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Observations on 'fade': a complication of the contractile response of smooth muscle to a large dose of an agonist

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Summary

1. A study has been made of the time course of contraction of guinea-pig isolated ileum when suddenly exposed to a high concentration of acetylcholine, carbachol or histamine. With a sufficiently large dose there was a 'fading' of the abrupt initial contraction. A sustained contraction followed, provided that the agonist was not washed out of the organ bath.

2. The fade produced by giving large equipotent doses of acetylcholine and histamine simultaneously was substantially greater than that obtained by giving either agonist alone. It was comparable to that produced by a double dose of acetylcholine or histamine. This result does not support any explanation of fade based on receptor occupancy.

3. The extent of fade and the level of the sustained contraction were strongly affected by the calcium concentration of the bath fluid: the higher the calcium concentration the less was the fade. It is suggested that fade occurs when there is such intense stimulation of receptors that excitation-contraction coupling becomes temporarily less efficient due to depletion of calcium from a store.

Introduction

In experiments with isolated intestinal strips the usual measure of the response to a stimulant drug is the maximum contraction obtained within a specified period. If this period is short (say, 30 s or less), the tone of the strip may still be increasing when the agonist is due to be washed out. With a large dose of the agonist, however, the contraction may 'fade' at least temporarily from a rapidly attained peak. Fade has been observed in experiments with the ileum and taenia coli of the guinea-pig (Fastier & Reid, 1952; Paton, 1961; Durbin & Jenkinson, 1961; Ariëns, Simonis & van Rossum, 1964). It has been obtained with isotonic recording, especially with heavy loading (Fastier & Reid, 1952; Ariëns *et al.*, 1964) and auxotonic recording (Paton, 1961). Similar phenomena have been noted in experiments on the rectal caecum of the fowl (Barsoum & Gaddum, 1935) and on vascular smooth muscle (Fara, 1971; Godfraind & Kaba, 1972).

From being merely a complication in the measurement of certain smooth muscle responses, fade became a phenomenon of intrinsic importance when Paton (1961)

put forward his rate theory of drug-receptor interaction. 'On rate theory, a stimulant action should be maximal at first exposure to a drug, when the receptors are all free. Then as they become occupied the rate of combination must fall off, until an equilibrium is reached, dictated primarily by the rate at which dissociation frees receptors.' As Paton further observed, fade should occur in some degree with all stimulants, and have a time course corresponding to that of the rising receptor occupancy to which he attributed it; but with strong stimulants, yielding low occupancies, the fade might be insignificant. Although no estimates of occupancy by strong agonists were then available, Paton also noted at the time that the fade seen with acetylcholine did not have properties that allowed it to be explained in terms of advancing occupancy; and this led to a study of non-specific desensitization of the ileum. Subsequent work on the affinity constants of agonists such as acetylcholine has confirmed the conclusion that advancing receptor occupancy by such agonist molecules would be insufficient to produce fade from this cause (Paton & Wand, 1964).

The experiments now to be described began with an attempt to test Paton's approach. If it be accepted that acetylcholine and histamine have different sites of action, it may be supposed that occupancy of acetylcholine receptors should not be affected by the presence of histamine, and *vice versa*. Thus, if the fade observed were due to receptor occlusion, the degree of fade should not be increased by combining the drugs; but if it were due to non-specific desensitization, then it should be of a degree appropriate to the total effector response.

Methods

Ileal strips 5–10 mm long were obtained from guinea-pigs shortly after they had been killed by cervical dislocation. Uterine fundus preparations were obtained from rats; pulmonary artery and portal vein preparations (spiral strips) from rabbits. The organ bath was of 5 ml capacity and kept at 37° C. It contained Krebs-bicarbonate solution (Ca^{++}, 2 mM) gassed with a 95% O_2: 5% CO_2 mixture. Since earlier experiments had indicated that fade was more evident the higher the tension applied to the muscle strips (Fastier & Reid, 1952), a tension equivalent to a 2 g weight load was applied to the strip. Either isometric or isotonic changes were recorded, the former with an isometric strain gauge, the latter with a simple lever. A pen recorder was used except for tracings of the type shown in Fig. 1: these were registered on a kymograph.

