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Authors
Weiss, JN
Lamp, ST

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Cardiac ATP-sensitive K^+ Channels

Evidence for Preferential Regulation by Glycolysis

JAMES N. WEISS and SCOTT T. LAMP

From the Department of Medicine and the American Heart Association, Greater Los Angeles Affiliate Cardiovascular Research Laboratory, University of California at Los Angeles, School of Medicine, Los Angeles, California 90024

ABSTRACT The ability of glycolysis, oxidative phosphorylation, the creatine kinase system, and exogenous ATP to suppress ATP-sensitive K^+ channels and prevent cell shortening were compared in patch-clamped single guinea pig ventricular myocytes. In cell-attached patches on myocytes permeabilized at one end with saponin, ATP-sensitive K^+ channels were activated by removing ATP from the bath, and could be closed equally well by exogenous ATP or substrates for endogenous ATP production by glycolysis (with the mitochondrial inhibitor FCCP present), mitochondrial oxidative phosphorylation, or the creatine kinase system. In the presence of an exogenous ATP-consuming system, however, glycolytic substrates (with FCCP present) were superior to substrates for either oxidative phosphorylation or the creatine kinase system at suppressing ATP-sensitive K^+ channels. All three groups of substrates were equally effective at preventing cell shortening. In 6 of 38 excised inside-out membrane patches, ATP-sensitive K^+ channels activated by removing ATP from the bath were suppressed by a complete set of substrates for the ATP-producing steps of glycolysis but not by individual glycolytic substrates, which is consistent with the presence of key glycolytic enzymes located near the channels in these patches. Under whole-cell voltage-clamp conditions, inclusion of 15 mM ATP in the patch electrode solution dialyzing the interior of the cell did not prevent activation of the ATP-sensitive K^+ current under control conditions or during exposure to complete metabolic inhibition. In isolated arterially perfused rabbit interventricular septa, selective inhibition of glycolysis caused an immediate increase in $^{42}K^+$ efflux rate, which was prevented by 100 μM glyburide, a known blocker of ATP-sensitive K^+ channels. These observations suggest that key glycolytic enzymes are associated with cardiac ATP-sensitive K^+ channels and under conditions in which intracellular competition for ATP is high (e.g., in beating heart) that act as a preferential source of ATP for these channels.

INTRODUCTION

ATP-sensitive K^+ channels are activated when cytosolic ATP concentration falls below a critical level, and have been implicated as a possible cause of the marked

Address reprint requests to Dr. James N. Weiss, Division of Cardiology, 47-123 CHS; UCLA School of Medicine, Los Angeles, CA 90024.

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increase in cellular K^+ efflux during ischemia and metabolic inhibition in heart (Noma, 1983; Trube and Hescheler, 1984). However, increased K^+ efflux can be detected within 30 s of inhibiting oxidative or glycolytic metabolism in heart at a time when total cellular ATP content is only mildly depressed or even normal (Rau et al., 1977; Weiss and Hiltbrand, 1985). In excised membrane patches, on the other hand, openings of ATP-sensitive K^+ channels are fully suppressed by submillimolar ATP concentrations (Noma, 1983; Findlay, 1988). Since the normal cytosolic ATP concentration in cardiac cells is 3–5 mM, it is difficult to postulate that activation of ATP-sensitive K^+ channels could account for the early increase in K^+ efflux during metabolic inhibition in heart unless (*a*) ATP-sensitive K^+ channels are much less sensitive to ATP in the intact cell than in excised membrane patches (Kakei et al., 1985; but see Nichols and Lederer, 1989), (b) the sensitivity of ATP-sensitive K^+ channels to ATP concentration is markedly reduced by certain sequellae of metabolic inhibition, or (*c*) the local ATP concentration in the vicinity of ATP-sensitive K^+ channels is depleted much more rapidly and completely than is reflected by the modest changes in total cellular ATP content, i.e., ATP is functionally compartmentalized. A number of reports have suggested that ATP derived from glycolysis is preferentially utilized to support membrane functions, in heart (McDonald and MacLeod, 1973; Bricknell and Opie, 1978; Bricknell et al., 1981; Higgins et al., 1982; Hasin and Barry, 1984; Hasin et al., 1984; Weiss and Hiltbrand, 1985), erythrocytes (Parker and Hoffman, 1967; Mercer and Dunham, 1981), vascular smooth muscle (Lynch and Paul, 1983), brain (Lipton and Robacker, 1983), and cultured cells (Balaban and Bader, 1984; Lynch and Balaban,1987). It has also been shown in heart that cellular K^+ loss is more sensitive to selective inhibition of glycolysis than selective inhibition of mitochondrial oxidative phosphorylation (Weiss and Hiltbrand, 1985). The present study was undertaken to investigate whether the responsiveness of cardiac ATP-sensitive K^+ channels to ATP was different depending on whether ATP originated from glycolysis or other metabolic pathways. Preliminary reports of the findings have appeared (Weiss and Lamp, 1987, 1988).

METHODS

Single Cell Experiments

Ventricular myocytes were isolated from adult guinea pigs weighing 300–400 g by the method of Mitra and Morad (1985) and studied using the gigaseal patch-clamp technique (Hamill et al., 1981). Whole-cell and single-channel recordings were made with fire-polished patch electrodes pulled from Corning 8161 glass (Corning Glass Works, Corning, NY) (tip resistance, 1–4 MΩ) sometimes coated with Sylgard 184 (Dow Chemicals, Midland, MI) and mounted to the headstage of a List EPC-7 patch-clamp amplifier (List Electronics, Darmstadt, FRG). Membrane current and voltage signals and a video image of the cell were recorded simultaneously on a modified videocassette recorder (model 420; A.R. Vetter Co., Rebersburg, PA) and later analyzed with a PDP 11–23 computer. Cell length measurements were made manually off a television screen from the recorded video image of the cell. The experimental chamber (0.5 ml) was mounted on the stage of an inverted microscope and was continuously perfused throughout the experiment at 1–4 ml/min. Single-channel currents were recorded from either excised inside-out patches or cell-attached patches on permeabilized myocytes. Myocytes were permeabilized after forming a cell-attached patch at one end of

the cell by briefly exposing the other end to a stream of bath solution containing 0.05–0.1% saponin. The saponin-containing solution was delivered by applying positive pressure through a second micropipette positioned adjacent to the cell (Kakei et al., 1985). In these experiments the standard bath solution contained (in millimolar): 150 KCl + KOH, 2 etyhylene glycol bis-(β-aminoether)-*N,N,N',N'*-tetraacetic acid (EGTA), 0.5 $CaCl_2$, 2 $MgCl_2$, and 10 *N*-2-hydroxyethylpiperazine-*n'*-2-ethanosulfonic acid (HEPES) to which MgATP or various metabolic substrates were added; the pH was adjusted to 7.1. The free Ca^{++} concentration was estimated to be ~40 nM. In some experiments hexokinase (10 μ/ml) with or without 2-deoxyglucose (10 mM) were added. Carbonyl cyanide(4-trifluoromethoxy) phenyl hydrazone (FCCP; Calbiochem Behring Corp., La Jolla, Ca), 1 μM, was used as a mitochondrial uncoupler in some experiments. The patch electrode filling solution contained 150 mM KCl + KOH, 5 mM HEPES, pH 7.35, with a free Ca^{++} concentration of 4–6 μM.

For experiments in which whole-cell currents and voltages were measured the standard bath perfusate was a modified Tyrode's solution containing (in millimolar): 136 NaCl, 9 NaOH, 0.33 NaH_2PO_4, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, pH 7.35. To inhibit metabolism, glucose was replaced with equimolar 2-deoxyglucose and 0.1 μM FCCP was added. The patch electrode solution contained (in millimolar): 150 KCl + KOH, 20 HEPES, 14 EGTA, 1 $CaCl_2$, 10 NaCl, 1 $MgCl_2$, 0 or 15 MgATP, pH 7.1 and the free Ca^{++} concentration was estimated at 10 nM. In the current-clamp mode, action potentials were elicited by 10-ms suprathreshold current-clamp pulses at 0.2 Hz. Current-voltage relations were obtained by measuring the current at the end of 100-ms voltage clamp pulses to various membrane potentials (10-mV increments) from a holding potential of −40 mV to inactivate the fast Na^+ current. Capacitance and series resistance compensation circuitry were both used.

All single-cell experiments were performed at room temperature (21–23°C). Chemicals were obtained from Sigma Chemical Co., St. Louis, MO unless otherwise indicated.

Isolated Rabbit Heart Experiments

Hearts were excised from anaesthetized New Zealand white rabbits weighing 2–3 kg. The septal branch of the left coronary artery was quickly cannulated and a well-perfused portion of the interventricular septum was dissected free and mounted in a constant temperature (37°C) nitrogen-filled chamber as described previously (Weiss and Shine, 1982). A ligature placed at the apex of the septum was sutured to a force transducer to measure tension. The preparation was paced at 75 beats per minute through a bipolar platinum electrode embedded at the base of the septum. A perfusion pump maintained a constant flow of perfusate (37°C) at a rate of 1.75 ml/min through the septal artery. Standard perfusate consisted of (in millimolar): 120 NaCl, 4 KCl, 1.5 $CaCl_2$, 25 $NaHCO_3$, 0.44 NaH_2PO_4, 1 $MgCl_2$, 5.6 dextrose, and 10 μm/ml insulin. pH was maintained at 7.3–7.4 by gassing with a mixture of 5% CO_2 and 95% O_2. In some experiments dextrose was replaced by equimolar pyruvate, and either 10 mM 2-deoxyglucose or 1 mM iodoacetate were added. Ethanol (0.25%) with and without potassium glyburide (10–100 μM; The Upjohn Co., Kalamazoo, MI) was also added to the perfusate in some experiments. Venous effluent was collected through a polyethylene tube anchored at the base of the septum. Intracellular potential was monitored with floating microelectrodes filled with 3 M KCl (Woodbury and Brady, 1956). Preparations were allowed to equilibrate for at least 1 h before experimental interventions.

