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INHIBITORY EFFECT OF A PYRROLIZIDINE ALKALOID, CROTALABURNINE, ON RAT PAW OEDEMA AND COTTON PELLET GRANULOMA

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1 The anti-inflammatory activity of crotalaburnine (=anacrotine) was investigated against increased vascular permeability and oedema produced by formalin, carrageenin, hyaluronidase, 5-hydroxytryptamine, dextran, bradykinin and prostaglandin, and against formation of granulation tissues by cotton-pellet in rats. The effect was compared with the activity of hydrocortisone, phenylbutazone, sodium salicylate and cyproheptadine against different types of inflammation.

2 Crotalaburnine (40 mg/kg s.c. x 5 alternate days) had no significant inhibitory effect against formalin-induced arthritis, while hydrocortisone (40 mg/kg s.c. x 10 days) was effective from the fifth day onwards.

3 Against carrageenin-induced oedema both crotalaburnine (10 mg/kg s.c.) and phenylbutazone (100 mg/kg oral) produced a similar degree of inhibition. Hydrocortisone (10 mg/kg s.c.) produced slightly greater inhibition.

4 In normal rats crotalaburnine (10 mg/kg s.c.), phenylbutazone (100 mg/kg oral) and sodium salicylate (500 mg/kg i.p.) inhibited hyaluronidase-induced oedema. However, in adrenalectomized rats, there was a reduction of the inhibitory effect of sodium salicylate but not of phenylbutazone or crotalaburnine.

5 Crotalaburnine (40 mg/kg s.c. and 30 mg/kg i.p., respectively) was ineffective against 5-hydroxytryptamine- and dextran-induced oedema but against bradykinin- and prostaglandin-induced oedema (in a dose of 20 mg/kg i.p.) it was quite effective. In a parallel series cyproheptadine (10 mg/kg oral and i.p., respectively) produced significant inhibition of 5-hydroxytryptamine- and dextran-induced oedema, while phenylbutazone (100 mg/kg i.p.) failed to produce any significant inhibition of prostaglandin-induced oedema.

6 Against cotton-pellet granuloma crotalaburnine, in half the dose of hydrocortisone, produced similar inhibition while phenylbutazone produced much greater inhibition in five times the dose of crotalaburnine given orally.

7 The possible mode of action of crotalaburnine as an anti-oedema agent is discussed.

Introduction

Crotalaburnine, an alkaloid isolated from the seeds of *Crotalaria laburnifolia* Linn. (Snehalata, Ghosh, Nagarajan & Subramanian, 1966) has since been shown to be identical with the pyrrolizidine alkaloid anacrotine (Crout, 1972). The alkaloid was found to have a papaverine-like activity as well as bronchodilator and local anaesthetic action (Snehalata & Ghosh, 1968). Since crotalaburnine (=anacrotine) showed antagonistc action against histamine and 5-hydroxytryptamine on guinea-pig ileum (Snehalata & Ghosh, 1968) and since histamine and 5-hydroxytryptamine can also be isolated from inflammatory exudate in rats and are probably partly responsible for pain and increased capillary permeability of inflammation (Keele & Neil, 1965), the present study was undertaken in order to see if crotalaburnine has any anti-inflammatory property. So far only hepatotoxic activity in livestock, carcinogenicity and anti-carcinogenicity, and some anti-spasmodic, curare-like and local anaesthetic actions of pyrrolizidine alkaloids have been reported (see Bull, Culvenor & Dick, 1968; Pomeroy & Raper, 1971; Atal & Sawhney, 1973).

Methods

Albino rats weighing between 100 and 200 g were used throughout the present investigation. The rats were divided into batches which included a control group receiving placebo treatment and groups treated with either crotalaburnine or some standard anti-inflammatory drug. Both control and treated groups in a particular series received the same oedema-inducing agent.

Formalin-arthritis

A subcutaneous injection of 0.1 ml 2% formalin was given under the plantar aponeurosis of both feet of the rat on the first and third days (Brownlee, 1950). Hydrocortisone (40 mg/kg) was injected subcutaneously daily for 10 days in one group and crotalaburnine (40 mg/kg) on alternate days for 5 days in the other. The linear cross-section immediately below the ankle-joint was measured daily with a vernier calliper and the mean values recorded.

