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The contractile action of slow reacting substance of anaphylaxis (SRS-A) on rat isolated fundic strip and guinea-pig isolated ileum and its antagonism by FPL 55712

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Piper & Vane (1969) reported that the rat superfused fundic strip preparation was contracted by slow reacting substance of anaphylaxis (SRS-A), now known to consist of a mixture of leukotrienes (Samuelsson 1981). We have investigated the contractile actions of SRS-A on rat fundic strip and compared them with those on guinea-pig isolated ileum, the tissue on which SRS-A is usually assayed (Brocklehurst 1962). Further, we have compared the actions of the SRS-A antagonist, FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-benzopyran-2-carboxylic acid) (Augstein et al 1973) on SRS-A-induced contractions of guinea-pig ileum and rat fundus.

Methods

Rat fundic strip and guinea-pig ileum preparations were prepared as described by Vane (1957) and Horton & Main (1965) respectively. Preparations were mounted for cascade superfusion (Vane 1964). This technique allowed four preparations to be dosed with the same solution of SRS-A thus ensuring the most efficient use of a limited amount of material. Preparations were superfused with Tyrode solution at 37 °C containing atropine (1×10^{-6} mol litre^{-1}), mepyramine (3×10^{-6} mol litre^{-1}) and indomethacin (3×10^{-6} mol litre^{-1}) as described previously (Coleman et al 1979). Changes in tension were measured isometrically. Doses of SRS-A were infused into the superfusion fluid in a volume of 150 μl over 15 s. FPL 55712 was infused into the superfusion fluid such that the first preparation served as an untreated control and the remaining three preparations received cumulatively increasing concentrations of antagonist to give the final concentrations quoted. Three concentrations of FPL 55712 were tested in each experiment and a pA_2 value was determined from the data obtained in individual experiments as described by Arunlakshana & Schild (1959).

The following drugs were used: atropine sulphate (Sigma), histamine acid phosphate (BDH), 5-hydroxytryptamine creatinine sulphate (Sigma), indomethacin (Merck, Sharp & Dohme) and prostaglandin E_2 (Ono). SRS-A was prepared from rat peritoneal anaphylactic fluid as previously described (Whelan 1980 a,b). FPL 55712 was kindly donated by Mr P. Sheard of Fisons Limited.

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Results

Responses of guinea-pig ileum and rat fundus to SRS-A. Partially purified SRS-A (1–1000 units) caused dose-related contractions of guinea-pig superfused ileum and rat superfused fundus. The sensitivity of the two preparations were similar (Fig. 1). Contractions of ileum and fundus were rapid in onset, recovery being complete within 10 min. Responses of both preparations were reduced or abolished following incubation of SRS-A with arylsulphatase (Orange et al 1974) or soybean lipoxygenase (Sirois 1979), procedures which inactivate SRS-A.

Effect of indomethacin on responses of preparations to SRS-A. In some experiments preparations were superfused with Tyrode solution containing no indomethacin. In these experiments SRS-A dose-effect curves were obtained in the presence and absence of indomethacin (1×10^{-6} mol litre^{-1}). The data obtained, demonstrate that indomethacin has no effect on SRS-A-induced contractions of guinea-pig ileum or rat fundus.

Effect of FPL 55712 on responses of preparations to SRS-A. In two preliminary experiments the SRS-A antagonist FPL 55712, at a concentration of 1×10^{-6} mol litre^{-1}, did not antagonize SRS-A-induced contractions of rat fundus preparations whereas those of ileum were antagonized (Fig. 2). In subsequent studies FPL 55712 (1×10^{-7}–3×10^{-5} mol litre^{-1}) blocked responses of both preparations to SRS-A. Owing to limited supplies of SRS-A, it was rarely possible to obtain complete dose-effect curves in the presence of FPL 55712. However, so far as could be determined, FPL 55712 produced parallel shifts of the dose-effect curve for SRS-A and therefore dose-ratios were measured on the linear part of the dose-effect curves and pA_2 values were calculated by the method of Arunlakshana & Schild (1959). When an antagonist contact time of 10 min was used a pA_2 value of 7·25 (95% confidence limits, 7·04–7·5, n = 5) was obtained on the ileum. However, the corresponding value on the fundus was only 5·74 (5·43–6·05, n = 5). When the contact time was increased to 60 min pA_2 values of 7·40 (7·10–7·70, n = 6) and 6·90 (6·73–7·05, n = 8) were obtained on ileum and fundus respectively. These

