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Effect of various drugs on carrageenin-induced oedema in the rat hind paw

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Local oedema in the rat hind paw is induced by subplantar injection of a 1% suspension of carrageenin. An assay procedure for the analysis of inhibitory effects of drugs on this inflammatory process is described. The results obtained with a single oral dose of 14 antirheumatic drugs (3 steroids and 11 non-steroids) and of 49 substances without established clinical antirheumatic value are reported. Only 8 compounds were found to be completely devoid of anti-carrageenin activity. Others were active at dose levels producing striking behavioural, autonomic or toxic effects. All clinically established antirheumatic substances were active in the carrageenin test at non-toxic doses producing no obvious behavioural or autonomic effects and data on dose-response relationship of these compounds are presented. It is concluded that the assay in its present form is an acceptable preliminary screening test for antirheumatic activity.

CARRAGEENIN is a sulphated polygalactose extracted from the marine alga *Chondrus crispus* (Irish sea moss). It is a complex mixture of at least 5 different polysaccharides. Its two main components have been designated kappa and lambda fractions (Smith, O'Neill & Perlin, 1955). Different authors found preparations of carrageenin to possess inflammatory properties in laboratory animals and the active fraction in inflammation has been identified as the lambda component (McCandless, 1962). Carrageenin-induced oedema in the hind paw of the rat, as an assay for anti-inflammatory drugs was introduced by Winter, Risley & Nuss (1962, 1963).

We describe the experimental details of the carrageenin-test in rats as used by us and report upon the inhibitory effects obtained with this procedure using a variety of well-known steroid and non-steroid antirheumatic drugs, and other pharmacodynamic agents without established clinical antirheumatic action.

Experimental

METHOD

A modification of the method described by Winter & others (1962) was used. Young male Wistar rats of 195 ± 10 g body weight were maintained in an air-conditioned room (temp. 22 ± 1°; relative humidity: 65 ± 15%). Food was withdrawn 16 hr before the start of the experiment. Tap water was withheld during the experiment only.

The carrageenin used is coded Seakem 402 AP,* it is a predominantly lambda carrageenin and, out of 10 commercially available carrageenins, it was found to be the most active in inducing inflammation (Atkinson, Jenkins, Tomich & Woollett, 1962). A 1% suspension in 0·9% saline was prepared 1 hr before each experimental session, and a volume of

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0·05 ml was injected into the plantar side of both hind paws of the rats.

Drugs in aqueous solution or suspension (. . . 0·31, 0·63, 1·25 . . . 160 mg/kg) were administered by stomach tube in a volume of 1 ml/100 g body weight, followed immediately by tap water to a total of 5 ml/rat. Controls received 5 ml tap water only. This ensured uniform hydration in all rats and minimised the variability of oedematous responses in the paws (Winter & others, 1962).

Drugs or solvent were given 1 hr before the carrageenin treatment and the degree of the carrageenin-induced swelling of the hind paws was measured 3 hr after the carrageenin-treatment.

The apparatus for measuring foot volume was a commercially available electric antiphlogmeter developed by Kemper & Ameln (1959).

The degree of swelling is the ratio a/b, where "b" is the total volume of both hind paws before, and "a" the total volume of both hind paws after carrageenin treatment. Since it was found that there was a lack of correlation between the volume of both paws before and after carrageenin treatment ($\chi^2 = 0{\cdot}28$; $P > 0{\cdot}50$) the mean value "b" was calculated for 500 control experiments and preferred to the true "b" value obtained in each animal. In 500 control rats (1,000 paws) the mean ratio a/b was 2·0 (swelling equal to 100%). A ratio a/b $\leqslant 1{\cdot}5$ (swelling less than or equal to 50%) after drug administration was considered as a significant inhibitory effect of the drug. On this basis using different dose levels and 6 rats per dose level (2 groups of 3 rats on different days), the usual quantal assay procedure was employed. The dose producing a ratio of a/b $\leqslant 1{\cdot}5$ in 50% of the treated animals (ED 50 in mg/kg), the 95% confidence limits (LL and UL), the slope (S) and the slope function (fS) were determined by the graphic log-probit method of Litchfield & Wilcoxon (1949).

