Positive inotropic effects of the endogenous Na⁺/K⁺-transporting
ATPase inhibitor from the hypothalamus

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ABSTRACT  Bovine hypothalamus contains a nonpeptidic
substance that inhibits purified Na⁺/K⁺-transporting ATPase
[ATP phosphohydrolase (Na⁺/K⁺-transporting), EC 3.6.1.37] reversibly
with high affinity by a mechanism similar to, but not
identical to, that of the cardiac glycosides. It possesses some of
the characteristics ascribed to a putative endogenous “digitalis-
like” compound that has been implicated in the control of renal
sodium excretion and the pathogenesis of essential hypertension
in man. To determine whether this hypothalamic Na⁺/K⁺-
transporting ATPase inhibitor might have physiologic proper-
ties in cardiac tissues, its effects on Na⁺ pump inhibition,
accumulation of cytosolic free calcium, and contractile response
were studied in cultured, spontaneously contracting neonatal rat
cardiocytes. The hypothalamic factor potently inhibited the Na⁺
pump in these cells, increased myoplasmic free calcium in a
dose-dependent manner, and reversibly enhanced myocyte con-
tractility by up to 40%, comparable in degree to maximal
positive inotropic effects caused by the cardiac glycoside oua-
bain. Comparative studies further indicate that cardiotoxic
effects of ouabain in the myocytes may be more complex than
simple progressive elevation of intracellular free calcium con-
centration because at a free calcium concentration in excess of
that produced by a toxic dose of ouabain, no toxicity with the
hypothalamic Na⁺/K⁺-transporting ATPase inhibitor oc-
curred.

By regulating the electrolyte balance of cells and organs, the
plasma membrane Na⁺/K⁺-transporting ATPase [ATP phos-
phohydrolase (Na⁺/K⁺-transporting), EC 3.6.1.37; Na⁺/K⁺-
ATPase] is responsible for many fundamental cellular and
physiologic processes such as cell volume regulation, the
setting of membrane potential, regulation of cardiac contracti-
tility, and renal Na⁺ reabsorption. The enzyme also serves as
the cellular receptor for the digitalis glycosides (1). Relatively
little is known about the endogenous regulation of the enzyme.
Catecholamines (2–4), thyroid hormone (5), aldosterone (6),
and vanadium (7) have all been linked to either direct or
indirect effects on enzyme activity. However, the fact that
the only known specific regulatory ligand, digitalis, is a substance
from the plant kingdom has given rise to the notion of the
existence of a specific endogenous physiologic regulator of
mammalian origin, which might functionally resemble the
cardiac glycosides. The concept of an “endogenous digitalis”
has been made more provocative by a growing body of
experimental evidence for a circulating Na⁺/K⁺-ATPase in-
hibitor that would play a physiologic role in the normal
natriuretic response to extracellular fluid volume expansion (8)
and a pathogenic role in a prevalent human disease, hyper-
tension (9). The postulated action of this substance is the
modulation of renal tubular Na⁺ reabsorption and vascular
smooth muscle tone by regulating Na⁺/K⁺-ATPase activity
(10). Indeed, the exogenous analogue of this putative regula-
tor, digitalis, a potent inhibitor of the Na⁺/K⁺-ATPase, has
been shown to cause both natriuresis (11) and an increase in
vascular resistance (12), although these are not its major
pharmacological effects.

Much effort has been expended in many laboratories to
identify and characterize a plasma, urinary, or tissue inhibitor
of Na⁺/K⁺-ATPase; still a dearth of functional studies exist,
and the definitive structure of such an inhibitor is not known
(13). Furthermore, the degree to which various candidate
compounds arc truly “digitalis-like” in either structure or
function remains controversial because even those sub-
stances characterized in the greatest biochemical detail (14–
16) manifest some differences from the cardiac glycosides.