Carrageenin-induced oedema

The method of Winter, Risley & Nuss (1962) with slight modifications was followed. Carrageenin (1%) in 0.9% w/v NaCl solution (saline) was injected in a volume of 0.1 ml into subplantar tissues in one hind-paw of the rat. Groups of rats were pretreated either with hydrocortisone or with crotalaburnine (10 mg/kg subcutaneously) 1 h before, or with phenylbutazone (100 mg/kg) suspended in gum acacia, orally 3 h before carrageenin. The foot volume was measured in unanaesthetized rats by the modified plethysmographic method described by Singh & Ghosh (1968) immediately after and again 3 h after carrageenin and the 'volume of oedema' was recorded as the difference between the two readings.

Hyaluronidase-induced oedema

Hyaluronidase (600 i.u. per ml of saline) was injected (0.4 ml) subcutaneously into the dorsum of one foot of the rat and oedema produced by the method of Ghosh, Banerjee & Mukherji (1963). Groups of rats were treated with phenylbutazone (100 mg/kg orally), sodium salicylate (500 mg/kg intraperitoneally) and crotalaburnine (10 mg/kg subcutaneously) 3 h, 0.5 h and 2 h respectively before hyaluronidase. The oedema was measured 20 min after hyaluronidase injection.

Adrenalectomized rats. Rats were adrenalectomized through the dorsal route under light ether anaesthesia. Drinking water was replaced by 1% saline and the animals maintained for about 3 weeks before they were used for anti-inflammatory studies. Hyaluronidase-oedema was induced in these rats and the same three treatments repeated in this series as in normal rats.

5-hydroxytryptamine-induced oedema

A volume (0.1 ml) of 5-hydroxytryptamine (1 mg/ml in saline) was injected into subplantar tissues in one hind-paw of the rat and oedema recorded after 20 minutes. Groups of rats were treated either with crotalaburnine 40 mg/kg subcutaneously or with cyproheptadine (10 mg/kg; in 1% methylcellulose) orally 2 h before 5-hydroxytryptamine. The control group was fed with 1 ml 1% methylcellulose.

Dextran-induced oedema

A volume (0.05 ml) of 6% dextran in saline was injected as above and oedema recorded after 30 minutes. Groups of rats were treated either with crotalaburnine (20 mg/kg intraperitoneally) or with cyproheptadine (10 mg/kg intraperitoneally) 1 h before dextran. The control group was treated with saline.

Bradykinin-induced oedema

One μg bradykinin in 0.1 ml saline was injected as above and oedema recorded after 30 minutes. Groups of rats were treated with either crotalaburnine (20 mg/kg intraperitoneally) or with saline (control) 1 h before bradykinin.

Prostaglandin-induced oedema

Prostaglandin (5 ng in 0.1 ml saline) was injected subcutaneously into the dorsum of one foot and oedema recorded after 30 minutes. Groups of rats were treated with either crotalaburnine (20 mg/kg intraperitoneally) or with phenylbutazone (100 mg/kg intraperitoneally) 1 h before prostaglandin. The control group was treated with saline.

Cotton-pellet granuloma

Cotton-pellets cut from dental rolls and weighing 50 ± 1 mg were sterilized, soaked in 0.2 ml distilled water containing penicillin (0.1 mg) and streptomycin (0.13 mg), and then inserted one in each axilla by the method of Winter & Porter (1957). Groups of rats were treated respectively with hydrocortisone (40 mg/kg subcutaneously), phenylbutazone (100 mg/kg orally) and crotala-

