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Biochemical Pharmacology 63 (2002) 2055–2062

Biochemical Pharmacology

3-Isobutyl-1-methylxanthine (IBMX) sensitizes cardiac myocytes to anoxia

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Received 15 August 2001; accepted 1 October 2001

Abstract

Cardiac myocytes incubated with 3-isobutyl-1-methylxanthine (IBMX), a nonspecific cyclic nucleotide phosphodiesterase inhibitor, formed rigor complexes under anoxic conditions more readily than cells incubated with other phosphodiesterase inhibitors. Cardiac myocytes were incubated for 1 hr with either (a) no additions, (b) 150 μM zaprinast, or (c) 1 mM IBMX, and then were rendered anoxic for periods up to 60 min. Cells were >80% viable throughout the anoxic period; viability was unaffected by either drug. Rod count decreased more rapidly after the onset of anoxia in the IBMX-treated cells than in control or zaprinast-treated cells (11% rods vs. roughly 47% rods after 30 min of anoxia). IBMX-treated cell groups also formed more ''contracted'' myocytes (box-like rods) than their untreated or zaprinast-treated counterparts (50% contracted vs. roughly 27% contracted after 30 min of anoxia). While nucleotide degradation patterns were similar in all experimental groups, the ratio of ATP to ADP was lower in IBMX-treated cells than in control or zaprinast-treated cells. The L-type calcium channel was apparently not involved in this phenomenon; while cyclic AMP was elevated in the IBMX-incubated cells, verapamil did not protect IBMX-incubated cells from premature damage by anoxia. Incubation with 8-cyclopentyl-1,3-dipropylxanthine (CDPX), an A_1 receptor antagonist, at concentrations up to 1 μM in place of 1 mM IBMX did not reproduce the IBMX effect. We concluded that IBMX sensitizes cardiac myocytes to anoxia through a mechanism related to its effect on ATP/ADP, and unrelated to an elevation of intracellular calcium or preconditioning phenomena. © 2002 Published by Elsevier Science Inc.

Keywords: Heart; Myocyte; Anoxia; 3-Isobutyl-1-methylxanthine; Zaprinast; Verapamil; 8-Cyclopentyl-1,3-dipropylxanthine; ATP/ADP ratio

1. Introduction

For many years, cardiac myocytes have been used as a model system for examining metabolic phenomena in the heart. While obvious differences exist between isolated cells and the intact heart, both the ability to control the environment of isolated muscle cells and the ease with which isolated cells can be extracted and analyzed have popularized this model system. The resulting studies have vastly extended our knowledge of the way heart muscle behaves under a variety of conditions.

Under anoxic conditions, cardiac myocytes undergo a series of biochemical and morphologic changes. After the onset of anoxia, many myocytes change morphology from elongated rod-shaped forms to a shortened box-like shape (''contracted'' or ''rigor complexes''; [1–5]). This morphologic form is thought to correspond to the state of reduced contractility and increased stiffening seen in intact myocardium just after the onset of ischemia [4,6]. The appearance of rigor complexes is related to low levels of ATP seen under ischemic/anoxic conditions, and apparently is unrelated to cytosolic calcium levels [1,7,8]. If anoxia is prolonged, or if the cells are reoxygenated, many myocytes hypercontract to a form that is round or cabbage-like in appearance [3,5]. The appearance of these forms is thought by some to be related to a sudden rise in intracellular calcium, but may be due to other mechanisms [9]. Finally, myocytes may be classed as viable or nonviable, as determined by trypan blue exclusion. [Nonviable cells do not have intact plasma membranes, and hence cannot exclude trypan blue (MW = 960) or related dyes of similar MW (Evan's Blue or the other Direct Blue dyes)]. While any one of the cellular forms mentioned above (rods, rigor complexes, or hypercontracted cells) may be nonviable, the overwhelming majority of nonviable cells in any sample are hypercontracted.

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Abbreviations: CDPX, 8-cyclopentyl-1,3-dipropylxanthine; IBMX, 3-isobutyl-1-methylxanthine.

0006-2952/02/$ – see front matter © 2002 Published by Elsevier Science Inc.
PII: S0006-2952(02)00901-2

Recently, we reported that anoxia inhibits the α-adrenergic stimulation of inositol phosphate turnover in cardiac myocytes, but that cyclic GMP production is enhanced under similar conditions [10]. Initially, we found that endogenous phosphodiesterases degraded a significant portion of the cellular cyclic nucleotides before they could be extracted and analyzed (the heart contains several different isoforms of cyclic nucleotide phosphodiesterase [11]). To prevent this degradation from occurring, phosphodiesterase inhibitors were included in the suspending medium. While screening these inhibitors, it was apparent that cells incubated with 100 μM or 1 mM IBMX, a general phosphodiesterase inhibitor, showed a greater tendency to form rigor complexes and to hypercontract than cells incubated with 150 μM zaprinast (formerly known as May and Baker 22948, an inhibitor of cyclic GMP-specific phosphodiesterase) or with no phosphodiesterase inhibitor at all.

