EVIDENCE THAT THE REDOX STATE HAS A ROLE IN MUSCULAR CONTRACTION AND RELAXATION

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• *The present work reports that simple oxidizing agents are capable of inducing isotonic contraction of rat aorta in vitro, and that the concentration of agent required depends on its oxidizing potential. Conversely a reducing agent will reverse a muscular contraction induced by oxidizing agents.*

INTRODUCTION

There is now considerable evidence that actin and myosin are among the most important cellular proteins and that they play an essential role in many cellular processes such as division, movement, morphology, adhesiveness, membrane stability and integrity, capping of surface membrane receptor and secretion in addition to their classical role as muscle proteins. Given the importance of these contractile proteins in cellular structure and function, understanding their regulation is necessary.

It is generally believed that the actomyosin system is regulated by intracellular free Ca^{++} concentration via the regulatory proteins[1]. However, there is evidence that;

(i) Contraction can take place in the absence of the regulatory proteins[2].

(ii) Contraction can take place in the absence of Ca^{++} or in the presence of low intracellular Ca^{++} concentration when the ATP and Mg^{++} concentration are low, or when oxidizing agents are present[3].

(iii) For Ca^{++} sensitivity of the actomyosin system, myosin SH moieties are essential[3]. Furthermore, the Ca^{++} theory of muscle contraction fails to elucidate the nature of the physicochemical interaction between actin and myosin.

One of us (E. P-E) has presented evidence elsewhere[3], that the interaction between actin and myosin is via a hydrogen bond, formed by a charge transfer from one of the functional sulphydryl groups of myosin to actin. According to this theory contraction takes place when the magnesium or ATP concentrations are low or when oxidizing or SH reagents, which block or oxidize the other functional myosin SH group, are present. Conversely, reduction of myosin sulphydryl groups, in the presence of magnesium will result in ATP synthesis and relaxation. Although both actin and myosin contain sulphydryl groups only the myosin functional SH groups are freely reactive whilst the SH groups of actin are protected by myosin when actomyosin is formed[3,4].

MATERIALS AND METHODS

To test this hypothesis, 3 mm segments of rat aorta were suspended between two thin glass rods in a 20 ml water bath, containing Ringer's solution at a constant temperature, 37°C ± 0.5°C, with 5% CO_2 and 95% O_2

continuously bubbling. Isotonic contraction was measured via an LVDT)Shaevitz) transducer and recorded by a polygraph. (Riken Denshi).

Following mounting, all aortic segments were pretensed to 2 g and following a stabilisation period of two hours, contractile activity of the aortic segment was confirmed using a 2×10^{-2}M KCl solution. Artery segments that did not produce adequate contraction were discarded.

RESULTS AND DISCUSSIONS

The ability of cumulative additions of potassium permanganate to induce isotonic contraction of rat aorta is shown in Figure 1, which also demonstrates that relaxation of the artery occurs after the addition of further oxidizing agents at the peak of contraction.

Cumulative dose response curves were then calculated from the mean dose response of 6 different aortic segments for each of the following oxidizing agents: Hydrogen Peroxide (H_2O_2), Silver Nitrate ($AgNO_3$), Potassium Permanganate ($KMNO_2$), Sodium Perchlorate ($NaClO_4$), and Sodium Nitrate ($NaNO_3$). Figure 2 shows that these simple oxidizing agents are capable of inducing muscular contraction. This strongly suggests that oxidation is the fundamental interaction responsible for contraction. Furthermore, as