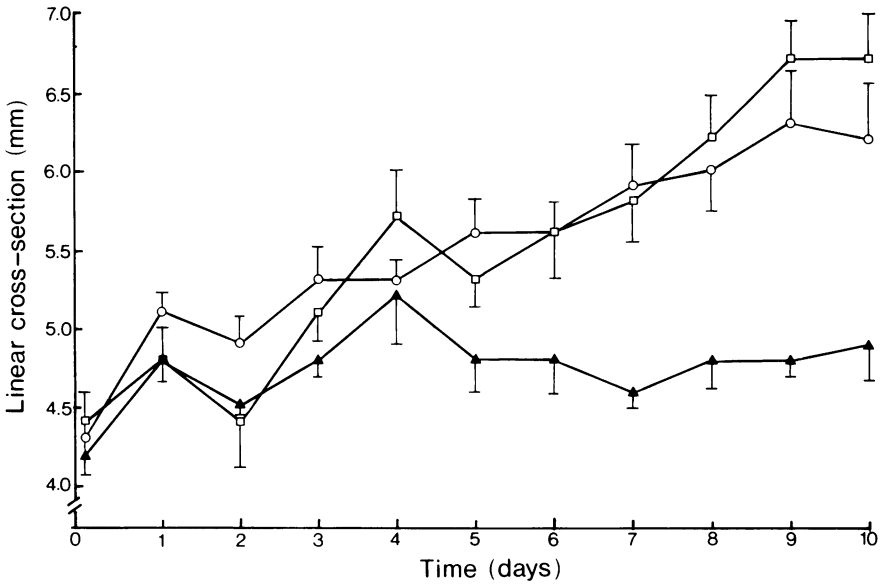

Fig. 1 Effect of crotalaburnine and hydrocortisone on formalin-arthritis in rats. Ordinates: linear cross-section immediately below the ankle joint (mm). (○) control rats; (▲) hydrocortisone 40 mg/kg s.c. x 10 days; (□) crotalaburnine 40 mg/kg s.c. x 5 alternate days. Each point represents the mean value from six rats. Vertical bars show s.e. mean.

burnine (20 mg/kg subcutaneously) daily for 6 days. The pellets together with the granuloma were dissected out carefully after 7 days and any increment in the dry weight taken as a measure of granuloma formation.

Drugs and chemicals

Formalin 2% in distilled water; carrageenin (Seakem 402, Marine Colloids); hyaluronidase (Hyalase, Tata Fison); 5-hydroxytryptamine creatinine sulphate (Serotonin, Roche); dextran; bradykinin (Sandoz); prostaglandin E_1 (Upjohn); hydrocortisone acetate (Roussel); phenylbutazone (Butazolidin, Irgapyrin, Suhrid Geigy); sodium salicylate B.P.; cyproheptadine (Periactin, Merck, Sharp & Dohme); crotalaburnine. Crotalaburnine was dissolved in distilled water with the addition of HCl and the pH adjusted to between 4 and 6.

Statistical procedures

All results quoted are means with standard error of the mean (s.e. mean) and tests for significance were performed with Student's *t* test.

Results

Formalin-induced arthritis

The mean linear cross-section values and their standard errors were plotted against time (in days) as in Figure 1. Hydrocortisone produced definite inhibition from the fifth day onwards, while there was little difference in the effects between the control and the crotalaburnine-treated animals except on the second day when crotalaburnine produced some inhibition but this was not statistically significant.

Carrageenin-induced oedema

The results of carrageenin-induced oedema experiments are summarized in Figure 2. Hydrocortisone produced about 40% inhibition and the result was statistically significant ($P < 0.01$). Both crotalaburnine and phenylbutazone produced about 30% inhibition but only the inhibition produced by crotalaburnine was statistically significant ($P < 0.05$).

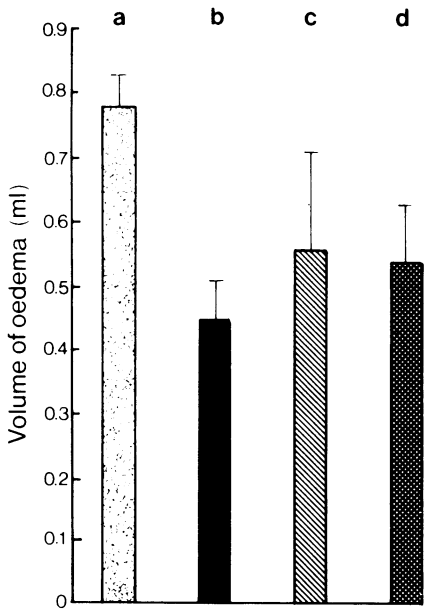

Fig. 2 Effect of crotalaburnine, hydrocortisone and phenylbutazone on carrageenin-induced oedema of the rat-paw. Ordinates: volume of oedema in ml. (a) control rats (n = 9); (b) hydrocortisone 10 mg/kg s.c. 1 h before (n = 8); (c) phenylbutazone 100 mg/kg oral 3 h before (n = 5); (d) crotalaburnine 10 mg/kg s.c. 1 h before carrageenin (n = 9). Vertical bars show s.e. mean.

