Effect of Varying K^+ Concentrations on Phosphorylase Activity, Lipolysis, and cAMP Levels in Perfused Rat Heart[1] (36710)

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The heart has two sources of stored endogenous fuel, lipids and glycogen, which can be mobilized by hormone stimulation; specifically by catecholamines (1–3) and possibly by glucagon (4). Increasing K^+ level in the perfused heart from 5.6 m*M*, the circulation physiological level, to greater than 11 m*M*, can block contraction of the perfused rat heart and also inhibit the effect of catecholamines on heart metabolism. Namm, Mayer and Malbie (5) found that elevation of K^+ to 56 m*M* in the perfused rat heart blocked the increase of cAMP in response to epinephrine. There was no increase in phosphorylase *a* activity, but phosphorylase kinase activity was found to be significantly elevated.

Challoner and Steinberg (6), using perfused rat hearts blocked by 31 m*M* K^+, suggested that epinephrine would cause a breakdown of endogenous myocardial lipid, releasing FFA intracellularly, which would result in an increased oxygen consumption independent of chronotropic and inotropic effects. Therefore, it was assumed that increased extracellular K^+ levels did not interfere with the lipolytic response to epinephrine.

The purpose of this study was to investigate the effects of hyperkalemia on glycerol release, phosphorylase activation, and cAMP elevation in hearts perfused with 5.6, 16 and 55 m*M* K^+. Previous evidence has suggested that varying the K^+ concentration may also vary the effects of catecholamines on glycogen and lipid mobilization as well as contractile properties. These effects may be mediated through adenyl cyclase with the result that varying the K^+ level will vary the depolarizing effect on the plasma membrane, and in turn, influence the level of intracellular messenger, cAMP, in response to catecholamines in the perfused rat heart.

Methods and Materials. Fed, male rats weighing between 250 and 350 g were decapitated and hearts were removed quickly and perfused by a modified Langendorf procedure (7). The perfusate was a Krebs–Henseleit bicarbonate solution containing the following concentrations: NaCl, 120 m*M*, KCl, 5.6 m*M*; $CaCl_2$, 1.22 m*M*; $MgSO_4$, 1.34 m*M*, Na_2PO_4, 1.21 m*M*; glucose, 5.5 m*M*, and Na_2HCO_3, 25.37 m*M*. The perfusing fluid was equilibrated with 95% O_2, 5% CO_2 and perfusate temperature maintained at 37°. The flow rate was maintained at 10 ml/min with a Harvard peristaltic pump. A nonrecirculating system was used and the perfusate collected for glycerol analysis.

A constant force was placed on the spontaneously beating hearts by passing a silk thread through the apex and attaching it to the lever arm of a Grass strain gauge; then adjusting to 10 g diastolic force as described previously (3). Drugs were dissolved in saline and infused at a rate of 0.197 ml/min using a Harvard infusion pump. The medium used for increased K^+ levels was identical with normal medium except that the KCl was present at 16 or 55 m*M* rather than 5.6 m*M*. The NaCl levels were reduced to maintain isotonicity.

Experimental design. All hearts were perfused with normal buffer (5.6 m*M* KCl) for 30 min in order that the high initial release of glycerol from the heart could return to stable basal levels. High K^+ buffer was infused into appropriate hearts for another 10

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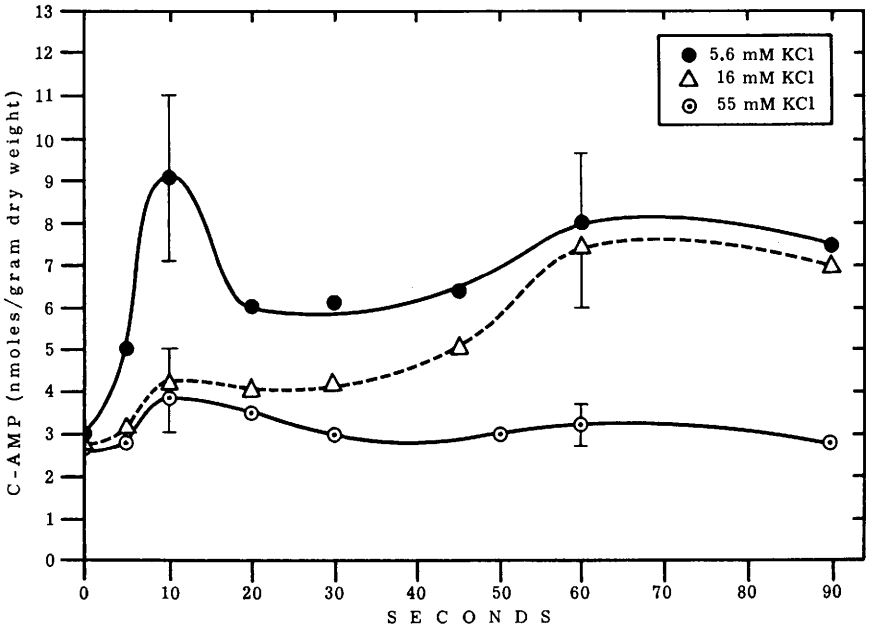