A more systematic examination of this unexpected effect of IBMX clearly was warranted, especially in view of the general utility of IBMX in second messenger studies. In addition to the obvious pharmacologic implications of this phenomenon, it seemed clear that a controlled study might shed further light on the mechanisms behind rigor contracture in myocytes. Therefore, we constructed a model of the system which described three possible causes of the observed effect of IBMX (Fig. 1).

One possible mechanism focused on the elevation of intracellular cyclic AMP levels by IBMX. The primary effect of elevated cyclic AMP in cardiac cells is the activation of cyclic AMP-dependent protein kinase [protein kinase A (PKA)]. PKA, in turn, phosphorylates any of four main target proteins in the heart (phosphorylase kinase, phospholamban, troponin I, and elements of the L-type calcium channel; see Bers [12], Opie [13], and Walsh and Van Patten [14] for reviews). Phosphorylation of phosphorylase kinase promotes glucose mobilization from glycogen; any resulting increase in ATP would tend to be cardioprotective (we observed an increase in rigor forms, a form of injury). Phosphorylation of phospholamban promotes sequestration of calcium in the sarcoplasmic reticulum *via* the Ca^{2+}-ATPase; phosphorylation of troponin I alters the affinity of troponin C for calcium, such that calcium binding is reduced. Both of these latter actions of PKA would, therefore, promote relaxation of the cardiac cell, rather than the increased contraction that was observed. Phosphorylation of the L-type calcium channel increases the probability that the channel will be in an open state at any given time, thereby promoting calcium influx and, theoretically, increased tension. Using this line of reasoning, we hypothesized that elevation of intracellular cyclic AMP by IBMX promoted opening of the L-type calcium channel, allowing resting cytosolic calcium levels to rise. Increased cytosolic calcium would lower the threshold of the myocytes for contraction, thus appearing to sensitize the myocytes to anoxia.

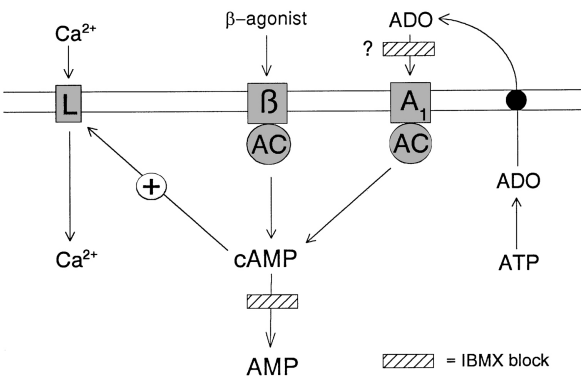

Fig. 1. Model of the possible effects of IBMX on cardiac myocytes. IBMX could cause metabolic disruptions at two points, as shown. L = L-type calcium channel; β = β-adrenergic receptor; A_1 = adenosine receptor; AC = adenylate cyclase; ADO = adenosine; AMP, ATP = adenosine mono- and triphosphate, respectively; cAMP = cyclic AMP.

A second possible mechanism stipulated that incubation of the myocytes with IBMX caused nucleotides (especially ATP or GTP) to be degraded at an accelerated rate under anoxic conditions. The lack of nucleotide would then allow these cells to be damaged more quickly than the controls.

The third possible mechanism dealt with preconditioning, defined as resistance to ischemia generated by prior small episodes of ischemia or by exposure to certain compounds (including adenosine) which generate a similar effect [15–17]. The anoxia protocol used in this study causes large-scale degradation of cellular adenine nucleotides, which are exported as adenosine and inosine into the medium [18]. The hypothesis of the third mechanism was that the myocytes in the control and zaprinast-incubated flasks were preconditioned by the presence of adenosine in the medium; relatively high levels of added IBMX would interact with the adenosine (A_1) receptor, acting as an adenosine antagonist. According to this hypothesis, the action of IBMX would be to "reverse-precondition" the cells by preventing the binding of adenosine to its receptor, thus making them more susceptible to anoxia than the controls.

To test these three hypotheses, adult cardiac myocytes were incubated with no drug, 1 mM IBMX, or 150 μM zaprinast, after which they were subjected to anoxia. The morphology of the cells was then observed, and cell extracts were analyzed for 5′-nucleotides and cyclic nucleotides. Myocytes were also incubated with CDPX, a specific A_1 receptor antagonist, at concentrations up to 1 μM, as well as IBMX plus verapamil (an L-type Ca^{2+} channel blocker). The data confirm that IBMX sensitizes cardiac myocytes to anoxia. The data also suggest that this effect is mediated by nucleotide depletion, specifically depletion of ATP (mechanism 2), and argue against the participation of elevated intracellular Ca^{2+} (mechanism 1) or reverse-preconditioning (mechanism 3) in the IBMX phenomenon.

