

Pharmacological reactivity of granulation tissue

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The contractile system of the myofibroblast has well defined characteristics. The findings suggest the presence of 5-hydroxytryptaminergic as well as α and β -adreno-receptors, although both appear to be immature. It does not have cholinergic or histaminergic receptors. The contractile system is inhibited by cytochalasin B and is not sensitive to colchicine. The contractile machinery is suggested to be the microfilament system, as it occurs in the contractile apparatus of primitive cells. This is in accordance with the role of myofibroblasts in granulation tissue neoformation.

In 1971, Gabbiani et al described fibroblasts with ultra-structural, chemical, immunological and functional characteristics in the granulation tissue of animals and man. The characteristics clearly distinguished these cells from the fibroblasts of normal tissue, i.e. a nucleus with multiple indentations or deep folds, a microfilament system (bundles of parallel fibrils resembling those of smooth muscle cells, arranged parallel to the long axis of the cell), numerous intercellular connections between granulation tissue fibroblasts (tight or gap junctions), and an abundant rough endoplasmic reticulum.

Immunofluorescence tests showed that microfilaments corresponded to actin filaments. These characteristics suggested the possibility that these cells (called by Gabbiani et al 1971 myofibroblasts) could be the cause of wound contraction. After different pharmacological tests, Gabbiani et al (1971, 1972) Majno et al (1971) and Ryan et al (1973, 1974) observed that granulation tissue strips contracted and relaxed in a similar way to smooth muscle cells.

The possibility of altering the natural course of wound healing (contraction) in those parts of the body where it could result in functional disturbances or unsightly results seemed pharmacologically feasible (Madden et al 1974). A complete study of granulation tissue reactivity has not been made up until now and as a knowledge of the problem will allow better comprehension of the wound healing process, we have attempted a systematic study of granulation tissue reactivity with the aim of revealing the mechanisms that cause wound contraction. This study we considered necessary before any speculation of wound healing contraction could be made.

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MATERIAL AND METHODS

The Sprague Dawley rats, 250-300 g, used were maintained at 22 °C and a humidity of 70-80%.

Granulation tissue was obtained following Selye's model (1953). A granuloma pouch was created in the rat dorsum by injecting 20 ml of air, and then 1 ml of 1% croton oil in olive oil. By the twenty-first day the pouch was removed and granulation tissue strips of 3 × 1 × 0.2 cm were obtained (10-12 strips).

Granulation tissue strips were suspended in an 8.3 ml organ bath containing Tyrode solution at 37 °C and bubbled with 95% O₂ + 5% CO₂. Responses to drugs were recorded with an isometric type transducer (LIAP) with a sensitivity up to 10 mg. The initial tension was 250 mg. The polygraph used was a two-channel Hewlett-Packard type HP-7702-B, with an amplifier type HP-8805-B. The rate used was 2.5 mm min⁻¹.

The substances assayed were (g ml⁻¹): 5-hydroxytryptamine (5-HT) (From 0.9 to 30 × 10⁻⁶), orni-pressin (0.015 to 0.024 I.U.), angiotensin (1.48 to 12 × 10⁻⁶), adrenaline (0.18 to 6 × 10⁻⁶), nor-adrenaline (6 to 12 × 10⁻⁶), histamine (1 × 10⁻⁴), acetylcholine (1 × 10⁻⁸ to 1 × 10⁻⁴), atropine (0.6 to 1 × 10⁻⁵), papaverine (5 × 10⁻⁸ to 1 × 10⁻⁴), cytochalasin B (6 × 10⁻⁶), antazoline (0.5 to 10 × 10⁻⁶), cimetidine (6 × 10⁻⁴), phenoxybenzamine (1.5 to 3 × 10⁻⁶), propranolol (5 to 15 × 10⁻⁶), verapamil (0.3 to 1 × 10⁻⁶), cyproheptadine (1 × 10⁻⁷ to 6 × 10⁻⁶), methysergide (1 × 10⁻⁷ to 1 × 10⁻⁶), PGF_{1 α} (0.5 to 1.5 × 10⁻⁵), colchicine (1 to 12 × 10⁻⁵), and arachidonic acid (0.5 × 10⁻⁵ to 1 × 10⁻⁴).

