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Inhibition by non-steroidal anti-inflammatory agents of the release of rabbit aorta contracting substance and prostaglandins from chopped guinea-pig lungs

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The release of prostaglandins (PGs) and rabbit aorta contracting substance (RCS) was investigated using mechanical agitation of chopped lung tissue from unsensitized guinea-pigs. Manual stirring of the lung tissue for 45 s produced maximal release of prostaglandins, a release corresponding to the effect of [20–40 ng ml^{-1} of PGE_2 × 45 s at 5 ml min^{-1}] about 100 ng PGE_2 on the rat stomach strip. Non-steroidal anti-inflammatory agents inhibited the release of the contracting substances. Indomethacin, the most potent, was active in concentrations of about 20 ng ml^{-1}, whereas flufenamic acid was twice, phenylbutazone about 60 times, acetylsalicylic acid 100 times and sodium salicylate about 6000 times less active than indomethacin. The method could prove to be a simple test for screening non-steroidal anti-inflammatory agents for inhibition of prostaglandin synthesis.

In 1969 Piper & Vane described the release of a rabbit aorta contracting substance (RCS) during anaphylaxis in guinea-pig isolated sensitized lungs and showed that this release could be antagonized by anti-inflammatory agents. RCS and prostaglandins (PGs) have since been shown to be released from different tissues by anaphylaxis, by mechanical agitation or by different pharmacologically active agents (Piper & Vane, 1969; Palmer, Piper & Vane, 1970; Gryglewski & Vane, 1972a). The biosynthesis and release of PGs is accompanied by the release of RCS (Piper & Vane, 1969). Gryglewski & Vane (1972a) concluded that RCS is an unstable intermediate in the biosynthesis of PGs. Samuelsson, Granström & Hamburg (1967) and Samuelsson (1973) have shown that a cyclic endoperoxide is an intermediate in biosynthesis of PGs, and to-day there is much evidence that the unstable RCS is a cyclic endoperoxide (Gryglewski & Vane, 1972b).

I have investigated a simple *in vitro* method (Palmer & others, 1970) in which isolated chopped unsensitized guinea-pig lungs were used for studying the release of RCS and PGs, and compared the ability of some non-steroidal anti-inflammatory agents to inhibit the release of RCS and PGs in this test model.

MATERIAL AND METHODS

Unsensitized guinea-pigs of either sex, 400–600 g, were used. The lungs were removed and perfused free of blood. The lung tissue was then chopped with scissors into pieces of approximately 2 mm^3, washed and placed in a stainless steel wire-mesh basket (mesh size: 0·5 mm). Krebs solution was dripped through the chopped lung at a rate of 5 ml min^{-1}.

The Krebs solution was gassed with oxygen containing 5% carbon dioxide and contained atropine sulphate (100 ng ml^{-1}), mepyramine maleate (100 ng ml^{-1}), methysergide bimaleate (200 ng ml^{-1}), phenoxybenzamine hydrochloride (100 ng ml^{-1}) and propranolol hydrochloride (2 μg ml^{-1}), to block the effects of acetylcholine, histamine, 5-HT and α- and β-adrenoceptor agonists (Gilmore, Vane & Wyllie, 1968).

The effluent from the lung tissue superfused a rabbit aorta (Furchgott & Bhadrakom, 1953) and a rat stomach strip (Vane, 1957). The spirally cut aorta strip from a male rabbit was used for detection of RCS (Piper & Vane, 1969) and the rat stomach strip for detection of PGs (Gilmore & others, 1968).

Release of spasmogenic substances was induced by mechanical agitation of the lung tissue with a blunt rod for 15 to 75 s.

The contraction produced by different concentrations of PGE_2 directly applied to the stomach strip was compared to that obtained after mechanical agitation of the lung tissue. The contractions of the isolated tissues were recorded by Ugo Basile isotonic transducers loaded with 1·2 g (the stomach strip) and 4 g (the aorta).

Drugs

The following compounds were investigated: acetylsalicylic acid, flufenamic acid, indomethacin, phenylbutazone, sodium salicylate and prostaglandin E_2. Indomethacin was dissolved in 0·03M sodium carbonate and the solution adjusted to pH 7·4.