2. Materials and methods

2.1. Myocyte isolation

Cardiac myocytes were isolated from adult rat hearts by collagenase digestion [19]. The resulting cell population was tolerant to 1 mM $CaCl_2$. Unless noted otherwise, the suspending buffer was Minimal Essential Medium (Joklik-modified) supplemented with 60 mM taurine, 20 mM creatine, 5 mM HEPES, and 1.5% (w/v) BSA.

2.2. Experimental protocol

The basic anoxia protocol was conducted as previously described [5]. All incubations were conducted at 37° in siliconized glass flasks that were agitated throughout the incubation period. After isolation, the myocytes were incubated for 1 hr in the suspending buffer (see preceding paragraph) with either (a) no additions, (b) 150 μM zaprinast, or (c) 1 mM IBMX. [These levels of zaprinast and IBMX are generally recognized as effective at inhibiting their respective phosphodiesterase targets (see [20] for zaprinast and [21–24] for IBMX). Our crude concentration responses tended to confirm literature values.] The experiment was initiated by settling the cells, removing the supernatant, and replacing it with appropriately gassed buffer (suspending buffers were equilibrated for at least 1 hr with 100% O_2 or N_2). The flasks were stoppered and incubated with a steady stream of either O_2 or N_2. Suspending buffers contained 1 mM IBMX, 150 μM zaprinast, 1 μM verapamil, or 100–1000 nM CDPX where appropriate. After incubating for 0, 15, 30, or 60 min, three 200-μL aliquots were removed and extracted for either 5′-nucleotide or cyclic nucleotide analysis. One 200-μL aliquot was removed at each time point for cell counting.

2.3. Extraction

Aliquots removed from each tube or flask were transferred quickly to 1.5-mL microcentrifuge tubes containing (top to bottom) 0.4 mL of ice-cold buffer, 0.4 mL of bromododecane (oil), and 0.1 mL of 2 M perchloric acid. The tubes were centrifuged immediately at 13,000 *g* for 30 s at room temperature (23–26°). The aqueous layer above the oil was removed, the tube above the oil was washed, and the extract was diluted and neutralized as previously described [25]. The neutralized extract was frozen at −70°.

2.4. Nucleotide analysis

Cell extracts were analyzed for 5′-nucleotides by anion exchange HPLC [18]. Cyclic AMP was assayed by radioimmunoassay (RIA) or enzyme immunometric assay (EIA), using kits designed for this purpose from New England Nuclear or from the Cayman Chemical Co.

2.5. Cell counting

Myocytes were stained and counted using light microscopy as previously described [10]. Individual cells were scored as (a) viable or nonviable, and (b) one of three possible morphologic forms. Rods are myocytes with a ratio of length to width (length/width) ≥ 3. ‘‘Contracted’’ myocytes have a length/width <3 (generally in the range of 1 to 1.5). ‘‘Hypercontracted’’ cells are rounded myocytes that display little if any rod-like character. (These morphological forms are described fully in both Brierley *et al.* [3] and Geisbuhler and Rovetto [5]).

2.6. Cell protein

Cell pellets were dissolved in NaOH and assayed for protein essentially as described by Lowry *et al.* [26].

2.7. Statistics

Mean values were compared for differences, using Student’s *t*-test. All comparisons were made within-time (for example, 30-min anoxic values were compared with 30-min aerobic values) and within-condition (for example, 30-min IBMX-incubated aerobic values were compared with 30-min zaprinast-incubated aerobic values).

3. Results

The anoxia protocol used in this study is well documented [5,10]. As described in Section 2, the experiment was initiated by settling the cells, removing the supernatant, and replacing it with buffer equilibrated with either O_2 (aerobic cells) or N_2 (anoxic cells). (Replacing aerobic buffer with aerobic buffer in the oxygenated controls assures that both populations of cells are handled in an identical manner.) The cells were then incubated for 0, 15, 30, or 60 min, with or without the appropriate drugs as additives. Aliquots of the cell preparation taken at each time point were scanned for morphology and extracted for analysis of nucleotides or other metabolites.