RESULTS

Cholinergic system

Acetylcholine, even at high doses of 1 × 10⁻⁴, did not produce any response, which agrees with the results of Gabbiani et al (1972). This is in contrast

to the behaviour of most smooth muscle tissues which respond (by contraction or relaxation) to doses as small as 1×10^{-7} . In accordance with the lack of response to acetylcholine, atropine had no effect on the base line. We therefore can say that granulation tissue does not seem to have cholinergic receptors.

5-Hydroxytryptaminergic system

5-HT produced a contractile effect at concentrations from 0.9 to 30×10^{-6} which is in accordance with responses obtained in other contractile tissues, for example: rat vas deferens or guinea-pig ileum (García-Valdecasas et al 1974). Nevertheless, the rate of contraction was slow, and the strip tended to remain contracted unless the preparation was washed, then the relaxation that occurred was very slow, and the tracing never returned to the base line. The tissue showed tachyphylaxis.

A dose-response curve was obtained evaluating only the first contraction. Every point on the curve is the average of seven tests. We have shown that the dose-response curve moved to the right in the presence of anti-5-HT drugs, like cyproheptadine and methysergide, at a dose of 1×10^{-7} . This also happened in the presence of atropine at 6×10^{-6} . The new dose-response curves maintained the same slope and maximum response, which suggests a competitive blocking action.

Papaverine between 0.6 and 20×10^{-6} , inhibited the action of 5-HT. The dose-response curve not only moved to the right but the maximum response also decreased (Fig. 1). Papaverine concentrations in the range 20×10^{-6} caused granulation tissue to be insensitive to 5-HT (Majno et al 1971; García-Valdecasas et al 1977).

Antazoline (H_1 blocking agent) and cimetidine (H_2 blocking agent) also inhibited the 5-HT contraction. The dose-response curves maintained the same slope and maximum response (Fig. 2).

α - and β -Adrenoceptor blocking drugs, such as phenoxybenzamine or propranolol, inhibited 5-HT contractions. Propranolol moved the dose response curve to the right, maintaining the same slope and maximal response. The curve obtained with phenoxybenzamine not only moved to the right, but also produced a change in the slope and maximum response.

Adrenergic system

Adrenaline had a weak two phase action on granulation tissue. Initially it produced a small contraction, but then the strip relaxed beyond the base line.

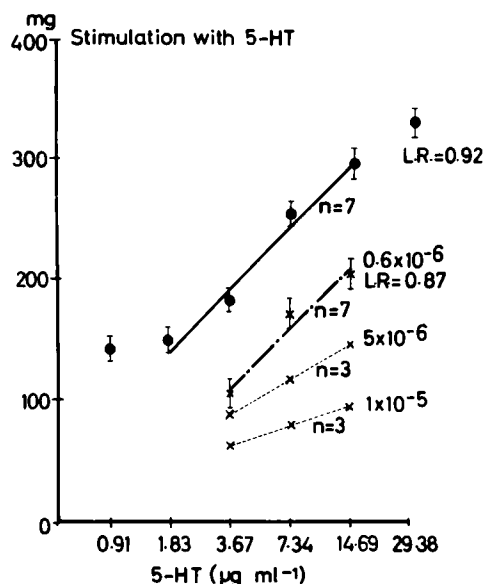


FIG. 1. Dose-response curve of granulation tissue by 5-HT. Papaverine moves the curve to the right and decreases the maximal response. Each point is the mean for a number of tests—(n), and the vertical bar represents s.e.m. L.R. = Linear regression coefficient. — without papaverine; - - - - and - · - · - with papaverine.

When the strip was contracted by 5-HT, the relaxant action was more obvious (Fig. 3).

Noradrenaline also had a small contractile effect, without any relaxing component as in the results of Gabbiani et al (1972) and García-Valdecasas et al (1977).

Histaminergic system

In contrast to the sensitivity of smooth muscle cells to histamine, the granulation tissue did not react to it at doses of up to 1×10^{-4} . Furthermore the histaminergic H_1 and H_2 blocking drugs had no action on the base line. These results agree with those obtained by Gabbiani et al (1972).

Polypeptides

Ornpressin and angiotensin stimulate granulation tissue strips. Ornpressin produced contractions at doses of 0.03 I.U. ml^{-1} , which is the weight equivalent of 1×10^{-7} . Angiotensin was less active, requiring concentrations of 1.48×10^{-6} .

Calcium blocking agents

This new group of substances has the characteristics of blocking the entrance of calcium into the cell. Verapamil and papaverine when added to the bath produced a relaxation of granulation tissue strips.