FIG. 1. Effect of iosoproterenol on cAMP levels in hearts perfused with varying concentrations of K^+. Hearts were continuously infused with isoproterenol (3×10^{-8} *M*). Points at 10 and 60 sec represent the mean of 5 animals ± SEM; other points are the mean of 2 observations.

min. Isoproterenol (IPR) was infused and hearts frozen by clamping with aluminum blocks cooled in liquid nitrogen (8). Hearts were stored at —70° until assayed for phosphorylase activity.

Biochemical measurements. Hearts were powdered in a stainless steel mortar and pestle previously cooled with dry ice. Phosphorylase *a* and total phosphorylase were assayed as described by Cori and Illingsworth as modified by Mayer, DeV Cotten and Moran (9). Glycerol was determined by the method of Wieland (10) as modified by Chernick (11) for fluorometric assay. Enzymes used in the assay were purchased from Boehringer Mannheim. Cyclic AMP was assayed by the method of Steiner *et al.* (12) with materials purchased from Collaborative Research, Inc., Waltham, MA.

Results. The effect of IPR infusion at a constant level of 3×10^{-8} *M* over a 2 min period produced a rapid increase in ventricular cAMP levels in hearts perfused with normal K^+ concentrations (Fig. 1). An initial 3-fold increase was observed in the first 10 sec and remained elevated throughout infusion of IPR. Infusing the same level of IPR to K^+ blocked hearts resulted in a diminished initial peak at 10 sec for the 16 m*M* K^+ perfused heart, but cAMP levels after this time appeared to be similar with those found in the normal K^+ perfused hearts. The effect of 55 m*M* K^+ in the perfusate blocked the large initial response in cAMP elevation and blocked cAMP elevation throughout the infusion period.

The activation of phosphorylase reached a peak 20 sec after continual infusion of IPR, then leveled off to a plateau of 50% phosphorylase *a* (Fig. 2). In the K^+ blocked hearts, both 16 and 55 m*M* perfused hearts had similar phosphorylase *a* values. The initial increase in phosphorylase *a* started 5 to 10 sec after perfusion of IPR, but the total amount of phosphorylase in the activated form remained lower than found in the beating heart.

The most striking difference between K^+ blocked hearts and beating hearts was found in glycerol release, presumed to be an index of lipolysis. In hearts perfused with physiological K^+ levels, there was a rapid increase in