Results

CONTROL EXPERIMENTS

The degree of swelling of the carrageenin-injected paws was maximal 3 hr after injection and the mean increase in volume at that time was about 100% (a/b $\sim 2{\cdot}0$). The time-effect curve obtained in 15 rats (30 paws) is shown in Fig. 1. The 100% increase of the paw volume 3 hr after carrageenin injection remained fairly constant; in 500 control rats (1,000 paws), injected over a period of about one year, the mean increase was exactly 100% (mean ratio a/b = 2·0). As shown in Fig. 2 there was no remarkable difference between the right and the left paws of the rats and an increase less than or equal to 50% (a/b $\leqslant 1{\cdot}5$) was observed in only 3·1% of the control animals. Furthermore for 75% of the control animals the degree of swelling after carrageenin treatment was between 80% (a/b = 1·8) and 120% (a/b = 2·2) compared to the uninjected paws of the same animals (Fig. 2).

ANTI-INFLAMMATORY COMPOUNDS

The results obtained with a series of 14 so-called antirheumatic drugs (3 steroids and 11 non-steroids) in the carrageenin-induced oedema test

are shown in Table 1. No obvious behavioural or autonomic effects were observed with these drugs at the highest dose levels tested (Fig. 3). All clinically established antirheumatic drugs seem to possess anti-carrageenin activity.

TABLE 1. ANTI-CARRAGEENIN EFFECTIVENESS OF 14 ANTIRHEUMATIC DRUGS

Substances	ED 50	LL*	UL	S	fS	n
Indomethacin	2·2	1·2	3·8	1·67	1·41	30
Mefenamic acid	9·0	4·3	19	3·20	1·85	48
Flufenamic acid	10	4·8	21	4·32	3·25	36
Phenylbutazone	25	14	47	2·15	1·56	36
Amidopyrine	31	20	49	1·77	1·35	30
Phenacetin	57	39	83	1·60	1·22	30
Aspirin	72	50	104	1·39	1·20	24
Phenazone	87	53	142	1·87	1·58	24
Acetaminophen	88	53	145	1·56	1·52	18
Cinchophen	92	70	121	1·27	1·14	18
Sodium salicylate	98	65	148	1·69	1·24	18
Paramethasone	0·057	0·042	0·077	1·46	1·18	30
Hydrocortisone	30	16	57	3·10	1·96	36
Cortisone	40	28	57	1·37	1·16	24

* See page 811.

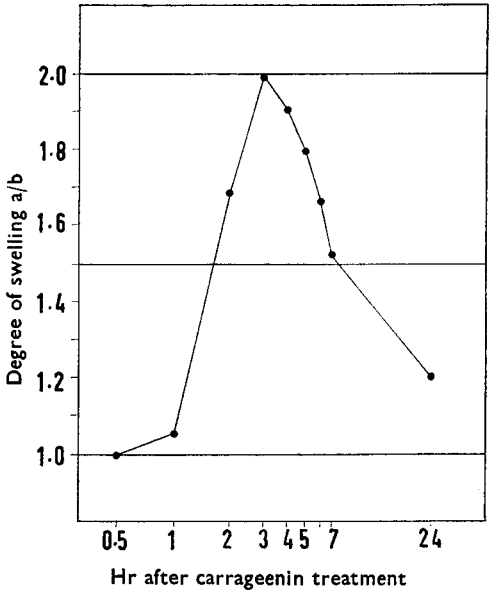

FIG. 1. Local carrageenin-induced oedema in the rat hind paw. Carrageenin 1% in 0·9% saline; 0·05 ml subplantar in both hind paws. Time effect curve obtained in 15 controls (30 paws).

OTHER DRUGS

To study the specificity of the carrageenin-test, 49 miscellaneous drugs of different pharmacological classes were administered orally at different

dose levels to groups of (2 × 3) rats. The results are summarised in Table 2.

Most of the active compounds of this group were found to antagonise the carrageenin-induced swelling reaction at dose levels producing overt