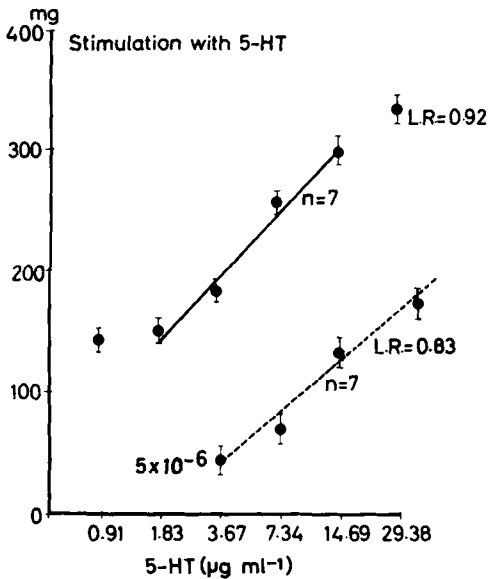


FIG. 2. Antazoline moves the dose response curve by 5-HT to the right. — without antazoline; - - - - with antazoline.

We have shown that papaverine inhibited the contractile effect of 5-HT by moving the dose-response curve to the right, changing the slope and the maximum response. The verapamil dose-response curve moved to the right, with the same slope and maximum response.

Cytochalasin B

Primitive cells have two contractile systems, microfilaments and microtubules. Cytochalasin B specifically inhibits microfilaments, while colchicine and vinblastine disrupt microtubules (Wessells et al 1971). Cytochalasin B therefore has a use in distinguishing if either of the contractile systems takes part in wound contraction.

The microfilament system has its more important role in morphogenesis. Recently White & Gerrard (1979), after studying blood-clot contraction, suggested that the microtubule system would form the skeleton of the true contractile system, the microfilament system. But Ehrlich et al (1977) have

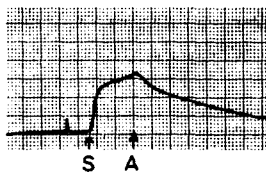


FIG. 3. Granulation tissue strip. S = 5-HT 7.34×10^{-6} A = Adrenaline 5×10^{-5} .

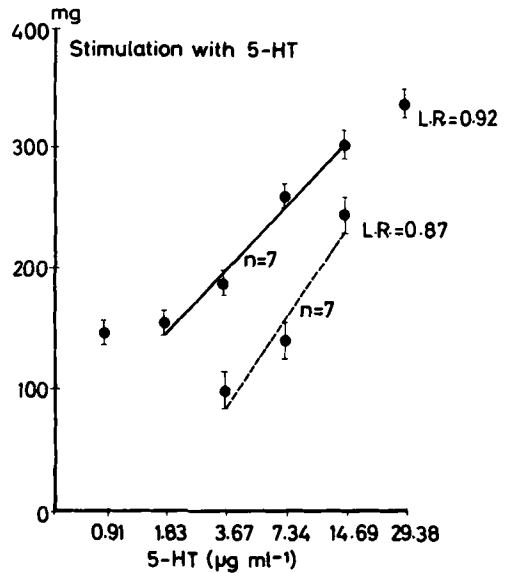


FIG. 4. Dose-response curve of granulation tissue to 5-HT. Cytochalasin B moves the curve to the right maintaining the slope. — without cytochalasin B; - - - with cytochalasin B, 6×10^{-6} .

suggested the role of microtubules is in wound contraction.

Some processes are sensitive to cytochalasin B (cytokinesis of fibroblasts, lymphocytes, axonal growth cone activity, blood-clot contraction), while other processes are insensitive (ciliary function, sperm tail function, karyokinesis) depending on the integrity of the microtubules (Wessells et al 1971; Clarke & Spudich 1977).

Cytochalasin B relaxed granulation tissue in basal conditions, in a similar way to papaverine or verapamil. In addition, cytochalasin B moved the dose-response curve of 5-HT to the right, maintaining the slope and maximum response (Fig. 4). The concentration needed was in the range of 1×10^{-6} . The results suggest that granulation tissue contraction is mediated by calcium and altered by cytochalasin B, and this, in turn, suggests that the contractile system arises from the presence of microfilaments in the myofibroblasts, as happens in other processes of morphogenesis. This is in accordance with the results of Van den Brenk & Stone (1974) and Gabbiani & Montandon (1977).

Prostaglandins

As previously reported (Gabbiani & Montandon 1977), prostaglandin $\text{PGF}_{1\alpha}$ contracted granulation tissue at concentrations of 12×10^{-8} . Arachidonic acid had no effect.