Typical results from studies using this system have been published (e.g. [10]), and are also seen in Fig. 2. Anoxia up to 60 min typically does not cause large-scale cell death, as evaluated by trypan blue exclusion [5,10]; incubation of the myocytes with phosphodiesterase inhibitors did not alter this established behavior (Fig. 2, top panel). Anoxia typically causes an increase in ‘‘contracted’’ cells (box-shaped cells, length/width = 2–3; bottom panel), apparently at the expense of normal rod-shaped cells (center panel). Hypercontracted cells (round) also appear with greater frequency under anoxic conditions. Zaprinast-treated cells were identical to untreated cells both morphologically and with respect to extractable nucleotides (morphological data shown in Fig. 2); that is, the presence

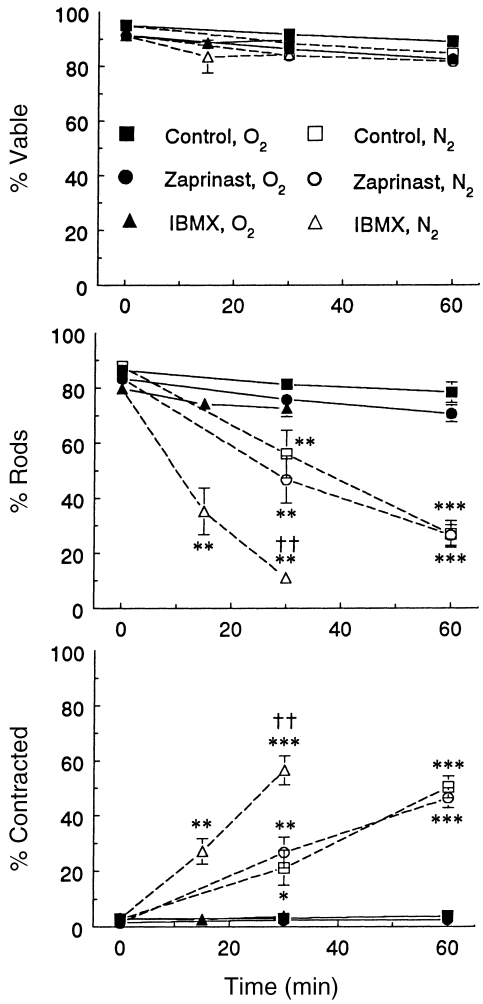

Fig. 2. Comparison of the morphology of zaprinast-incubated and IBMX-incubated myocytes under anoxic conditions. Myocytes were preincubated with no additions, 150 μM zaprinast, or 1 mM IBMX for 1 hr, and then subjected to 0–60 min of anoxia as described in Section 2. Values are expressed as a percentage of the total cells counted, and represent the means ± SEM of at least 5 preparations (each mean is the result of triplicate determinations). Asterisks indicate a significant change from aerobic controls at the same time point [$P \leq 0.05$ (∗), 0.01 (∗∗), or 0.001 (∗∗∗)]. Daggers appearing over points representing IBMX- or zaprinast-treated cells indicate a significant difference of treated cells from untreated cells [$P \leq 0.01$ (††)].

of zaprinast was neither protective nor deleterious, under either aerobic or anoxic conditions.

Fig. 2 also illustrates the basic phenomenon that precipitated this study. IBMX decreased the time necessary for anoxia to produce morphologic changes to the myocytes. Fifteen minutes of anoxia produced a 40% decrease in rod-shaped cells when the preparation was incubated with IBMX; 45 min of anoxia was required for a similar decrease when the cells were untreated or incubated with zaprinast (Fig. 2, center panel). In a similar fashion, morphological forms associated with anoxic damage (contracted and hypercontracted cells) appeared more rapidly in preparations incubated with IBMX than in untreated cells or in myocytes exposed to zaprinast (Fig. 2, bottom panel).

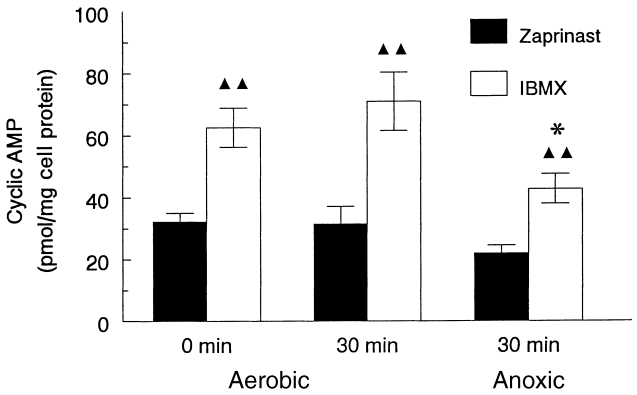

Fig. 3. Comparison of cyclic nucleotide content of zaprinast-incubated and IBMX-incubated myocytes under anoxic conditions. Myocytes were preincubated with 150 μM zaprinast or 1 mM IBMX for 1 hr, and then were subjected to 0 or 30 min of anoxia as described in Section 2. Cyclic AMP was extracted from the cells and quantitated by RIA or EIA. Values are expressed as pmol cyclic AMP/mg cell protein, and represent the means ± SEM of at least 4 preparations (each mean is the result of triplicate determinations). An asterisk indicates a significant change from aerobic controls at the same time point [$P \leq 0.05$ (∗)]. Closed triangles appearing over bars representing IBMX-treated cells indicate a significant difference of IBMX-treated cells from zaprinast-treated cells [$P \leq 0.01$ (▲▲)].