One cardinal criterion for biological activity functionally
analogous to the cardiac glycosides would be the demonstra-
tion of positive inotropic effects in cardiac muscle. We have
isolated and partially purified from bovine hypothalamus a
low-molecular-mass, nonpeptidic substance that satisfies a
number of essential criteria for a physiologic regulator of Na⁺
pump activity in vivo (17). It inhibits the purified Na⁺/
K⁺-ATPase reversibly, with high affinity (Kᵢ = 1.4 nM) (18);
it’s effects are specific for the Na⁺/K⁺-ATPase in the plasma
membrane (14), and it acts only from the extracellular surface
of the cell (14, 17), consistent with the concept of a circulating
inhibitor of the Na⁺/K⁺-ATPase. Thus, while certain of the
biologic effects of this hypothalamic substance are the same
as those of the cardiac glycosides, important differences in its
mechanism of inhibition of the Na⁺/K⁺-ATPase have been
discovered (14, 18). Its potential role as regulator of renal
Na⁺ excretion was strengthened by the demonstration of
binding and dissociation reactions in intact renal tubular cells
consistent with physiologic regulation in vivo (19). But phys-

eologic effects in cardiac cells remained unknown, although
preliminary experiments documented inhibition of purified

cardiac Na⁺/K⁺-ATPase (20). To further explore the possi-

bility that this hypothalamic inhibitory factor (HIF) could
have physiologic actions in cardiac tissues, its effects on Na⁺
pump activity, cytosolic free Ca²⁺ ([Ca²⁺]c) accumulation,
and contractility were studied in spontaneously contracting,
neonatal rat myocytes in culture.

We report here that HIF is a potent inhibitor of the Na⁺
pump in these cells, with effects on [Ca²⁺]c changes and
contractile response parallel to those of the cardiac glycoside
ouabain. Our results also show that at the same [Ca²⁺]c in the
myocytes, ouabain is toxic, whereas the endogenous factor is
not.

MATERIALS AND METHODS

Preparation of HIF. The Na⁺/K⁺-ATPase inhibitor was
prepared from bovine hypothalamus as described (14).

Abbreviations: Na⁺/K⁺-ATPase, Na⁺/K⁺-transporting ATPase;
[Ca²⁺]c, cytosolic free calcium concentration; HIF, factor isolated
from hypothalamus; ASM, amplitude of systolic cell motion.
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Briefly, hypothalami collected fresh and frozen immediately on dry ice were thawed, homogenized, and extracted in methanol/water (4:1, vol/vol). Methanol was removed by flash evaporation, and lipids were removed by extraction of the remaining aqueous phase with petroleum ether and chloroform. Initial separation of HIF was done by using lipophilic gel chromatography (14). Further purification was accomplished by using successive cation- and anion-exchange chromatographies (21). Purified HIF is free of vanadate (emission spectroscopy), 

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\text{Na}^+\text{K}^+\text{-ATPase (reductive amination, Sigma kit 170-A), and free fatty acids and lysophospholipids (GC/MS), which have been shown to interfere in pertinent bioassays.}
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One unit of HIF in the myocyte studies is defined as that amount of inhibitor that inhibits ouabain-sensitive K\textsuperscript{+} transport by 50\%, as determined by \(^{86}\text{Rb}^+\) uptake into human erythrocytes (14). One unit of HIF in this assay also inhibits the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase purified from canine renal medulla by 50\%, as measured spectrophotometrically in a kinetic coupled-enzyme assay (18). With the latter assay, it could be estimated that one unit per 50 \(\mu\)l equals \(15 \text{nM HIF (18).}\)

**Tissue Culture.** Myocardial cells were isolated from ventricular fragments of the hearts of 1-day-old Sprague–Dawley rats by serial trypsinizations in a Ca\textsuperscript{2+} - and Mg\textsuperscript{2+}-free Hank’s solution as described (22). For measurements of \([\text{Ca}^{2+}]_i\), the cells were plated on rectangular (13 \(\times\) 30 mm) glass coverslips and for measurements of cell contractility, cells were plated on circular (12 mm) glass coverslips; both types of coverslips were placed inside Petri dishes. For measurements of \(^{86}\text{Rb}^+\) uptake, cells were plated in Petri dishes at \(1-1.5 \times 10^6\) cells per 35-mm dish. All cultures were incubated in humidified 5\% CO\textsubscript{2}/95\% air at 37°C. Confluent monolayers in which an estimated 80\% of the cells exhibited spontaneous synchronous contractions developed by 3 days, at which time experiments were done. \(^{86}\text{Rb}^+\) Influx Measurements. Na\textsuperscript{+} pump activity was estimated in the cultured cardiac cells as the difference in \(^{86}\text{Rb}^+\) uptake seen with and without 5 mM ouabain, following the method of Panet et al. (23). Incubations were continued for up to 10 min (period of linear uptake of the isotope) (24).