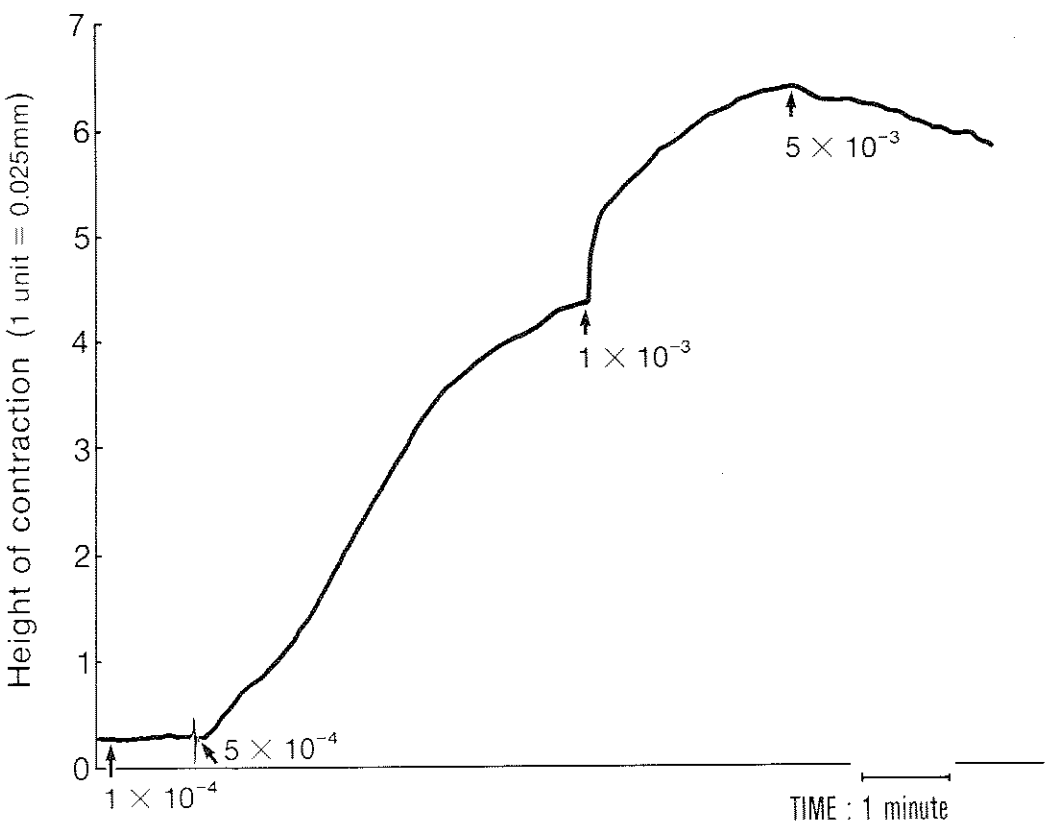

FIGURE 1. The dose response curve of a segment of rat aorta subject to increasing concentration of potassium permanganate ($KMNO_4$). The ordinate represents the displacement due to contraction and the abscissa represents time after addition of the oxidizing agent. The curve represents increasing contraction with successive additions of $KMNO_4$ from 1×10^{-4} to 5×10^{-3} molar. At the position of maximum contraction the addition of further $KMNO_4$ resulted in spontaneous relaxation after which the artery was unresponsive to further stimulation.

shown in Figure 3, the ability to induce contraction is highly correlated with the oxidation potential[5] of the reagent, E_o. The exception, $AgNO_3$ which causes contraction at a lower than expected concentration, may be due to the observed precipitation of silver around the artery causing permanent inactivation of the sulphydryl groups.

To elucidate the relaxing ability of reducing agents we tested the ability of Dimethylsuphoxide (DMSO) to relieve a contraction induced by 1×10^{-3} molar $KMNO_4$. We found that a 1 molar solution of DMSO relieved a maximum contraction within 20-30 minutes, while a 5×10^{-1} molar solution relieved 90% of maximum contraction within 40-50 minutes.

It is evident from the above results that relaxation may occur either as a result of oxidation or reduction at the peak of contraction. To clarify the important difference between these mechanisms, two arteries were maximally stimulated by 5×10^{-2} $KMNO_4$, following which one was left in oxidizing agent while the other was rapidly reduced with 1M DMSO. In both cases the arteries relaxed. They were then washed repeatedly and subsequently treated with 2×10^{-2}M KCl. Only the artery subject to DMSO treatment showed response to the addition of KCl. This behaviour was repeated on 3 pairs of arteries and is strong evidence that following contraction the actomyosin system must be reduced before further contraction is possible. This type of behaviour is also observed with other vasoactive compounds (e.g. serotonin) in which following peak contraction, relaxation will result after addition of further serotonin, leaving the artery in a "desensitized state" from which further contraction is inhibited[6].