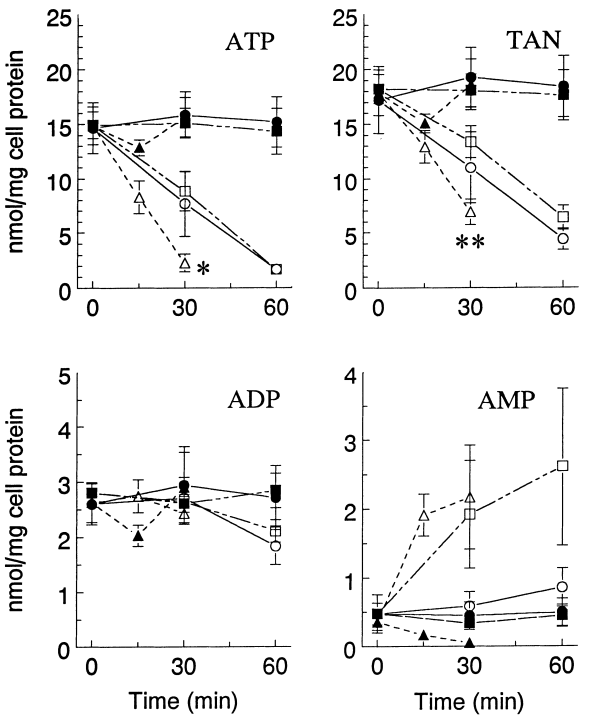

Fig. 4. Adenine nucleotide content of myocytes under anoxic conditions. Myocytes were preincubated with no additions, 150 μM zaprinast, or 1 mM IBMX for 1 hr, and then subjected to 0–60 min of anoxia as described in Section 2. Nucleotides were extracted from the cells and quantitated by HPLC. Values are expressed as nmol nucleotide/mg cell protein, and represent the means ± SEM of at least 7 preparations (each mean is the result of triplicate determinations). Closed symbols represent aerobic incubations (control = ■, zaprinast = •, IBMX = ▲); open symbols represent anoxic incubations (control = □, zaprinast = ○, IBMX = △). Asterisks indicate a significant change from aerobic controls at the same time point [$P \leq 0.05$ (∗) or 0.01 (∗∗)]. TAN = total adenine nucleotides.

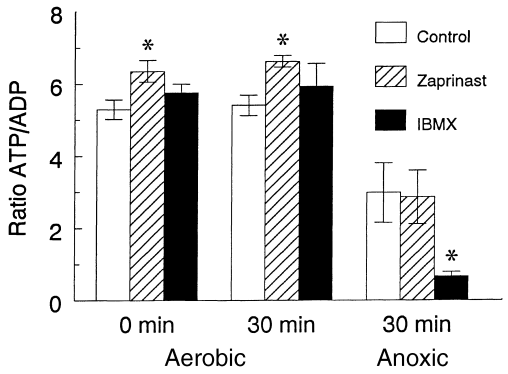

Fig. 5. Ratio of ATP to ADP in myocytes under anoxic conditions. Daily mean values of adenine nucleotides (see Fig. 4) were used to calculate a ratio of ATP to ADP. These daily ratios were used, in turn, to calculate the values shown (means ± SEM of at least 7 preparations). Asterisks indicate a significant change from time- and condition-matched untreated controls [$P \leq 0.05$ (∗)].

Since the purpose of both zaprinast and IBMX in this study was to block degradation of cyclic GMP by native phosphodiesterases, cyclic nucleotides were measured in cell suspensions containing these inhibitors. Basal levels of cyclic GMP were unaffected by either inhibitor (data not shown; cyclic GMP data for zaprinast-incubated cells has been published [10]). Incubating the myocytes with IBMX elevated cyclic AMP up to 2-fold as compared with zaprinast-treated cells (Fig. 3).

