# ROLE OF $Ca^{++}$ IN VASODILATOR REACTIONS

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When vascular reactivity of resting dog skeletal muscles was tested by determining the intensity of reactive hyperemia (RH) in response to occlusion of the femoral artery for 10 sec, after intravenous bolus injection of small doses (10 μg) of noradrenalin (NA), the action of NA was found to be phasic: an increase in RH to 301 ± 26.8% (M ± m) if the artery was occluded 20-25 sec after injection of NA, and absence of RH in the case of later (40-60 sec) occlusion of the artery [1]. Simultaneous recording of the blood flow in the femoral artery and of the oxyhemogram of venous blood flowing from the skeletal muscles, and also analysis with the use of α- and β-adrenergic agonists and blockers led to the conclusion that the phase of increased RH is due mainly to stimulation of metabolism in skeletal muscles by catecholamines through the α-receptors of the skeletal muscles, whereas the next phase (absence of RH) is connected with the direct α-adrenergic constrictor effect of NA on vascular smooth muscles. The phasic character of the response of the vessel wall to intravenous injection of NA arose from the fact that the time constant of activation of α-adrenoreceptors, increasing the permeability of cell membranes for $Ca^{++}$, was shorter in skeletal muscles than in smooth-muscle cells (SMC).

In this investigation vasodilator effects mediated through α-adrenoreceptor stimulation, were studied, paying particular attention to changes in vascular reactivity connected with α-adrenergic stimulating of metabolism in skeletal muscles, which is usually not taken into account when nervous influences on vascular reactivity are studied. To rule out β-adrenergic stimulation by catecholamines, whose physiological effect on SMC is opposite to that of α-stimulation (it is largely similar in its ultimate metabolic effect in skeletal muscles, although realized by different biochemical mechanisms) the effect of α-adrenergic stimulation was stimulated by intra-arterial injection of $CaCl_2$. This method had advantages over the use of β-blockers, for in the course of one experiment the injection of $CaCl_2$ could be repeated several times, while the metabolic background in the skeletal muscles remained stable.

## EXPERIMENTAL METHOD

Vascular reactivity of resting skeletal muscles to intra-arterial injection of 0.6 mmole $CaCl_2$ in 6 ml of blood together with 1500 U heparin was investigated *in situ* in the hind limb of 19 mongrel dogs, anesthetized with hexobarbital (50 mg/kg, intravenously). Vascular reactivity was tested by measuring the intensity of RH in response to occlusion of the femoral artery for 10 sec, starting 8-10 and 30-40 sec after the end of injection of $CaCl_2$. The blood flow in the femoral artery was recorded by means of a "Delalande Électronique" ultrasonic flowmeter, and the blood pressure (BP) in the femoral artery was recorded on a "Hewlett Packard 1280" electromanometer, the transducer of which was applied distally to the point of temporary occlusion of the artery, so that during occlusion, the vascular resistance in the test limb could be judged from the steepness and degree of fall of pressure. Oxygen saturation of the venous blood was recorded on the 0.36 M oxyhemograph, the transducer of which was located on a continuous-flow cuvette, $\alpha_1$-Adrenoreceptors were blocked with prazosin (0.1 mg), $\alpha_1$- and $\alpha_2$-adrenoreceptors by phentolamine (2 mg), and $\alpha_2$-adrenoreceptors by yohimbine (1 mg), which were injected intra-arterially. NA and isoprenaline were injected intravenously by the bolus method in a dose of 10 μg.

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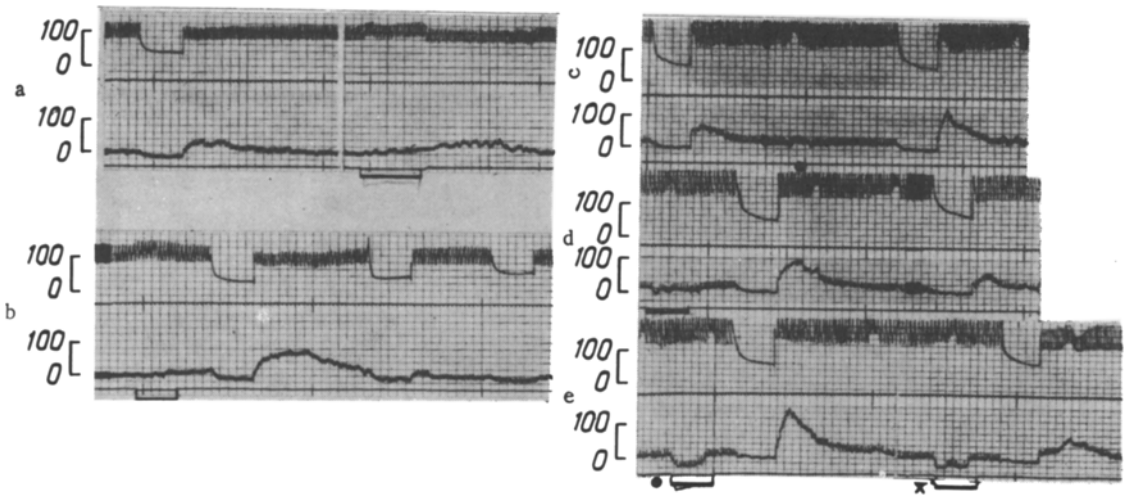


