RELATION OF BIOCHEMICAL EFFECTS OF EPINEPHRINE TO ITS MUSCULAR EFFECTS[1]

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This paper is an attempt to bring together many ideas that have been developing around epinephrine in that twilight zone in pharmacology—the cellular mode of action. On one side of this twilight zone is the almost complete darkness in which the pharmacologist usually finds himself when he asks questions beyond those dealing with the effect of a drug and the site of this action; on the other side is the bright glow of early morning light shed by the studies of Sutherland and Cori (75) and the more recent investigations outlined for us by Rall and Sutherland (68a) on the epinephrine-stimulated biochemical events at the cellular and subcellular levels.

The question is whether the known metabolic effects of epinephrine on cells can explain the pharmacological effects of epinephrine on those cells; and it is made more complex by the fact that epinephrine has several known metabolic effects and, most likely, other metabolic effects about which we know little or nothing at present. It would appear that epinephrine does influence the oxygen consumption of several muscular organs, the carbohydrate metabolism of smooth and striated muscle and the electrolyte exchange in certain muscles.

Since two prominent metabolic effects of epinephrine are its calorigenic and hyperglycemic effects, it has been tempting to attribute the pharmacological effects of epinephrine on several organs to its action on oxygen metabolism, on glycogenolysis or on lactic acid production.

Oxidative metabolism and epinephrine effects on muscular tissues. In early studies on mammalian tissues a requirement for oxygen in the action of epinephrine appears to be explained by the severe demands imposed on the anaerobic energy-producing systems under the conditions of the experiments. Thus, Labes (54) reported that strips of calf carotid artery would not respond to epinephrine in Ringer's solution under anaerobic conditions. More recently, however, Furchgott (35) found that epinephrine would contract or relax (36) aortic strips in glucose-containing solution at 37.5° C in the absence of oxygen. Also, enzyme inhibitors which interfere with oxidative metabolism did not prevent the vasoconstrictor action of epinephrine on the perfused rabbit ear (50). The ability of epinephrine to stimulate the frog heart (23), the rat diaphragm (27) and the rabbit uterus (24), and to relax the rabbit intestine (83), the frog stomach and the guinea pig uterus (72) during anaerobic metabolism appears to eliminate an essential role of oxidative metabolism in the pharmacological action of epinephrine.

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The above conclusion appears true despite excellent evidence that epinephrine, under certain experimental conditions, can increase the oxygen consumption of the liver (1), salivary glands (48), contracted smooth muscle (14, 20) and of the beating heart (4, 39, 66) even under conditions in which only the inotropic effect is present (53, 57).

Carbohydrate metabolism and epinephrine effects on muscular tissues. The prominent glycogenolytic action of epinephrine suggests a potentially important role of carbohydrate metabolism in the pharmacological effects of epinephrine. Glycogenolysis in response to this agent has been demonstrated in skeletal muscle, heart, rat and rabbit uterus, seminal vesicles, bladder and spleen (26). Indirect evidence of increased glycogenolysis is the elevated lactic acid formation in the presence of epinephrine by arterial strips (74), bovine tracheal muscle, guinea pig uterus and rabbit intestine (61, 62, 63). The accumulated evidence indicates a glycogenolytic effect of epinephrine on smooth muscles which are inhibited (or relaxed), as well as on muscles which are excited by epinephrine.

Since the glycogenolytic effect of epinephrine appears to accompany its pharmacological effect, the interrelation of the two effects has been explored. Indirect evidence obtained by using the glycolytic inhibitor iodoacetate indicates that the energy-producing steps of the Embden-Meyerhof pathway are not essential for the characteristic effects of epinephrine on the frog heart (23), on the rat diaphragm (27), on constriction of blood vessels (52) and on the rabbit uterus (24). The evidence obtained in the iodoacetate-poisoned rat diaphragm appears most critically opposed to a mechanism of action of epinephrine which involves energy derived from carbohydrate metabolism. This is so because Bloom *et al.* (9) found that the Embden-Meyerhof chain is the main pathway of carbohydrate metabolism in this tissue. There is, however, the disturbing fact that in this tissue the relative potencies of a series of amines on amplitude of contraction and on glycogenolysis were similar (29). We shall return to this matter subsequently.