Adenine nucleotides were degraded after the onset of anoxia (Fig. 4), in agreement with previous studies

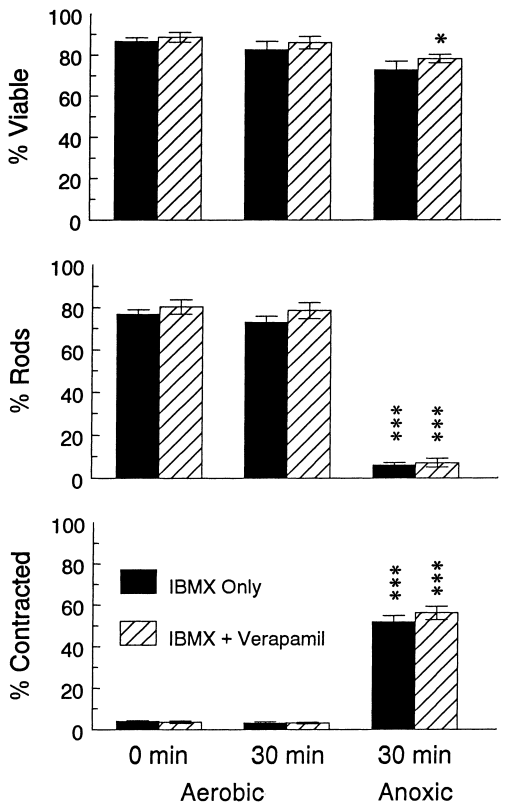

Fig. 6. Effect of verapamil on the IBMX-induced sensitivity of cardiac myocytes to anoxia. Myocytes were incubated with 1 mM IBMX or 1 mM IBMX/1 μM verapamil for 1 hr prior to the onset of anoxia, and continuously throughout the anoxic period. The cells were sampled, extracted, and counted as described in Section 2. Values are expressed as a percentage of total cells counted, and represent the means ± SEM of at least 5 preparations (each mean is the result of triplicate determinations). Asterisks indicate a significant change from aerobic controls at the same time point [$P \leq 0.05$ (∗) or 0.001 (∗∗∗)].

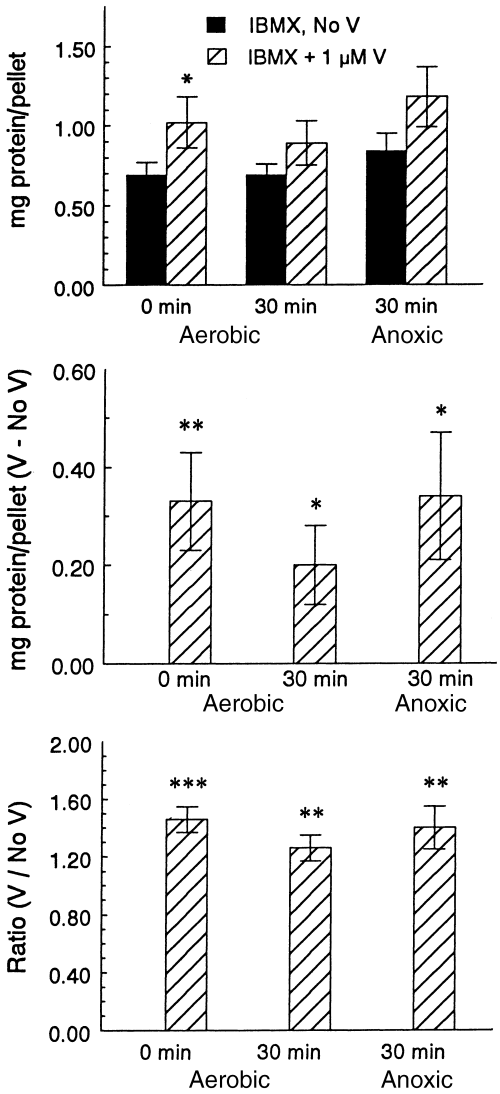

Fig. 7. Effect of verapamil on the yield of cardiac myocytes. Myocytes were incubated as described in the legend of Fig. 6. Top panel: values are expressed as mg protein/cell pellet obtained from 300 μL of incubation buffer. Middle panel: values are expressed as the numerical difference between cell pellets obtained from verapamil-containing (IBMX + V) and verapamil-free (IBMX, No V) incubations. The difference obtained is compared to (No V) − (No V), or zero. Bottom panel: values are expressed as the numerical ratio of cell pellets obtained from verapamil-containing (IBMX + V) and verapamil-free (IBMX, No V) incubations. The ratio obtained is compared to the ratio No V/No V, or 1. All results were compared within-time and within-group, so that verapamil was the only variable. Values represent the means ± SEM of 7 preparations (each mean is the result of triplicate determinations). Asterisks indicate a significant change from verapamil-free controls (top panel), from zero (middle panel), or from 1 (bottom panel) at the same time point [$P \leq 0.05$ (∗), 0.01 (∗∗), or 0.001 (∗∗∗)].

[1,5,9,10,18,19]. Both the pattern and rate of adenine nucleotide degradation were similar in all myocyte populations regardless of treatment, except that ATP degradation was enhanced in the IBMX-containing cells ($P < 0.05$).

The ratio of ATP to ADP (ATP/ADP) appears to be an important factor in the formation of rigor complexes [8]. ATP and ADP were measured in each cell preparation under each condition of incubation. The ratios were calculated, and the numbers from 7 preparations were used to calculate the data in Fig. 5. ATP/ADP was roughly the same in all cell populations under aerobic conditions; under anoxic conditions, ATP/ADP was depressed significantly in the IBMX-treated cells as compared with the untreated or zaprinast-treated myocytes.