**Measurement of \([\text{Ca}^{2+}]_i\).** Changes in \([\text{Ca}^{2+}]_i\) were detected using the fluorescent probe fura-2 (25). Rectangular glass coverslips with attached myocytes were placed in buffered salt solution (BSS; 140 mM NaCl/5 mM KCl/1 mM CaCl\textsubscript{2}/1 mM MgCl\textsubscript{2}/10 mM glucose/1 mM Na\textsubscript{2}HPO\textsubscript{4}/10 mM Heps-Tris, pH 7.4) to which was added 5 \(\mu\)M fura-2/AM and incubated for 1 hr in humidified 5\% CO\textsubscript{2}/95\% air at 37°C. Additional loading medium was added, and incubation was continued for 15 min to complete the hydrolysis of fura-2/AM. The cells were washed and incubated an additional 30 min in BSS (26). Coverslips with loaded myocytes were inserted into a thermostated (37°C) cuvette containing 2 ml of BSS and various additions of HIF or ouabain as indicated. The fluorescence was continuously recorded using a Photon Technology International (South Brunswick, NJ) DeltaScan II scintillation fluorometer. Values of \([\text{Ca}^{2+}]_i\) were calculated from the ratio \(R = F_{390}/F_{340}\) by using the formula: \([\text{Ca}^{2+}]_i = K_d B (R - R_{\text{min}})/(R_{\text{max}} - R)\), where \(K_d\) is 225 nM, \(F\) is fluorescence, and \(B\) is the ratio of fluorescence of free dye to that of Ca\textsuperscript{2+}-bound dye measured at 380 nm. \(R_{\text{max}}\) and \(R_{\text{min}}\) were determined in separate experiments by using digitonin to equilibrate \([\text{Ca}^{2+}]_i\) with ambient \([\text{Ca}^{2+}]_i\) \((R_{\text{max}})\), and addition of MnCl\textsubscript{2} (0.1 mM) and EGTA (1 mM) \((R_{\text{min}})\). Background autofluorescence was measured in unloaded cells and subtracted from all measurements.

**Measurements of Myocyte Contractility.** Contractility, determined as amplitude of systolic cell motion (ASM) and beating frequency were measured in individual cells by using a phase-contrast microscope video motion-detector system according to the method of Barry et al. (27). A glass coverslip with attached cultured myocytes was placed in a chamber provided with inlet and exit ports for medium perfusion. The chamber was enclosed in a Lucite box at 37°C and placed on the stage of an inverted phase-contrast microscope. The cells were covered with 1 ml of medium containing HIF or ouabain. Motion was monitored by a low-light-level TV camera attached to the microscope and calibrated with 262 raster lines. The motion-detector monitors a selected raster line segment and provides new position data every 16 msec for an image border of a microsphere within the cell layer moving along the raster line. The analog voltage output from the motion detector is filtered at low-band pass filter and calibrated to indicate actual \(\mu\)m of motion, and the derivative is obtained electronically and recorded as velocity of motion in \(\mu\)m/sec. Rate, amplitude, and velocity of contraction remained stable for several hours during control perfusions. The changes in the contractility induced by ouabain or HIF were calculated in comparison with the contractility of the same cells before addition of ouabain or HIF. Position of maximal relaxation and shifts of this baseline (AMR) which indicate toxicity can be reliably measured by the video method used (26, 28).

**RESULTS**

**HIF Effects on Myocyte Na\textsuperscript{+} Pump Activity.** Fig. 1 shows the effects of HIF and ouabain on total \(^{86}\text{Rb}^+\) uptake into the cultured cardiac cells. In this experiment, to ensure saturation binding, myocytes were preincubated for 20 min with HIF (2 units/ml) or ouabain (5 mM), or both before adding \(^{86}\text{Rb}^+\) to run the 10-min flux. Ouabain decreased uptake from control levels of 810 nmol/mg of protein to 210 nmol/mg (74\%), whereas HIF at 2 units/ml decreased the uptake from control levels to 377 nmol/mg (54\%). The combination of ouabain plus HIF was not additive, indicating that HIF inhibitory effects are specific for K\textsuperscript{+} transport through the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase. In this experiment, HIF at 2 units/ml thus inhibited ouabain-sensitive K\textsuperscript{+} transport by 74\%. In subsequent experiments, Na\textsuperscript{+} pump activity is defined as the difference in \(^{86}\text{Rb}^+\) uptake seen with and without 5 mM ouabain.