Fig. 1. Dilator response of dog's femoral artery to intra-arterial injection of 0.6 mmole $CaCl_2$ and phasic nature of response of femoral artery, reflected in intensity of RH in response to occlusion of artery for 10 sec at different times after injection of Ca. From top to bottom: BP (in mm Hg), averaged blood flow (in ml/min), marker of injection (period of injection of $CaCl_2$ indicated by rectangle, bolus injection of NA by dot, and bolus injection of isoprenoline by cross). One small square corresponds to 2 sec. *a*: Left — control RH after occlusion of artery for 10 sec, right — increase in blood flow after intra-arterial injection of $CaCl_2$. b: On left — increase in RH in response to occlusion of artery for 10 sec, starting 8 sec after intra-arterial injection of $CaCl_2$ (vascular resistance reduced), right — repeated occlusion of artery (RH absent, vascular resistance increased); c: left — control RH after occlusion of artery for 10 sec. Right — increase in RH after occlusion of artery 25 sec after intravenous injection of NA (vascular resistance unchanged); d: left — increase in RH after occlusion of artery starting 12 sec after intra-arterial injection of $CaCl_2$, right — control RH; e: left — summation of dilator responses during RH after occlusion of artery preceded by combined injection of NA and $CaCl_2$, right — occlusion of artery after combined injection of isoprenaline and $CaCl_2$.

## EXPERIMENTAL RESULTS

Intra-arterial injection of $CaCl_2$ was accompanied by a transient increase, to a varied degree, in the blood flow in the limb (Fig. 1a, right), which began immediately after the end of $CaCl_2$ injection and lasted 20-30 sec, or occasionally longer (but not more than 40 sec). The increase in blood flow in response to intra-arterial injection of $CaCl_2$ occurred regularly, and it could not be obtained in only one experiment, in which there was total areactivity of the vessels even in response to intra-arterial injection of 0.25 ml of a 2% solution of papaverine and 0.3 ml of a 20% solution of caffeine, because of the low systemic BP in the dog. The dilator response of the femoral artery to injection of $CaCl_2$ was potentiated against the background of intravenous injection of 20 ml of a 40% solution of glucose, $\alpha_1$-Adrenoblockade regularly inhibited the vasodilator response to intra-arterial injection of $CaCl_2$. Yohimbine, unlike $\alpha_1$-adrenergic blockers, abolished the vasodilator response to $CaCl_2$ in only half of the cases, and in the rest it had little effect on it, and in one case it actually potentiated the vasodilator response to intra-arterial injection of $CaCl_2$. Vasodilatation in response to injection of $CaCl_2$ apparently contradicts the view which has arisen mainly as a result of research on isolated vessels that an increase in the extracellular $Ca^{++}$ concentration must always lead to contraction of SMC in the vessel wall and, consequently, to narrowing of the lumen of the vessels. However, the reaction of a vessel *in vivo*, with a complex geometry of the direction of the SMC in the vessel wall and endothelial layer, which plays an important role in the synthesis and degradation of humoral factors, cannot be regarded as equivalent to the response of isolated SMC [5]. The role of the blood cells, especially platelets, which like the endothelium, can participate in a cascade of reactions, stimulated by Ca and hormones ($\alpha$-adrenergic agonists and acetylcholine), with activation of phospholipase A and lipoxygenase, with the formation of hydroperoxides of unsaturated fatty acids, activation of guanylate

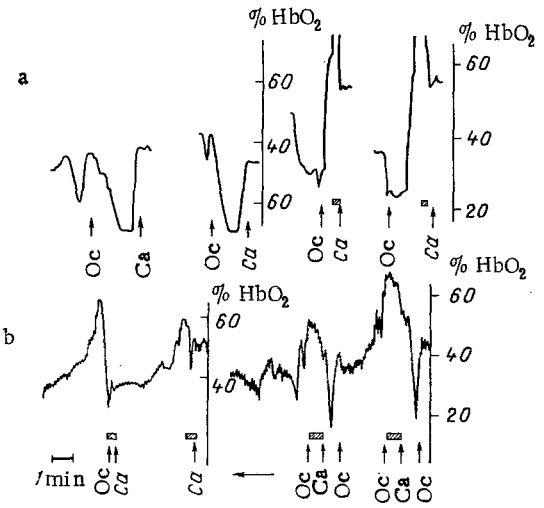


Fig. 2. Oxyhemograms of responses to intra-arterial injection of $CaCl_2$: *a*) Predominance decreased in oxygen saturation of venous blood, b) predominant increase in oxygen saturation of venous blood. Oc) Occlusion. Explanation in text. Here and in Fig. 3, oxyhemograms are to be read from right to left.

cyclase, and accumulation of cyclic guanosine monophosphate which, like hydroperoxides, can perhaps exert a vasodilator action [6, 9], must also be taken into account.