Radioisotope Techniques and Metabolic Assays

To measure unidirectional K^+ efflux rates, rabbit septa were loaded with perfusate containing $^{42}K^+$ (1.75 μCi/ml) for 45 min and then washed out with nonradioactive perfusate for at least 20 min to establish the control rate of $^{42}K^+$ efflux before any intervention. Venous

effluent was collected for 30 s of each 1-min interval and analyzed for radioactivity with a gamma counter (5500; Beckman Instruments, Inc., Palo Alto, CA). Counts per minute were corrected for background and decay. By measuring the time constant of $^{42}K^+$ washout during the control washout period, and using the assumption that the $^{42}K^+$ uptake and washout rate are identical during control perfusion (Langer and Serena, 1970), the specific activity of the tissue at the start of $^{42}K^+$ washout was calculated. The rate of unidirectional $^{42}K^+$ efflux (in micromoles per minute) at any time during the washout was then calculated by dividing the effluent counts per minute by the product of the specific activity corrected for that time and the duration of the effluent collection period.

The venous effluent not used for radioactivity measurements was collected separately over 2.5-min segments, frozen, and later analyzed for lactate content by standard spectrophotometric techniques (Guttman and Wahlefeld, 1974).

Data Analysis

Results are presented as mean ± SE. Student's *t* test for paired and unpaired observations were used to evaluate statistical significance.

RESULTS

Responsiveness of ATP-sensitive K^+ Channels and Cell Length to Metabolic Substrates in Permeabilized Myocytes

We have previously shown that ventricular myocytes permeabilized by the saponin technique remain metabolically intact and capable of generating sufficient ATP endogenously to suppress ATP-sensitive K^+ channels when supplied with appropriate substrates for glycolysis, oxidative (mitochondrial) metabolism, or the creatine kinase reaction (Weiss and Lamp, 1987). Furthermore, substrates for one or both of the ATP-producing steps of glycolysis (Fig. 1) remained effective at suppressing ATP-sensitive K^+ channels in the presence of a mitochondrial inhibitor, indicating that oxidative metabolism of glycolytically-generated pyruvate was not essential. Activation of ATP-sensitive K^+ channels in permeabilized cells was always accompanied by progressive cell shortening. When the cell was resupplied with exogenous ATP or any of the metabolic substrates described above, cell shortening reversed partially but usually not completely, concomitant with closure of ATP-sensitive K^+ channels. The degree of cell length recovery was generally inversely proportional to the duration of exposure to solution lacking metabolic substrates or ATP.

The intrinsic rate of ATP consumption in relaxed nonbeating permeabilized cells at room temperature is very low compared with the beating heart under physiologic conditons. Permeabilized myocytes were therefore exposed to an exogenous ATP-consuming system consisting of 2-deoxyglucose (10 mM) and hexokinase (10 μ/ml). In the presence of ATP and hexokinase, 2-deoxyglucose is phosphorylated to 2-deoxyglucose-6-phosphate, which is nonmetabolizable, converting ATP to ADP in the process. Thus ATP generated endogenously in permeabilized myocytes is consumed in the hexokinase-2-deoxyglucose reaction, simulating ATP consumption by the contractile machinery, sarcoplasmic reticulum, and other ATP-dependent processes in the intact beating heart. Fig. 2 compares the effects of mitochondrial (*MSS*) and glycolytic substrates (*GSS*) on ATP-sensitive K^+ channels and cell length in a permeabilized myocyte with hexokinase (*HK*) and 2-deoxyglucose (*DG*) present.

In this and subsequent figures, both the bath and patch electrode solutions contained 150 mM KCl, and the membrane potential was held continuously at −40 mV (i.e., patch electrode voltage +40 mV with respect to the bath). At the beginning of the continuous recording mitochondrial substrates and hexokinase were present without 2-deoxyglucose. No ATP-sensitive K^+ channels were active in the cell-attached patch and the cell was relaxed, indicating a sufficient level of endogenous ATP production by mitochondria to suppress the channels and prevent cell shortening. Inwardly rectifying K^+ (i_{K1}) channels, although not present in this tracing, were often observed in the presence of ATP, but could be distinguished from ATP-sensitive K^+ channels by their smaller single-channel conductance (39 ± 4 pS vs. 76 ± 5 pS in symmetrical 150 mM KCl, determined from the linear portion of the current-voltage relation). At the first arrow mitochondrial substrates were removed and 2-deoxyglucose was added to the hexokinase, activating ATP consumption by

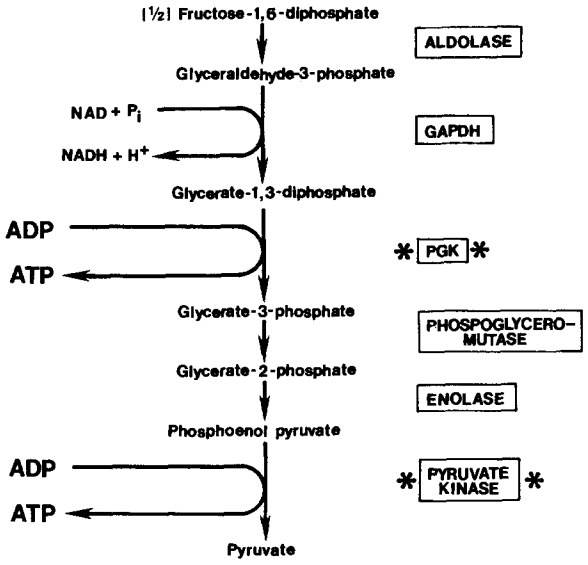

FIGURE 1. Summary of the glycolytic pathway beyond the ATP-consuming steps. Asterisks indicate glycolytic enzymes catalyzing ATP production. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase, *PGK*, phosphoglycerate kinase; P_i, inorganic phosphate.

the hexokinase-2-deoxyglucose reaction and causing ATP-sensitive K^+ channels in the cell-attached patch to open and the cell to shorten. Reexposure to mitochondrial substrates in the presence of both hexokinase and 2-deoxyglucose (second arrow) failed to suppress the ATP-sensitive K^+ channels but initiated reversal of cell shortening. Glycolytic substrates (third arrow), however, were effective at suppressing ATP-sensitive K^+ channels completely and promoted further recovery of cell length in the presence of hexokinase and 2-deoxyglucose. When 1 μM FCCP was added to uncouple mitochondrial metabolism (fourth arrow), a low level of activity of ATP-sensitive K^+ channels returned and cell length decreased slightly. Reexposure to mitochondrial substrates in the presence of hexokinase and 2-deoxyglucose (fifth arrow) again resulted in full activation of the ATP-sensitive K^+ channels, despite improving further recovery of cell length.

Fig. 3 summarizes the effects of mitochondrial substrates and glycolytic sub-

strates + FCCP on ATP-sensitive K^+ channels and cell length in a total of 15 permeabilized cells. Current through ATP-sensitive K^+ channels was time-averaged under each condition and normalized to the time-averaged value in the absence of substrates. Average current and cell length in the presence of mitochondrial substrates shown in Fig. 3 include only values before exposure to FCCP. In 11 cells 2 mM

