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The Schultz–Dale reaction of the depolarized guinea-pig uterus

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1. The sensitized guinea-pig uterus contracted on addition of antigen (egg-albumin), even after it had been completely depolarized in an isotonic potassium sulphate solution.

2. The contractile response of the depolarized preparation started at a later time after addition of the antigen, and had a smaller amplitude, than the Schultz–Dale contraction induced in the ordinary Ringer solution.

3. A contracting substance was released from the depolarized uterus. The average histamine equivalent of this substance was 89% of the histamine concentration which reproduced the anaphylactic contraction and probably proportional to the mechanical response.

4. The main component of the material released from the uterus in the ordinary Ringer was histamine. The material released from the depolarized uterus did not contain any demonstrable histamine activity.

The mechanisms underlying the Schultz–Dale reaction are still not completely known. It is generally held that the first step in this reaction, the antigen-antibody coupling, occurs on the cell surface. Recent evidence for this view has been presented in studies of the cytotoxic antigen-antibody reaction (Easton, Green & Goldberg, 1962 ; Rosenau, Moon & McIvor, 1962). As a rule the release of a biologically active substance has been shown to occur in parallel with the Schultz–Dale reaction. It is reasonable to believe that the contraction is induced by virtue of such a substance (Feldberg, 1961). Nevertheless it is possible that the antigen may initiate the contraction by a more direct effect ; in other words, without being mediated by a biologically active substance. Thus, as proposed by Dale (1920) and later discussed by Mongar & Schild (1962), it is possible that the antigen-antibody reaction may induce a depolarization of the cell membrane, leading to activation of the muscle cell. It has been the aim of the present investigation to test this point by studying the Schultz–Dale reaction of the guinea-pig uterus in the completely depolarized state as well as in normal polarized conditions. It will be shown that the Schultz–Dale reaction can be elicited in the fully depolarized muscle and that there is a release of a biologically active substance in this state, which is quantitatively related to the contractile response.

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Methods

Sensitization

Female guinea-pigs (weight: 250–450 g) were sensitized 2–14 weeks before the experiments by duplicate injections of 100 mg of egg albumin, the first dose subcutaneously and the second dose, given 3 days later, intraperitoneally. The uterus horns of these animals were used for studies of the anaphylactic response.

Preparation, mounting and stimulation of the guinea-pig uterus

The uterus was removed from the animal immediately after it had been killed and exsanguinated. In most experiments both horns were used simultaneously, one for the experiment in ordinary NaCl-Ringer and the other for the experiment in isotonic K_2SO_4-Ringer. The lumen of the horn was split open longitudinally, and the base and the tip were removed, so that a strand of about 15 mm was obtained. The uterine horn was mounted vertically in a jacketed thermostated bath of 3 ml. The upper end of the preparation was connected to a tension transducer. All experiments were done at 37° C. The bathing fluid was stirred by constant bubbling with pure oxygen. The solutions were added after complete emptying of the bath except for the antigen which was carefully injected into the bath. The contractions were recorded isometrically by means of a strain-gauge transducer connected by means of a carrier amplifier (Elema, EMT 460) to a double-channelled Varian G-22 ink-writer.

Before the start of the actual experiment the organ was stimulated regularly with acetylcholine hydrochloride 10^{-5} g/ml. until constant responses were obtained. Between the stimulations the horn was stretched until it retained a constant resting tension, about 500 mg. During the actual experiment the responses to acetylcholine, 10^{-5} g/ml., was determined at given intervals. These contractions were used as standards, and other responses compared with them. The tension response of the muscle was considered maximal at a histamine concentration of 10^{-4} g/ml. Stimulation of the depolarized uterus was not begun until the potassium induced contracture had subsided. The bathing fluid during the Schultz–Dale reaction was collected for assay 15 min after the antigen had been added to the bath.

Assay procedure

The amount of contracting substance liberated during the Schultz–Dale reaction was measured biologically in the following ways:

1. Samples from experiments carried out in NaCl-Ringer were assayed according to the standard technique on the atropinized guinea-pig ileum.

2. Samples from experiments on the depolarized uterus were assayed on a guinea-pig uterus in K_2SO_4-Ringer.

Histamine was used as the reference substance in the assays. The results have been expressed in g histamine equivalents (free base)/g wet weight tissue.