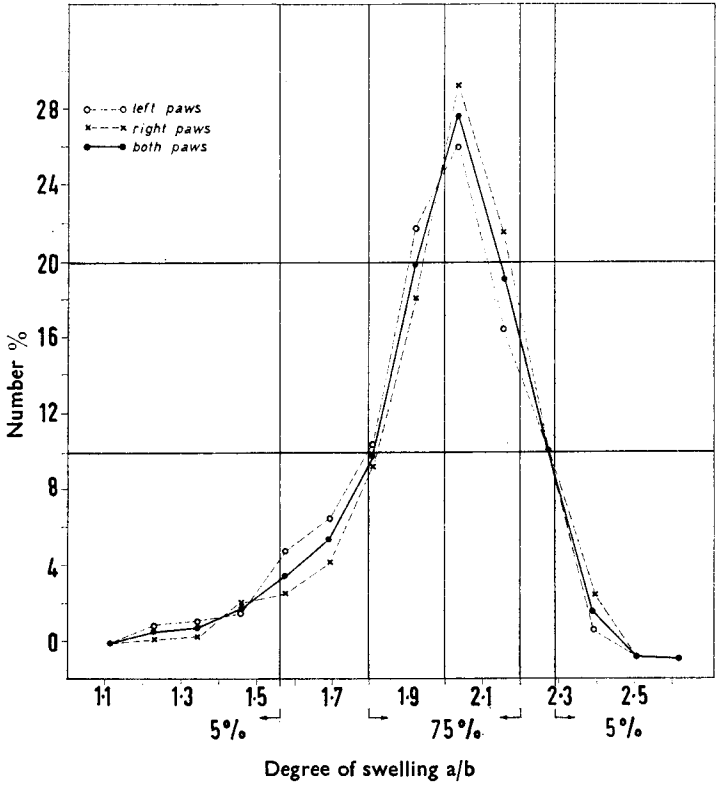

FIG. 2. Local carrageenin-induced oedema in the rat hind paw. Carrageenin 1% in 0·9% saline; 0·05 ml subplantar. Frequency distribution of the degree of swelling obtained in 500 control rats, i.e. 1000 paws.

behavioural changes or various autonomic effects such as mydriasis or both. Obviously anti-carrageenin activity is by no means a rare pharmacological property and as such insufficient evidence for potential usefulness in the treatment of rheumatic disorders.

Discussion

Oral administration was adopted for all compounds, thus largely avoiding the so-called "counter-irritant effect" a phenomenon described by Benitz & Hall (1963), defined by them as a competitive reaction to two or more equal or different stimuli at two or more different locations.

Only 8 compounds were found to be virtually devoid of anti-oedema

TABLE 2. ANTI-CARRAGEENIN EFFECTIVENESS OF 49 MISCELLANEOUS DRUGS, WITHOUT ESTABLISHED CLINICAL ANTIRHEUMATIC VALUE, BELONGING TO DIFFERENT PHARMACOLOGICAL CLASSES

Substances	Class	ED 50	LL	UL	S	fS	n
Diphenoxylate	Analgesics	2·5	1·6	4·0	1·52	1·25	30
Fentanyl		3·5	2·6	4·7	1·44	1·12	24
Dextromoramide		3·6	2·7	4·7	1·41	1·30	12
Piritramide (1)		17	14	21	1·21	1·08	18
Morphine		20	13	32	2·07	1·62	24
Codeine		40	22	72	1·70	1·45	30
Triperidol	Neuroleptics	4·2	2·7	6·5	1·74	1·44	24
Haloperidol		4·6	2·6	8·2	2·43	1·79	30
Reserpine		9·2	4·9	17	1·74	1·66	30
Chlorpromazine		14	9·4	21	1·67	1·48	18
Droperidol (2)		17	11	27	1·76	1·60	18
Fluphenazine		19	12	30	1·55	1·37	24
Spiroperidol		29	16	51	2·05	1·81	24
Pentobarbitone	Hypnotics	40	not linear				12
Phenobarbitone		>160	—	—	—	—	6
Diphenhydramine	Antihistamines	75	46	121	1·86	1·58	24
Pyrilamine		80	not linear				30
Cinnarizine		⩾160	—	—	—	—	6
Chlordiazepoxide	Tranquillising muscle relaxants	73	56	95	1·26	1·11	18
Meprobamate		>160	—	—	—	—	6
Hydroxyzine	Sedatives	160	—	—	—	—	6
Thalidomide		>160	—	—	—	—	6
Norimipramine	Antidepressants	42	not linear				30
Imipramine		70	not linear				30
Amitryptiline		92	57	149	1·86	1·80	18
Phencyclidine	Halucinogenics	7·1	4·2	12	1·94	1·46	30
Mescaline		67	52	86	1·25	1·11	18
Atropine	Anti-cholinergics	61	54	111	2·14	1·95	24
Benactyzine		160	—	—	—	—	6
Benzetimide (3)		⩾160	—	—	—	—	6
Tranylcypromine	MAO inhibitors	5·0	2·3	11	3·31	2·12	42
Iproniazid		94	49	182	2·32	2·23	24
Diphenylhydantoin	Anticonvulsant	>160	—	—	—	—	6
Cyclopenthiazide	Diuretics	65	40	105	1·84	1·77	18
Hydrochlorothiazide		68	31	145	3·93	2·66	36
Chlorothiazide		⩾160	—	—	—	—	6
Amphetamine	CNS-stimulants	1·8	1·0	3·2	2·07	1·75	36
Caffeine		66	not linear				30
Tryptamine		160	—	—	—	—	6
Apomorphine		>160	—	—	—	—	6
Aceperone (4)	Hypotensive drugs	26	18	37	1·58	1·37	18
Guanethidine		52	33	81	1·76	1·44	24
Acoxatrine (5)		80	60	107	1·30	1·17	18
Hexamethonium		>160	—	—	—	—	6
Cocaine	Local anaesthetics	40	19	84	1·94	1·72	36
Xylocaine		90	69	118	1·27	1·08	24
Procaine		>160	—	—	—	—	6
Prozapine (6)	Papaverine-like compounds	160	—	—	—	—	6
Papaverine		⩾160	—	—	—	—	6