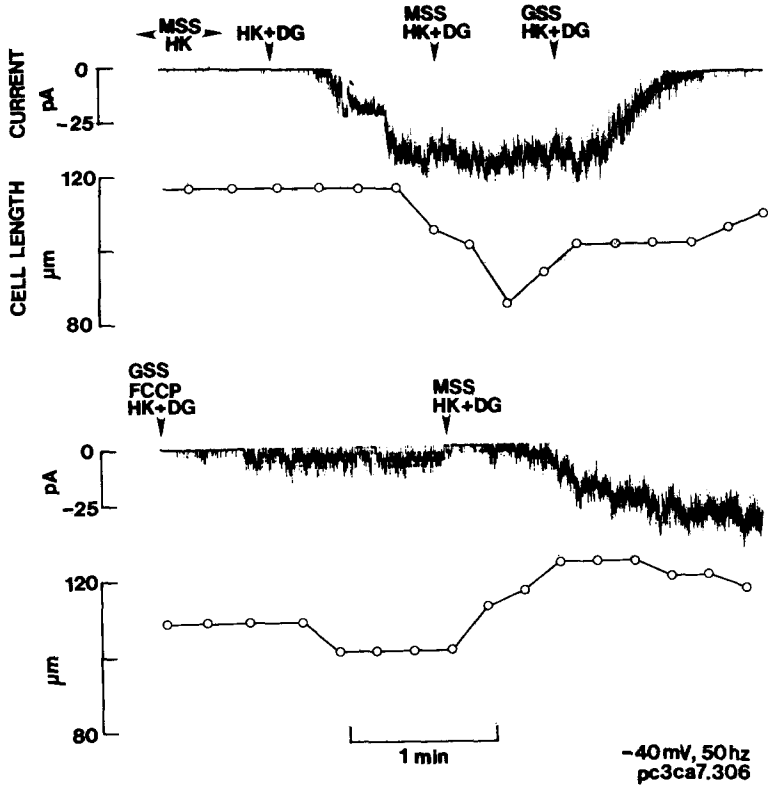

FIGURE 2. Effects of glycolytic (*GSS*) vs. mitochondrial substrates (*MSS*) on ATP-sensitive K^+ channels and cell length in a permeabilized ventricular myocyte exposed to hexokinase (*HK*, 10 IU/ml) and 2-deoxyglucose (*DG*, 10 mM). See text for description of traces. Patch electrode contained (in millimolar) 150 KCl + KOH, 5 HEPES, pH 7.3, and the substrate-free bath solution contained 150 KCl + KOH, 5 HEPES, 2 EGTA, 0.5 $CaCl_2$, 2 $MgCl_2$, pH 7.1. MSS included (in millimolar) 2 pyruvate, 2 glutamate, 2 creatine, 1 K_2HPO_4, and 0.5 ADP. GSS included 2 fructose-1,6-diphosphate, 2 phosphoenol pyruvate, 1 NAD, 1 K_2HPO_4, and 0.5 ADP. FCCP (1 μM) was used to uncouple mitochondria. The patch electrode was held at +40 mV throughout (equivalent to a membrane potential of −40 mV). Inward current is downward. Filter setting, 50 Hz.

creatine (*Cr*) was included with the mitochondrial substrates to facilitate energy transfer utilizing the creatine phosphate shuttle. The results show that in the presence of hexokinase and 2-deoxyglucose mitochondrial substrates with and without creatine present suppressed current through ATP-sensitive K^+ channels to 82 ± 10% and 81 ± 22%, respectively, of the control value without any substrates present

($P = 0.963$). Glycolytic substrates + FCCP reduced current to 34 ± 7% of control in the same group of cells ($P < 0.004$ by paired t test). Conversely cell length was slightly better preserved by mitochondrial substrates than by glycolytic substrates + FCCP in the nine cells in which accurate measurements were technically feasible. Cell length decreased on the average to 67 ± 2% of the control value during the first episode of ATP depletion and recovered to 83 ± 4% with mitochondrial substrates and 77 ± 3% with glycolytic substrates and FCCP. The differences was not statistically significant.

The ability of creatine phosphate + ADP to suppress current through ATP-sensitive K^+ channels and preserve cell length in the presence of hexokinase and 2-deoxyglucose was also compared to glycolytic substrates + FCCP. At the beginning of the continuous recording from the permeabilized cell in Fig. 4, 2 mM creatine phos-

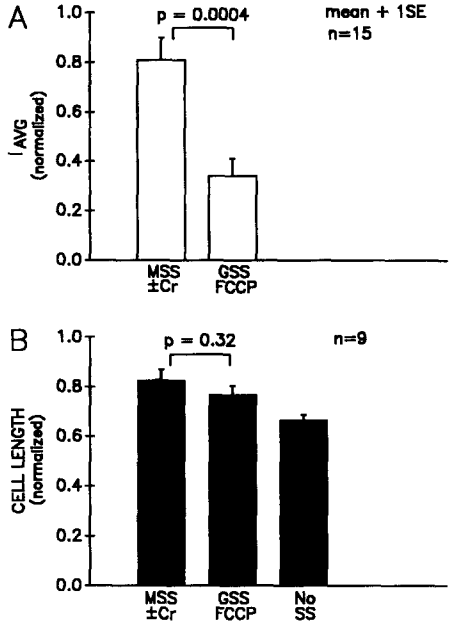

FIGURE 3. Summary of effects of GSS with FCCP present vs. MSS with and without 2 mM creatine (*Cr*) on time-averaged current through ATP-sensitive K^+ channels (*A*) and cell length (*B*) in 15 permeabilized cells exposed to HK and DG. Conditions and composition of substrates were the same as in Fig. 2. In *B*, "*No ss*" indicates cell shortening when all substrates were removed from the bath solution.

phate (*CP*), 0.5 mM ADP and hexokinase (*HK*) were present in the bath; ATP-sensitive K^+ channels in the cell-attached patch were closed and the cell was relaxed. Replacing the bath solution with perfusate containing hexokinase and 2-deoxyglucose without substrates (first arrow) caused ATP-sensitive K^+ channels to open and the cell to shorten. Readdition of creatine phosphate + ADP in the presence of both hexokinase and 2-deoxyglucose (second arrow) failed to suppress ATP-sensitive K^+ channels completely although the cell lengthened to nearly the control level. Glycolytic substrates with 1 μM FCCP present, however, did completely suppress ATP-sensitive K^+ channels although the cell length decreased slightly (third arrow). The sequence was repeated in the lower trace. Although glycolytic substrates with FCCP no longer completely suppressed the ATP-sensitive K^+ channels in the patch (sixth arrow), they remained superior to creatine phosphate + ADP at reducing current through the channels (fifth and seventh arrows).

Fig. 5 compares the results in a total of nine permeabilized cells exposed to both creatine phosphate + ADP and glycolytic substrates + FCCP in the presence of hexokinase and 2-deoxyglucose. The time-averaged current through ATP-sensitive K^+ channels was reduced by creatine phosphate + ADP to 55 ± 1% of the control value without any substrates present, and by glycolytic substrates + FCCP to 18 ±

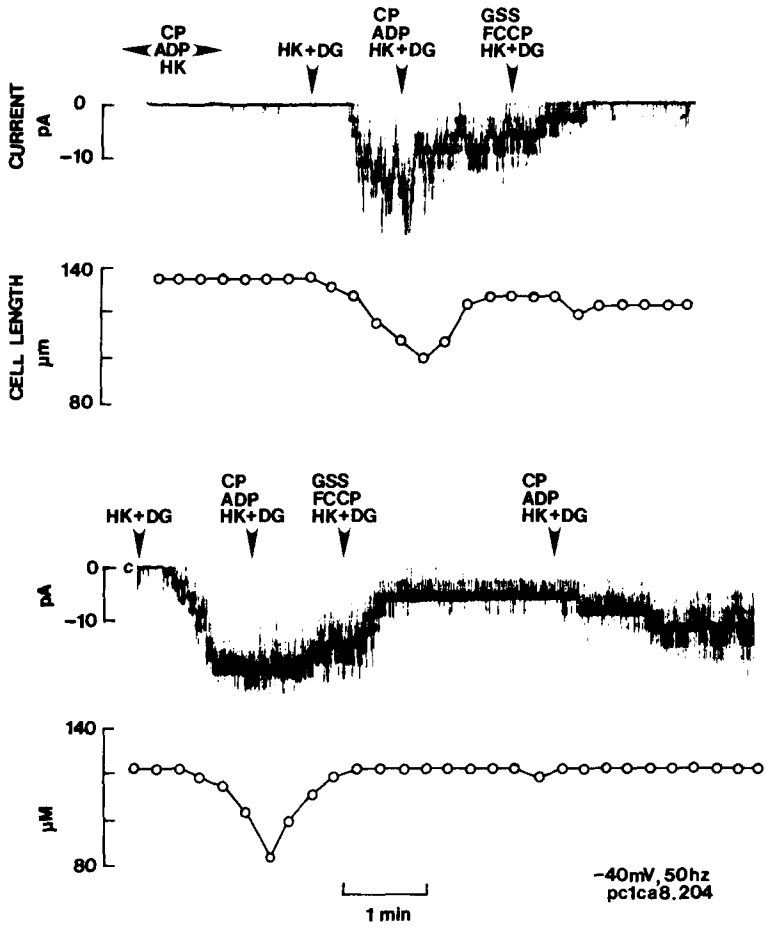

FIGURE 4. Effects of GSS vs. creatine phosphate (*CP*) and ADP on ATP-sensitive K^+ channels and cell length in a permeabilized myocyte exposed to HK and DG. See text for description of traces. Patch electrode and substrate-free bath solution, GSS, membrane potential, and filter settings were the same as in Fig. 2. CP concentration was 2 and [ADP] was 0.5 mM. FCCP (1 μM) was used to uncouple mitochondria.

7% of control, $P = 0.015$ by paired t test (Fig. 5 *A*). In contrast, creatine phosphate + ADP were slightly more effective at reversing cell shortening than glycolytic substrates + FCCP. Cell length, which upon removing substrates decreased to 62 ± 2% of control, recovered to 81 ± 3% of control with creatine phosphate + ADP, and to 78 ± 3% with glycolytic substrates + FCCP (Fig. 5 *B*). The difference was not statistically significant ($P = 0.41$).

These results indicate that despite having comparable effectiveness at preventing cell shortening, ATP generated by glycolysis was more effective at suppressing ATP-sensitive K^+ channels than ATP generated by mitochondrial oxidative phosphorylation or by the creatine kinase reaction in permeabilized cells exposed to an exogenous ATP-consuming system.