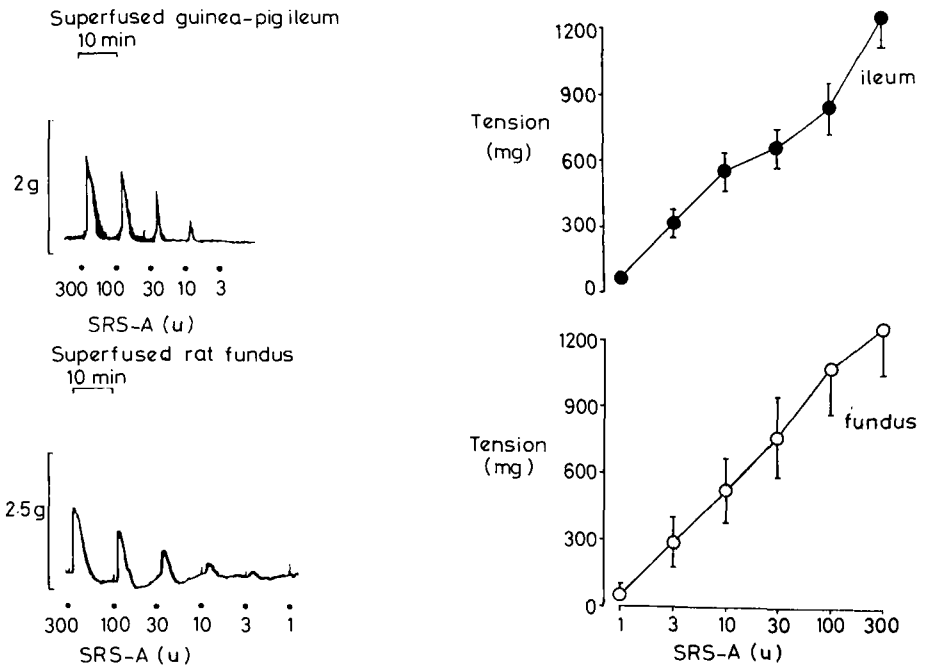

FIG. 1. The contractile actions of SRS-A on the guinea-pig isolated ileum and the rat isolated fundic strip. Each point on the graphs represents the mean ± s.e. from four preparations.

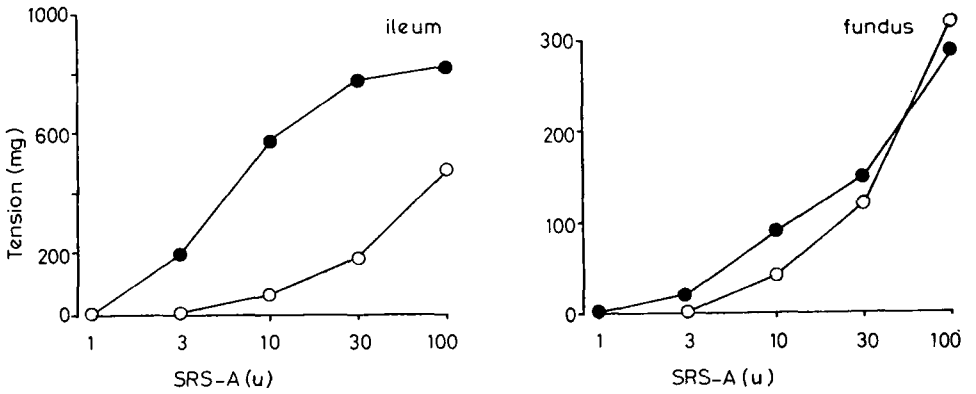

FIG. 2. Antagonism of SRS-A-induced contractions of guinea-pig ileum and rat fundus by FPL 55712 (10^{-6} mol litre^{-1}) following a 10 min antagonist contact time (—●— control and —○—10^{-6} mol litre^{-1} FPL 55712). Each point is the mean of two determinations.

values are significantly different ($P < 0.05$). In one further experiment on the fundus using a contact time of 120 min no further increase in the pA_2 was seen, a value of 6·5 being obtained. In all cases cited above the slopes of the Schild plot were not significantly different from unity ($P > 0.05$).