Solutions

The following solutions were used for experiments on the guinea-pig uterus (mM): NaCl-Ringer: NaCl 154, KCl 5.6, $NaHCO_3$ 3.6, sodium phosphates 8.0, $CaCl_2$ 1.0, $MgCl_2$ 1.0, glucose 5.5. K_2SO_4-Ringer: K_2SO_4 126, KCl 1.0, $KHCO_3$ 3.6, sodium phosphates 8.0, $CaCl_2$ 1.0, $MgCl_2$ 1.0, glucose 5.5.

The pH of the solutions was 7.2–7.5. The expression " depolarized uterus " refers to a preparation depolarized in K_2SO_4-Ringer. The chemicals used in the Ringer solutions were of analytical grade. Crystalline egg albumin as well as human and porcine albumin were obtained from AB Kabi, Sweden. Histamine (free base, Sigma), acetylcholine hydrochloride (crystallized, Roche), mepyramine maleate (May & Baker) and atropine sulphate (Ph.Nord) was used.

Results

Figure 1 shows that a sensitized uterus horn in spite of complete depolarization reacted specifically with a Schultz–Dale contraction on addition of the specific antigen, egg albumin. The reaction started after a latency and was specific for egg

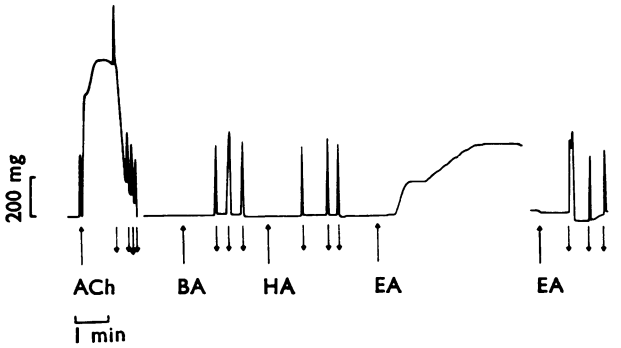

FIG. 1. Schultz–Dale reaction of a guinea-pig uterus horn in depolarizing K_2SO_4-Ringer. Unspecific antigens gave no contractions. Repeated addition of egg albumin had no effect (desensitization). ACh, Acetylcholine hydrochloride (10^{-5} g/ml.); HA, human albumin (10^{-3} g/ml.); BA, bovine albumin (10^{-3} g/ml.); EA, egg albumin (10^{-3} g/ml.).

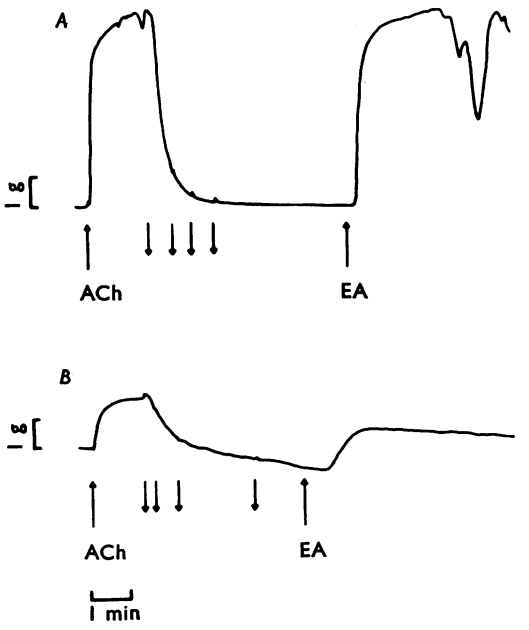

FIG. 2. Comparison of the Schultz–Dale reaction in two different guinea-pig horns in ordinary Ringer (A) and depolarizing K_2SO_4-Ringer (B). ACh, Acetylcholine hydrochloride (10^{-5} g/ml.); EA, egg albumin (10^{-3} g/ml.).

albumin and could not be elicited by other proteins. Repeated addition of egg albumin failed to contract the muscle, which had become desensitized. A comparison between the Schultz–Dale reaction in NaCl-Ringer and K_2SO_4-Ringer is illustrated in Fig. 2. The reaction was performed on the same uterus, one horn in NaCl-Ringer and the other in K_2SO_4-Ringer. As can be seen the response was much smaller in the depolarizing solution. On the average in ten experiments it was 33.5% of the maximal contraction. In the ordinary Ringer the anaphylactic reaction was maximal. It is noteworthy in these experiments that the depolarized muscle contracted after a comparatively longer latency—mean value 28 sec—after the addition of the antigen. In the ordinary Ringer the muscle contracted after 15 sec. It therefore seems clear that the chain of events preceding the contractile response takes longer in the depolarizing solution, while the time course of the contraction itself is not strikingly different.

