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Short communication

The in vitro enhancement of rat myofibroblast contractility by alterations to the pH of the physiological solution

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Abstract

Wound contraction achieved by myofibroblast contraction is vital for the repair of cutaneous wounds. Many changes occur during tissue repair one of which is a lowering of pH. This study was designed to determine if myofibroblast contractility, as mimicked by using in vitro preparations, was sensitive to alterations of the pH. The responses of strips of rat superficial fascia when stimulated in vitro by adenosine, calcium and potassium ions, and mepyramine in physiological solutions at pH 5.5, 6.1, 7.3 and 8.1 were clearly pH dependent with acidic media producing an enhanced in vitro contractility. Perhaps modifying the pH of the wound environment could enhance wound contraction.

Keywords: Superficial fascia; (Rat); Wound healing; Adenosine; Mepyramine

1. Introduction

Wound healing is an orchestrated phenomenon involving interaction of many cells and complex factors (Clark, 1996). There is now extensive evidence from a variety of experimental methods that a cell central to the contractile phase of wound repair namely the myofibroblast exists and is contractile (Gabbiani et al., 1972). It appears that existing fibroblasts are transformed into myofibroblasts by as yet unknown factors so as to produce cells with marked enhanced power of contractility (Sappino et al., 1990; Schurch et al., 1992). Could a simpler explanation to account for this transformation lie in the 'environmental' changes which the fibroblast is exposed to during the process of inflammation? Such changes are of course multifactorial ranging from the products of blood coagulation to factors released from phagocytic cells. However throughout the initial stages of the inflammatory process there occurs changes in pH, in tissue oxygenation and lactic acid production to mention only three relevant factors.

To determine if changes in tissue pH could affect the contractility of myofibroblasts this in vitro study used strips of rat subcutaneous fascia a tissue with a resident population of myofibroblasts, to investigate the effect of changes of pH on their contractility. Sammak et al. (1993) found that acidification of the intracellular pH of the cells at the wound edge were only reversed when the pH of the media was increased. In addition, Lengheden and Jansson, 1995 reported an increase in fibroblast migration and DNA synthesis with reduction in the pH. Added together, these data suggest that the pH may be an important factor in the differentiation and termination of the contraction in the wound healing process. In this study, the influence of change in the pH of the physiological solution media was achieved using bicarbonate free Krebs–Hensleit solution, addition of lactic acid or an excess sodium bicarbonate to Krebs–Hensleit solution. The responses to mepyramine, adenosine, calcium chloride and potassium chloride are investigated on strips of the superficial fascia.

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2. Materials and methods

2.1. Preparation of the superficial fascia

Eighteen male Lister Hooded rats (University of Bradford strain) weighing 250–350 g were randomly divided into three groups of six. The rats were sacrificed by an overdose with sodium pentobarbital (200 mg/ml) and cervical dislocation. Two full-thickness incisions were made on the lower dorsum and the subcutaneous fascia was exposed. With aid of a fine forceps and scissors a small semitransparent layer of subcutaneous superficial fascia was removed and immediately placed in aerated (95% O_2 and 5% CO_2) Krebs–Henseleit solution (KH). One subcutaneous superficial fascia from identical site (5×20 mm) from each rat was prepared. The tissues were suspended for superfusion (Gaddum, 1953) and preloaded under 2 g tension and allowed to equilibrate for a period of at least 2 h until the baseline isometric tension remained constant. Normal Krebs– Hensleit solution at 37°C was pumped at rate of 3 ml/min over the tissues. Control responses to mepyramine (64 and 128 μM), adenosine (24–48 μM), calcium chloride (300 μM) and potassium chloride (400 μM) in constant volume of 100 μl bolus doses were added to the superfusate. The responses were again recorded using the same tissues either with acidic or alkaline KH solutions. KH solution was made acidic by removal of sodium bicarbonate (pH 5.5) and replaced it with equimolar concentration of sucrose. Under these conditions, in order to prevent further reduction of the pH, the solution was aerated with pure oxygen. Another acidic KH solution was prepared by the addition of approximately 1 ml of lactic acid per litre of KH solution (pH 6.6). The solution was made alkaline by a dropwise addition of approximately 20 ml sodium bicarbonate 8% per litre of KH solution (pH 8.1). No adjustment of electrolyte contents were thought necessary, due to the small amount of lactic acid or sodium bicarbonate necessary to produce the required changes. After equilibration with the second superfusate media test responses were recorded for each tissue in either acidic or alkaline media.

