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Changes in blood flow, histamine and prostaglandin E_2 in rabbit skin grafts

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When skin is transplanted either as autografts or homografts, distinct vascular changes occur during healing in and in the case of homografts also at the onset of rejection (Medawar, 1944). The question arises, do these vascular changes reflect the activity of pharmacological mediators? To investigate this possibility an attempt was made to correlate blood flow changes with the presence of mediators in the graft tissue.

Full thickness skin grafts (Jasani & Lewis, 1971) were used and blood flow changes were measured using a ^{133}Xenon clearance technique (Lewis, Peck, Williams & Young, 1976). Grafts were homogenised in 60% alcohol, dried and assayed for histamine and prostaglandin (PG) E_2. Histamine was measured fluorimetrically (Evans, Lewis & Thomson, 1973) and PGE_2 by radioimmunoassay (Hennam, Johnson, Newton & Collins, 1974).

Blood flow was first detected in the grafts on day 3 after grafting, becoming maximal by 12.00 h on day 4 in homografts and between days 4 and 6 in autografts. The blood flow continued in the autografts but could no longer be detected by day 5 in the homografts.

There is a striking similarity between the pattern of changes of histamine and of blood flow in both autografts and homografts. In both, the histamine content was low for the first 3 days. In autografts it rose on day 4, reaching a peak by day 5 (4.4 μg/g tissue) which was maintained until day 8, and fell sharply on day 9 to control level (2.3 μg/g tissue). In the homograft the histamine content reached two peaks. The first on day 4 at 12.00 h (12.5 μg/g tissue) corresponded with the maximum increase in blood flow. The second peak was after the onset of rejection (i.e. stoppage of blood flow) on day 5 (14.5 μg/g tissue). Between the two peaks, at a time which corresponded to the fall in blood flow, there was a significant fall in the level of histamine (5.8 μg/g tissue).

In both autografts and homografts the level of PGE_2 was raised above control immediately after grafting (87–125 ng/g tissue) but fell to control level by day 4 (15–28 ng/g tissue). It remained low in autografts throughout the experiment but rose sharply on day 4 at 12.00 h in homografts. This short-lived plateau of PGE_2 (90–95 ng/g tissue) occurred during the first peak of histamine and the peak of high blood flow. The level of PGE_2 increased further after the onset of rejection.

Histamine H_1-receptor antagonist mepyramine (but not H_2-receptor antagonist, metiamide), prolonged the increased blood flow in the homografts and increased their survival time from 5 days to 7–9 days. Indomethacin, on the other hand, did not prolong the increased blood flow and had no effect on rejection time but caused a decrease in the maximum blood flow at a time when there was an increase in PGE_2 content in the homografts. Neither antagonist affected the blood flow changes in autografts.

These findings suggest that: (a) histamine, via an H_1-receptor, is involved in the fall in blood flow associated with the rejection process; (b) although changes in histamine correspond with those in blood flow in autografts, it is present in a form which is not accessible to antagonists (see Kahlson, 1962; Schayer, 1962); (c) a prostaglandin, probably PGE_2, accounts

partly for the maximum peak of blood flow attained in homografts; and (d) the increase of mediators after the onset of rejection is the result of non-specific tissue breakdown.

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Lysosomal enzyme release from leucocytes by N-formyl-L-methionyl-L-leucyl-L-phenylalanine *in vitro:* effect of some anti-inflammatory drugs

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When rabbit peritoneal leucocytes are incubated, *in vitro*, with low concentrations of N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) in the presence of cytochalasin B (5μg/ml), β-glucuronidase (a lysosomal marker enzyme) is released (Becker, 1976). Neither FMLP nor cytochalasin B alone induce β-glucuronidase release. This process is not accompanied by release of the cytoplasmic marker enzyme lactate dehydrogenase (LDH) (Becker, 1976). We have studied the effect of some anti-inflammatory drugs on the process.

Human leucocytes were exposed to cytochalasin B and drug for 15 min before the addition of FMLP. All agents were dissolved in dimethylsulphoxide (DMSO) to give a final concentration of DMSO of 0.75% in a saline medium. After 15 min incubation at 37°C the leucocytes were centrifuged at 12,000*g* for 30s and the supernatant removed for enzyme assay. β-Glucuronidase was measured by the method of Ringrose, Parr & McLaren (1975), and LDH by the method of Wroblewski & LaDue (1955). Total enzyme present in the leucocytes was determined by addition of Triton X-100 to give a final concentration of 0.2%. Controls were included to determine basal enzyme release as well as any effects of the drugs on the activity of β-glucuronidase and LDH.

The log dose-response curve for release of β-glucuronidase by FMLP in the presence of cytochalasin B (5μg/ml) was sigmoid, as described by Becker (1976) for rabbit peritoneal granulocytes. The EC50 in our conditions (2.3×10^{-8}M) was about one hundredfold greater. Human cells are similarly less sensitive to FMLP as chemotactic stimulant (Williams, Snyderman, Pike & Lefkowitz, 1977).

Release of enzyme was inhibited by hydrocortisone (10^{-3}M). At this concentration of hydrocortisone, FMLP, even at 10^{-4}M, released only about 5% of the control amount; whereas at 10^{-4}M hydrocortisone, release was barely inhibited. The antagonism therefore appeared to be non-competitive or insurmountable.

Indomethacin and phenylbutazone also inhibited release, moving the dose response curve to the right without loss of parellelism, and giving pA_{10} of about 4.05 and 4.96 respectively. Aspirin was inactive at 10^{-3}M but papaverine (5×10^{-5}M) and theophylline (10^{-3}M) were inhibitory. Neither LDH release nor the activity of β-glucuronidase was affected at any of these concentrations of drug.

These results suggest that treatment of leucocytes with FMLP and cytochalasin B activated an enzyme, which was competitively inhibited by indomethacin and phenylbutazone. If the enzyme were involved in prostaglandin synthesis, the effect of hydrocortisone could be attributed to inhibition of release of prostaglandin precursors, and that of inhibitors of phosphodiesterase to increase of tissue cyclic AMP.

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