Metabolic Intermediates and the Sensitivity of ATP-sensitive K^+ Channels to ATP

One possible explanation for the superiority of glycolytic ATP at suppressing ATP-sensitive K^+ channels is that metabolic intermediates may have altered the sensitivity of the channels to ATP. ADP can be excluded since the concentration was identical in all three groups of substrates. Intermediates generated during mitochondrial

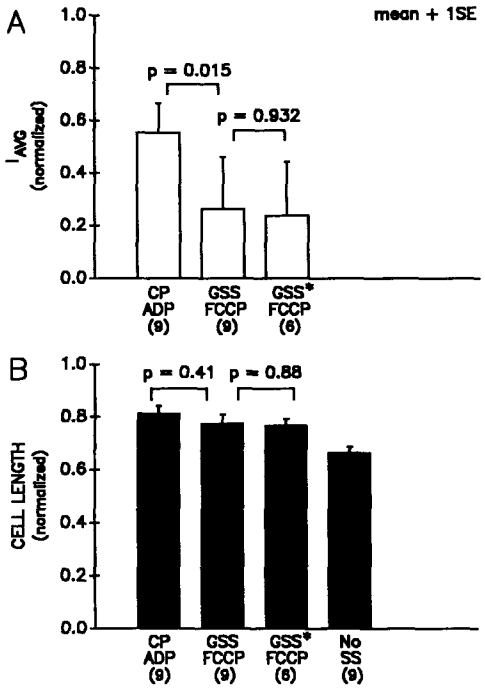

FIGURE 5. Summary of effects of GSS and GSS* with FCCP present vs. CP and ADP on time-averaged current through ATP-sensitive K^+ channels (*A*) and cell length (*B*) in permeabilized myocytes exposed to HK and DG. Conditions were the same as in Fig. 4. GSS consisted of substrates for both ATP-producing steps of glycolysis as in Fig. 2, whereas GSS* included only 2 mM phosphoenol pyruvate and 0.5 mM ADP, substrates for the last ATP-producing step of glycolysis. In *B* "*No ss*" indicates cell shortening when all substrates were removed from the bath solution.

metabolism and the creatine-kinase reaction are unlikely to have reduced the sensitivity of ATP-sensitive channels to ATP since these pathways have no intermediates in common except ADP. It is possible that glycolytic intermediates increased the sensitivity of ATP-sensitive channels to ATP so that they remained closed in the presence of comparable or even lower ATP concentrations than those generated by mitochondrial metabolism or creatine kinase. We did not formally test the effects of various glycolytic intermediates on the ATP sensitivity of ATP-sensitive K^+ channels in permeabilized cells. However, the effectiveness of substrates for the last step of glycolysis involving conversion of phosphoenol pyruvate to pyruvate, which generates one ATP molecule (Fig. 1), at suppressing ATP-sensitive K^+ channels in the presence of hexokinase and 2-deoxyglucose was investigated. Under these condi-

tions the only glycolytic intermediate present that was not present during exposure to mitochondrial substrates is phosphoenol pyruvate.

Fig. 6 shows a continuous tracing from a cell-attached patch on a permeabilized myocyte in which the effects of creatine phosphate + ADP were compared with phosphoenol pyruvate + ADP + FCCP on ATP-sensitive K^+ channels in the presence of hexokinase and 2-deoxyglucose. ATP-sensitive K^+ channels were much more effectively suppressed by phosphoenol pyruvate and ADP (*GSS**) with FCCP (third and fifth arrows) than by creatine phosphate + ADP (second and fourth arrows). The results from six permeabilized cells are summarized in Fig. 5. In three cases the effects of phosphoenol pyruvate + ADP + FCCP on ATP-sensitive K^+ channels and cell length were directly compared in the same cell to the full complement glycolytic substrates used previously (labeled GSS in Fig. 5). With FCCP present, GSS* and GSS were equally effective, both at suppressing time-averaged current through ATP-sensitive K^+ channels (to 24 ± 21% and 26 ± 20%, respectively, of the control value in the absence of substrates) and at recovering cell length (to 76 ± 3% and 76 ± 6% of the control length, respectively). These findings make it unlikely that any glycolytic intermediates, with the possible exception of phosphoenol pyruvate, increased the ATP sensitivity of ATP-sensitive K^+ channels sufficiently enough to account for the superiority of glycolysis at suppressing these channels in the presence of hexokinase and 2-deoxyglucose.

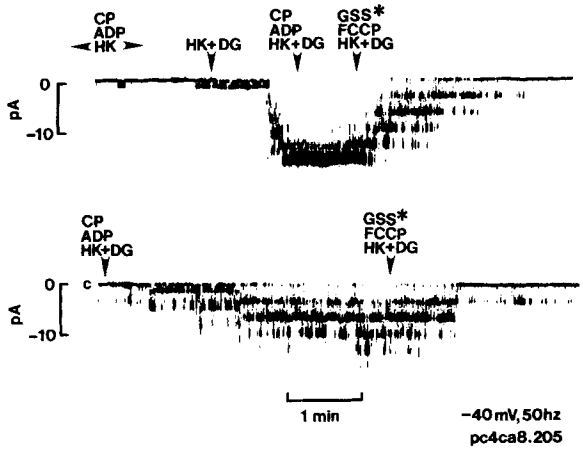

FIGURE 6. Effects of phosphoenol pyruvate and ADP (GSS*) with FCCP present vs. CP and ADP on ATP-sensitive K^+ channels in a permeabilized myocyte exposed to HK and DG. At the beginning of the trace, occasional openings of an i_{K1} channel were observed but ATP-sensitive K^+ channels remained closed in the presence of CP, ADP, and HK. When the substrates were removed and DG added (*first arrow*) ATP-sensitive K^+ channels opened. Readdition of CP + ADP in the presence of both HK + DG (*second arrow*) now failed to close the channels, but they closed immediately upon exposure to GSS* + FCCP (*third arrow*). Replacing GSS* + FCCP with CP + ADP (*fourth arrow*) again caused the ATP-sensitive K^+ channels in the patch to open. Patch electrode and substrate-free bath solutions, membrane potential and filter settings were the same as in Fig. 2. CP concentration was 2 and ADP 0.5 mM. FCCP (1 μM) was used to uncouple mitochondria.

Glycolytic Enzymes Localized to ATP-sensitive K^+ Channels

Another possible explanation for the superiority of glycolytically-generated ATP at suppressing ATP-sensitive K^+ channels is that ATP generated by glycolysis may have

preferential access to the channels. For example, glycolytic enzymes might be located in close physical proximity to the channels so that when provided with the appropriate substrates they still could maintain a local concentration of ATP adequate to suppress the channels despite the presence of hexokinase and 2-deoxyglucose. This hypothesis was tested by examining the effects of glycolytic substrates on the activity of ATP-sensitive K^+ channels in excised (cell-free) inside-out patches,

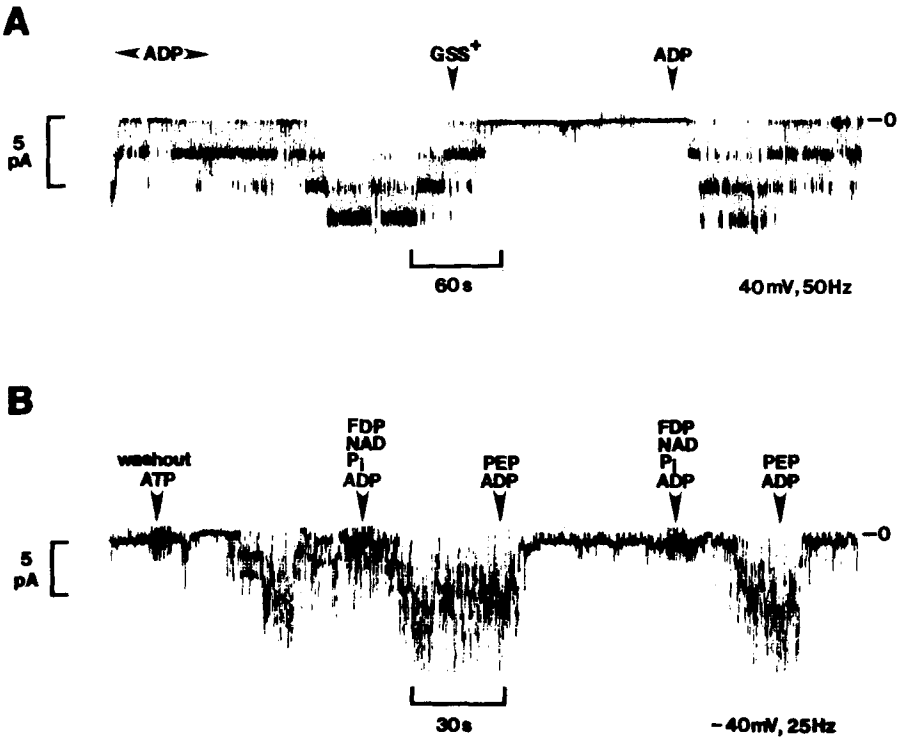

FIGURE 7. Suppressant effects of glycolytic substrates on ATP-sensitive K^+ channels in two excised inside-out membrane patches. Patch electrode solution, substrate-free bath solution and membrane potential were the same as in Fig. 2. (*A*) At the start of the trace, the bath solution perfusing the cytoplasmic surface of the patch contained 0.5 mM ADP and frequent openings of three ATP-sensitive K^+ channels were observed. Addition of substrates for both ATP-producing steps of glycolysis (arrow labeled GSS^+) caused ATP-sensitive K^+ channels to close reversibly. GSS^+ consisted of (in millimolar) 2 glyceraldehyde-3-phosphate, 2 phosphoenol pyruvate, 1 NAD, 1 K_2HPO_4, 0.5 ADP, and 1 μ/ml glyceraldehyde-3-phosphate dehydrogenase. Filter setting was 50 Hz. (*B*) In this patch, when 2 mM ATP was removed from the bath (*first arrow*), ATP-sensitive K^+ channels in the patch opened and were not suppressed by a combination of substrates for the proximal ATP-producing step of glycolysis (*second arrow*) including (in millimolar) 2 fructose-1,6-diphosphate (FDP), 1 NAD, 1 K_2HPO_4 (P_i), and 0.5 ADP. However, substrates for the second ATP-producing step of glycolysis, consisting of 2 phosphoenol pyruvate (*PEP*) and 0.5 ADP, did completely suppress the ATP-sensitive K^+ channels, unmasking the persistent activity of the smaller i_{K1} channels (*third arrow*). The effects were reproducible (*fourth and fifth arrows*). High frequency deflections beneath the arrows were artifacts caused by rapid solution flow. Filter setting was 25 Hz.

