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Importance of the redox state in vasoconstriction induced by adrenaline and serotonin

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ABSTRACT The actin myosin system and the redox state have been implicated in a number of cellular functions and in many pathological conditions. To study the predictions of a new redox theory of actin-myosin interaction we examined the relationship between the redox state and vasoconstriction of rat aorta and dog basilar artery by adrenaline and serotonin. The results show that the contraction induced by these physiological agents can be inhibited and reversed by reducing agents.

There is considerable evidence that the contractible proteins, actin and myosin, are found in all cells and cellular subunits, including the surface of the plasma membrane.[1–3] In fact these proteins form the basis of the cytoskeleton, which is an integrated system of proteins interconnecting all cellular organelles.[4] Apart from their structural role, these proteins have been found to play a major role in many cellular phenomena, including movement, adhesiveness, membrane stability and integrity, secretion and capping of surface membrane receptors. However their best known role still remains that in muscle structure and function. Thus any attempt to explain cardiovascular diseases must involve an understanding of the cyclic contraction-relaxation of the actin-myosin system. It is generally believed that the actomyosin system is regulated by intracellular free Ca^{++} concentration via the regulatory proteins.[5] However, there is evidence that (1) contraction can take place in the absence of the regulatory proteins;[5] (2) contraction can take place in the absence of Ca^{++} or in the presence of low Ca^{++} concentration when the ATP and Mg^{++} concentration are low, or when oxidising agents are present;[6] and (3) for Ca^{++} sensitivity of the actomyosin system, myosin SH moieties are essential.[7]

One of us (E P-E), has proposed the theory that both actin-myosin interaction and Ca^{++} concentration are regulated by oxidation of the functional sulphydryl (SH) groups of myosin and by a charge transfer between actin and myosin. In some instances the redox changes in the functional SH groups of surface and intracellular myosin induced by the oxidising and reducing agents may be direct. In other instances they may interact only with the SH groups of the surface myosin and this in turn may lead to redox changes of the SH groups of intracellular myosin. They could also interact with other myosin amino acids, amino acids of other proteins (receptors), or non-protein groups such as lipids or sugars, and these in turn could induce either oxidation or reduction of the functional SH groups of surface and intracellular myosin SH groups. A further indirect method of inducing changes in intracellular redox is by oxidising elements of the extracellular medium which are capable of diffusing into the cell. Thus even if the contractile proteins do not come into direct contract with oxidising or reducing agents we would expect that their influence would be manifest by indirect methods. We have presented experimental evidence elsewhere[8] that (1) simple oxidising agents (peroxide, silver nitrate, potassium permanganate, sodium nitrate) produce muscle contraction; (2) the concentration of agents required depends on their oxidising potential; and (3) the contraction induced by the above agents can be inhibited and reversed by reducing agents. The present experiments were

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designed to see whether the physiological vasoconstrictors adrenaline and serotonin behave like oxidising agents as the theory predicts.

Methods

We used male Wistar rat aorta and mongrel dog basilar artery.

In the rat, an appropriate dose of thiopentone was given to maintain respiration while producing anaesthesia. The abdominal wall was incised, the peritoneum was divided and the abdominal contents and connective tissue separated from the abdominal aorta which was then transected and removed.

In the dog, animals weighing between 15 and 25 kg were given a lethal dose of thiopentone, the calvarium removed and the basilar artery dissected free from the brain stem.

After removal the vessels were divided into 3 mm segments and suspended between two glass hooks in a water bath containing 20 ml Krebs-Ringer buffer solution (NaCl 120 mM, KCl 4.5 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.0 mM, $NaHCO_3$ 27.0 mM, KH_2PO_4 1.0 mM, Na_4EDTA 0.01 mM, glucose 10.0 mM), at pH 7.4. To keep the volume constant when testing, the volume of buffer was decreased by the volume of reagent added.

The vessels were pretensioned with a force of 2×10^{-2} N and allowed to stabilise for 2 h while the bath was bubbled with 5% CO_2 and 95%O_2 and maintained at a constant temperature of 37±0.5°C.

Isotonic contraction was measured with an LVDT (Shaevitz) transducer and recorded on a chart Recorder (Ricken Denshi, Tokyo) model 7858A polygraph. After stabilisation, contractile activity of the vessel segments was confirmed using potassium chloride with a bath concentration of 2×10^{-2} M. Segments that did not produce adequate contraction were discarded, the others were repeatedly washed with 200 ml of buffer and allowed to restabilise.