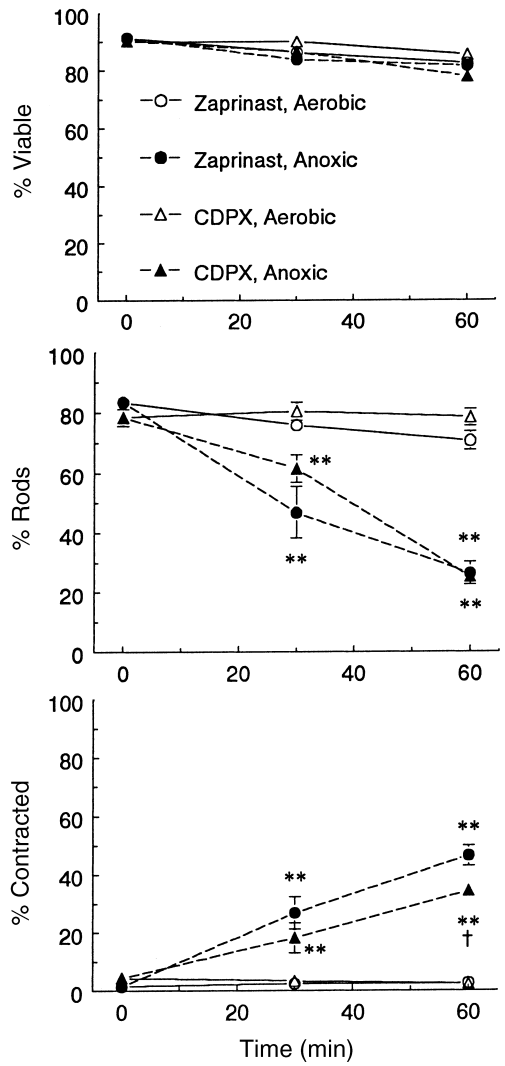

Fig. 8. Comparison of the morphology of zaprinast-incubated and CDPX-incubated myocytes under anoxic conditions. Myocytes were incubated with 150 μM zaprinast or 100 nM CDPX as described in Section 2. Values are expressed as a percentage of the total cells counted, and represent the means ± SEM of at least 4 preparations (each mean is the result of triplicate determinations). Asterisks indicate a significant change from aerobic controls at the same time point [$P \leq 0.01$ (**)]. A dagger appearing over points representing CDPX-treated cells indicates a significant difference of CDPX-treated cells from zaprinast-treated cells [$P \leq 0.05$ (†)].

Since elevation of basal cyclic AMP content could impact the activity of the L-type calcium channel (i.e. allow more calcium into the cell), we examined the effect of IBMX on myocyte morphology in the presence and absence of 1 μM verapamil. We hypothesized that verapamil would eliminate the effect of IBMX by blocking the L-type channel. Instead, verapamil had no effect on myocyte morphology in the presence of IBMX (Fig. 6), and hence had no impact on the effect of IBMX. Since verapamil was added before calcium tolerance steps were initiated, it protected the cells from calcium overload and increased cell yield (Fig. 7).

The amount of IBMX used in this study (100 μM, 1 mM) represents a pharmacologic concentration of inhibitor to the myocytes; such high levels of inhibitor could have unintended consequences. One of these consequences could be called "reverse-preconditioning"; that is, our normal anoxia protocol could release enough adenosine into the medium to partially precondition the myocytes and make them resistant to anoxia. IBMX at 1 mM could conceivably block the A_1 receptor and eliminate this effect. To test this possibility, CDPX, a genuine A_1 antagonist, was added to the medium at concentrations up to 1 μM. We postulated that CDPX would produce the same kind of sensitivity to anoxia in the myocytes as IBMX. Instead, this inhibitor had no effect on myocyte morphology (Fig. 8).

4. Discussion

Cardiac myocytes rendered anoxic after incubation with IBMX show a larger percentage of damaged myocytes (contracted and hypercontracted cells) than cells preincubated with zaprinast. In cell populations incubated with IBMX, damaged cell forms associated with anoxia (especially contracted cells) appeared in approximately half the time required for similar forms to appear in zaprinast-incubated populations. Incubation of cardiac myocytes with IBMX, therefore, sensitizes the cells to the damaging effects of anoxia.