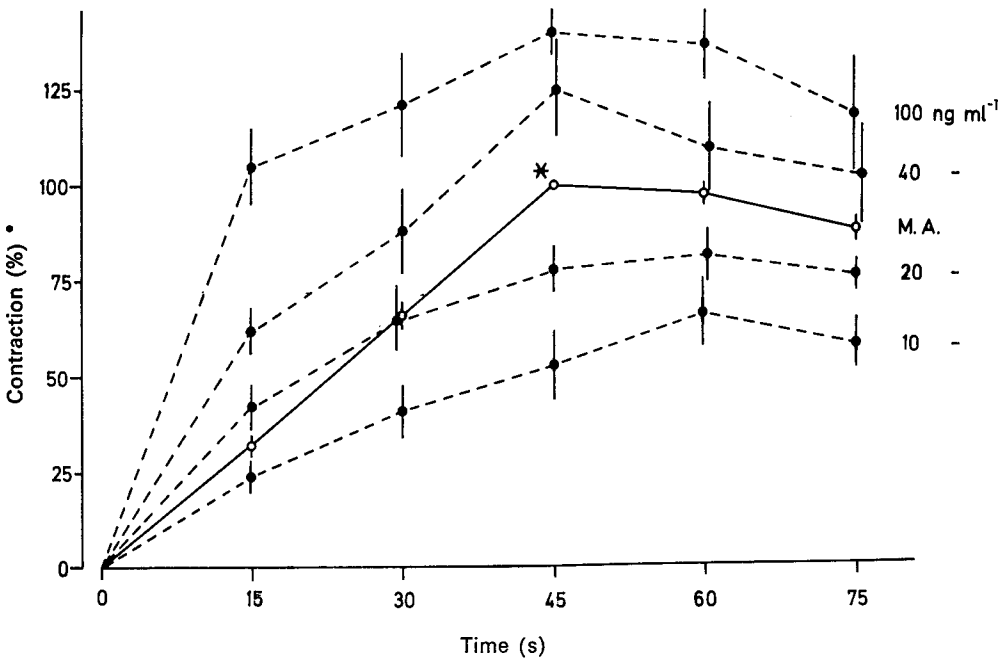

FIG. 1. The response of rat stomach strip to the effluent from mechanically agitated lung tissues from unsensitized guinea-pigs and to PGE_2. The chopped lung tissue was manually agitated for periods of 15 to 75 s and the effluent superfused a rat stomach strip. PGE_2 was directly applied to the stomach strip in concentrations of 10, 20, 40 and 100 ng ml^{-1} for 15 to 75 s. ○——○ Manual agitation ●- - ● PGE_2. *All values are expressed as per cent of the contraction of the stomach strip to 45 s of mechanical agitation. Vertical bars indicate s.e. Each point represents the mean of 4–21 experiments.

Table 1. *The effect of some anti-inflammatory agents on the* in vitro *release of RCS and PGs, caused by mechanical agitation for 45 s of chopped lung tissue from unsensitized guinea-pigs.*

	Concentration $\mu g\ ml^{-1}$	Rabbit aorta % inhibition in response	Rabbit aorta IC50* $\mu g\ ml^{-1}$	Rat stomach strip % inhibition in response	Rat stomach strip IC50* $\mu g\ ml^{-1}$
	0·010	42		19	
	0·020	65	0·012	28	0·034
Indomethacin	0·040	76	(0·008–0·018)	66	(0·028–0·040)
	0·080	79		72	
	0·160			86	
	0·016	31		13	
	0·031	50		33	
Flufenamic acid	0·062	74	0·030	59	0·069
	0·125		(0·025–0·035)	63	(0·056–0·084)
	0·250			73	
	0·50			36	
Phenylbutazone	1·00	40	1·23	50	0·91
	2·00	74	(1·07–1·41)	71	(0·72–1·16)
	8·00	100		91	
	1·00	43	1·53	27	1·93
Acetylsalicylic acid	2·00	50	(1·17–2·00)	62	(1·56–2·40)
	4·00	75		64	
	16	20		16	
	64	39	106	41	105
Sodium salicylate	128	46	(80–140)	50	(81–135)
	256	73		71	

* IC50 values (the concentration of drug which inhibits the contraction of the rabbit aorta or the rat stomach strip by 50%) were determined by probit analysis and the 95% confidence limits are listed in brackets. Each value represents the mean of 4–6 experiments.

Phenylbutazone was purchased as ampoules. Flufenamic acid was dissolved in the equivalent amount of sodium hydroxide. The other compounds studied (Table 1) were water soluble in the concentrations used. The final solutions were made with Krebs solution.

Results

Fig. 1 shows that a PG-like substance was released from the lung tissue by agitation of the lung tissue for different lengths of time (15–75 s). In the figure the contraction of the stomach strip by PGE_2 applied for different periods is expressed relative to that induced by a 45 s agitation period (*) taken as 100. The contraction is maximal after 45 s of agitation and when this is done at 20 min intervals it is possible to obtain 10–12 equal contractions of the stomach strip.

Agitation of the lung tissue for between 30 and 60 s causes a contraction of the stomach strip which is bracketed by those produced by perfusion with PGE_2 20–40 $ng\ ml^{-1}$.

Five known non-steroidal anti-inflammatory agents inhibit the release of PGs and RCS in the above-mentioned test, as shown in Table 1. The agents were added to the superfusion fluid for a period of 20 min before agitation of the chopped lung and their ability to inhibit release of PGs and RCS was calculated as an IC50 after probit conversion of the changes in contraction of the test tissues.

Indomethacin is very potent in inhibiting release of both RCS and PGs, flufenamic acid is about half as potent, phenylbutazone 60 times, acetylsalicylic acid 100 times and sodium salicylate 6000 times less active than indomethacin. The dose-response relation for all substances tested showed no significant difference in slope suggesting a similar mode of action.

DISCUSSION

Mechanical agitation of chopped lung tissue from unsensitized guinea-pigs induces aorta and stomach strip contraction, which is assumed to be due to RCS and PGE_2 as shown by Gryglewski & Vane (1972a). Mechanical agitation for 45 s produces a maximal release of activity, which corresponds to the contracting activity of about 100 ng of PGE_2.

Anti-inflammatory agents have been shown to antagonize the release of RCS and PGs in a variety of tissues by different stimuli, these effects are due to an inhibition of the synthesis of the prostaglandins (Ferreira, Moncada & Vane, 1971) at the stage before formation of RCS (Gryglewski & Vane, 1972b; see also Piper & Vane, 1969; Palmer & others, 1970; Vane, 1971; Gryglewski & Vane, 1972a; Tomlinson, Ringold & others, 1972; Piper & Walker, 1973; Ferreira, Moncada & Vane, 1971, 1973).

In the present study non-steroidal anti-inflammatory agents, which are active in the usual laboratory models of inflammation as well as clinically, have been assigned an IC50 for their ability to block the release of active agents from guinea-pigchopped lung. According to other authors this effect is probably an index of inhibition of the synthesis of RCS and PGs. The present data suggest that the method employed might be suitable for screening new compounds for ability to inhibit prostaglandin synthesis.

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