Specificity of FPL 55712. The highest concentration of FPL 55712 tested on guinea-pig ileum preparations (3×10^{-6} mol litre^{-1}) had no effect on contractions induced by PGE_2 or 5-HT. Similarly 3×10^{-5} mol litre^{-1} FPL 55712 and a 10 min contact time or 3×10^{-6} mol litre^{-1} FPL 55712 and a 60 min contact time had no effect on PGE_2 or 5-HT-induced contractions of rat fundus preparation.

Discussion

The rat fundic strip resembles the guinea-pig ileum in that it is contracted by SRS-A, the sensitivities of the two preparations being similar. The cyclo-oxygenase inhibitor indomethacin (Vane 1971) had no effect on SRS-A-induced responses of ileum or fundic strip preparations indicating that these responses are not

mediated indirectly via the release of cyclo-oxygenase products unlike some other SRS-A induced responses (Piper et al 1981).

Responses of both preparations to SRS-A were blocked by FPL 55712, however, our results demonstrate two differences in the effects of FPL 55712 on these preparations. Firstly, FPL 55712 is a less potent antagonist on the fundus than on the ileum and secondly, whereas a contact time of 10 min was sufficient to achieve equilibrium on the ileum, 60 min was necessary on the fundus. One possible explanation for the potency difference is that the receptors in the ileum are different from those in the fundus. However, there are many factors unrelated to receptor differences which can influence the potency of antagonists, for example the presence of inactivation processes for the agonist (Furchgott 1972), and further quantitative studies with synthetic leukotrienes will be necessary to resolve this question.

The finding that FPL 55712 equilibrates more rapidly on the ileum than on the fundus has implications for the use of this compound as a pharmacological tool. FPL 55712 has been shown to be a potent antagonist of the actions of SRS-A on guinea-pig ileum using contact times as short as 15 s (Augstein et al 1973). Our data show that not only does the potency of FPL 55712 vary between tissues but the equilibration time also varies. Therefore when this compound is used as a tool to investigate the involvement of leukotrienes in anaphylactic reactions (Chand 1979) it will be necessary to establish both the potency and contact time for the tissue under study before valid conclusions can be drawn.

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Behavioural actions of neuroleptics are not reduced by hypophysectomy

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The behavioural effects and biochemical changes in cerebral dopamine systems caused by sulpiride treatment testify to benzamide action on the central nervous system. Yet, notwithstanding these indices of changed brain activity, sulpiride has a very low penetration into brain tissue (Benakis & Rey 1976) and it has been considered that sulpiride may influence structures outside the blood-brain barrier, such as the pituitary, to indirectly modify central events (Portaleone et al 1978). Thus, we have studied the effects of hypophysectomy on both the behavioural effects and brain penetration of neuroleptic drugs.

Method

Normal, sham-operated and hypophysectomized male Sprague-Dawley (CD) rats were obtained from Charles River UK Ltd and were maintained under identical conditions except that the hypophysectomized rats received a supplement of sodium chloride in their drinking water. The success of hypophysectomy was indicated by the maintenance of a constant body weight by the lesioned rats (127·7 ± 2·6 g; the weights of sham-operated animals being 329·6 ± 4·3 g), and absence of pituitary tissue on histological examination at completion of the studies. For behavioural testing, carried out between 08.00 and 18.00 h, animals were taken from normal housing of 6 per cage and allowed 30 min to adapt to individual observation/testing cages. The presence of catalepsy was detected by placing animals' front limbs over a horizontal bar; the intensity of catalepsy was determined as the time an animal

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