In the absence of stimulants or inhibitors, cardiac myocytes contain from 2–5 pmol cyclic AMP/mg cell protein [22,23,27,28]. The data of Fig. 3 show that preincubation of the myocytes with IBMX elevates unstimulated levels of cyclic AMP to 60–80 pmol/mg cell protein, which is well above operational levels seen in cells stimulated with isoproterenol in the absence of phosphodiesterase inhibitors [27]. Zaprinast-treated cells, which were not sensitized to anoxia in this protocol, showed a more modest elevation in basal cyclic AMP levels. We, therefore, initially speculated that the anoxia-sensitizing effects of IBMX were somehow associated with elevated intracellular cyclic AMP.

The L-type calcium channel is the most obvious target of cyclic AMP in cardiac cells; elevated cyclic AMP acts to

open the channel, and allow intracellular calcium to rise. Initially, it seemed likely that this mechanism was responsible for the damaging effects of IBMX shown in Fig. 2. However, blocking this channel with 1 μM verapamil provided no relief from IBMX treatment (Fig. 6). With negative results, one always wonders whether higher levels of the inhibitor might have a salutary effect; However, the effect of 1 μM verapamil on reported cell yield (Fig. 7) was striking enough to allay this concern. The L-type calcium channel, therefore, does not mediate the observed effect of IBMX on cardiac myocytes. This finding concurs with at least one other previous report [4], which showed that formation of rigor complexes (''contracted'' myocytes) precedes calcium influx under anoxic conditions.

The formation of rigor complexes has been associated with the ratio of ATP to ADP in the cell. Stapleton and Allshire [8] found that higher levels of ADP (as might be encountered under anoxic conditions) promoted the formation of these shortened cells. If IBMX stimulated ATP degradation, intracellular conditions would favor the phenomenon observed in Fig. 2. Fig. 4 does indeed show enhanced ATP degradation in the IBMX-treated cells. The ratio of ATP to ADP (an important indicator in the Stapleton and Allshire study [8]) was also depressed in IBMX-treated cells after 30 min of anoxia (Fig. 5). It is somewhat surprising that the phenomenon was observable (that is, not masked by standard error), given that the present study was not designed to finely control ATP and ADP concentrations in individual myocytes. As one might infer from Fig. 2, the myocyte extracts used to determine nucleotide levels in this study represent an average of rod-shaped cells, contracted (box-like) cells, and hypercontracted cells. Even so, the results of this study substantially agree with previous studies on rigor contracture. Bowers *et al*. [7] found that rigor contracture began at about 2 mM cytosolic ATP and was essentially complete at 5–25 μM ATP (similar data are shown in [1]). Similar levels of ATP can be observed in suspended myocytes after 30–60 min of anoxia without IBMX (Fig. 4; also [18] and unpublished data [29,30]) or after 15–30 min of anoxia with IBMX (Fig. 4). Since IBMX-treated cells were damaged more significantly than control or zaprinast-treated cells during the initial anoxic period, enhanced nucleotide depletion in IBMX-treated cells likely explains the sensitization to anoxia observed in the IBMX-treated cells.

Short periods of ischemia separated by periods of reflow render the heart resistant to a subsequent prolonged ischemic episode. This behavior of heart tissue has been termed ''preconditioning'' [15–17]. Preconditioning can be induced in the heart by any one of several diffusible substances; adenosine was of particular concern in this study. Preconditioning by adenosine is mediated by the A_1 adenosine receptor; cyclic AMP is produced, and (by a mechanism which is still unclear) opens the ATP-gated potassium channel. This potassium current increases extracellular potassium, which appears to be cardioprotective.

In this study, cardiac myocytes were exposed to anoxia for up to 60 min. Under these conditions, large amounts of adenine nucleotides are degraded and released into the medium as adenosine and inosine [18]. The incubation system is closed, so that no degradation of adenosine or inosine occurs. It was possible, therefore, that the myocytes in the control and zaprinast-incubated flasks were actually preconditioned (that is, control or ''normal'' cell responses represented a protected state). In this scenario, IBMX at high concentrations would compete for the A_1 receptor with adenosine, returning the cell to a less protected state (in effect, a sort of ''reverse-preconditioning''). If this hypothesis were correct, a genuine A_1 receptor antagonist (CDPX) should have the same effect as IBMX. However, the data of Fig. 8 clearly show that CDPX cannot duplicate the effects of IBMX, even at high concentrations. Thus, preconditioning is not a significant feature of this anoxia protocol.

In summary, we concluded that IBMX sensitizes myocardial cells to the damaging effects of anoxia by a mechanism that involves ATP degradation (that is, the ratio of ATP to ADP). While cyclic AMP could somehow be involved in this sensitization to anoxia, the action of cyclic AMP on the L-type calcium channel is clearly not part of the mechanism. Finally, adenosine release and preconditioning were excluded as an explanation for the observed phenomenon.

Acknowledgments

This work was supported by grants from the National Institutes of Health (HL 39025), the American Heart Association-Missouri Affiliate, and the KCOM Warner-Fermaturo Research Fund.

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