The finding that a Schultz–Dale contraction can occur in a uterus which is already depolarized suggests that depolarization cannot be the immediate cause of the observed contraction. Histamine and other biologically active substances are known to contract a fully depolarized smooth muscle (Evans, Schild & Thesleff, 1958; Edman & Schild, 1962; Edman & Schild, 1963; Schild, 1967), so it was of interest to find out whether the anaphylactic contraction was associated with a release of biologically active material. The following experiment was performed to investigate this point. Two uterine horns were mounted together in the same organ bath, one from a sensitized and the other from a non-sensitized guinea-pig, their mechanical responses being recorded by separate transducers. When antigen was added there was first a contraction of the sensitized uterus and 7 sec later a small contraction of the non-sensitized muscle. An unspecific contraction from the egg albumin could be excluded, so the experiment proves that a smooth-muscle stimulating substance was liberated from the sensitized preparation.

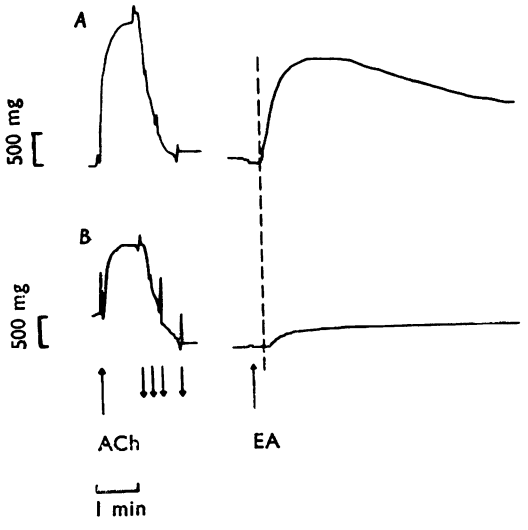

FIG. 3. Schultz–Dale reaction of a sensitized depolarized uterus horn (A) and a 7 sec delayed contraction of a non-sensitized uterus horn (B) in the same organ bath. ACh, Acetylcholine hydrochloride (10^{-5} g/ml.); EA, egg albumin (10^{-3} g/ml.).

It is thus evident that the addition of antigen to the sensitized guinea-pig uterus gives rise to a Schultz–Dale reaction and a simultaneous liberation of a biologically active substance regardless of the electrical state of the muscle. Because the Schultz–Dale contractions in the depolarizing Ringer were submaximal, approximately one-third of the maximal contractile force, conditions were favourable for assessing whether the amount of biologically active material released from the muscle was quantitatively related to the mechanical output. For this purpose the following parameters were determined in each muscle: (1) The dose of histamine necessary for reproduction of the Schultz–Dale contraction (x). The dose was determined graphically as indicated in Fig. 4; (2) the amount of biologically active material, expressed in histamine equivalent, liberated during the Schultz–Dale reaction (y).