Fig. 2 shows the effects of increasing doses of HIF on Na\textsuperscript{+} pump activity in the myocytes. The largest dose of HIF tested (4 units/ml) inhibited ouabain-sensitive K\textsuperscript{+} uptake by 90\%, whereas the ID\textsubscript{50} for pump inhibition occurred at an HIF concentration of 0.55 unit/ml. Inhibition of transport by HIF progressed as a function of exposure time of cells to inhibitor.

![Fig. 1. Inhibition of uptake of Rb\textsuperscript{+} by neonatal rat cardiac myocytes exposed to HIF (2 units/ml), ouabain (5 mM), or both. HIF inhibited 54\% of total Rb\textsuperscript{+} uptake and 74\% of the ouabain-sensitive uptake. Incubation of the cells with both ouabain and HIF produced no additive inhibition. Values are means \pm SEM for \(n = 6\) determinations for each condition. Concentration of HIF is in arbitrary units as described in the text.](https://example.com/fig1.png)
Hypothalamic Factor (units/ml)

**Fig. 2.** Concentration dependence of HIF inhibition of ouabain-sensitive Rb⁺ uptake in neonatal rat cardiac myocytes. Ninety percent of ouabain-sensitive K⁺ (Rb⁺) uptake was inhibited by the maximal dose of HIF tested. Values are means ± SEM for n = 4 determinations at each concentration. (**Inset**) Inhibition of active K⁺ transport as a function of exposure time of myocytes to HIF (2 units/ml), n = 4 determinations at each point.

**Table 1.** Changes in steady-state [Ca²⁺] in cardiac myocytes treated with ouabain or various concentrations of HIF

<table>
<thead>
<tr>
<th>[Ca²⁺], nM</th>
<th>Ouabain (10⁻⁶ M)</th>
<th>HIF</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.25 U/ml</td>
<td>0.5 U/ml</td>
</tr>
<tr>
<td>138 ± 3</td>
<td>287 ± 15</td>
<td>197 ± 9</td>
</tr>
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</table>

Values are mean ± SEM; n = three determinations at each concentration. U, units.

to a level significantly greater (432 ± 18 nM) than that induced by 1 µM ouabain. Thus, like ouabain, inhibition of Na⁺/K⁺-ATPase activity in these cardiac cells was accompanied by progressive elevation, in [Ca²⁺].

**HIF Effects on Myocyte Contractility.** The effects of different doses of HIF on cell contractility, measured as ASM, and beating frequency were measured using the phase-contrast video-motion-detection system and compared to the effects of ouabain. Results of a typical experiment are shown in Fig. 4. Ouabain (5 × 10⁻⁷ M) (Fig. 4B), a maximally inotropic but nontoxic dose in these cells (26), increased ASM by 41% and decreased beating frequency compared to the same cells in the control period (Fig. 4A). There was no change in the position of maximal relaxation, indicating "therapeutic range" but nontoxic effects with this dose of the cardiac glycoside. Ouabain at 1 µM (Fig. 4C) showed a decrease in ASM, an elevation in the position of maximal relaxation, and an increase in beating frequency, all indicating "toxic-range" effects of this higher dose on the contracting myocytes. HIF at 1 unit/ml (Fig. 4E) increased ASM 39% compared to control (Fig. 4D), with a decrease in beating frequency and no change in the position of maximal relaxation. After a 1-min "washout," during which the same cell was perfused with HIF-free buffer, ASM and beating frequency returned to control levels (Fig. 4F), indicating rapid reversibility of the positive inotropic effects caused by HIF. Table 2 summarizes changes in ASM and beating frequency as a function of various HIF doses. Parallel to changes in [Ca²⁺], increased HIF concentrations caused progressive increases in ASM and decreases in beating rate. Maximal increase in ASM occurred at an HIF concentration of 0.5 unit/ml (39 ± 6%), a level equal to the maximal nontoxic dose of ouabain (0.5 µM, Table 2). It was striking that at the maximal dose of HIF tested (1.0 unit/ml), elevation of [Ca²⁺] went in excess of the level induced by a toxic dose of ouabain (1 µM) occurred (Table 1, Fig. 4C), although the cells showed no manifestations of toxicity at this HIF dose (Table 1, Fig. 4E).