We have also obtained sufficient evidence that iodoacetate does not interfere with the relaxing action of epinephrine on guinea pig tracheal rings, rat uterus and rabbit intestine, or with epinephrine contraction of the rabbit aortic strips until the respective tissue becomes generally unresponsive to other agents as well. When glucose is the only substrate presented to the tissue, failure occurs rapidly and the response to epinephrine disappears. When lactate or pyruvate is present, the tissue survives for longer periods and requires severe poisoning with iodoacetate before it eventually becomes unresponsive to stimulants and to epinephrine. In this state oxidative, as well as glycolytic, energy production is severely depressed and there is most likely some poisoning of the contractile mechanism.

Lactic acid production and the relaxation of smooth muscles by epinephrine. Despite the evidence that blockade of the Embden-Meyerhof chain does not prevent either the stimulating or the inhibitory actions of epinephrine, we must consider the very interesting results published by Mohme-Lundholm (61) and Lundholm (60). On the basis of their results, the Swedish workers have proposed

that the relaxing effect of epinephrine is caused by the increased tissue production of lactic acid. They obtained significant correlations of lactic acid production and relaxation to support this concept and found several conditions under which neither effect occurred. This is an extremely important suggestion and, therefore, we must weigh the evidence carefully.

Furchgott (36) has raised several points which oppose the theory that the relaxing action of epinephrine is mediated by the increased accumulation of lactic acid. His evidence that epinephrine acts in the presence of only nonglycolyzable substrate after depletion of endogenous substrates is insufficient to eliminate lactic acid as an intermediate. Bentley (7) has found that under these conditions the substrates may rapidly form glycogen. It has been the general experience that one cannot completely deplete glycogen from a tissue and expect the tissue to remain functional. However, a recent report claims a complete removal of glycogen from bullfrog hearts (induced by anoxia and epinephrine), followed by recovery of the heart and resynthesis of glycogen (22). Furchgott's experiments with DL-glyceraldehyde, which prevented glucose utilization for energy but did not block the subsequent relaxing action of epinephrine in the poisoned muscle supplied with pyruvate or butyrate, offer a much stronger argument against any theory involving glycolytic formation of lactate as a step in the relaxing action of epinephrine.

Bentley (7) has taken issue with the results of Mohme-Lundholm (61) on the rabbit intestine since he found no increase in lactic acid of the tissue with concentrations of epinephrine which completely relaxed the intestine. His concentrations were in the neighborhood of 1:10,000,000. Since Mohme-Lundholm found only slight (but significant) effects at a similar concentration and a marked increase at 1:1,000,000, the apparent discrepancies in these results may be reconcilable. On the other hand, the fact that Mohme-Lundholm (61) found that iodoacetate, 1:250, prevented the relaxant action of epinephrine as well as the increased production of lactic acid tends to confuse the issue. It is well established that a high concentration of iodoacetate interferes with aerobic metabolism as well as glycolysis, and that this substance can produce irreversible contracture of most muscular tissues.

The correlation of vascular dilation with lactic acid production as presented by Lundholm (60) must be analyzed in more detail. He did not find that venous blood lactate increased during the vasodilator action of epinephrine; thus, his results are in accord with those of Bell and Stead (6) and Barcroft and Cobbold (3) who measured blood flow and venous lactic acid concentration of human limbs during the primary dilator effect of epinephrine. None of these workers found increased lactate during early vasodilation. Lundholm also determined the arteriovenous difference in blood lactate. This also was not increased by epinephrine. However, since the blood flow increased markedly, the calculated lactic acid production from the limb also increased. Griffith *et al.* (47) had observed similar effects when low concentrations of epinephrine were infused intraarterially in the cat. A recent report by Honig and Gabel (51) suggests that epinephrine causes greater blood flow through muscles by closing arteriovenous

channels and decreasing skin flow. Thus, it is conceivable that the more complete perfusion of the muscle allows more lactate to be removed without changing the venous concentration of lactate.

Since it is the concentration of lactic acid in or around the blood vessel which appears to be important in the mechanism proposed by Lundholm, we must consider the influence of muscle cell lactic acid on the vessel wall. Although we might argue that the direction of fluid flow on the arterial side of the capillary bed is out of, and away from, the vessel wall and that the reverse is true on the venous side, we must grant that the dilator effect of epinephrine must be in the arteriolar region before the point at which fluid passes rapidly out of the vessels. These points could be neglected completely if Lundholm conceived of the lactic acid as being produced in the vascular smooth muscle, but to account for certain facts he suggests that it is the skeletal muscle which produces the lactate which influences the vessels to relax. The fact that certain vessels *in vitro* can dilate in response to epinephrine makes it essential that lactic acid production in the vessels be related to the relaxation, or Lundholm's conception takes on too limited an application.

