis of the amount of the leaked dye. The two last columns show the local effect on the dye exudation when the paws were painted with an ointment containing 3% samarium sulphosalicylate 1 and 3 h before the intradermal injection of 0·5 μg bradykinin or 2 μg bothrops venom. Each column represents the mean value of 5–10 experiments. Azovan blue dose: 50 mg/kg.

The fact that the rare earth metal complex produced approximately the same inhibition on the inflammation produced by kallikrein or bothrops venom and that induced by bradykinin suggests that the inhibition of kinin formation does not play a decisive role in the antiphlogistic effect of these compounds.

Inhibition of the thermic oedema

The thermic oedema of 46·5° is mediated (Rocha e Silva & Antonio, 1960), partly mediated (Starr & West, 1967) or at least accompanied (Urbanitz, Wiegand & Habermann, 1969) by release of bradykinin. This type of oedema can only be inhibited with very high doses of anti-inflammatory agents (Starr & West, 1967).

The weight increase of 6 paws immersed for 30 min in water at 46·5° was 703 mg (53%) over the control while the 6 paws of animals given Phlogodym (250 mg/kg, i.v.) 20 min before the experiment showed a weight increase of 342 mg, a 26% increase over the control. Thus the agent inhibited the oedema formation by 51%. This finding parallels the previous studies on the experimental burn of the rabbit ear at 54° (Oyvin & others, 1964) and provides experimental support for the claim that the rare earth containing ointment (Phlogosam) is of value in the treatment of superficial burns in man (Dömötör, 1969).

The marked effect of rare earth complexes with anticoagulant action in inflammations resistant to other agents support the hypothesis of Jancsó that the blood clotting system plays an important role in the development of inflammation.

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