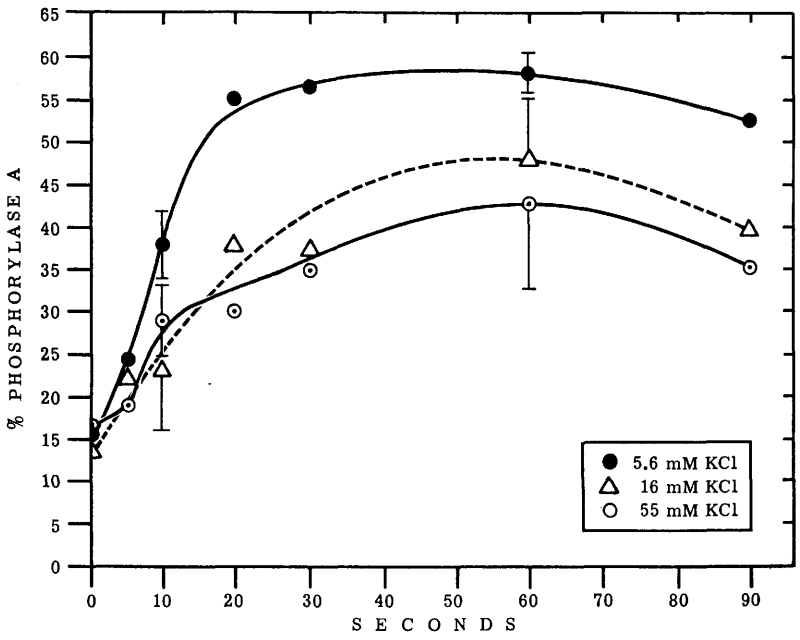

FIG. 2. Effect of isoproterenol on phosphorylase activation with varying concentrations of K^+ in perfused heart. Hearts were the same as those in Fig. 1.

glycerol release which peaked at 2 times control value 40 sec after beginning infusion of IPR (Fig. 3). However, the glycerol value dropped to control value 120 sec after beginning of infusion. In the 16 m*M* K^+ perfused hearts, glycerol release remained at control values for the first minute, then rose to values 50% above control 2.5 min after infusion of IPR. However, 55 m*M* K^+ perfused hearts showed no increase in glycerol release throughout the experimental period.

The assay for glycerol involved 2 enzymatic reactions:

$$\text{ATP} + \text{glycerol} \xrightarrow[\text{GK}]{\text{Mg}^{2+}} \text{ADP} + \alpha\text{-glycerolphosphate and}$$

$$\alpha\text{-glycerolphosphate} + \text{NAD} \xrightarrow[\text{GDH}]{} \text{dihydroxyacetonephosphate} + \text{NADH} + \text{H}^+.$$

By sequentially adding glycerolphosphate dehydrogenase (GDH), alpha-glycerolphosphate (αGP) can be quantitated in the perfusate. Upon addition of glycerolkinase (GK), the glycerol value in the perfusate was obtained.

The αGP concentration in the perfusate revealed a similar pattern when compared to glycerol after IPR infusion (Fig. 3). A sharp spike appeared at 40 sec followed by a rapid decline to basal values when the hearts were perfused with 5.6 m*M* K^+. Perfusing the hearts with 16 m*M* K^+ delayed the onset of αGP release and 55 m*M* K^+ buffer completely blocked the release of αGP after infusion of IPR.

Discussion. The effect of K^+ on inhibiting the rise of cAMP in response to IPR is apparently concentration dependent. Hearts perfused with 16 m*M* K^+ lost the ability to produce a large initial rise in cAMP in response to IPR. However, after 30 sec of continued IPR infusion, the cAMP levels were similar to those of normal hearts and significantly different from the 55 m*M* hearts ($p < .05$). The perfusion of 55 m*M* K^+, however, had the effect of completely blocking the rise of cAMP in response to IPR and were not significantly different from zero time controls.

The action of increased K^+ in the perfused

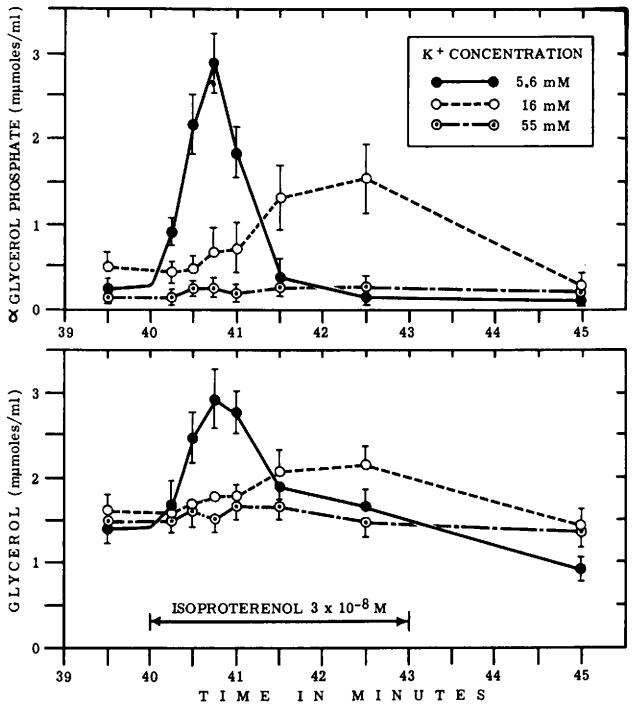

FIG. 3. (top) Hearts were perfused with 5.6 m*M* K^+ buffer for 30 min. They were then perfused with 5.6, 16 or 55 m*M* K^+ buffer for 10 min before beginning infusion of IPR at 40 min. Perfusate was collected for 5 sec durations. Alpha-GP is given as the mean of 8 observations ± SEM. (bottom) The glycerol content of perfusate collected from the same hearts as described above.