reasoning that if glycolytic enzymes for the ATP-producing steps of glycolysis (Fig. 1) were bound to the sarcolemma or cytoskeleton close to the channels, they might sometimes remain present and functional when the patch was excised (Weiss and Lamp, 1987). In 5 of 27 excised inside-out patches, a combination of all the necessary substrates for the two ATP-producing steps of glycolysis (GSS^+) reversibly and reproducibly suppressed the time-averaged current through ATP-sensitive K^+ channels in the patch, to 1.6, 3.3, 7.1, 13.6, and 64% of the control value with only 0.5 mM ADP present. Fig 7 *A* illustrates an example. In 1 of 11 patches, substrates for the second ATP-producing step of glycolysis (phosphoenol pyruvate + ADP) suppressed the time-averaged current through ATP-sensitive K^+ channels completely, whereas substrates for the proximal ATP-producing step of glycolysis (FDP, NAD, P_i, and ADP) were ineffective, suggesting that pyruvate kinase, but not all of the more proximal glycolytic enzymes, were functional in the patch (Fig. 7 *B*). In the other 32 patches, glycolytic substrates had no conclusive effects on ATP-sensitive K^+ channels, possibly because associated glycolytic enzymes were either damaged during patch excision or the anatomy of the patch did not permit ATP generated by the enzymes to accumulate sufficiently to interact with the channels. The effects of various glycolytic intermediates were also tested individually or in various combinations that were insufficient to provide all of the substrates necessary for phosphoglycerokinase or pyruvate kinase to generate ATP endogenously. The intermediates tested included (in 1–2 mM concentrations) glyceraldehyde-3-phosphate + NAD + K_2HPO_4 + 2–10 μ/ml glyceraldehyde-3-phosphate dehydrogenase (to generate glycerate-1,3-diphosphate), NADH, fructose-1,6-diphosphate, glycerate-3-phosphate, glycerate-2-phosphate, phosphoenol pyruvate, and pyruvate. No exogenous ATP was present. None of the substrates reversibly suppressed ATP-sensitive K^+ channels in the patches tested.

Effect of Patch Electrode ATP Concentration on Whole-Cell ATP-sensitive K^+ Currents

The findings above suggest that when the rate of ATP consumption in the cytosol is intrinsically high, ATP generated by glycolytic enzymes closely associated with ATP-sensitive K^+ channels may be critical for maintaining the channels in a closed state. To investigate this issue further, we examined the effectiveness of ATP supplied exogenously through the patch electrode at suppressing ATP-sensitive K^+ channels in the whole cell after the patch was ruptured. Large patch electrodes were deliberately used with an outer tip diameter of 3–5 μm and a resistance of 0.5–1.5 MΩ when filled with the standard internal solution. Under similar conditions other investigators have estimated that the time required for the cytoplasm to reach 90% equilibration with molecules the size of ATP present in the patch electrode is 2–3 min (Kameyama et al., 1985). Fig. 8 shows changes in the action potential and current-voltage relations after rupturing the patch in a cell in which the patch electrode contained 15 mM ATP (and 0.84 mM free $[Mg^{++}]$). With standard glucose-Tyrode's solution in the bath action potential duration gradually shortened, decreasing to 81% of the control value after 30 min (not shown) and to 58% of control after 60 min (Fig. 8 *A*, middle trace). The current-voltage relations at both 30 and 60 min showed an outward current shift (Fig. 8 *C*), due to both rundown of the Ca^{++} cur-

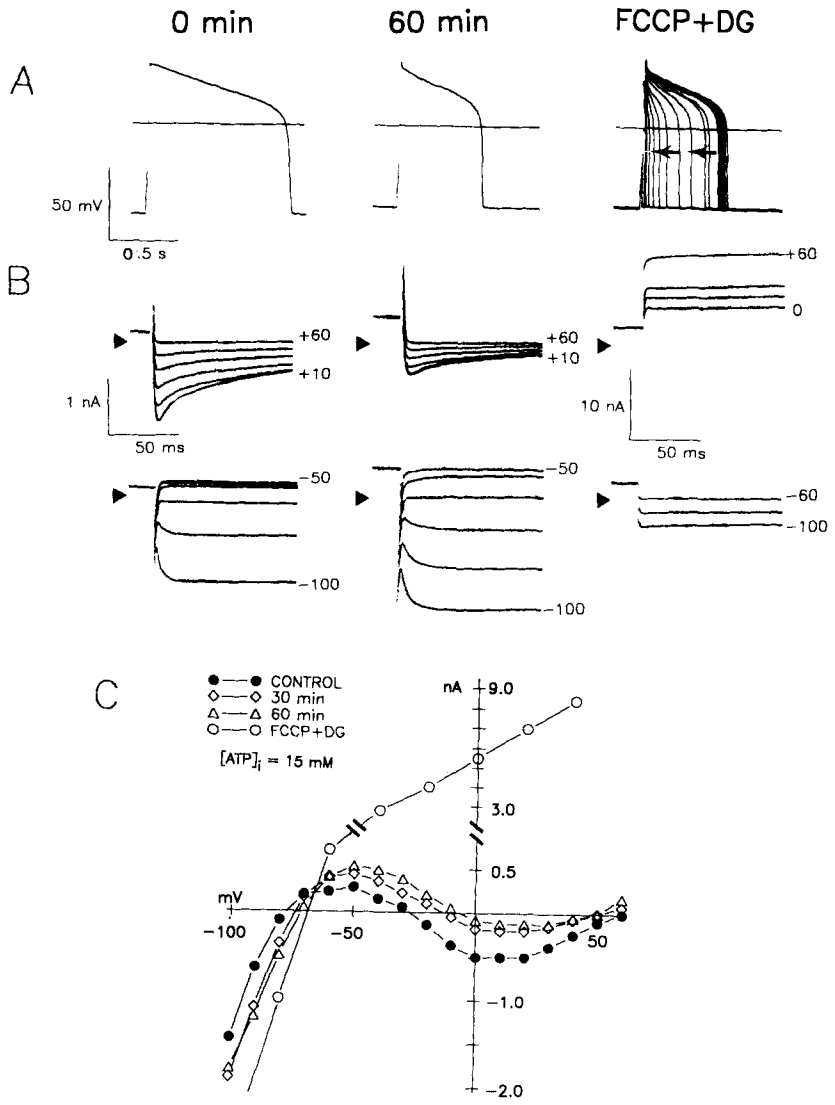

FIGURE 8. Effects of patch electrode ATP concentration on the action potential and whole-cell currents in a myocyte under control conditions and during metabolic inhibition. The patch electrode solution contained 15 mM ATP and 0.84 mM free Mg^{++}. (*A*) Recordings of action potentials shortly after rupturing the patch (0 min), after 60 min of superfusion with standard glucose-Tyrode's solution, and during exposure to glucose-free Tyrode's solution containing 10 mM DG and 0.1 μM FCCP to inhibit metabolism. (*B*) Corresponding whole-cell currents during voltage-clamp pulses of 100 ms duration to the potentials indicated, in 10 mV (0- and 60-min traces) or 20 mV increments (FCCP + DG traces). The far right traces were taken shortly after the cell became inexcitable during exposure to FCCP and DG. Holding potential was −40 mV, zero current levels indicated by arrowheads. Note the change in current gain in the far right traces. (*C*) Current-voltage relations at 0, 30, and 60 min and after exposure to DG and FCCP. Current was measured at the end of the 100-ms voltage-clamp pulses.

rent and activation of a time-independent outward K^+ current. Under similar conditions, this outward current has been attributed to activation of ATP-sensitive K^+ channels, since it can be induced by ATP depletion (Taniguchi et al., 1983; Noma and Shibasaki, 1985) and blocked by tolbutamide, a selective blocker of these channels (Belles et al., 1987). After 60 min the glucose-containing Tyrode's solution in the bath was replaced with glucose-free solution containing 10 mM 2-deoxyglucose and 0.1 μM FCCP to inhibit metabolism. Action potential duration rapidly shortened over 2 min until the cell became inexcitable (Fig. 8 *A*, far right trace), at which time the current-voltage relations revealed full activation of the ATP-sensitive K^+ current (Fig. 8 *B*, far right traces, and 8 *C*).