Results

After stabilisation a control dose-response curve was obtained using an adrenaline concentration ranging from 2×10^{-8} M to 2×10^{-5} M with rat aorta and a serotonin concentration ranging from 1×10^{-9} M to 1×10^{-5} M with dog basilar artery (fig 1). To test the relationship between the contraction induced by the physiological vasoconstrictors and the redox state the following reducing agents were added to the water bath (concentration in bath is given in brackets): citric acid (1×10^{-2} M), succinic acid (1×10^{-2} M), reduced glutathione (3×10^{-2} M) and dimethyl sulphoxide (1×10^{-1} M). After the addition of the reducing agents a repeat dose-response curve for adrenaline and serotonin was performed. Each control and test was performed sequentially on the same vessel segment after repeated washing with 200 ml of buffer. Each reagent and control test was repeated three times on three different vessels. The test results were expressed as a percentage of the control contraction induced by 1×10^{-5} M serotonin and 2×10^{-5} M adrenaline. As seen in fig 1 the reducing agents induced a significant inhibition of adrenaline and serotonin contraction. Reduced glutathione (not

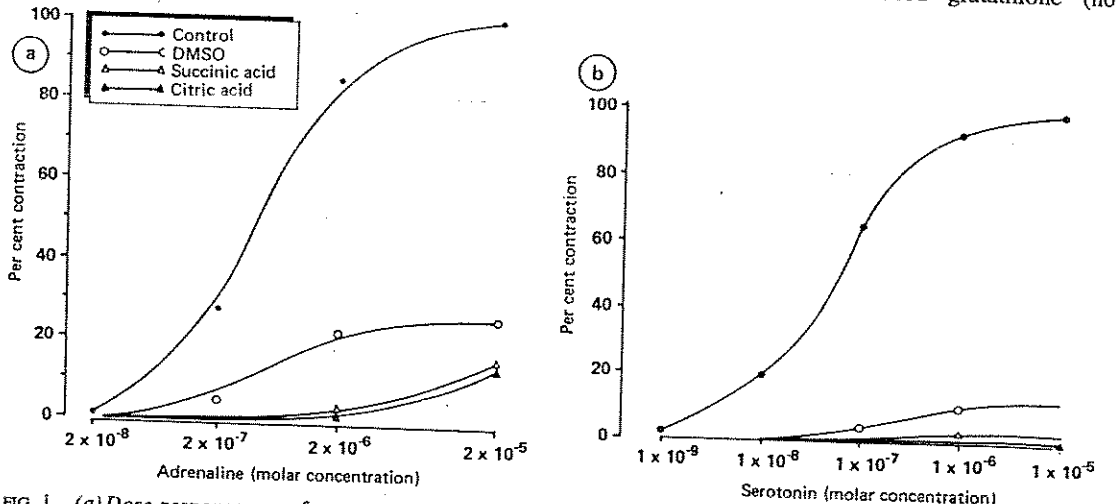

FIG 1 *(a) Dose-response curve for rat aorta treated with adrenaline. Separate curves show the control response and the response with 10^{-1} M dimethyl sulphoxide, (DMSO) 10^{-2} M succinic acid and 10^{-2} M citric acid respectively. (b) Dose-response curve for dog basilar artery treated with serotonin. Separate curves show the control response and the response with 10^{-1} M dimethyl sulphoxide, 10^{-2} M succinic acid and 10^{-2} M citric acid respectively.*

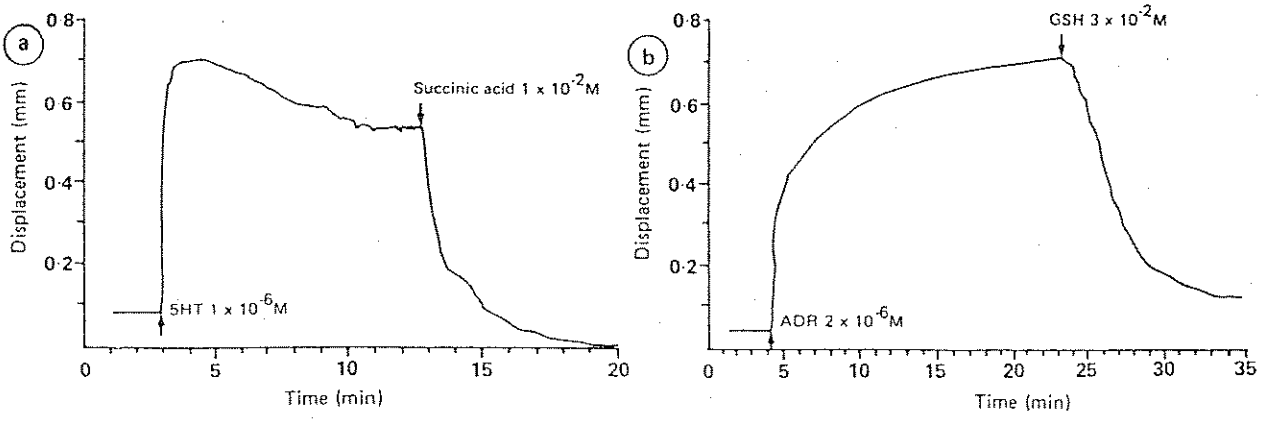

FIG 2 *(a) Contraction of dog basilar artery with 10^{-6} M serotonin and its subsequent relaxation following addition of 10^{-2} M succinic acid. (b) Contraction of rat aorta with 2×10^{-6} M adrenaline and its subsequent relaxation following addition of 3×10^{-2} M glutathione.*