A theory relating epinephrine-stimulated lactic acid formation to relaxation contains the implied assumption that muscles which are caused to contract by epinephrine do not also produce an increased amount of lactic acid. This matter must be investigated by the proponents of the lactic acid relaxation hypothesis for epinephrine.

Glycogenolysis and the hexosephosphates in the effects of epinephrine on muscular tissues. Some time ago we concluded that the action of epinephrine was not dependent on the metabolic energy derived from oxidative metabolism or that derived from carbohydrate metabolism through the Embden-Meyerhof glycolytic pathway (27). It would appear that these conclusions apply equally well to actions of epinephrine on skeletal muscle, heart, excited smooth muscle and inhibited smooth muscle.

The available evidence, however, would be compatible with a mechanism of action of epinephrine on muscle activity based upon the activation of the glycogenolytic system so long as it included only the steps in glycogenolysis prior to the energy-yielding reactions of the Embden-Meyerhof pathway. Although the general tendency has been to assume rather uncritically that glycogenolysis is a constant effect of epinephrine in all tissues and, therefore, an important basic fact to be considered in any mechanism of action for epinephrine, Morsa and Goffart (65) have come to the conclusion that increased glycogenolysis cannot be involved in the action of epinephrine on skeletal muscle. The basis for their conclusion is not completely acceptable. They cite their own work on the rat diaphragm in which ergotamine did not prevent the potentiation of the twitch by epinephrine (41) and the work of Tuerkischer and Wertheimer (77) which indicated that ergotamine inhibited the glycogenolytic action of epinephrine on the diaphragm. In the papers by Goffart (41, 42) the tables state that epinephrine potentiation of contraction was prevented by ergotamine 1:20,000 (50 mg/l). Since Goffart prevented the effect on contraction of 1:1,000,000 epinephrine

with 1:20,000 ergotamine, his experiment required 50 times as much ergotamine as epinephrine. This is not a very different ratio of concentrations from the use of 1:100,000 ergotamine to block the glycogenolytic action of 1:3,000,000 epinephrine, the ratio of 30:1 used by Tuerkischer and Wertheimer (77). There are also sufficient data on muscles *in situ* showing that the improvement of contraction in response to epinephrine or to sympathetic stimulation can be inhibited by ergotamine and other adrenergic blocking agents. One example of this was presented by Corkill and Tiegs (18) in the frog muscle in which 1:50,000 ergotamine prevented the action of epinephrine, 1:1,000,000, or of sympathetic stimulation. We have collected further examples of this blockade of epinephrine action on rat diaphragm and cat gastrocnemius muscle (unpublished experiments). These observations give no firm ground for eliminating the glycogenolytic effect of epinephrine from the group of possible mechanisms of action of epinephrine on muscle activity.

Since the experimental evidence limits a potentially important effect to the early steps in the phosphorylitic decomposition of glycogen, it was of interest to look into the changes in cellular concentration of the hexosephosphates in response to epinephrine and into the pharmacologic effects of the hexosephosphates.

If our conclusions to this point are correct, we are left with an action of epinephrine which is not dependent on metabolic energy-producing systems, but which may involve glycogenolysis. It is conceivable that the effect of epinephrine on contraction may not be on the transfer of energy to or from the contractile system or on the electrical-mechanical coupling system. It may be that epinephrine influences contraction by changing the physico-chemical environment of the contractile system. Such an effect could come about by a change in the cellular content of the hexosephosphates caused by an increased rate of glycogenolysis.

In this connection it is of considerable interest that Cori and Cori (17) found that epinephrine caused a marked increase in muscle hexosemonophosphate. This effect of epinephrine on cellular content of hexosemonophosphate has been found in mammalian muscle (16) and more recently in liver (75). We have confirmed these effects of epinephrine on rabbit diaphragm and liver and have found an increased hexose-6-phosphate in epinephrine-treated heart muscle and uterus (30).

There are three lines of evidence which suggest that the hexosephosphate concentration may play an important role in muscle contraction.

1) A short tetanus or a series of rapid twitches of skeletal muscle lead to a post-stimulation potentiation of contraction which is dissipated over a period of several minutes. Cori and Cori have shown that the elevation of the hexosephosphate concentration which follows a period of stimulation also diminishes toward the resting level over a similar period of time.

2) Epinephrine-like effects have been observed in response to hexosephosphates. Freeman (34) observed an increased rate and amplitude of beat of the frog heart in response to fructosediphosphate. This was confirmed by Lindner and Rigler (58). Glucose-1-phosphate increased the amplitude of contraction

and the coronary flow (and reduced slightly the rate) of the isolated rabbit heart (40). Russian workers (73) reported that hexosephosphates improved the response of frog rectus abdominis to acetylcholine. The hexosephosphates improved the contraction of the rat diaphragm (25). Epinephrine potentiates the contractions of frog muscle and rat diaphragm under these conditions.