heart may be due to simply a depolarization effect which in some manner changes the adrenergic receptor, or may directly inhibit adenyl cyclase activity. Mayer (2) has reported that K^+ stimulated control adenyl cyclase activity in a particulate preparation from rat heart which would indicate that the effect of hyperkalemia is at the receptor site, since it blocked the epinephrine stimulation of adenyl cyclase.

The effect of K^+ depolarization on cAMP values in other tissues is quite different. In isolated diaphragm, K^+ depolarization increased the cAMP concentration and epinephrine caused a further increase (13). Similar findings were revealed in guinea pig cerebral cortex slices when increased K^+ (40 m*M*) levels caused an increased accumulation of cAMP (14). The combined effects of the elevated K^+ levels plus biogenic amines, such as norepinephrine, histamine, or serotonin, were more than additive. Therefore, the depolarizing effect of elevated K^+ levels on cAMP levels appears to be very different in heart than in other tissues.

Several investigators have reported that cAMP is the mediator of the effects of catecholamines on cardiac phosphorylase through activation of phosphorylase kinase (5, 15). In all the literature that has been reported, the increase in cAMP was found to precede the rise in phosphorylase activation in response to catecholamine (5, 16) or glucagon (4). The rise in cAMP levels in the heart has also been shown to precede lipolysis in the heart as measured by glycerol release into the perfusate (3). However, the mechanism of activation of the tissue lipase is still uncertain.

The effect of increased K^+ levels on phosphorylase activation and lipolysis in response to IPR was very different. In hearts perfused with normal K^+ buffer, both phosphorylase activation and glycerol release rose rapidly to a peak about 30 sec after infusing IPR. When perfusing with 16 m*M* K^+

buffer, the phosphorylase activity still rose rapidly, but to a lower level than when infusing with normal buffer. Lipolysis, however, was delayed and increased glycerol levels in the perfusate did not occur until after 120 sec of continual IPR infusion. Increasing the perfusion K^+ to 16 or 55 m*M* had no differential effect on the phosphorylase activity. Glycerol release from the heart increased only in 16 m*M* K^+ perfused hearts after continual infusion of IPR. The mechanism by which phosphorylase is activated in the K^+ blocked heart may be different than that for initiating glycerol release. On the other hand, phosphorylase activation may occur with only slight increases in tissue cAMP levels. Also, other mechanisms may be important in activating phosphorylase in the heart that are not mediated by cAMP. It has been shown that hypoxia (17) in cardiac muscle and stimulation of motor nerves in skeletal muscle (18) can both mediate phosphorylase activation through mechanisms which apparently are not cAMP dependent. Recent evidence indicates that IPR also can activate skeletal muscle phosphorylase *a* formation *in vivo* without cAMP formation or activation of phosphorylase kinase (19). This type of phosphorylase *a* formation occurred at submaximal doses of IPR. Since such doses were used in the present experiments, a similar phosphorylase activation may occur in high K^+ perfused hearts where no elevation of cAMP was detected.

The release of αGP from the heart with infusion of IPR cannot be explained at this time. Since the heart has little glycerol kinase activity (20), the most logical explanation is that it may come from a sudden increase in glycolytic intermediates with the activation of phosphorylase in the heart. At any rate, the importance of assaying the glycerol as a 2-step procedure reveals that a sizable amount of αGP can be released from the heart with infusion of catecholamine. This to some extent correlates with tissue levels of cAMP, but not with phosphorylase activation.

Other investigators have reported that with Ca^{2+} free media, epinephrine did not induce phosphorylase activation (5), apparently because phosphorylase kinase required Ca^{2+} for activity (21). A recent paper has revealed that Ca^{2+} in the presence of 60 m*M* K^+ could activate phosphorylase in smooth muscle from rabbit thoracic aorta (22). This effect was apparently due to the depolarization of the muscle cell membrane which allowed Ca^{2+} to enter the cell and activate phosphorylase kinase. However, in heart muscle, depolarization of the plasma membrane in the presence of Ca^{2+} does not activate phosphorylase (5). Our results in heart indicate that perfusate containing Ca^{2+} with 16 or 55 m*M* K^+ did not activate the phosphorylase system. Only when isoproterenol was also infused did activation of phosphorylase occur.

