Br. J. Pharmac. (1979), **66**, 385–389

INVOLVEMENT OF SRS-A IN THE SCHULTZ-DALE RESPONSE OF THE GUINEA-PIG SMALL INTESTINE

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1 The anaphylactic reaction of the guinea-pig ileum, the so called Schultz-Dale reaction, shows a biphasic response: a short rapid contraction followed by a partial relaxation and a slow contractile response.

2 Dose-response curves with ovalbumin as an antigen were obtained for the quick and slow contraction of this anaphylactic reaction.

3 Mepyramine (1 μg/ml) blocked the rapid first contraction, but failed to abolish the slow one in about 50% of the animals studied.

4 The SRS-A antagonist, FPL 55712, significantly depressed the slow sustained contraction during the Schultz-Dale reaction. Disodiumcromoglycate was without effect on both phases when it was added 5 min before addition of the antigen. However, when added simultaneously with the antigen it produced a 30% suppression of the slow phase in the highest concentration used.

Introduction

Dale & Zilletti (1970) reported a specific pattern of contraction of the whole ileum from guinea-pigs sensitized to ovalbumin during the Schultz-Dale reaction: a short rapid contraction followed by a partial relaxation and a slow contractile response. The first phase is mainly due to the liberation of histamine since it can be specifically blocked by H_1-antagonists (Hawkins & Rosa, 1956). However, the possible mediators of the slow response are not yet fully understood although it was recently reported that acetylcholine and 5-hydroxytryptamine are not involved (Laekeman, Herman & Van Nueten, 1977; Laekeman & Herman, 1978).

Slow reacting substance of anaphylaxis (SRS-A) is released from sensitized guinea-pig lungs (Brocklehurst, 1960; Piper & Vane, 1969) and produces a slow contraction of the guinea-pig ileum resembling the slow second phase of the Schultz-Dale reaction. We studied the release of this mediator from the guinea-pig ileum during anaphylaxis and its possible relationship with the different phases of the contractile response of this organ.

Methods

Anaphylactic reaction

Guinea-pigs (350 to 450 g) of either sex were sensitized to ovalbumin as previously described (Laekeman *et al.*, 1977). From the fourteenth day on, they were killed by a blow on the head; pieces of terminal ileum (approximately 6 cm long) were removed and suspended in a 50 ml organ bath containing Tyrode solution at 37°C and bubbled with 5% CO_2 in O_2. The Tyrode solution had the following composition in g/l (mM): KCl 0.2 (2.7), $MgSO_4.7H_2O$ 0.26 (1.06), $NaH_2PO_4.2H_2O$ 0.065 (0.42), $CaCl_2.2H_2O$ 0.503 (3.42), NaCl 8 (136.8), $NaHCO_3$ 1 (11.9) and glucose monohydrate 1 (5.55).

Contraction of the intestinal segment was detected by an auxotonic lever (Paton, 1957) attached to a Harvard Smooth Muscle Transducer (load 0.75 g) and recorded on a Bryans 28000, 2 channel recorder.

The tissues were allowed to stabilize for 20 min. Histamine (0.5 μg/ml) was then given until reproducible contractions were obtained. Drugs were given before or during the challenge with ovalbumin. The height of the last histamine contraction was taken as 100% contraction, and the two phases of the Schultz-Dale reaction were calculated as a percentage of this contraction. These values were compared with simultaneously studied control strips which did not receive any antagonist either before or during the challenge with ovalbumin.

Extraction and assay of SRS-A

The method of Burka (1976), slightly modified, was

0007-1188/79/070385-05 $01.00

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used for incubation and challenge of the tissues with ovalbumin. Guinea-pigs were sensitized as described previously. After killing the animals, lungs and small intestine were removed, washed with Tyrode solution, cut into cubes of about 5 mm^3, weighed and put into 50 ml conical flasks filled with Tyrode solution at room temperature and bubbled with 5% CO_2 in O_2. From each animal the following preparations were taken and incubated in a shaking water bath: (a) 6.0 ml Tyrode + total lung tissue (±3 g wet weight); (b) 6.0 ml Tyrode + small intestine (3 to 4 g wet weight); (c) 6.0 ml Tyrode + small intestine (3 to 4 g wet weight). After 10 min of incubation at 37°C, 5 mg ovalbumin was added to (a) and (b), while (c) was used as a control. After 20 min of incubation of the organ in the presence of the antigen the Tyrode was filtered through cambric gauze and the residues were rinsed with 1 ml of Tyrode; 30 ml of ice cold ethanol was then added to the filtrate. This mixture was centrifuged for 30 min at 0°C and 40,000 *g*.