3) Insulin was reported to increase muscle hexosemonophosphates in rat diaphragm (49, 69). Under conditions in which epinephrine produces a marked stimulation of the rat diaphragm, insulin produced a similar stimulation (25). Other interesting sympathomimetic effects of insulin have been reported. Insulin has a stimulating action on the frog heart (5) and on the dog heart-lung preparation (32, 46, 81), and a depressing effect on the rabbit intestine (32). The effects observed on the dog heart and the rabbit intestine may be due to a contaminant of insulin (32). Nonetheless, the biochemical and pharmacological effects are of interest.

The above observations suggest an important role of the cellular concentration of the hexosephosphates in the control of contractility. Whether this action is directly on the contractile proteins, on the electrolyte concentrations, or on other controlling mechanisms is not known.

Muscle potentials and potassium changes. The epinephrine-induced changes in cell potassium vary from tissue to tissue. Recent work on electrolyte changes, transmembrane potentials and activity potentials in smooth muscle suggest some generalizations relative to the actions of epinephrine. By studying the intracellular potentials of the *taenia coli* muscles of guinea pigs, Bülbring (15) extended the earlier work of Bacq and Monnier (2) and Bozler (11) with extracellular electrodes. The increased tone induced by acetylcholine and other agents, including an occasional contraction induced by epinephrine, was accompanied by a decreased membrane potential and an increased frequency of spike potentials; the relaxation induced by epinephrine was accompanied by an elevation of membrane potential and a decreased frequency of spike potentials. The elevated membrane potential was accompanied by a net increase in potassium entering the cell (10).

Although oversimplification and extrapolation may be disastrous, let us for the moment accept in a more general sense the observations that in smooth muscle increased tone is accompanied by a diminished membrane potential, an increased frequency of spike potentials and an increased loss of potassium, and that relaxation of smooth muscle is accompanied by the reverse changes. At present, we need not concern ourselves with the question of which is cause and which is effect.

Elevated membrane potential, reduced frequency of activity potentials and net increase in potassium accompany epinephrine relaxation of the *taenia coli* (11, 15), as mentioned above. A diminished membrane potential is suggested by the reduced demarcation potential in the nictitating membrane and other epinephrine-excited smooth muscles (2). Loss of potassium accompanied the epinephrine-induced contraction of arteries (19, 76) and of the rabbit uterus (19).

In striated muscle (heart and voluntary) epinephrine improves contraction, but in other ways the responses of the cells appear more closely akin to epineph-

rine-inhibited smooth muscle. Thus, in voluntary muscle epinephrine induced the retention of potassium (45), and an increase in the demarcation potential (13). The effects of epinephrine on cardiac muscles are less consistent with this generalization. Epinephrine caused a very slight reduction in resting potential in the isolated rat atrium (82) and Witt and his associates have found that epinephrine caused no change in potassium loss from the isolated rabbit atrium either at rest (84) or during activity (85). A further resemblance of the heart and skeletal muscle to inhibited smooth muscle is the fact that isoproterenol, which is a potent relaxant and a poor stimulant for smooth muscle, is more potent than epinephrine in stimulating the heart (55) and skeletal muscle (29). Another generally accepted rule is that the adrenergic blocking agents are very effective antagonists in smooth muscles which are excited by epinephrine, but rather poor epinephrine antagonists in epinephrine-inhibited smooth muscles, in the heart or in skeletal muscle. There is an obvious need for further investigation into the interesting effects of epinephrine on electrical and electrolyte changes in various muscles.

Influence of cation concentration on responses to epinephrine. a) *Striated muscle.* The hypothesis that the potentiation of the muscle twitch by epinephrine is related to changes in potassium or calcium was investigated and reviewed by Goffart (43). He found that raising the potassium concentration in the medium surrounding the isolated rat phrenic-diaphragm preparation reduced its response to epinephrine, whereas lowering the external potassium concentration potentiated the response to epinephrine. These findings do not lend themselves to a simple interpretation of the importance of potassium in the action of epinephrine. Firstly, they are complicated by the additional observations that the early effect of low potassium is to diminish the amplitude of contraction, and that the early effect of high potassium is to increase the amplitude of contraction. These effects upon the amplitude of contraction would of themselves tend to increase and decrease, respectively, the responses to epinephrine. Secondly, prolonged exposure to either low or high potassium concentration modifies the epinephrine response in a direction opposite to that observed during the early period of exposure. Thus, prolonged exposure of the diaphragm to a potassium-free medium reduced or reversed the epinephrine effect (43), and prolonged exposure to a high potassium concentration depressed the rat diaphragm and potentiated the response to epinephrine (29, 64). On the basis of the mechanical responses alone one could only speculate about the unifying principle which might account for these varied responses with potassium changes. When studies are made of the electrical properties of the muscle membranes and of the chemical changes which occur under these varied conditions, the explanation may be self-evident. Epinephrine itself elevates the resting potential of skeletal muscle (12, 13), reduces the loss of potassium from muscle (45, 59, 68) and delays paralysis when excess potassium is applied to the muscle (56, 68).