The drugs used were acetylcholine perchlorate (B.D.H.), histamine dihydrochloride (Sigma), carbachol (carbamylcholine chloride, B.D.H.), 5-hydroxytryptamine creatinine sulphate (B.D.H.) and (−)-noradrenaline bitartrate (Koch-Light). The doses stated are those calculated for the free bases.

Drugs were dissolved in Krebs-bicarbonate solution and added to the bath by a rapid injection technique. The injected volume was always 0·5 ml. It was found by following the distribution of a tritium-labelled histamine solution that the mode of injection enabled a uniform concentration to be attained throughout the organ bath within 2 s, which was well before the time taken for the tissue to reach a peak initial contraction in response to the added histamine.

Results

Occurrence of fade

In preliminary experiments on guinea-pig isolated ileum the agonist was given over a very wide range of doses (Figure 1). Dosage size was randomized and each dose was given at least twice to check the consistency of the response to it.

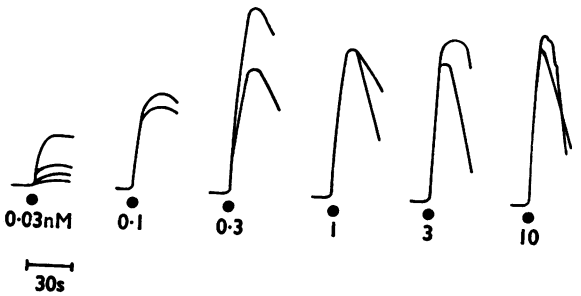

FIG. 1. Facsimiles of kymograph recordings showing the response over a 30 s period of guinea-pig ileal strips exposed to a wide range of concentrations of acetylcholine. Each dose (stated in nanomoles) was tested at least twice on the strip over the course of several hours.

With some of the smaller doses of acetylcholine, carbachol or histamine the contraction did not reach a peak within the 30 s period of exposure to the drug. With large doses, however, there was obvious fade sometimes accompanied by small fluctuations in tone. Although the abrupt relaxation suggested some compensatory process, fade was not obtained when the ileum was suddenly stretched by increasing the load.

When the period of exposure to the agonist was made longer (i.e. more than 30 s), it was observed that the degree of contraction steadied ; either a plateau was reached or there would be only a slight rise or fall in tone (Figure 2). The height of this

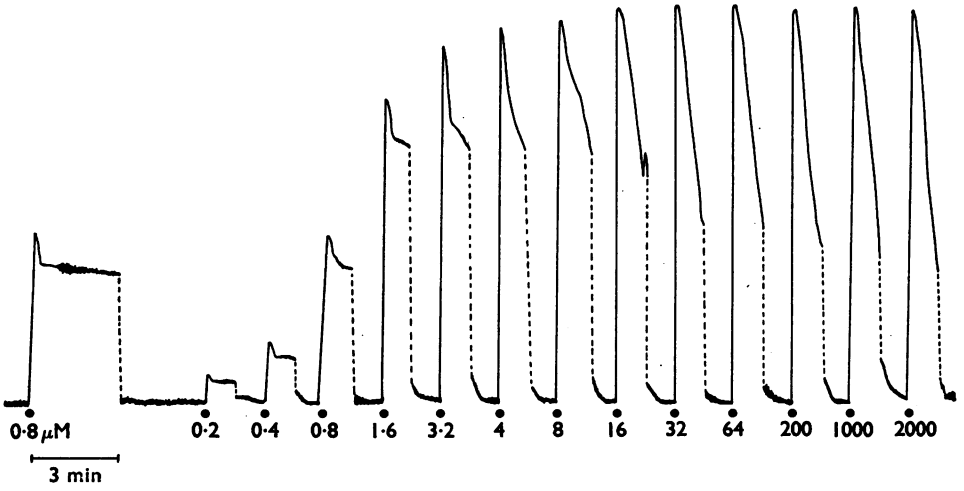

FIG. 2. Isotonic recordings of the responses of a guinea-pig ileal strip to increasing concentrations of histamine. The figures record the dose of histamine (in μM of free base) injected into the 5 ml organ bath. Dotted lines indicate the points at which the chart recorder was stopped and the preparation washed twice with Krebs-bicarbonate solution.