Despite the vasodilatation induced by intra-arterial injection of $CaCl_2$, occlusion of the femoral artery 8-10 sec after the end of injection of $CaCl_2$ was accompanied in half of the cases by an increase in RH. It will be clear from Fig. 1b that the vasodilator response after occlusion of the artery, 8 sec after injection of $CaCl_2$, was considerably stronger than the vasodilator responses to $CaCl_2$ (see Fig. 1a, right) and the control RH (see Fig. 1a, left). Occlusion of the artery 40 sec after the end of injection of $CaCl_2$ and later (see Fig. 1, 2nd and 3rd occlusions of the artery) were not accompanied by the development of RH. Vascular resistance during this period, as the curve of the drop of perfusion pressure during occlusion of the artery shows, was increased. Thus against the background of injection of $CaCl_2$, just as when small doses of NA were given, a phasic vascular response was observed: a phase of increase and a phase of absence of RH during occlusion of the artery at different times after intra-arterial injection of $CaCl_2$. Moreover, as will be clear from Fig. 1, if the phase of increase of RH in response to injection of NA (Fig. 1c, right, control RH on left) and the phase of increase of RH in response to injection of $CaCl_2$ (Fig. 1d on left, control RH on right) are superposed in time, the dilator responses undergo summation during RH preceded by combined injection of NA and $CaCl_2$ (Fig. 1e, on left). Replacement of NA by isoprenaline (Fig. 1e, on right) did not lead to an additive dilator effect. The fact that after injection of $CaCl_2$ a phase of increased RH followed occlusion of the artery, during which the supply of oxygen and energy-yielding substrates went into deficit, confirms the previous hypothesis [1]: α-adrenergic activation of metabolism in the skeletal muscles has vasodilator influences on the vessels supplying the muscles.

Changes in the oxyhemogram of venous blood flowing from skeletal muscles in response to injection of $CaCl_2$, unlike the more uniform changes to injection of NA, varied considerably from animal to animal, although the individual response to repeated injections of $CaCl_2$ was stable, as will be clear from Fig. 2 (the oxyhemograms are to be read from right to left), where two traces of responses to $CaCl_2$ are given in each case. Responses with a predominant increase in oxygen saturation of the blood (Fig. 2b) were observed in 10 cases, responses with a predominant decrease of saturation (Fig. 2a) in eight cases. In one case there was no change in the oxyhemograms in response to injection of $CaCl_2$. We know that Ca stimulates respiration and activates several dehydrogenases in the mitochondria [4]. This state of affairs can explain the increase in oxygen extraction by the muscles after injection of $CaCl_2$. The increase in oxygen saturation of the venous blood after injection of $CaCl_2$ can be partly explained by the vasodilator response to injection of $CaCl_2$ (excessiveness of the blood flow). However, if the time of the dilator response to injection of $CaCl_2$ (see Fig. 2, shaded rectangle) is compared with the duration of the rise of the oxyhemogram, it will be clear that at a certain

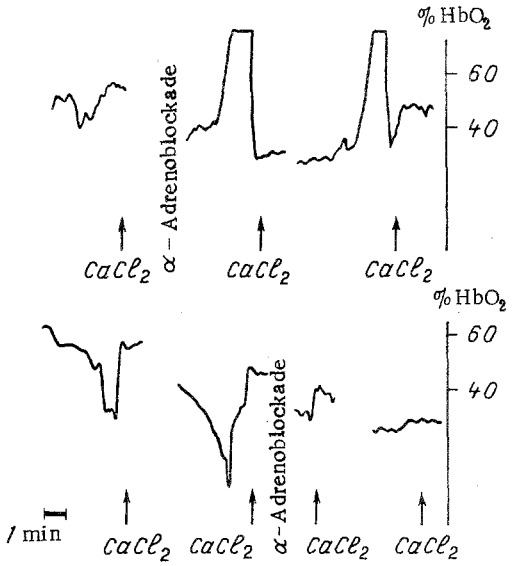


Fig. 3. Shift of response of oxyhemogram of venous blood toward increased oxygen extraction from blood by muscles after α-adrenergic blockade.