These results are compatible with the theory previously published[3], which claims that myosin is found in two states, an energized state in which myosin forms a sulphydryl-magnesium-ATP ring, and a de-energized state in which the ring is broken. This ring can be broken by decreasing the

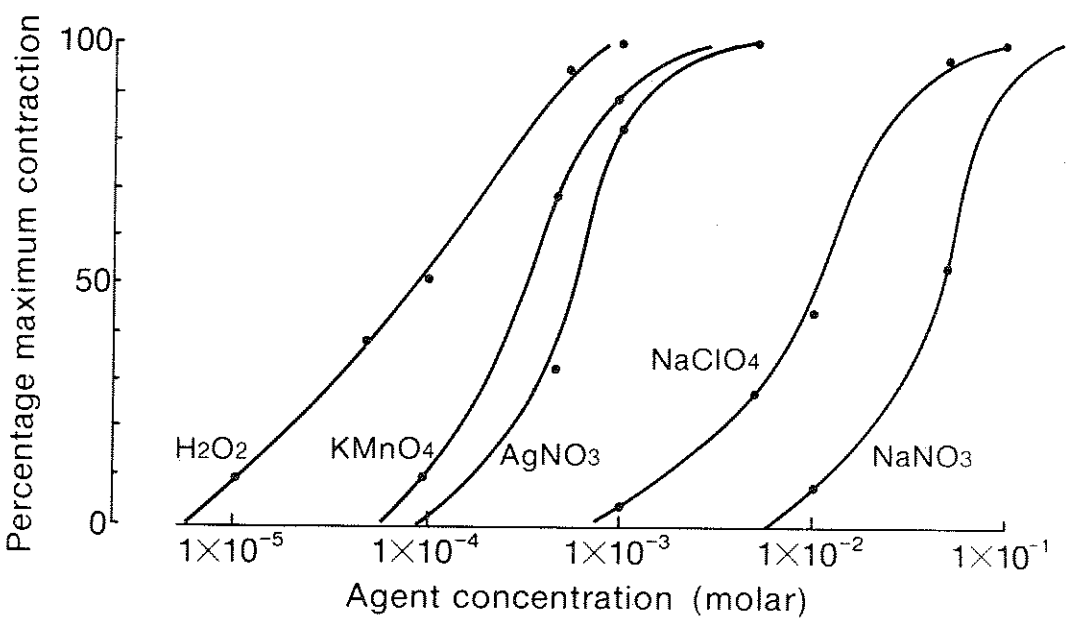

FIGURE 2. The figure demonstrates the cumulative dose response curves of the 5 oxidizing agents tested — H_2O_2, $KMNO_4$, $AgNO_3$, $NaClO_4$, $NaNO_3$. The ordinate is the percentage of maximum arterial contraction for each of the individual oxidizing agents. The abscissa is the molar concentration of each agent.

magnesium, or ATP concentration, or oxidation or blocking of the sulphydryl group SH_2, following which a charge transfer from the myosin sulphydryl group SH_1 to actin occurs, resulting in ATP hydrolysis and resultant contraction. For contraction to persist, the hydrogen bond between myosin and actin must be maintained, however in the presence of excessive concentration of oxidizing agent, this bond also will be oxidized, and myosin and actin will dissociate, but without formation of the energized ring, leaving myosin in an unresponsive state. Physiological relaxation takes place by reduction of myosin, with ATP synthesis, in the presence of magnesium and formation of the energized ring.

The redox changes in the functional SH groups of myosin induced by the oxidizing and reducing agents do not have to be direct but could be induced indirectly through what Ling calls an association-induction effect[7]. The oxidizing agent could directly interact with the myosin ATP or some foci in the protein which will activate the myosin ATPase. They could also interact with other myosin amino acids, amino acids of another protein, non-protein groups such as lipids or sugars and these, in turn, induce oxidation of the functional myosin SH groups, charge transfer from myosin to actin, ATP hydrolysis and thus contraction.