Hyaluronidase-induced oedema

Table 1 presents the values for different groups both in normal and in adrenalectomized animals at the peak oedema (20 min) following hyaluronidase injection. In general there was evidence of inhibition of hyaluronidase-induced oedema in the normal rats after all three compounds (phenylbutazone, sodium salicylate and crotalaburnine). In adrenalectomized rats also phenylbutazone produced almost similar inhibition although this failed to reach the level of statistical significance because of wider variation and employment of small number of animals. In similar groups of animals sodium salicylate produced significantly less ($P < 0.05$) though definite inhibition when compared with the non-adrenalectomized rats. Crotalaburnine, on the other hand, produced slightly more inhibition in adrenalectomized than in normal rats. However, this difference in effect was not statistically significant.

5-Hydroxytryptamine, dextran, bradykinin and prostaglandin-induced oedema

The results are summarized in Table 2. Crotalaburnine produced about 15-20% inhibition of 5-hydroxytryptamine- and dextran-induced oedema which was not statistically significant. Cyproheptadine, however, produced significant inhibition of both oedemas. Against bradykinin- and prostaglandin-induced oedemas crotalaburnine produced about an equal degree of inhibition

Table 1 Effects of crotalaburnine, phenylbutazone and sodium salicylate on hyaluronidase-induced oedema of the rat-paw in normal and adrenalectomized rats

Drug	*No. of rats*	*Dose and route*	*Mean volume oedema (ml) with s.e.*	*% Inhibition*
Control	9		4.1 ± 0.27	
Phenylbutazone (normal rats)	6	100 mg/kg oral, 3 h before	2.9 ± 0.50	29***
Phenylbutazone (adrenalectomized)	5	100 mg/kg oral, 3 h before	3.2 ± 0.59	22 (NS)
Sodium salicylate (normal rats)	6	500 mg/kg i.p., 0.5 h before	1.6 ± 0.24	61*
Sodium salicylate (adrenalectomized)	6	500 mg/kg i.p., 0.5 h before	2.6 ± 0.32	37**
Crotalaburnine (normal rats)	6	10 mg/kg s.c., 2 h before	2.4 ± 0.50	42**
Crotalaburnine (adrenalectomized)	6	10 mg/kg s.c., 2 h before	1.8 ± 0.39	56*

Oedema was measured 20 min after hyaluronidase injection.
* $P < 0.001$; ** $P < 0.01$; *** $P < 0.05$; NS, not significant.

Table 2 Oedema induced in rat's hind paw by injection of various agents and inhibition by drugs

Oedema-inducing agents	*Drug*	*Dose and route*	*Mean volume oedema (ml) with s.e.*	*% Inhibition*
5-hydroxytryptamine	Saline		2.5 ± 0.25	
	Cyproheptadine	10 mg/kg oral, 2 h before	1.4 ± 0.32	44***
	Crotalaburnine	40 mg/kg s.c., 2 h before	2.0 ± 0.29	20 (NS)
Dextran	Saline		6.1 ± 0.33	
	Cyproheptadine	10 mg/kg i.p., 1 h before	1.8 ± 0.40	70*
	Crotalaburnine	20 mg/kg i.p., 1 h before	5.2 ± 0.46	15 (NS)
Bradykinin	Saline		0.9 ± 0.07	
	Crotalaburnine	20 mg/kg i.p., 1 h before	0.4 ± 0.08	56**
Prostaglandin	Saline		2.4 ± 0.37	
	Phenylbutazone	100 mg/kg i.p., 1 h before	2.2 ± 0.53	8 (NS)
	Crotalaburnine	20 mg/kg i.p., 1 h before	1.0 ± 0.29	58**

Oedema was recorded 20 min after 5-hydroxytryptamine and 30 min after dextran, bradykin and prostaglandin. There were 6 animals per group except with prostaglandin, where 10 animals were used.
* $P < 0.001$; ** $P < 0.01$; *** $P < 0.05$; NS, not significant.

which was statistically significant. Phenylbutazone, however, failed to produce any significant inhibition of prostaglandin-induced oedema.