stage after injection of $CaCl_2$, the oxygen consumption of the muscles was reduced in half of the cases. It has been shown that glucose transport into the muscle cell and phosphofructokinase activity are controlled through α-adrenergic mechanisms [3]. The distinguishing features of the changes in the oxyhemogram in response to injection of $CaCl_2$, namely stability of the individual response and variation of the time course of the oxyhemogram from animal to animal in response to injection of $CaCl_2$, are evidently connected with individual differences in metabolism in the skeletal muscles, the degree of activation of respiration and glycolysis by calcium, differences in the time of beginning and ending, and nonsynchronization of the return to the original level of activity of respiration and glycolysis. However, both processes — respiration and glycolysis — are activated in the resting muscle during an increase in the blood Ca concentration. An indirect indication that an increase in oxygen saturation of the venous blood is linked with activation of glycolysis is given by the decrease in the oxygen consumption observed during occlusion of the artery. As will be clear from Fig. 2b (right), occlusion of the artery before injection of $CaCl_2$ was accompanied by a marked fall in the oxygen saturation of the venous blood. Occlusion of the artery for the same length of time, but carried out during the period of rise of the oxyhemogram curve, was accompanied by a much smaller fall in oxygen saturation of the venous blood. However, even in the case of predominant activation of respiration by Ca (Fig. 2a), occlusion of the artery immediately after the end of the period of increased extraction of oxygen by the muscles (2.5 min after injection of CA), at the time of rise of the oxyhemogram curve above its initial level was accompanied by a less deep fall of the oxyhemogram than when the artery was occluded later — 3.5 min after injection of Ca.

The high frequency of responses of increased oxygen saturation of the blood to injection of Ca compared with injection of NA can be explained on the grounds that activation of β-adrenoreceptors by NA blocks the entry of extracellular glucose into the muscles [8]. Against the background of intravenous injection of glucose, the oxyhemogram after $CaCl_2$ was shifted to some degree or other toward an increase in oxygen saturation of the blood. α-Adrenergic blockage (yohimbine and, to a greater degree, phentolamine), on the other hand, changed the time course of the oxyhemogram in response to injection of Ca toward an increase in extraction of oxygen from the blood by the muscles (Fig. 3). This can be explained on the grounds that α-adrenoreceptor blockade reduced permeability for extracellular glucose in the muscles, but Ca, whose pathways of entry are not receptor-stimulated, continued to stimulate respiration. α-Adrenoblockade considerably weakened or completely abolished the constrictor phase to injection of $CaCl_2$, from which it follows that permeability for Ca in vascular SMC of resting skeletal muscles is largely determined by α-adrenoreceptor activity.

It is difficult to explain in the animal by what mechanism glucose potentiates vasodilator responses to injection of $CaCl_2$. Not all links in the chain of Ca- and hormone-dependent reactions responsible for vasodilatation have yet been investigated completely. A no less

difficult problem is that of the mechanisms of adaptive (i.e., determined by changes in metabolism in skeletal muscles) vasodilator reactions. However, it can be postulated on the basis of the results that disturbance of vasodilator influences, due to α-adrenergic stimulation in effector tissues, and counteracting the direct α-adrenoreceptor constrictor effect on SMC of the vessel wall, may lead to systemic disturbance of vascular tone. Further investigation of adrenergic influences on vasodilator adaptive reactions, and also of Ca- and α-adrenergically dependent relations of SMC of the vessel wall with the endothelium and blood cells, may shed some light on the mechanisms of the hypotensive action of Ca in a high proportion of patients with hypertension [2, 7].

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# ROLE OF THE ENDOTHELIUM IN CONTRACTILE RESPONSES OF VASCULAR SMOOTH MUSCLES WITH DIMINISHED OXYGENATION


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Many new data have been obtained to broaden significantly our traditional views on the functional role of the vascular endothelium. Participation of the endothelium in relaxation of smooth muscles (SM) of arteries in response to injection of acetylcholine [6], histamine [10], ATP and ADP [4], bovine thromin and arachidonic acid [5], bradykinin [3], substance P [11], the calcium ionophore A 23187 [3], etc., has been established. Information on the role of the endothelium in the formation of vascular responses in hypoxic states of varied genesis is scanty and contradictory [2, 5].

The aim of this investigation was to study the role of the endothelium in the development of contractile reactions of vascular SM (VSM) when their oxygenation is diminished.

## EXPERIMENTAL METHOD

Experiments were carried out on isolated preparations of the rat thoracic aorta. The endothelial layer was removed mechanically, by gently rolling the vascular preparation on filter paper [6]. This is one of the gentlest ways of removing endothelium, for it causes no damage to the muscular layer and the inner elastic membrane is preserved [5]. Complete removal of the endothelial layer was tested by noting the absence of relaxation of the preactivated aortic SM on application of acetylcholine (from Merck, West Germany) in a concentration of $10^{-7}$ M. Segments weighing 1-2 mg were placed in a constant temperature chamber (36-37°C), perfused


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