sustained contraction bore no obvious relationship to the height of the initial peak response and was not affected by the rate of addition of the drug to the organ bath. The rate of decline from the initial peak upon washing the preparation with plain Ringer (Krebs-bicarbonate) solution was faster than the fade obtained in the presence of the drug and no resting tension remained. With guinea-pig ileum the dose of acetylcholine or histamine needed to produce the maximum secondary contraction was less than that needed to produce the maximum primary response. Fade increased with dosage (Figures 2, 5).

During the 'plateau period' after a maximal initial response, the strip was relatively refractory to further doses of the original or other agonist (Fig. 3), but the initial sensitivity was regained after the wash-out. If a maximum primary

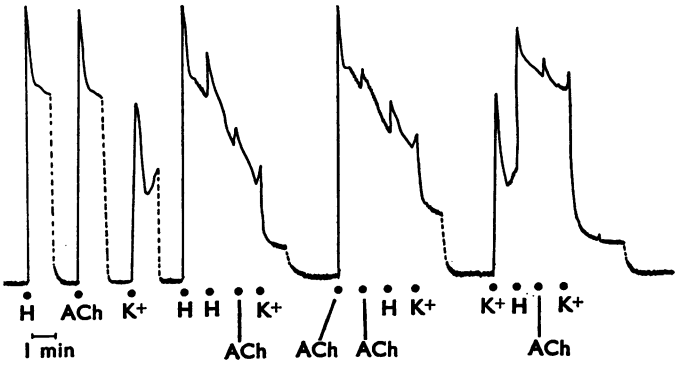

FIG. 3. Isotonic recordings of the responses of a guinea-pig ileal strip to maximal doses of histamine (8 μM at each H), acetylcholine (0·56 μM at each ACh) and potassium (20 mM at each K), given either with or without washing of the strip before the giving of another agonist. Dotted lines each indicate a double washing of the strip.

response had not been attained, further additions of the agonist would still produce a greater contraction. After repeated moderate doses of an agonist without a washing out of the bath, the sustained secondary but not the primary response would build up to a maximum, which was the same as that obtained after a single, larger dose of the agonist (Figure 4). There was often a wobbling of the trace during the plateau period. This was not an artifact of the potentiometric recording system, since wobbling was sometimes evident in kymograph records of ileal contractions made with a simple lever system.

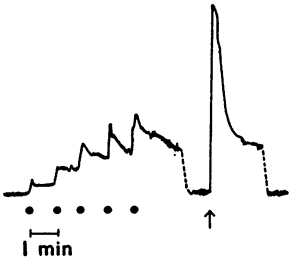

FIG. 4. Isotonic recordings comparing the cumulative response of a guinea-pig ileal strip to 5 doses of histamine (0·2 μM) at each dot given at 1 min intervals with that of the responses to the same total dose (1 μM) given all at once. Each dotted line shows where the strip was doubly washed.

