contractions at 25°C in MgATP could occur without any Ca\(^{2+}\) [62.7% ± 9.2% of that in Ca\(^{2+}\) (mean ± s.d., n = 9)]. The 'desensitized' preparation is suitable for the investigation of the effects of caldesmon.

A typical experiment is illustrated in Fig. 1. The preparation is bathing in 'rigor' solution (composition: 5 mM-Pipes (pH 7.1, 25°C), 5 mM-EGTA, 50 mM-KCl, 1.5 mM-Na\(_2\)SO\(_4\)), contraction is initiated by addition of 4 mM-MgATP to this solution which also included 4 μM-caldesmon at the times indicated. Maximum tension was obtained when 4 mM-CaCl\(_2\) together with MgATP was added to the 'EGTA' solution.

Additional information about the effect of caldesmon on cross-bridge interactions can be obtained by imposing a shortening (0.2–0.3%) of the muscle length and monitoring the ensuing tension changes in conditions with or without caldesmon. Data from four preparations indicate that caldesmon, at concentrations of 4 and 6 μM, does not alter the specific muscle stiffness ([d(I/dl)/T], the instantaneous change in tension with length) nor the rate of recovery of force following the length change.

Hence we conclude that caldesmon has the ability to control steady-state isometric force of contraction and seems to do so by reducing the number of interacting cross-bridges rather than altering the individual properties of the cross-bridges. The thin filament regulatory system of smooth muscle can therefore control contractility.

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Synthesis, release and action of leukotrienes in the isolated rat heart

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Leukotrienes are a group of molecules which are synthesized from arachidonic acid by the action of a 5-lipoxygenase. They are produced by many different types of cells in response to various stimuli and they cause a variety of effects (reviewed by Hammarstrom, 1983). Infusion of leukotrienes LTC\(_3\) and LTD\(_3\) into isolated perfused guinea-pig or rat hearts causes vasoconstriction (Lettis & Piper, 1982; Roth & Lefer, 1983; Britil et al., 1985). This effect is blocked by leukotriene receptor antagonists and also by indomethacin, a cyclo-oxygenase inhibitor; it has been suggested that indomethacin is effective because leukotrienes act partly by stimulating thromboxane synthesis.

In an earlier publication (Garlick & Steinhardt, 1984) we reported that when the coronary effluent from a buffer-perfused heart was used as the perfusion medium for the same or a different heart, a profound vasoconstriction was observed. We found that this effect was blocked by indomethacin and also by the more specific cyclo-oxygenase inhibitor, flurbiprofen. We now report the results of further experiments we have carried out which suggest that this effect is mediated by leukotrienes.

Male Wistar rats (250–300 g) were lightly anaesthetized and heparinized, and their hearts were then excised and perfused in a non-recirculating Langendorff system at constant pressure (125 cmH\(_2\)O) with a modified Krebs buffer containing 11 mM-glucose and gassed with 95% O\(_2\) + 5% CO\(_2\) (v/v). After collecting the coronary effluent for 2 h, glucose was re-added and the effluent was then re-filtered (0.22 μM filter) and stored for up to 48 h at 4°C. In a separate series of experiments, a perfusion system with two matched reservoirs (A and B) was used. Hearts were perfused for a 2 h stabilization period with buffer from reservoir A, during which time the coronary flow was recorded every 10 min for the first 110 min and then every minute for the following 10 min. At 120 min, the perfusing medium was changed from buffer to coronary effluent by first releasing the clamp on the inlet from reservoir B and then clamping the inlet from reservoir
A change from buffer to coronary effluent; (ii) a change from buffer to effluent containing FPL 55712 (3.8 μM); (iii) a change from buffer to effluent collected from hearts perfused with buffer containing AA861 (1 μM); (iv) infusion of a mixture of leukotrienes LTC₄, LTD₄, and LTE₄ into a buffer-perfused heart. Data are given as mean ± S.E.M. (n = 6 or 8). CFR, Coronary flow rate.

The results obtained are illustrated in Fig. 1. When the perfusion medium is changed from buffer to coronary effluent, a transient increase is observed (possibly due to pressure changes caused by unclamping the inlet from reservoir B) followed by a sustained vasoconstriction, such that by 10 min the flow is only 78 ± 2% (n = 8, S.E.M.) of its original value. When the leukotriene receptor blocker, FPL 55712 (3.8 μM) is added to the effluent, a larger transient increase is observed, but at 10 min the flow is still 104 ± 3% (n = 6) of its original value. Leukotriene-depleted effluent was collected in the presence of the leukotriene synthesis inhibitor AA861 (1 μM). When this effluent is used as the perfusing medium, the coronary flow at 10 min is 98 ± 2% (n = 6) of its original value.

The effluent was analysed for leukotrienes LTC₄, LTD₄, and LTE₄ by radiimmunoassay after separation by h.p.l.c. (Beaubien et al., 1984). The analysis showed that the total amounts released over 2 h were 9, 5 and 32 pmol of LTC₄, LTD₄ and LTE₄, respectively. A leukotriene 'cocktail' was therefore made from these three synthetic leukotrienes such that when it was infused into a buffer-perfused heart at 1/20 of the coronary flow rate, the concentrations reaching the heart would be the same as the average concentration measured in the effluent; as can be seen from Fig. 1, the infusion resulted in a vasoconstriction which is almost identical to that obtained with the 'control' effluent.

In conclusion, we have shown that isolated, unstimulated, buffer-perfused rat hearts synthesize leukotrienes LTC₄, LTD₄, and LTE₄ in significant amounts. We would suggest that the vasoconstriction caused by these compounds may play a role in the deterioration of isolated, recirculating heart preparations such as the working heart.

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