The supernatants were carefully decanted and evaporated under negative pressure at 60°C. The dried extracts were stored at −30°C until further purification. Extracts from different experiments were pooled and hydrolysed with 0.1 N NaOH for 30 min at 37°C. Column chromatography was performed according to Stechschulte, Orange & Austen (1973). The solution was desalted by non-ionic chromatography on Amberlite XAD-2. The water wash was reapplied to the column, similarly eluted with 80% ethanol and evaporated to dryness. The residue was resuspended in 1 ml of absolute ethanol and applied to a silica gel column (70–230 mesh). Different solvents (15 ml of each) were added consecutively: *n*-hexane, methylenechloride, acetone and *n*-propanol.

SRS-A was finally eluted with ethanol:concentrated ammonia:water (6:3:1 v/v) and the eluate was evaporated to dryness. The residue was resuspended in Tyrode solution and bioassayed on segments of guinea-pig ileum suspended in a 15 ml organ bath filled with Tyrode solution at 37°C and bubbled with 5% CO_2 in O_2 to which mepyramine (1 μg/ml) and atropine (0.1 μg/ml) had been added. The contraction equivalent to 10 ng/ml histamine was defined as 'two' histamine equivalents (HE).

Chemicals

Acetone, Amberlite XAD-2 (Serva), ammonia, disodiumcromoglycate (Fisons), ethanol, FPL 55712 or sodium 7-(3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxypropoxyl)-4-oxo-8 propyl-4H-chromene-2-carboxylate (Fisons), *n*-hexane, histamine dihydrochloride (Aldrich Europe), methylene chloride, ovalbumin (2× crystallized Worthington), *n*-propanol and silica gel 70-230 mesh (Merck Darmstadt) were used. Solutions of FPL 55712 and disodiumcromoglycate were made up daily in distilled water. Concentrations of salts are expressed in terms of base/ml bath fluid.

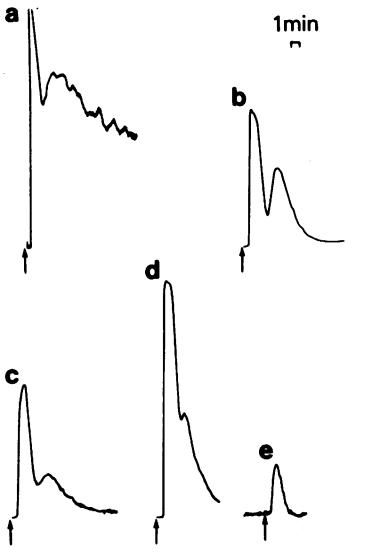

Figure 1 Responses of pieces of ileum from sensitized guinea-pigs to ovalbumin 100 μg/ml. At the arrows, ovalbumin was added. In (a) and (b), a clearcut second phase can be observed; in (c), (d) and (e) responses are insufficient for further evaluation.

Results

The biphasic response of the Schultz-Dale reaction

Figure 1 shows the different types of reactions produced by the ileal segments from sensitized animals after the addition of ovalbumin (100 μg/ml) to the organ bath. About 75% (n = 81) of the sensitized segments displayed a clearcut slow phase (Figure 1a and b), which however was less marked or absent in the remainder of the experiments (Figure 1c, d and e). Segments whose first phase was less than 90% of the histamine-induced contraction or whose slow phase did not reach 40% of that contraction were discarded. The intensity of both phases is dose-dependent as shown in Figure 2. Mepyramine, in a concentration which completely antagonized the histamine (0.5 μg/ml) contraction, was able to suppress the first phase of the anaphylactic reaction, but in 6 out of 12 experiments failed to block the second phase.

Effect of some SRS-A antagonists

Incubation of the ileum for 5 min with disodiumcromoglycate (0.1 to 100 μg/ml) which inhibits the release

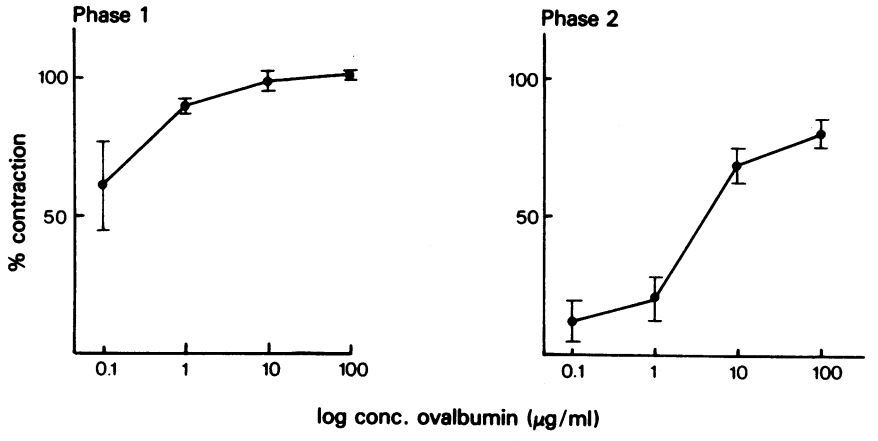

Figure 2 Schultz-Dale reaction of the guinea-pig ileum. Dose-response curves for increasing concentrations of ovalbumin ($n = 5$ for each concentration). All results are expressed as a percentage of the contraction obtained to histamine 0.5 μg/ml.