Fig. 9 summarizes the time required for the action potential duration to shorten to <10% of the initial value after rupture of the patch or exposure to metabolic inhibitors in myocytes in which the patch electrode solution contained either 15 mM

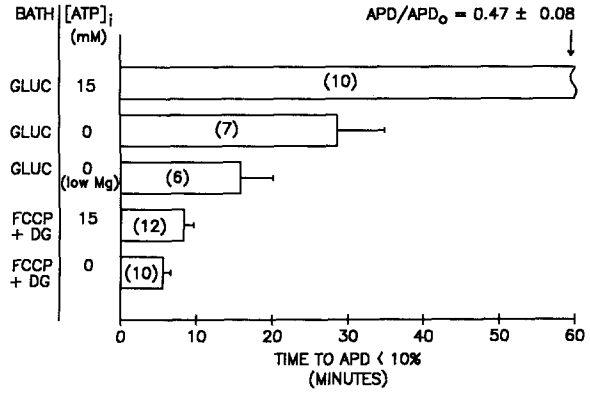

FIGURE 9. Effect of patch electrode ATP concentration on action potential duration (*APD*) shortening in whole-cell clamped myocytes under control conditions and during exposure to metabolic inhibitors. The time required for ADP to shorten to <10% of the control value is compared when the patch electrode contained either 15 mM ATP or no ATP and the bath contained either standard glucose-Tyrode's solution (*GLUC*) or glucose-free Tyrode's solution containing 10 mM DG and 0.1 μM FCCP (FCCP + DG). The free Mg^{++} concentration in the patch electrode was ~1 mM except in the case labeled "*low Mg*" in which Mg^{++} was omitted from the patch electrode solution. Values represent the mean + 1 SE for the number of cells indicated in parentheses.

or no ATP. Fig. 10 shows the corresponding current-voltage relations. With 15 mM internal ATP and the standard glucose-Tyrode's solution in the bath, myocytes remained excitable for >60 min, at which time action potential duration had shortened to 47 ± 8% of the initial value. The current-voltage relations after 30 and 60 min showed an outward current shift (Fig. 10 *A*) as in Fig. 8. With no ATP in the patch electrode solution and glucose-Tyrode's solution in the bath, the action potential duration shortened more rapidly, but did not decline to <10% of the initial value until an average of 28.6 ± 6.3 min. Omitting Mg^{++} from the patch electrode solution caused action potential duration to decline more rapidly, reaching <10% of control in 15.8 ± 4.3 min. When metabolism was inhibited by 2-deoxyglucose and FCCP, the presence of 15 mM ATP in the patch electrode delayed but did not prevent activation of the ATP-sensitive K^+ current. The time from exposure to metabolic inhibitors to reduction of action potential duration to <10% of control

averaged 5.6 ± 1.0 min with no internal ATP and 8.3 ± 1.3 min with 15 mM internal ATP (Fig. 9). Fig. 10, *C* and *D* shows the current-voltage relations at this point. The magnitude of the outward current was larger in the absence of internal ATP.

These findings indicate that even in the absence of metabolic inhibitors diffusion of ATP from the patch electrode to the cytoplasm is insufficient to prevent at least

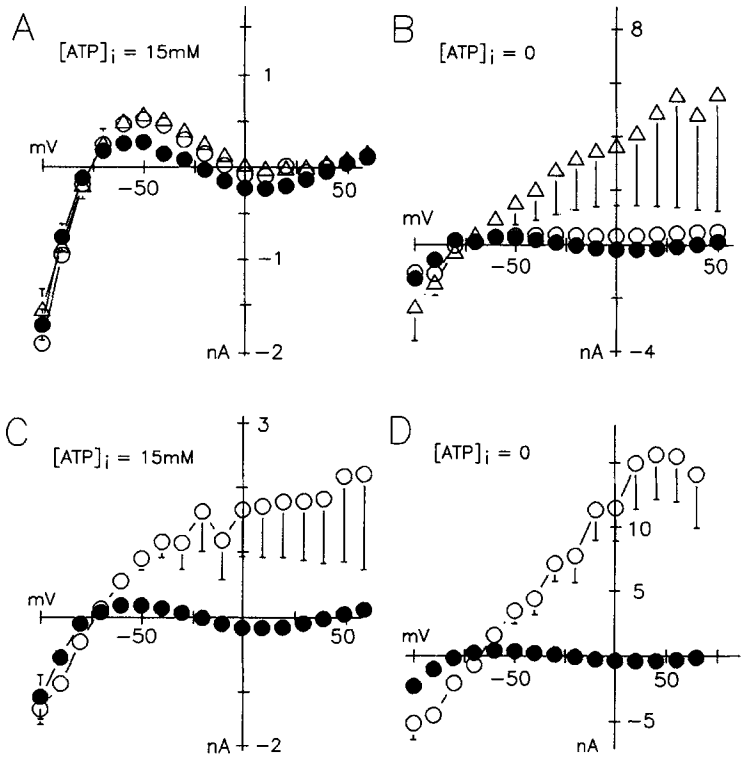

FIGURE 10. Effect of patch electrode ATP concentration on current-voltage relations under control conditions and during exposure to metabolic inhibitors. Current was measured at the end of a 100-ms voltage clamp from a holding potential of −40 mV to the voltages indicated. (*A*) Initial (●), 30 (○), and 60 min (△) after rupture of the patch, with 15 mM ATP in the patch electrode and standard glucose-Tyrode's solution in the bath. (*B*) Initial (●), 15 min (○), and after action potential duration (*ADP*) had shortened to <10% of the initial value (△), with no ATP in the patch electrode and glucose-Tyrode's in the bath. (*C* and *D*) Initial (●) and after APD had shortened to <10% of control (○) during exposure to glucose-free Tyrode's solution containing 0.1 μM FCCP and 10 mM DG (FCCP + DG), with 15 mM ATP (*C*) or no ATP (*D*) in the patch electrode solution. Values represent the mean ± SE for the number of cells indicated.

partial activation of ATP-sensitive K^+ channels, even though the concentration of ATP in the patch electrode was 1 to 2 orders of magnitude greater than the concentration necessary to completely suppress the channels in excised patches (Noma, 1983; Findlay, 1988). Conversely, when the patch electrode contained no ATP, the washout of endogenous metabolic substrates such as ADP into the vast reservoir of

the patch electrode was slow enough to prevent full activation of the ATP-sensitive K^+ current for almost 30 min. It is relevant that Mg^{++}, an essential cofactor in metabolic reactions, delayed activation of the ATP-sensitive K^+ current when ATP was omitted from the patch-electrode solution. In these experiments, we could not exclude the possibility that the major limitation to diffusion of ATP from the patch electrode to the sarcolemma was at the electrode tip rather than in the cytoplasm (Oliva et al., 1988). However, EGTA clearly diffused into the cytoplasm rapidly enough to abolish contraction in a few seconds.

Inhibition of Glycolysis and $^{42}K^+$ Efflux Rate in Isolated Rabbit Ventricle

To determine whether the superiority of glycolytic substrates at suppressing ATP-sensitive K^+ channels in permeabilized myocytes exposed to hexokinase and 2-de-

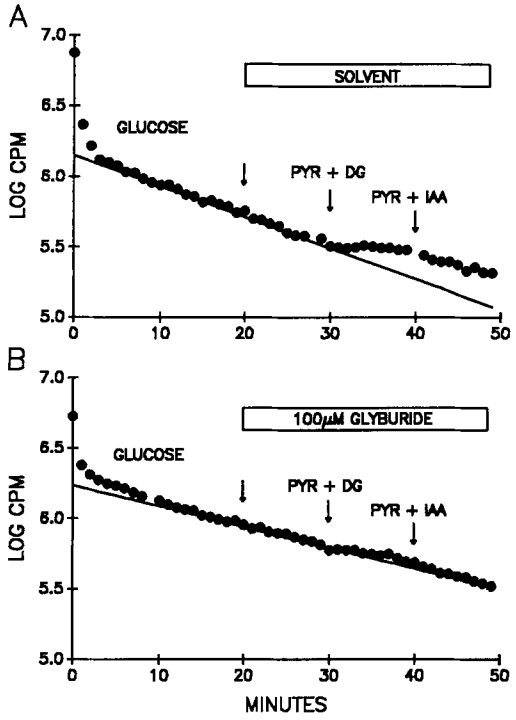

FIGURE 11. Effect of selectively inhibiting glycolysis on $^{42}K^+$ efflux (LOG CPM in venous effluent) in two representative rabbit interventricular septa, in the absence (*A*) and presence (*B*) of 100 μM glyburide. See text for details. Solvent (0.25% ethanol) was added at the first arrow in *A* and was present throughout in *B*. Glycolysis was selectively inhibited by replacing glucose with equimolar pyruvate (*PYR*) and adding either 10 mM DG or 1 mM iodoacetate (*IAA*).

oxyglucose is physiologically relevant to the intact beating myocardium, the effect of selectively inhibiting glycolysis on $^{42}K^+$ efflux rate was studied in isolated rabbit interventricular septa, arterially perfused at 37°C and paced at 75 beats per minute (Fig. 11 *A*). During perfusion with Tyrode's solution containing insulin and 5.6 mM glucose, the rate of unidirectional $^{42}K^+$ efflux was constant (0–20 min) and did not change when the solvent ethanol (0.25%) was added to the perfusate (20–30 min). When glucose was replaced by 10 mM 2-deoxyglucose (to inhibit glycolysis) and 5.6 mM pyruvate (as substrate for oxidative metabolism) there was an immediate increase in the $^{42}K^+$ efflux rate (30–40 min), which increased even further when the more potent glycolytic inhibitor iodoacetate (1 mM) was substituted for 2-deoxyglu-

cose (40–50 min). The presence of ethanol had no effects on the results. Action potential duration did not change significantly during exposure to ethanol and decreased slightly from 200 ± 12 ms to 190 ± 11 ms during exposure to 2-deoxyglucose. During exposure to iodoacetate action potential duration shortened further to 155 ± 16 ms. We have previously shown that neither of these interventions reduced total cellular ATP or creatine phosphate levels (Weiss and Hiltbrand, 1985).