Experiments with paired agonists

Acetylcholine, carbachol and histamine produced similar changes in tone when tested on the one ileal strip. Large doses of each drug elicited fade and a secondary, relatively steady contraction. It was therefore possible to match some of the responses to large doses of acetylcholine fairly closely by giving histamine over a range of doses (Figure 5). The effects of these matched doses were compared with

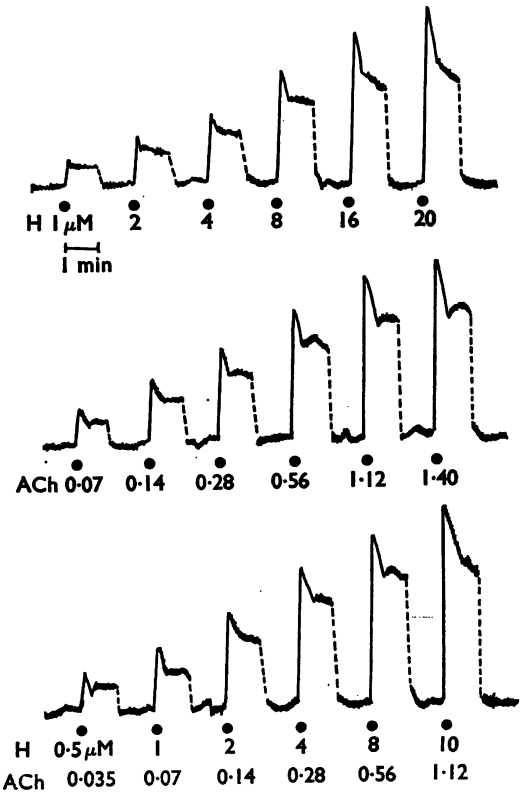

FIG. 5. Facsimiles of isometric recordings of the responses of a guinea-pig ileal strip to increasing doses of histamine (H) and acetylcholine (ACh). The dose (in μM) of the agonist is given beneath each response. The bottom set of tracings represents the responses when half doses of histamine and acetylcholine were added to the bath simultaneously. A dotted line indicates a double washing of the strip.

those obtained by giving in admixture equipotent amounts of acetylcholine and histamine. As may be seen from Fig. 5, the degree of fade produced by the agonists given in combination is comparable to that produced by a double dose of either agonist given alone. This was observed with both isotonic and isometric recording.

While a maximum sustained contraction was being obtained with histamine, the strip was found to be refractory (in other words, there was a non-specific desensitization) to further doses of acetylcholine and potassium, as well as of histamine, and *vice versa*. Whether histamine, acetylcholine or potassium was used as agonist, the same maximal sustained contraction was produced by giving either a sufficiently large dose of the one agonist or a series of smaller doses without a wash-out.

Experiments with modified Ringer solutions

The extent of fade and the level of the secondary contraction were strongly affected by altering the concentration of calcium (but not that of potassium or sodium) in the Ringer solution. When the calcium concentration was increased, a fixed dose of the agonist produced less fade and a larger sustained contraction (Figure 6). Conversely, exposing a strip of guinea-pig ileum to a calcium-free solution resulted in the test doses of the agonist producing greater degrees of fade and decreasing sustained contractions. Altering the calcium ion concentration did not alter the minimum dose of an agonist needed to produce a maximal primary contraction though it affected the height of this.

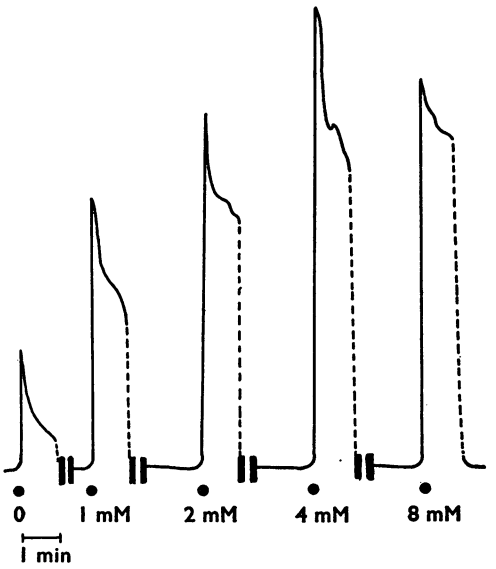

FIG. 6. Isotonic recordings of the responses of a guinea-pig ileal strip to histamine (1·6 μM) obtained when the calcium content of the Ringer solution in the bath was varied. Each dotted line indicates a double washing of the strip. Each break in the baseline indicates a 20 min period of equilibration with Ringer containing calcium in the concentration specified beneath.