Also known as (1) pirinitramide (Janssen, 1961); (2) dehydrobenzperidol (Janssen, Niemegeers, Schellekens, Verbruggen & Van Nueten, 1963); (3) dioxatrine (Niemegeers & Janssen, 1964); (4) acetabuton (Schaper, Jageneau, Xhonneux, 1962); (5) acetoxatrine (Schaper, Jageneau & Janssen, 1963); (6) hexadiphane (Gaussen, 1962).

properties at the highest dose levels tested (ED 50 >160 mg/kg oral). These were: apomorphine, diphenylhydantoin, hexamethonium, meprobamate, papaverine, phenobarbitone, procaine and thalidomide.

Three compounds possessed a slight inhibitory effect (ED 50 $\geqslant$160 mg/kg oral): chlorothiazide, cinnarizine and benzetimide (a/b $\leqslant$1·5 in 2/6 animals). For 4 other drugs—benactyzine, prozapine, hydroxyzine and tryptamine—a significant inhibitory effect was found in 50% of the animals at the 160 mg/kg dose level (ED 50 ~160 mg/kg oral; a/b $\leqslant$1·5 in 3/6 animals).

As seen in Table 1 all compounds of interest in the treatment of inflammation were found to be effective in the carrageenin-test at non-toxic

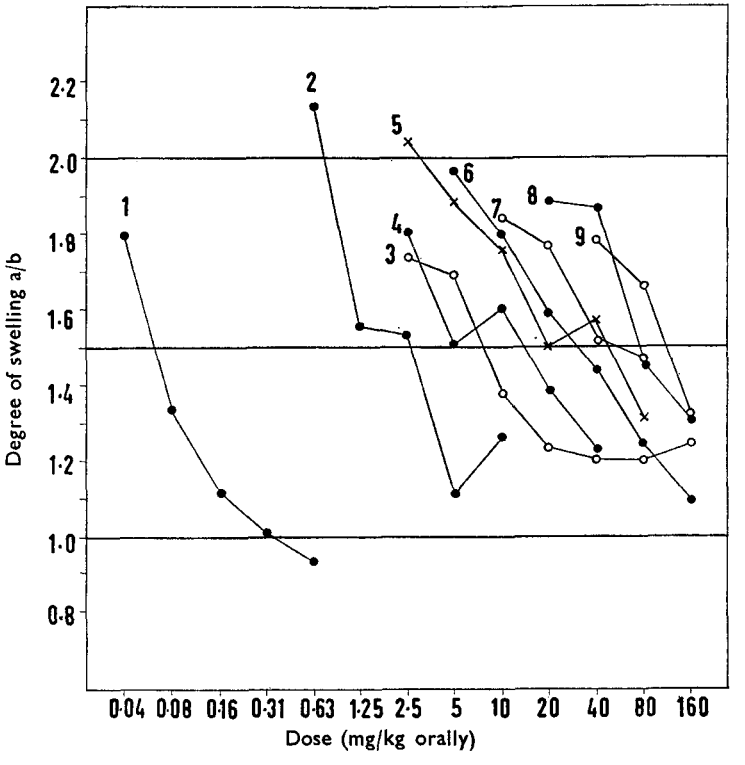

FIG. 3. Dose-effect curves for anti-carrageenin activity. Paramethasone (1), indomethacin (2), mefenamic acid (3), flufenamic acid (4), hydrocortisone (5), phenylbutazone (6), cortisone (7), acetylsalicylic acid (8), sodium salicylate (9).

dose levels. Satisfactory dose response curves (Fig. 3) were obtained with a relatively small number of rats.

It is concluded that the carrageenin-test in its present form is an acceptable screening assay for antirheumatic activity. Compounds showing effectiveness at low atoxic dose levels and producing no obvious behavioural or autonomic effects at relatively high (e.g. 4 times ED 50) dose levels merit further investigation as potentially useful antirheumatic drugs.

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