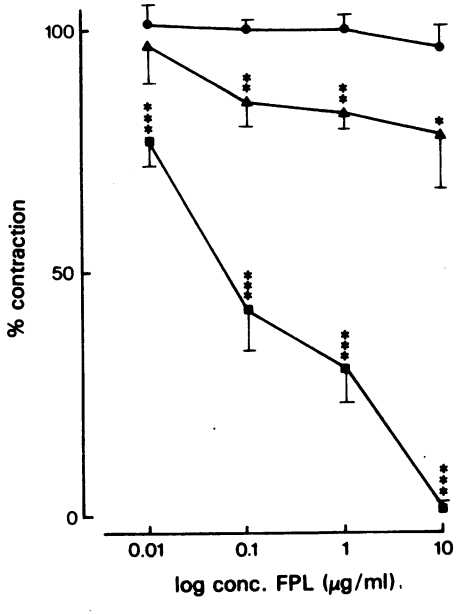

Figure 3 Effect of FPL 55712 ($n \geqslant 5$ for each concentration) on the rapid (●) and slow (▲ and ■) contraction of the ileum during the Schultz-Dale response. FPL was given 30 s before ovalbumin (100 μg/ml) and the responses were evaluated 1 min (●), 2 min (▲) and 10 min (■) after administration of the antigen. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.005$.

of SRS-A from lungs (Cox, 1967) did not influence the biphasic response. A higher concentration (100 μg/ml) depressed the slow phase by 30% when the drug was added to the organ bath simultaneously with the antigen. FPL 55712, a selective antagonist of SRS-A (Augstein, Farmer, Lee, Sheard & Tattersal, 1973), given 30 s before ovalbumin, produced a clear-cut inhibition of the second phase (Figure 3): all the FPL-treated segments returned to their baseline faster than the controls.

This effect of FPL 55712 was even more pronounced when the first phase had been blocked by the previous addition of mepyramine (1 μg/ml). Despite the use of high doses of FPL (0.1 to 10 μg/ml) a complete suppression of the slow contraction could never be obtained (Figure 4).

Extraction and detection of SRS-A

Pooled extracts from lungs and small intestine of 8 guinea-pigs were tested for SRS-A activity and the results are given in Table 1.

All the animals gave satisfactory Schultz-Dale reactions in the isolated organ bath with a clearcut slow response. The lungs challenged with antigen liberated large amounts of SRS-A. No SRS-A could be detected in the extract prepared from the intestine which had not been challenged with ovalbumin. However, the challenged intestine liberated some SRS-A which equalled 5.5% of the amount of SRS-A liberated by an equivalent amount of lung tissue.

The contractions of the ileum observed after the addition of the purified extracts to the organ bath could be blocked by FPL 55712 (0.1 μg/ml), indicating that the contractile substance present in the extracts was most probably SRS-A (Figure 5).

Discussion

The Schultz-Dale response of the guinea-pig ileum

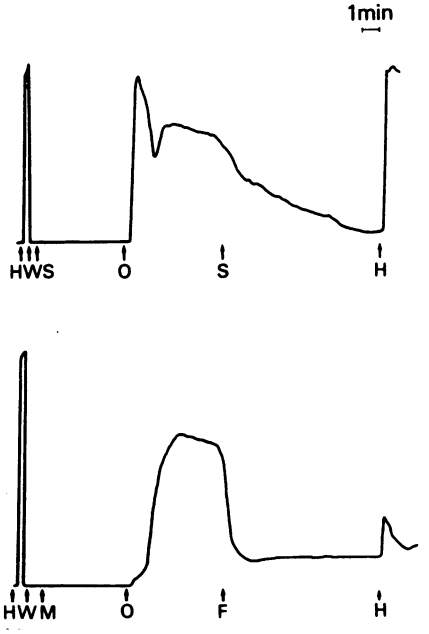

Figure 4 Effect of FPL 55712 on the Schultz-Dale response of the guinea-pig ileum. H = histamine 0.5 μg/ml; W = wash; S = solvent (twice-distilled water) 0.5 ml; M = mepyramine 1 μg/ml; O = ovalbumin 100 μg/ml; F = FPL 55712 in a concentration of 0.1 μg/ml.