Colchicine

Colchicine had a contracting action, which agrees with the results of Van den Brenk & Stone (1974). Activity was low, requiring doses of 1×10^{-4} and a long time (Fig. 5). No relaxation appeared, and contraction capability remained. This excludes the participation of microtubules in granulation tissue contraction which was suggested by Ehrlich et al (1977).

DISCUSSION

In agreement with Gabbiani et al (1971) our results showed granulation tissue to be a contractile organ, the contraction of which we found to have characteristics that differed from those of smooth muscle. The rate of contraction is low, up to 3 min being required for maximal response with 5-HT (the most active contracting substance) and tachyphylaxis soon occurs with all the substances tested, the second or third contraction always being smaller.

We have found that the most active substance producing contraction was 5-HT (0.1 to 30×10^{-6}). Ornipressin and angiotensin produced good contractions at low doses, but the maximum response was smaller (200 mg tension instead of the 300 mg with 5-HT). $\text{PGF}_{1\alpha}$ also contracted the granulation tissue, so did noradrenaline and adrenaline but weakly. Adrenaline in a second phase caused a relaxation which was also slight. All these drugs stimulate smooth muscle cells.

On the other hand, histamine at doses up to 1×10^{-4} did not produce any contraction or relaxation, which is in contrast to smooth muscle responses, nor did acetylcholine suggesting that the myofibroblasts differ from other adult contractile fibres in not having developed cholinergic or histaminergic receptors, thus placing them nearer to embryonic type, which are responsible for morphogenesis (Wessells et al 1971).

Among the relaxing substances tested, some were effective even on the base line (verapamil, papaverine and cytochalasin B), while others only inhibited contractions induced by agonists (atropine, cyproheptadine, methysergide, adrenaline, antazoline, cimetidine, phenoxybenzamine, and propranolol).

We suggest that granulation tissue has some kind of tonic contraction. Relaxation with verapamil suggests that contractions are mediated by calcium (Wessells et al 1971). The relaxation induced by adrenaline is too small to be considered.

The action of atropine and antazoline could be surprising, but it has been shown previously that although these drugs are supposed to be very specific,

they inhibit contractions induced by 5-HT in guinea-pig ileum (Rapport & Koelle 1953; Erspamer 1954) and other smooth muscle organs (Gaddum & Hameed 1954).

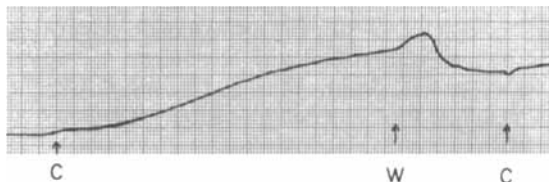


FIG. 5. Granulation tissue strip. Contractile effect obtained with colchicine. C = Colchicine 1.19×10^{-4} . W = Washing.

In view of the response to 5-HT the possibility of the presence of 5-HT receptors must be considered. The dose-response curves (Figs. 2, 4) presented semilogarithmically are straight, with a linear relation near to 1. Antazoline and atropine moved the curve to the right, maintaining the slope and maximum response, as did cyproheptadine and methysergide, which are typical competitive antagonists of 5-HT. The presence of 5-HT receptors has been recognized in rat uterus, guinea-pig ileum, rabbit ear artery (Gaddum & Hameed 1954) and in guinea-pig vas deferens (Puig et al 1974). In all those preparations atropine and histaminergic antagonists have a competitive action, so the evidence for 5-HT receptors in granulation tissue is supportive.

Although microtubules do not seem to have a contracting action (White & Gerrard 1979), but rather a structural function, Ehrlich et al (1977) have suggested they have a role in wound contraction. We found myofibroblasts contraction to be inhibited by cytochalasin B, while colchicine caused a contraction (Fig. 5).

These results agree with those obtained by Van den Brenk & Stone (1974), and they suggest the presence of microfilaments as described by Gabbiani et al (1971, 1972). This is all in agreement with Wessell's et al (1971) concept that portions of the complex striated muscle contractile system may be present in more primitive contractile machinery, and also with the idea that microfilament networks are associated with the behaviour of individual cells, such as migration, wound contraction, and cytokinesis. The myofibroblast would then be like a primitive cell which contributes to morphogenesis. Neo-formation needed for a second intention wound healing is actually a form of morphogenesis.

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