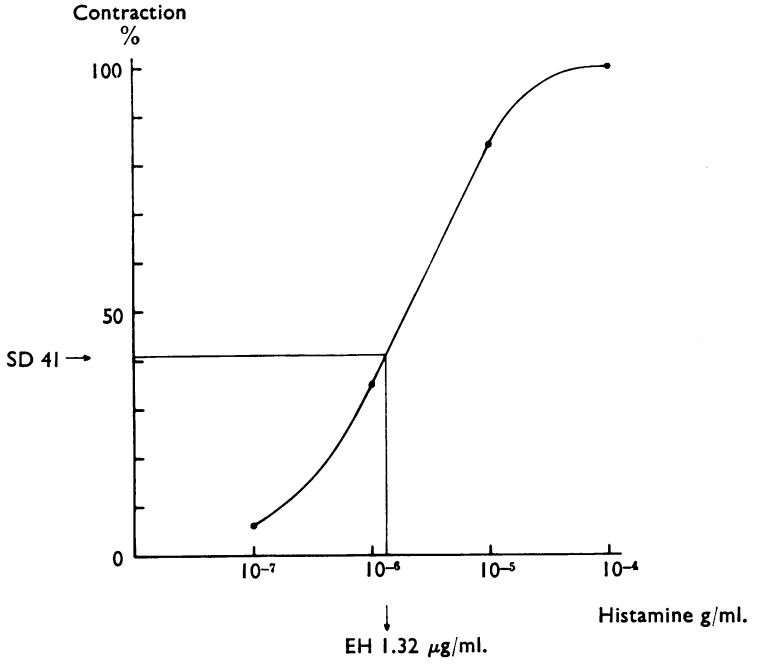

FIG. 4. Example of a graphical determination of the dose of histamine, which caused a mechanical response equal to the Schultz–Dale contraction. All contractions represented as a percentage of the response to histamine 10^{-4} g/ml. giving maximal response. SD, Schultz–Dale contraction, % of maximal response; EH, external histamine μg/ml. which reproduced the SD.

TABLE 1. *Schultz–Dale reaction of the depolarized guinea-pig uterus horn*

Schultz–Dale contraction (% of maximal response)	x Concentration (μg/ml.) of external histamine for production of mechanical response equivalent to the Schultz–Dale contraction	y Biologically active material, histamine equivalent (μg/g wet weight tissue)	y/x (%)
41	1·32	0·76	58
31	0·69	0·39	57
51	0·69	0·75	109
19	0·30	0·46	153
61	1·26	0·76	60
11	0·10	0·12	120
35	0·76	0·49	64
			89±14·6
			Mean±S.E.M.

The results of seven experiments have been summarized in Table 1, and plotted graphically in Fig. 5. There was a positive correlation ($r=0.83$, $0.05>P>0.01$) which indicated that the expressions for the Schultz–Dale contractions (x) probably were dependent on the liberated amounts of active substance (y). The true concentration of the released active substance at its site of action is unknown, but it was considered that an approximate estimate of this concentration might be obtained from the total amount of activity released per wet weight of tissue expressed as histamine equivalent. Calculated in this way the mean histamine equivalent released amounted to 89% of the concentration of histamine which reproduced the Schultz–Dale contraction. The relatively large amount of activity released supports the view that the mechanical response during the Schultz–Dale contraction of the depolarized uterus is mediated by the active material liberated in connexion with the antigen–antibody reaction.

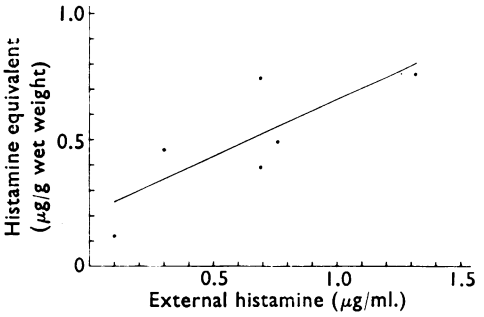

FIG. 5. Schultz–Dale reaction in the depolarized guinea-pig uterus horn. Relation between x (external histamine in μg/ml. which reproduced the contraction) and y (liberated biologically active material, histamine equivalent, μg/g wet weight tissue). $y=0.45\ x+0.21$; ($r=0.83$, $0.05>P>0.01$).

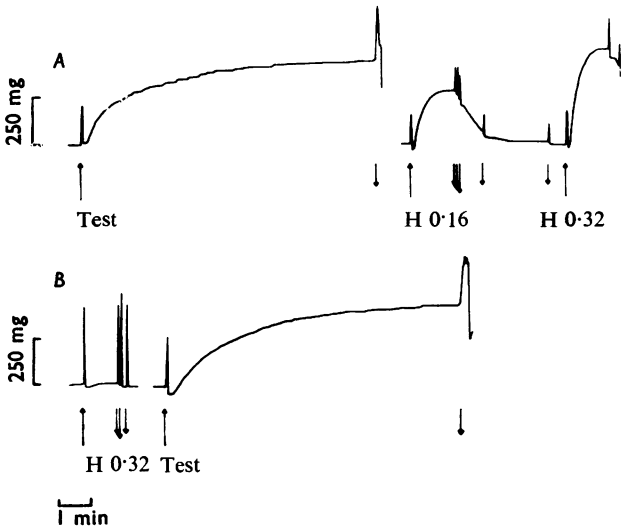

FIG. 6. A: Contractile responses produced by the bathing fluid collected after the Schultz–Dale reaction on the depolarized guinea-pig uterus. B: Lack of inhibition of the contractile response by mepyramine-maleate 10^{-6} g/ml. H 0.16 and H 0.32, histamine 0.16 and 0.32 μg/ml.