To determine whether the increase in $^{42}K^+$ efflux rate during selective inhibition of glycolysis was due to the activation of ATP-sensitive K^+ channels, the same protocol was repeated in the presence of glyburide, a known blocker of these K^+ channels (Schmid-Antomarchi et al., 1987). Fig. 11 *B* shows that 100 μM glyburide (in 0.25% ethanol solvent) had no significant effect on the $^{42}K^+$ efflux rate during perfusion with glucose-containing Tyrode's solution, but almost completely blocked the

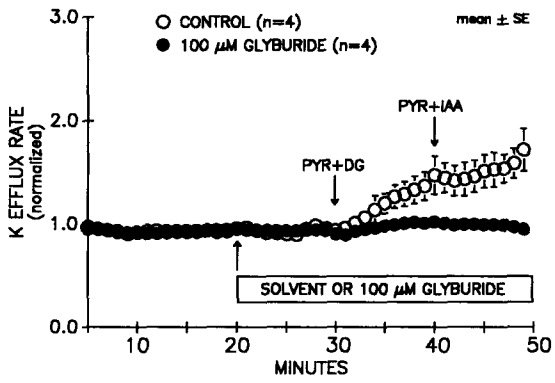

FIGURE 12. Effect of glyburide on K^+ efflux rate during selective inhibition of glycolysis in rabbit septa. The protocol was the same as in Fig. 11. Values are the mean ± SE for four preparations in each group. SE bars are shown only if larger than the symbols. In the control group (*open circles*) 0.25% ethanol solvent was added at the first arrow. In the glyburide group (*closed circles*) solvent was present throughout and 100 μM glyburide was added at the first arrow. The respective control rates of K^+ efflux averaged 2.58 ± 0.36 μmol/min in the control group and 3.06 ± 0.10 μmol/min in the glyburide group.

increase in $^{42}K^+$ efflux rate when glucose was replaced by pyruvate and either 2-deoxyglucose or iodoacetate. Fig. 12 summarizes the effects in eight septal preparations. In four preparations without glyburide present (*open circles*), K^+ efflux rate increased by 23 ± 4% during exposure to 2-deoxyglucose and pyruvate, and by 58 ± 9% during exposure to iodoacetate and pyruvate. In four preparations with 100 μM glyburide present (*closed circles*), the respective values were reduced to 5 ± 2% and 7 ± 3%. In one preparation in which 10 μM glyburide was used, K^+ efflux rates increased by 16% and 50%, respectively, during the two interventions. Effluent lactate increased by roughly 70% when glyburide was washed in for unknown reasons, but subsequent changes in effluent lactate during glycolytic inhibition paralleled those in the absence of the drug. 100 μM glyburide had no significant effect on control action potential duration (213 ± 7 ms) and did not prevent

action potential duration shortening during exposure to 2-deoxyglucose (203 ± 11 ms) or iodoacetate (123 ± 4 ms).

DISCUSSION

Evidence Supporting Functional Compartmentation of Metabolism

Evidence that ATP derived from anaerobic glycolysis preferentially supports different cellular functions than ATP derived from oxidative phosphorylation has been presented in a number of tissues, including erythrocytes (Parker and Hoffman, 1967; Mercer and Dunham, 1981), vascular smooth muscle (Lynch and Paul, 1983), brain (Lipton and Robacker, 1983), cultured cells (Balaban and Bader, 1984; Lynch and Balaban, 1987), and heart. In noncardiac tissues several of these studies concluded that key glycolytic enzymes associated with membrane-bound Na^+-K^+ ATPase preferentially provided ATP to the Na^+-K^+ pump. In heart the observation that elevating extracellular glucose concentration to supraphysiologic levels reversed shortening of the action potential duration during hypoxia suggested that glycolytic ATP may be preferentially used to preserve membrane function in this setting (McDonald and McLeod, 1973). Inhibition of glycolysis during low flow ischemia resulted in worse recovery of cardiac function upon reperfusion than inhibition of oxidative phosphorylation during ischemia despite identical tissue high energy phosphate levels (Bricknell and Opie, 1978; Bricknell et al., 1981). Similar findings were obtained in cultured neonatal myocytes with respect to their susceptibility to damage by phospholipase (Higgins et al., 1982). In cultured neonatal myocytes selective inhibition of glycolysis with metabolic inhibitors caused marked membrane depolarization and depression of $^{42}K^+$ uptake but had mild effects on contractility. Conversely, selective inhibition of oxidative metabolism caused severe depression of contractility but had mild effects on membrane function (Hasin and Barry, 1984; Hasin et al., 1984). Similar results were obtained in rabbit interventricular septa, in which extracellular K^+ accumulation was more sensitive to selective inhibition of glycolysis than selective inhibition of oxidative phosphorylation irrespective of total cellular high energy phosphate content (Weiss and Hiltbrand, 1985).

The present study extends these observations by demonstrating that glycolysis is a preferential source of ATP for cardiac ATP-sensitive K^+ channels and that the basis for this phenomenon may be related to the close physical proximity of key glycolytic enzymes to the channels. The lines of evidence are as follows: (*a*) in permeabilized myocytes exposed to an exogenous ATP-consuming system glycolytic substrates in the presence of a mitochondrial inhibitor were superior to either mitochondrial substrates or creatine phosphate + ADP at suppressing ATP-sensitive K^+ channels, although all three had a similar effectiveness at preventing cell shortening, (*b*) the presence of glycolytic enzymes bound to sarcolemma or cytoskeleton in the vicinity of ATP-sensitive K^+ channels could be inferred in some excised membrane patches in which the ATP-generating substrates for these enzymes were effective at closing the channels in the absence of exogenous ATP, (*c*) in whole-cell clamp experiments exogenous ATP delivered via the patch electrode had very limited effectiveness at preventing activation of the ATP-sensitive K^+ current, and (*d*) in intact beating rab-

bit ventricle, selective inhibition of glycolysis, which has no effect on total tissue levels of high energy phosphates (Weiss and Hiltbrand, 1985), caused an increase in $^{42}K^+$ efflux that was markedly reduced by glyburide, a known blocker of ATP-sensitive K^+ channels (Schmid-Antomarchi et al., 1987).

Several alternative interpretations of this evidence should be considered. It is possible that in the cells permeabilized with saponin, mitochondria and creatine kinase were damaged so that their capacity to generate ATP was reduced in comparison to anaerobic glycolysis. Although this possibility cannot be excluded, it seems unlikely for several reasons. The ATP concentration necessary to prevent rigor is similar to or greater than that necessary to suppress ATP-sensitive K^+ channels (White, 1970). Yet both mitochondrial substrates and creatine phosphate + ADP were as effective if not slightly superior to glycolytic substrates at reversing cell shortening despite their ineffectiveness at suppressing ATP-sensitive K^+ channels. Also, the ATP-generating capacity of mitochondria per molecule of substrate is intrinsically much greater than that of anaerobic glycolysis by a factor ranging from 5 to 24 under these conditions. Thus the mitochondria would have to be severely damaged in these cells for their ability to generate ATP to fall below that of glycolysis. Finally mitochondrial membranes are considered to be more resistant to saponin than sarcolemma and the exposure to saponin was brief.

Another possibility is that the exogenous ATP-consuming system was distributed in a nonuniform manner in the cytoplasm. However, since hexokinase is an endogenous glycolytic enzyme presumably located in close proximity to other glycolytic enzymes that generate ATP in the myocyte, it seems unlikely that the hexokinase-2-deoxyglucose reaction would degrade ATP originating from mitochondria or the creatine kinase reaction more efficiently than glycolytic ATP.

A third possibility is that glycolytic intermediates increased the sensitivity of the channels to ATP so that they remained closed in the presence of comparable or even lower ATP concentrations than those generated by mitochondria or creatine kinase. However, substrates for the last step of glycolysis, which included only phosphoenol pyruvate and ADP, were as effective as suppressing ATP-sensitive K^+ channels as substrates for the more proximal steps of glycolysis. This leaves phosphoenol pyruvate as the only possible glycolytic intermediate that might have modified the sensitivity of the channels to ATP. ADP has been shown to modify the ATP sensitivity of ATP-sensitive K^+ channels (Kakei et al., 1986; Misler et al., 1986; Ribalet and Ciani, 1987), but ADP concentrations were identical with all three sets of substrates. Differences in the quantities of H^+ ions generated by the various metabolic pathways are unlikely to have been important in the presence of 10 mM HEPES buffer. The possibility that intermediates generated during mitochondrial or creatine kinase–mediated ATP production reduced the ATP sensitivity of ATP-sensitive K^+ channels is unlikely because the mitochondrial and the creatine kinase systems have no intermediates in common with the exception of ADP.