These results and their explanation have important implications in the mechanisms of

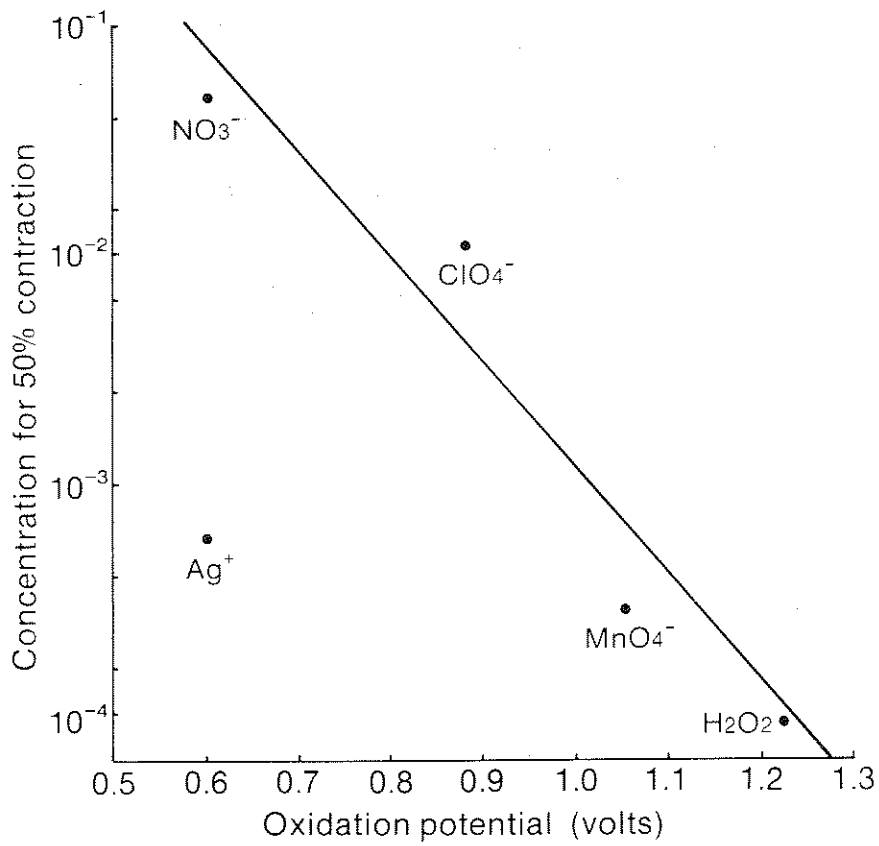

FIGURE 3. The figure demonstrates that the ease with which an oxidizing agent induces contraction is dependent on its oxidation potential. The ordinate is the molar concentration of the oxidizing agent that produces 50% contraction (obtained from Figure 2). The abscissa is the oxidation potential in volts calculated for the oxidizing agent at pH 7.4 using tables of electrochemical equilibria[5].

action of clinically relevant vasoactive compounds and in the management of pathological conditions.

There is already evidence that diverse pathological agents including cigarette smoke induce oxidation of cellular SH and that the concentration of SH in such diverse pathological conditions as cancer, rheumatoid arthritis, muscular dystrophies, cardiomyopathies and cerebral ischaemia is decreased[8]. Cerebral vasospasm resulting from progressive narrowing of cerebral vessels following subarachnoid haemorrhage is also known to be associated with an oxidized environment[9].

There exists experimental evidence that vasoactive compounds such as serotonin, noradrenalin, acetylocholine and prostaglandin are dependent on functional sulphydryl groups on the cellular membrane and it is found that oxidation of these sulphydryl groups will inhibit the binding and the physiological function of these compounds[10–18]. Furthermore it has been shown that the protein of the acetylocholine receptor, like myosin, is formed by related subunits, and has the same molecular weight as heavy meromyosin and copurifies with actin[19,20]. It is therefore proposed that these vasoactive compounds induce their effect through a direct or indirect oxidation of the myosin sulphydryl groups within the cell. It is evident that, although oxidation and reduction are fundamental mechanism leading to contraction and relaxation respectively, steric effects will be relevant in increasing the efficiency with which a given compound is capable of oxidizing or reducing the appropriate sulphydryl site.

This explains the greater effectiveness of some compounds in inducing muscular changes and the fact that although some compounds are regarded as specific for certain muscle receptors, this specificity is never more than an enhanced sensitivity over comparable reagents.

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