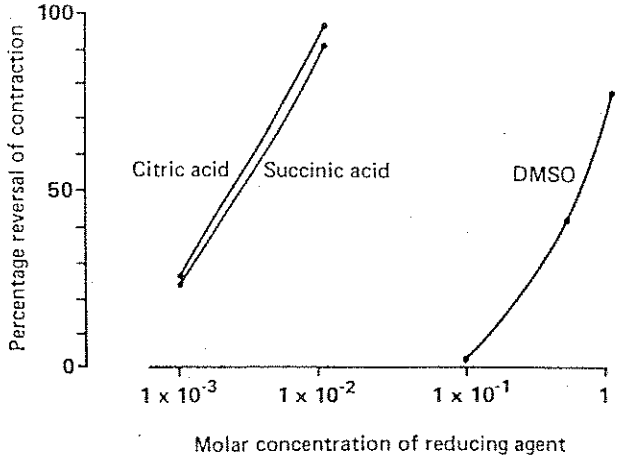

FIG 3 *The relationship between concentration of the reducing agents dimethylsulphoxide (DMSO), and succinic acid and reversal of rat aorta contraction induced by 2×10^{-6} adrenaline.*

shown) was the most potent, in that only 1% of the control contraction was produced. After testing and repeated washing with 200 ml of buffer all vessels responded normally to stimulation with 2×10^{-2} M potassium chloride.

To test the ability of reducing agents to reverse contraction induced by the physiological compounds, rat aorta was contracted by 2×10^{-6} M adrenaline and dog basilar artery by 1×10^{-6} M serotonin. At peak stable contraction, succinic acid, citric acid, reduced glutathione or dimethyl sulphoxide were added to the water bath. Each test was repeated three times on three different vessel segments. Following testing with the reducing agents and washing with buffer all vessels responded normally to a repeated potassium chloride stimulation.

All reducing agents tested reversed the contraction induced by 1×10^{-6} M serotonin. Reversals of 115%, 100%, 45% and 99% occurred with succinic acid at 1×10^{-2} M, citric acid at 1×10^{-2} M, dimethyl sulphoxide at 1×10^{-1} M and reduced glutathione at 3×10^{-2} M respectively.

The reducing agents were also able to reverse a stable contraction induced by adrenaline. Dimethyl sulphoxide at 5×10^{-1} M concentration produced 41% reversal of contraction. Succinic acid and citric acid at 1×10^{-2} M concentration produced 91% and 97% respectively. Reduced glutathione had a very specific dose (3×10^{-2} M) at which 91% reversal occurred (fig 2). Lesser doses of glutathione had minimal effect. Figure 3 shows the relationship between reversal of adrenaline induced contraction and the concentration of citric acid, succinic acid and dimethylsulphoxide.

Discussion

These results show that contraction induced by adrenaline and serotonin, in the same way as contraction induced by single oxidising agents, can be inhibited and reversed by reducing agents. Other workers have shown that both adrenaline and serotonin cause tissue oxidation and that their binding, active uptake and metabolism depend on cellular sulphydryl groups.[9–15]

In our previously reported experiments with oxidising agents[8] we found that the vessel's maximum contraction with these agents could be relaxed not only by reduction but also by further addition of those agents. We (results not shown) and others have observed the same effects in vitro and in vivo with the physiological vasoconstrictors.[16 17] However, there is a very important physiological difference between the two different methods of relaxation. The muscle relaxed by reduction will respond to a further

stimulation while that relaxed by high concentration of simple oxidising agents and physiological vasoconstrictors is in a "desensitised" state and does not respond to stimulation.

It appears, therefore, that the above physiological vasoconstrictors behave like oxidising agents, and by this oxidative property induced their physiological effects. Although not constituting a proof, nevertheless these results are compatible with the main prediction of the theory that oxidation leads to contraction and reduction to physiological relaxation.

Irrespective of the theory, these results as well as those of other workers may have important implications in understanding the mechanism of action of clinically relevant vasoactive compounds and in the prevention and management of cardiovascular pathologies. There is evidence that the function of other physiological vasoconstrictors is also redox dependent. For example oxidation of the cellular sulphydryl groups inhibits the binding and the effects of acetylcholine and prostaglandins.[18–20]

Thus we propose that vascular response is determined by the redox state of the system and that conventional vasoactive compounds exert their influence by oxidising or reducing of the actin-myosin system either directly or indirectly.

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