An additional consideration is the arbitrary conditions under which the permeabilized myocytes were studied. The concentrations of the substrates may not have maximized ATP production by the different metabolic pathways, although the mitochondrial substrates were deliberately chosen to simulate those typically used to study isolated mitochondria (Peng et al., 1977; Asimakis and Contri, 1984). Also, ATP consumption rates by the exogenous ATP-consuming system consisting of

hexokinase and 2-deoxyglucose may have grossly over- or underestimated realistic physiologic rates of ATP utilization by the contractile machinery, sarcoplasmic reticulum, membrane pumps, and other intracellular ATPases in the intact beating heart. However, the observation that selective inhibition of glycolysis caused an immediate increase in $^{42}K^+$ efflux in rabbit interventricular septa is consistent with the hypothesis that the regulation of ATP-sensitive K^+ channels is critically dependent on glycolysis even under conditions in which total cellular levels of ATP and creatine phosphate are normal. Although it is not certain that the increase in $^{42}K^+$ efflux in rabbit ventricle was due to activation of ATP-sensitive K^+ channels, it was prevented by glyburide, a selective blocker of the channels. The concentration of glyburide (100 μM) required to block the increase in $^{42}K^+$ efflux was quite high compared to the nanomolar-range concentrations that block ATP-sensitive K^+ channels in pancreatic beta cells (Schmid-Antomarchi et al., 1987), but cardiac ATP-sensitive K^+ channels have been reported to be significantly less sensitive to sulfonylureas (Belles et al., 1987). We also have observed that in whole-cell voltage-clamped myocytes 100 μM glyburide only partially blocked the ATP-sensitive K^+ current activated by exposure to 2-deoxyglucose and FCCP (Venkatesh et al., 1989). In the septum, glyburide did not prevent shortening of the action potential duration during exposure to glycolytic inhibitors. However, changes in action potential duration in this setting cannot necessarily be exclusively attributed to the ATP-sensitive K^+ current, since alterations in other ionic currents may also have been involved. Another concern is whether glyburide at this high dose may have affected other cardiac K currents such as the inward rectifier i_{K1} or the delayed rectifier i_K. It is unlikely that if present such effects were important since during control perfusion with glucose-containing Tyrode's solution, 100 μM glyburide had no significant effect on action potential duration or the rate of $^{42}K^+$ efflux.

Possible Mechanisms of Functional Compartmentation of Glycolysis

The superiority of glycolytic ATP at suppressing cardiac ATP-sensitive K^+ channels may be related to two factors: the close physical proximity of key glycolytic enzymes to the channels, and a functionally slow rate of ATP diffusion in the cytoplasm of beating heart. The diffusivity of ATP through the cytoplasm of skinned skeletal muscle fibers has been reported to be about half that in aqueous solution, and comparable to that of other molecules of similar size (Kushmeric and Podolsky, 1969). However, under the same conditions, the diffusion of Ca^{++} was ~50 times slower in the cytoplasm than in aqueous solution because of its sequestration by the sarcoplasmic reticulum. It is plausible to expect that the functional diffusivity of ATP in cytoplasm is subject to similar constraints so that when ATP utilization by the cell is intrinsically high (unlike the relaxed skinned skeletal muscle fiber), the functional rate of diffusion of ATP is reduced. Under these conditions the proximity of ATP production sites to ATP-sensitive K^+ channels may be a critical determinant of their ability to maintain a high local ATP concentration in the vicinity of the channels. ATP generated by mitochondria or creatine kinase at a distance from ATP-sensitive K^+ channels would be more likely to be consumed by the contractile machinery, sarcoplasmic reticulum, and other organelles as it diffuses towards channels than would ATP generated locally at the site of the channels by glycolytic enzymes. Inhi-

bition of glycolytic ATP production could result in a gradient in ATP concentration between the mitochondrial or creatine kinase production sites and ATP-sensitive K^+ channels in the sarcolemma, the magnitude of which would increase with increasing rates of ATP utilization in the cytoplasm. The results in the isolated myocytes support this hypothesis. In permeabilized myocytes, mitochondrial substrates or creatine phosphate + ADP were as effective as glycolytic substrates at suppressing ATP-sensitive channels when the intrinsic level of ATP utilization by the cells was low, but became ineffective when the level of ATP utilization was increased by adding an exogenous ATP-consuming system. In nonpermeabilized myocytes under whole-cell voltage-clamp conditions, the presence of an ATP concentration in the patch electrode 2 orders of magnitude greater than the concentration needed to suppress ATP-sensitive K^+ channels in excised patches was ineffective in preventing activation of the channels when endogenous ATP production by the myocyte was inhibited. Although this mechanism is still speculative, significant cytosolic ATP gradients have been inferred in hepatocytes during hypoxia (Aw and Jones, 1985; Jones, 1986), and could be quantitatively predicted by a model in which the distribution of ATP-generating and ATP-consuming systems was nonuniform without the need to invoke special cytoplasmic ATP diffusion barriers. Large oxygen gradients in the cytoplasm, an analogous situation, have also been measured in hepatocytes and cardiac myocytes and quantitatively explained by a similar mechanism (Jones, 1986; Jones and Kennedy, 1986).

Other studies in heart have suggested that contractile function may be preferentially dependent on ATP generated by oxidative phosphorylation (Hasin and Barry, 1984; Hasin et al., 1984; Weiss and Hiltbrand, 1985) and the creatine kinase system (Bessman and Geiger, 1981). In the permeabilized cells in these experiments, ATP generated by glycolysis, oxidative metabolism, or the creatine kinase reaction were essentially equally effective at reversing cell shortening induced by ATP depletion. With 2 mM EGTA present in the bath solution, cell shortening was unlikely to be Ca^{++}-induced and probably resulted from the gradual formation of rigor bonds in the myofilaments due to ATP depletion. Since glycolytic enzymes are distributed through the cytoplasm and are possibly bound to mitochondria (Gots and Bessman, 1974), it is reasonable that glycolysis alone was capable of generating sufficient ATP to prevent cell shortening in permeabilized cells in the presence of hexokinase and 2-deoxyglucose. However, the ATP concentration necessary to prevent rigor is much lower than that necessary to maintain normal contractile function and it is well established that in the absence of oxidative phosphorylation anaerobic glycolysis cannot provide enough ATP to support normal contractile activity (Rovetto et al., 1973). Therefore no conclusions can be drawn from the present study about whether oxidative phosphorylation or creatine kinase are preferential sources of ATP for contracting myofilaments.

Relevance to Myocardial Ischemia and Other Tissues

K^+ efflux increases within 15–30 s of the onset of myocardial ischemia and the resulting accumulation of K^+ in the extracellular compartment is a major factor contributing to electrophysiological changes that predispose the heart to serious arrhythmias (Harris et al., 1954). The role of ATP-sensitive K^+ channels as a poten-

tial cause of the increased K^+ efflux is questionable because total cellular ATP content, normally 3–5 mM, falls by only about one third during the first 10 min of ischemia (Hearse, 1979). The present study suggests that the source of ATP, rather than the overall cellular contents, may be the most important determinant of when the channels begin to open. Even so, since glycolytic flux increases during early ischemia (Rovetto et al., 1973), it seems unlikely that ATP-sensitive K^+ channels would be activated during the early stages of ischemia. However, total glycolytic flux is composed of both exogenous glucose utilization and glycogenolysis. Glycogenolysis might not be a preferential source of ATP for ATP-sensitive K^+ channels unless the necessary glycogenolytic as well as glycolytic enzymes were associated with the channels. In this case, ATP-sensitive K^+ channels might be activated during ischemia once ATP production from exogenous glucose utilization fell below a critical level, despite an increased level of total glycolytic ATP production due to glycogenolysis. Although this possibility is highly speculative, differences in the effectiveness of exogenous glucose metabolism and glycogenolysis at supporting cardiac function during ischemia and hypoxia have been noted previously (Dennis et al., 1982; Runnman and Weiss, 1988). It is also possible that an as yet unidentified sequella of ischemia and metabolic inhibition reduces the sensitivity of ATP-sensitive channels to ATP. Other mechanisms of increased K^+ efflux, such as anion-coupled K^+ loss (Kleber, 1984), may also be important during early ischemia and hypoxia.

Our findings may also be relevant to the regulation of insulin release by beta islet cells in the pancreas. In this tissue, ATP-sensitive K^+ channels, which are responsible for the high resting membrane K^+ conductance of beta cells in the absence of extracellular glucose, are suppressed when glucose concentration is increased, causing membrane depolarization which triggers Ca^{++}-mediated spikes and insulin release (Ashcroft et al., 1984, Cook and Hales, 1984). However, cytosolic ATP concentration is normally ~5 mM in beta cells (Ashcroft et al., 1973; Malaisse and Sener, 1987), and although removal of glucose causes a modest fall in this concentration the level still remains considerably above the threshold concentration of ATP that suppresses channel activity, since 50% suppression typically occurs at 10–20 μM ATP in excised patches. Changes in cytosolic ADP concentration may be important in reducing the sensitivity of ATP-sensitive channels to ATP into the physiological range (Kakei et al., 1986; Misler et al., 1986; Ribalet and Ciani, 1987). However, a preferential dependence of the channels on ATP derived from anaerobic glycolylsis would be an attractive mechanism linking the metabolism of glucose directly to the activity of ATP-sensitive K^+ channels.

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