Goffart (43) also reported that elevated calcium in the medium potentiated the response to epinephrine. The importance of calcium transport in muscular contraction has received renewed interest by the recent work of Neidergerke

(67) and Shanes and Bianchi (70). Okamoto (68) found no significant effect of epinephrine on calcium exchange in frog muscle. This study should be repeated with modern, sensitive techniques.

Reduction of sodium content to 25 % of normal by replacement with isoosmotic sucrose solution reduced the amplitude of contraction of the rat diaphragm but did not influence the sensitivity to epinephrine or the percentage elevation in contraction (28).

b) *Heart.* Although several kinds of potassium changes have been observed when epinephrine acted on the heart *in situ*, epinephrine did not modify significantly the potassium loss from the electrically stimulated rabbit auricle (85) or from the resting rabbit auricle (84). These findings of Witt and Jaeger were in contrast to the effect of the cardiac glycosides which increased potassium loss under both the above conditions (85).

The effect of changes in external electrolyte on the response of the heart to epinephrine has been studied extensively. The literature on this subject is too large to allow its review here.

c) *Excited smooth muscle.* Since several studies indicate that agents which cause smooth muscle to contract also increase the frequency of spike potentials (11, 15), it may be well to use the phrase "excited smooth muscle" to indicate a muscle excited to contract.

An increased concentration of potassium in the external medium potentiated the response to epinephrine of isolated rabbit aorta strips (37) and of human uterine strips (78). However, potassium depletion alone did not change the reactivity of the mesoappendix vessels to epinephrine, but potassium depletion plus cortisone administration increased the reactivity to epinephrine (33). The stimulating action of epinephrine on the nonpregnant human uterus was converted to a relaxing action when the tissue was placed in a potassium-free medium (78). An increase in response of blood vessels was found when the cellular sodium was elevated and the bathing fluid contained the usual concentration of sodium (80).

Calcium depletion *in vivo* reduced the reactivity of the blood vessels to epinephrine (79), and an excess of calcium converted the stimulating effect of epinephrine on human nonpregnant uterus to a relaxing action (78).

d) *Inhibited smooth muscle.* The response of the rectal caecum of chickens to epinephrine was increased by a lowered potassium concentration in the medium and reduced by an elevated potassium concentration (38).

There was a net uptake of potassium by the epinephrine-inhibited guinea pig *taenia coli* (10). An early report showed a slight increase in potassium and calcium in epinephrine-relaxed guinea pig intestine (21). An excess of calcium obliterated (63) or reversed (78) the relaxing effect of epinephrine.

Bentley (7) has been unable to find marked changes in the sensitivity of the rabbit intestine to epinephrine when large changes in sodium, potassium, calcium or magnesium were made.

Two recent communications (31, 71) claim that the responses of smooth muscles to epinephrine were present when the muscle was completely depolarized by re-

placing the sodium chloride of the medium with potassium chloride or potassium sulfate. These reports should stimulate further investigation in view of the obvious importance of a method which can separate the electrical and mechanical responses of smooth muscle to a neurohumoral agent.

Summary and conclusions

The actions of epinephrine on muscles are not dependent on either oxidative metabolism or on the energy produced from carbohydrate through the Embden-Meyerhof glycolytic pathway.

Nonetheless, the activation of glycogenolysis may be important for the muscular effects of epinephrine.

The apparent discrepancies of the two statements relating to carbohydrate metabolism may be resolved by findings which implicate the hexosemonophosphates in the control of muscle contractility.

In their responses to epinephrine, muscles appear to fall into two groups: 1) excited smooth muscles which lose potassium, and 2) inhibited smooth muscle, skeletal muscle and cardiac muscle which retain or increase their potassium.

Changes in the ionic environment of various muscle cells modify the degree of response and, in some instances, the duration of the response to epinephrine.

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