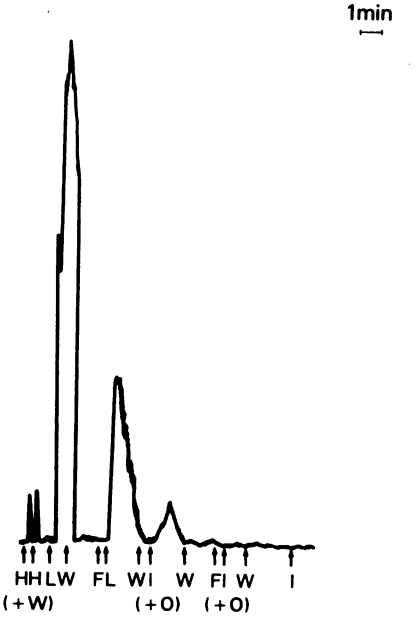

Figure 5 Bioassay of SRS-A extracted from lungs and small intestine of sensitized guinea-pigs. H = histamine 10 ng/ml; W = wash; L = extract from 6 g lung tissue challenged with antigen; F = FPL 55712 (0.1 μg/ml) I (+O) = extract from 6.4 g small intestine tissue challenged with antigen; I = extract from 4.8 g unchallenged intestine tissue.

is characterized by a clearcut biphasic pattern in about 75% of the animals. Our results suggest that, apart from histamine, other mediators are involved in this reaction, a theory also proposed by Cirstea & Suhaciu (1968). Indeed, although the first phase could be completely abolished by high doses of mepyramine, the latter failed to block the slow response in about 50% of the experiments. Similar results were obtained by Hawkins & Rosa (1956). By the use of disodiumcromoglycate and FPL 55712 and by chemical extraction procedures, we were able to establish a possible role of SRS-A in the anaphylactic reaction of the guinea-pig ileum.

Disodiumcromoglycate inhibits the antigen-induced release of histamine and SRS-A from rat peritoneal mast cells (Garland & Monger, 1974). However, the action is extremely time-dependent, since, in contrast to its prophylactic beneficial activity in allergic disorders, disodiumcromoglycate must be added to the medium simultaneously with the antigen in order to block the release of these mediators (Thomson & Evans, 1973). Only high concentrations of disodiumcromoglycate slightly suppressed the slow contraction of the ileum. FPL 55172 significantly diminished this response and this effect was even more pronounced after the first phase had been abol-

Table 1 Amount of SRS-A extracted from different preparations and expressed as histamine equivalents (2HE = 10 ng/ml histamine)

Preparation	*Total amount of SRS-A present in pooled extracts from 8 different animals*	*Amount of SRS-A/g tissue*
Control intestine	0	0
Intestine + ovalbumin	5.8	0.18
Lung + ovalbumin	98.75	3.29

ished by mepyramine. These results suggest that SRS-A, possibly liberated at a later stage of the anaphylactic reaction, helps to sustain the slow contraction. Our observations correspond fairly well with those obtained by Adams & Lichtenstein (1977) on guinea-pig and human airways.

The amount of SRS-A extracted from the guinea-pig ileum was small: only 1/20 of that extracted from lungs under the same conditions. These results fit very well with those obtained by Cirstea & Suhaciu (1968). It seems unlikely that SRS-A should be the only mediator involved in the slow phase. However, when liberated *in situ* small amounts of SRS-A could give higher contractions than they do after extraction and purification, which could have modified some of the components of SRS-A. Furthermore, the percentage recovery in the extraction of SRS-A should also be taken into account, since recovery diminishes dramatically when small amounts are extracted and purified (Stechschulte *et al.*, 1973). It may be that more SRS-A was liberated in the intestine during anaphylaxis but that greater amounts were lost during the extraction and purification procedure as compared with the loss of SRS-A extracted from the lungs. A direct biological quantitation (Liebig, Bernauer & Peskar, 1974) whereby the effluent from the continuously superfused guinea-pig ileum is brought over a series of assay organs (Piper & Vane, 1969) has been unsuccessful so far, presumably due to the fact that SRS-A is released so slowly that threshold levels for detection could never be obtained.

The mechanism by which SRS-A sustains the slow contraction is not yet fully understood. However, since some of its activities resemble those of some of the members of the prostaglandin series, the possibility exists that SRS-A, apart from having its own contractile activity, sensitizes the membrane of the smooth muscle cells to the action of other mediators released. This could be an explanation for the variability in the intensity of the slow response observed, since the effect obtained will depend on the relative amounts of SRS-A and other mediators released during the anaphylactic reaction.

We thank Dr P. Sheard of Fisons Ltd., Loughborough for the gift of disodiumcromoglycate and FPL 55712, and Dr D. Vandenberghe for preparing the *M. smegmatis* suspension. This work was supported by grant no. 3.0077.76 of the F.G.W.O.

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(Received September 9, 1978. Revised December 15, 1978.)