Table 1
Effect of pH of the physiological solution on the responsiveness of rat superficial fascia (mean mg tension ± S.E.M.)

Drug (μM)	KH (pH 7.3)	pH 6.6	pH 5.5	pH 8.1
Mepyramine (64)	97 ± 6	127 ± 8[a]	143 ± 16[b]	95 ± 9
Mepyramine (128)	145 ± 12	205 ± 14[b]	265 ± 16[c]	130 ± 15
Adenosine (12)	32 ± 1.6	52 ± 2.5[b]	360 ± 43[c]	37.5 ± 5
Adenosine (24)	87 ± 5	154 ± 11[c]	533 ± 88[c]	90 ± 10
Calcium chloride (300)	109 ± 10	205 ± 5[b]	225 ± 58[c]	130 ± 12
Potassium chloride (400)	−81 ± 5.5	−119 ± 4.5[a]	−508 ± 85[c]	−80 ± 8

[a] $P < 0.05$; [b] $P < 0.01$ and [c] $P < 0.001$ from control at pH 7.3 in Krebs–Hensleit (KH) solution.
Paired Student's *t*-test, $n = 6$, − = relaxation.

2.2. Recording device and experimental set up

The responses were recorded using 4 channel Grass recorder (D7 Polygraph, Grass Instrument, Quincy, MA, USA) via sensitive transducers (Type 5016, Palmer Bio-Science, Sheerness, Kent, UK). The tissues were arranged and placed in a superfusion arrangement adopted from that suggested by Gaddum (1953).

2.3. Statistical analysis

Quantitative results are expressed as mean ± S.E.M., and analysis of significance was made with paired Student *t*-test with $P < 0.05$ was considered significant.

3. Results

3.1. Effects of acidic media on the responses in the superficial fascia

When the superficial fascia were superfused with lactic acid containing KH solution (pH 6.6) the responses to both those agents that induced contraction (mepyramine, adenosine and calcium chloride) and that to potassium chloride which induced relaxation (Pipelzadeh and Naylor, 1996) were significantly increased (Table 1). However, under sodium bicarbonate free KH solution (pH 5.5) both the contractile and relaxation responses were further increased relative to the control responses in KH solution (pH 7.4). This may suggest that acidic pH may have induced changes in the receptor sensitivities towards the agents used.

One interesting observation is the profound increase in the responsiveness to adenosine in the acidic condition of low pH of 5.5 with a mean increase of at least 6 fold, whereas under pH 6.6 this increase was approximately 60% (Table 1). In addition, the relaxation responses to potassium chloride where also increased by 6-fold under pH 5.5 and only increased by 47% for pH 6.6.

3.2. Effects of alkaline conditions on the responsiveness of the superficial fascia

In contrast to the above observation, the responses to all the agents used were not significantly different from the controls.

In summary these observations suggest a modulatory role for acidic pH on the responsiveness of the superficial fascia.

4. Discussion

The experiments reported above show that influencing the pH of the physiological solution modifies the responses to a diverse range of agonists on myofibroblasts. Clearly, the responses to all the agonists, was pH dependent and this suggests that myofibroblasts may be sensitive to agonist-induced contractions in acidic environment. Do such environments exist in wounds and so have implications for the healing of wounds in vivo?

The evidence for this is not extensive. Schilling et al., 1959, using stainless steel wire mesh in mongrel dogs, reported the wound to be acidic as had Hunt and Halliday, 1980 who reported that the level of lactic acid in the wound fluid to be extremely high. Both these studies suggested that it was anaerobic metabolism, which had reduced the pH, but never gave an actual numerical value for the pH. Another group (Chakkalakal et al., 1994), who studied healing of bone fractures in rat, also reported an acidic pH in the initial stages of tissue repair followed by alkaline changes in the latter stages of fracture healing. The values given were between 7.28 to 7.52.

In a study by Konstantinov and Zatsepina (1985), carried out on 42 patients values were given for the pH changes around healing tissues for women suffering from purulent lactation mastitis, where the pH was initially 5.4 and then went to 8.2 at the end of the healing process. They used ultrasonic devices to actively lower the pH and then allowed it to become more alkaline.

These experiments suggest that changes to the wound environment may enhance the rate at which wounds contract and may be a simple explanation as to why this process proceeds at different rates under different conditions. Consequently, pH may be a contributing factor in addition to the more complex suggestions that wound contraction is more involved with growth factors (Rothe and Falanga, 1989) and complex phenotypic transformations of fibroblasts. Further experiments in progress may provide more evidence for such a suggestion.

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