Attempts have been made to investigate whether or not the substances liberated in the two solutions were identical. In Fig. 6 an assay of two samples from the bathing fluid of a depolarized uterus is shown. In the upper section the response to the test sample and two matching histamine responses are represented. The lower section illustrates the mechanical response to the test solution and histamine in the presence of mepyramine maleate 10^{-6} g/ml. As can be seen, while the responses to histamine were completely abolished by the antihistamine, the contraction produced by the test solution was virtually unaffected. It is evident from these results that a substance other than histamine was released from the depolarized muscle during the Schultz–Dale reaction. This view is further supported by the shape of the isometric myogram produced by the unknown substance as compared with the histamine response. The contractions produced by the unknown substance exhibited a slower initial rise and reached maximum later than the histamine-induced response. By contrast, the biologically active substance released in the ordinary Ringer was completely inhibited by mepyramine maleate 10^{-6} g/ml., and its mechanogram was very similar to that produced by histamine.

Discussion

During the Schultz–Dale reaction the smooth muscle is usually found to release a biologically active substance in parallel with the contraction. It is generally believed that the contractile response is induced by action of this material. As an alternative mechanism, proposed by Dale (1920), the antigen-antibody reaction itself might cause a change of the muscle cell membrane leading to depolarization and mechanical activity (see also Mongar & Schild, 1962). The present experiments, performed on the fully depolarized sensitized smooth muscle have provided evidence which makes this theory questionable. The results have shown that a specific Schultz–Dale response can be obtained even after the preparation has been equilibrated in an isotonic potassium sulphate solution. This shows that a depolarization process induced by the antigen is not a necessary step in the initiation of the anaphylactic response of the smooth muscle cell.

Activation of the depolarized muscle can be accomplished in two ways:

1. The antigen stimulates the depolarized muscle cells directly in a way different from a depolarization (compare Dale, 1920).

2. The antigen acts primarily on some type of cells other than the muscle fibres—the mast cells, for example—liberating a smooth muscle stimulating substance. The validity of this assumption is shown by the fact that the smooth muscle responds specifically to autonomic drugs even after complete depolarization (Evans, Schild & Thesleff, 1958; Edman & Schild, 1962; Edman & Schild, 1963; Schild, 1967).

The present experiments provide support for the second alternative. The results have shown that the size of the anaphylactic contraction of the depolarized uterus was probably proportional to the released amount of a biologically active substance. Expressed in terms of histamine equivalent, the activity released was 89% of the histamine concentration which reproduced the Schultz–Dale contraction. It is therefore likely that the second alternative plays an essential part in the mechanism of anaphylaxis in the depolarized guinea-pig uterus. The finding that there is a relation between the contractile response and the amount of release of biologically active substance is in accord with results obtained in similar studies of polarized

smooth muscles of the guinea-pig (vesicula seminalis, Schild, 1939; ileum, Liacopoulos, 1961).

It is of interest to note that the smooth muscle can liberate a biologically active substance even after immersion in the isotonic potassium sulphate solution. There are reasons to believe that in these conditions all the preparation, including cells other than the muscle fibres (Evans, Schild & Thesleff, 1958) is depolarized. The findings are in good agreement with recent investigations on partially depolarized nerves (Elmquist, 1965) and fully depolarized glandular tissue (Douglas & Poisner, 1964). These structures have been shown to release acetylcholine and vasopressin respectively in spite of increased potassium concentrations in the extracellular medium. If the mast cells are the source of the biologically active substance collected from the sensitized depolarized smooth muscle, the results would seem to indicate that depolarized mast cells *in situ* maintain the ability to release their content. The fact that isolated peritoneal rat mast cells, immersed in isotonic potassium Ringer, release histamine (my unpublished observations) provide further support for this idea.

So far only histamine and a slow reacting substance (SRS) have been identified in the effluent from the guinea-pig uterus during the anaphylactic reaction. The results have confirmed that the principal activity of the biologically active material from the depolarized uterus was not histamine. It is not likely that the substance was SRS either, because this substance does not contract the guinea-pig uterus in ordinary Ringer (Brocklehurst, 1956; Chakravarty, 1959). The active principle is therefore, probably, a substance which so far has not been identified during the anaphylactic reaction of the guinea-pig uterus.

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