Namm, Mayer and Maltbie (5) reported that perfusion of rat heart with 56 m*M* K^+ produced no elevation of cAMP or phosphorylase activity in response to a pulse dose of 2 μg epinephrine. However, after 30 sec phosphorylase kinase activity was elevated to about half the value of that observed with epinephrine stimulation at normal K^+ levels (5). It was suggested by these authors that the catalytic activity of kinase may be dependent upon additional factors other than its transformation to a new molecular form. Our studies agree that there is no elevation of cAMP in 55 m*M* K^+ perfused hearts upon infusion of a catecholamine, but contrary to these authors, we do find elevation of phosphorylase activity. This may be explained by the fact that rather than a pulse dose of catecholamine we continuously infused IPR over a span of 3 min. Since it was previously shown that phosphorylase kinase activity could be increased even with a pulse dose of epinephrine, continuous infusion of a catecholamine may bring about additional factors required for phosphorylase activation.

The different metabolic effects that several investigators have reported using different concentrations of K^+ might be due to morphological changes occurring in the cell. Electron microscope pictures have revealed that increasing the K^+ level in the perfusate between 5.5 to 70 m*M* will result in ultrastructural changes in adult rat ventricular muscle (23). The predominant feature was a disten-

sion of transverse tubules when perfusing with 12, 20, 30 or 70 mM K^+. Increasing the K^+ concentration resulted in an increased number of T-tubules which were distended and also in the longitudinal diameters of the distension; however, no changes in the plasma membrane were observed. Small changes in the plasma membrane may possibly occur with increasing K^+ concentrations which could alter the effects of catecholamines on adenyl cyclase but would not be visualized on electromicrographs.

The effect of hyperkalemia on electrical and contractile properties of isolated perfused mammalian heart is well documented (24, 25). Spontaneous activity in rabbit heart occurs when the K^+ concentration is < 11 mM (physiological concn, 5.6 mM); K^+ concentration between 11 and 30 mM results in blockage of spontaneous contraction, but the K^+ blockade in the heart can be overcome, and the activity restored by addition of epinephrine or Ca^{2+}. When the K^+ level is above 30 mM, the addition of epinephrine does not overcome the K^+ block (25). Our observations were similar in that the 16 mM K^+ blocked heart could be overcome with spontaneous contractions occurring with infusion of IPR. However, heart activity could not be restored with IPR when hearts were perfused with 55 mM K^+.

It would appear from our data and work from previous investigators that the effect of K^+ on the heart may be based on several discrete concentration ranges. K^+ concentrations between 11 and 30 mM blocked heart contractions, but infusion of catecholamines can overcome this block and cause sporadic contractions. Also, cAMP can be increased to values found in 5.6 mM K^+ perfused hearts only after 30 sec of continuous IPR infusions. The early rise in cAMP found within 15 sec after infusion of catecholamines, is absent in hearts perfused with 16 mM K^+.

Infusion of catecholamines in hearts perfused with K^+ concentrations > 30 mM could not overcome the K^+ block. Also, no increase in cAMP could be found. There appears to be a close relationship between the ability of the heart to overcome the K^+ block and for adenyl cyclase to be stimulated by catecholamines. The depolarization of the plasma membrane and the inhibition of adrenergic stimulation of adenyl cyclase appears to be closely aligned.

Summary. Rat hearts were perfused with 5.6, 16, or 55 mM K^+ buffer for 10 min. IPR (3×10^{-8} M) was infused and hearts assayed for cAMP and phosphorylase activation while the perfusate was assayed for glycerol and αGP. Both 16 and 55 mM K^+ buffer blocked contraction of the heart. With infusion of IPR spontaneous contractions occurred, a delayed elevation of cAMP was observed, and also a delayed release of glycerol and αGP into the perfusate was detected when compared to 5.6 mM K^+ perfused hearts. Hearts perfused with 55 mM K^+ buffer revealed no increase in cAMP and no increase in the perfusate of glycerol and αGP after IPR infusion. Phosphorylase was elevated in 5.6, 16 and 55 mM K^+ perfused hearts.

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