Experiments with other preparations

Strips of the fundal portion of rat uterus exhibited fade when exposed to large concentrations of acetylcholine, histamine or 5-hydroxytryptamine. As with guinea-pig ileum, an initial peak response was followed by fading to a plateau. Both the extent of fade and the plateau height depended on the calcium ion concentration of the bath fluid. In experiments with paired agonists (acetylcholine and histamine or 5-hydroxytryptamine), the extent of fade produced by the agonists in combination suggested that this effect was additive. Strips of rat ileum were also found to behave like guinea-pig ileum.

The effect of noradrenaline on two isolated blood vessel preparations was studied. With rabbit portal vein a rapidly achieved contraction faded back very slowly to the baseline. The fading was retarded by increasing the calcium concentration of the Ringer solution. The response of rabbit isolated pulmonary artery to a large

dose of noradrenaline was a slowly increasing contraction without fade. The same maximum response was obtained with a series of moderate doses given in quick succession as with a single large dose.

Discussion

When large equipotent doses of acetylcholine and histamine were given simultaneously, the response of an isolated strip of guinea-pig ileum or of rat uterus showed substantially more fade than that obtained with either agonist alone. The degree of fade was comparable to that produced by a double dose of either agonist given alone. This result endorses Paton's conclusion that the fade produced by agonists such as acetylcholine and histamine is not due to receptor occupancy by these drugs. It suggests that fade is an intrinsic property of the tissue, dependent on the sum total of chemical stimulation.

An ileal strip is a complex tissue. It is conceivable that fade might be due to local reflexes mediated by the intramural autonomic plexuses, although it has been shown that treatment with hexamethonium does not prevent the occurrence of fade (Fastier & Reid, 1952). We have been able to elicit fade using the isolated uterus fundus of the rat. We performed only preliminary experiments with isolated vascular smooth muscle preparations because these appeared inconvenient for the study of fade.

If fade is not to be explained in terms of drug-receptor interaction, it must arise at some later step in the coupling between stimulation and mechanical response. We think it significant that fade could be profoundly affected or even prevented by altering the external calcium concentration. Although the effect on the primary contraction varied, raising the calcium concentration increased the sustained contraction produced by a single large dose or by repeated moderate doses of an agonist. Since the concentration of agonist needed to produce a maximal primary contraction was not affected by changing the calcium concentration, it would seem that calcium is not affecting the binding of the agonist to the receptor but rather the response of the muscle to receptor stimulation.

For smooth muscle, as for skeletal and heart muscle, it is now thought that calcium is probably the trigger for the musle contractile mechanism (Bozler, 1969; Ebashi & Endo, 1968). The availability of calcium might therefore be the intrinsic factor in smooth muscle which limits the muscle response to receptor stimulation and can thus give rise to fade. The intracellular calcium is maintained at a lower concentration than the extracellular calcium against an electrochemical gradient. Durbin & Jenkinson (1961) suggested that the carbachol contraction of depolarized smooth muscle is a consequence of the net movement of calcium into the muscle cells, following the increase in permeability produced by the drug. They noted the dependence of the 'maintained phase' of the contraction on the concentration of calcium in the bathing fluid. It is difficult to extrapolate from tissue responses to molecular events initiating or otherwise affecting contraction. Nevertheless it is tempting to explain the occurrence of fade by supposing (i) that smooth muscle cells may maintain a small store of intracellular or membrane-bound calcium sufficient to permit a brief contraction; (ii) that prolonged excitatory stimuli deplete this store and, in so doing, lead to fade; and (iii) that the secondary, maintained contraction is dependent on the external calcium concentration and reflects a steady influx of calcium ions (see Urakawa & Holland, 1964).

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