Cotton-pellet granuloma

The results of the experiments on cotton-pellet granuloma are summarized in Figure 3. Both crotalaburnine and hydrocortisone produced about 45% inhibition of the granuloma formation ($P < 0.05$) while phenylbutazone produced about 70% inhibition ($P < 0.01$).

Discussion

Results suggest that crotalaburnine is effective only against acute oedema induced by a number of substances, such as carrageenin and hyaluronidase, but not against subacute formalin-induced arthritis. However, in the cotton-pellet granuloma test crotalaburnine was as effective as hydrocortisone. After adrenalectomy, the inhibitory effect of sodium salicylate against hyaluronidase-induced oedema was significantly lowered but not abolished. The inhibitory effect of phenylbutazone was almost the same in both groups although it failed to reach the level of significance because of the small number of

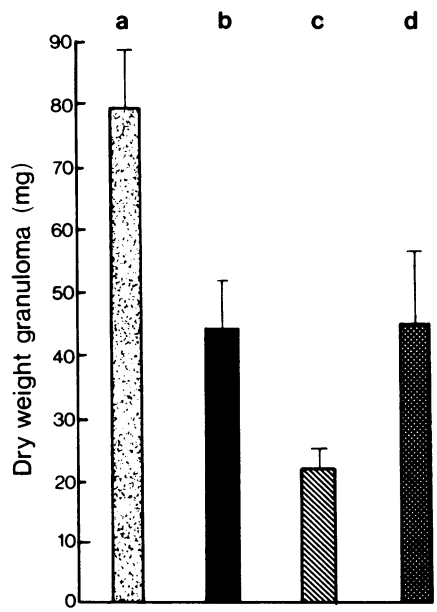

Fig. 3 Effect of crotalaburnine, hydrocortisone and phenylbutazone on cotton-pellet granuloma in rats. Ordinates: dry weight of granuloma in mg. (a) control rats; (b) hydrocortisone 40 mg/kg s.c. x 6 days; (c) phenylbutazone 100 mg/kg oral x 6 days; (d) crotalaburnine 20 mg/kg s.c. x 6 days. Six rats in each group. Vertical bars show s.e. mean.

animals in the adrenalectomized group as compared with the control. The effect of crotalaburnine, on the other hand, instead of being reduced was slightly but not significantly enhanced following adrenalectomy. These results suggest that in the dose-range used, neither phenylbutazone nor crotalaburnine act via the pituitary-adrenal axis, while sodium salicylate exerts its anti-hyaluronidase effect partly by a direct mechanism and partly through the pituitary-adrenal axis.

Although Snehalata & Ghosh (1968) demonstrated antagonism of crotalaburnine against histamine and 5-hydroxytryptamine-induced spasm of guinea-pig ileum, we failed to observe any significant inhibition of 5-hydroxytryptamine-induced oedema in the rat by the same compound. Similar failure of inhibition was also observed against dextran oedema which is supposed to be mediated in the rat through release of histamine and 5-hydroxytryptamine. However, it is interesting to note that crotalaburnine produces significant inhibition of both bradykinin- and prostaglandin-induced oedema while phenylbutazone in a dose five times greater than that of crotalaburnine failed to produce any significant inhibition of the latter. It has been suggested that a substance, which suppresses rat-paw oedema produced by a number of phlogistic agents, probably does so by virtue of its 'nonspecific irritant' effect at the site of injection rather than by a specific anti-inflammatory effect (Adams & Cobb, 1967). Although crotalaburnine possesses some local irritant property (personal observation), it seems an unlikely mechanism since it produced inhibition of the oedema produced by carrageenin, hyaluronidase, bradykinin and prostaglandin but not of that produced by 5-hydroxytryptamine or dextran.

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