**DISCUSSION**

The HIF possesses some of the characteristics ascribed to an endogenous "digitalis-like" compound that has been proposed to act as a natriuretic hormone and a pathogenetic factor in human essential hypertension and, perhaps, as an even more general regulator of Na⁺/K⁺-ATPase activity in mammalian cells (for review, see ref. 29). HIF satisfies several essential biochemical criteria for a physiologic regulator of the Na⁺ pump, including high-binding affinity, re-
versatility of effects, and specificity for the Na\(^+\)/K\(^+\)-ATPase in the plasma membrane (14, 18). Studies of the mechanism of inhibition of Na\(^+\)/K\(^+\)-ATPase by HIF showed similarities and important differences when compared with the effects of the cardiac glycoside ouabain. Like ouabain, HIF binds to the extracellular domain of the enzyme, stabilizes it in an E\(_2\)-like conformation as judged by fluorescence labeling, and inhibits p-nitrophenylphosphatase activity, a partial reaction of the Na\(^+\)/K\(^+\)-ATPase (14). Significantly different from ouabain, HIF does not support phosphorylation of the Na\(^+\)/K\(^+\)-ATPase from inorganic phosphate and magnesium (14) and has ligand requirements for optimal activity quite different from those of the cardiac glycosides (18). Based on the available biochemical data, the hypothesis has been advanced that HIF may inhibit Na\(^+\)/K\(^+\)-ATPase by influencing the phosphate-binding site on the enzyme and may alter active Na\(^+\) transport by preventing the cycling of the enzyme between the E\(_1\) and E\(_2\) conformations, thus preventing the translocation of Na\(^+\) and K\(^+\) ions across the cell membrane (30).

Thus, while differences in mechanism of action exist for HIF and ouabain, ultimate biological effects on cation transport in blood cells and ion-transporting epithelia are shared by the two substances. The extent to which HIF might be truly digitalis-like in its biological effects \textit{in vivo} remained uncertain because HIF actions in cardiac tissues, the primary target for digitalis glycosides, were unknown. The present work addresses this question by studying the effects of HIF on Na\(^+\) pump activity, [Ca\(^{2+}\)]\(_{\text{cyt}}\), accumulation, and contractile responses in spontaneously beating neonatal rat myocytes in culture.

Na\(^+\) pump activity was measured in the myocytes using \(^{86}\)Rb\(^+\) as a tracer for active K\(^+\) transport into the cells. HIF significantly decreased ouabain-inhibitable K\(^+\) transport into the cells, and the combination of HIF and ouabain produced no additive inhibition of K\(^+\) transport, indicating HIF inhibitory effects to be specific for K\(^+\) transport through the Na\(^+\)/K\(^+\)-ATPase (Fig. 1). The specificity of HIF acting only via pump-mediated cation transport in the myocytes parallels previous findings in human erythrocytes (14) and cultured renal tubular cells (19).

HIF-mediated Na\(^+\) pump inhibition was dose-dependent and related to exposure time of myocytes to inhibitor (Fig. 2). Ninety percent of Na\(^+\) pump activity in the myocytes was inhibited by the maximal dose of HIF used in these studies. The neonatal rat myocytes appear to have a relatively high affinity for HIF. Thus, the ID\(_{50}\) for these cells was \(\approx 30\)-fold less than that for cultured porcine renal tubular cells (Fig. 2; ref. 19).

The shape of the dose–response curve for pump inhibition in the myocytes suggests a single population of HIF-binding sites, closely resembling the hyperbolic curve characterizing ouabain binding and \(^{86}\)Rb\(^+\) transport inhibition in cultured chicken embryo myocytes (31). This is of interest, as it suggests another significant difference between HIF and ouabain because the ouabain-binding curve in neonatal rat myocytes is much flatter and bimodal, reflecting two binding sites of different affinities (31).

Na\(^+\) pump inhibition in the myocytes was accompanied by increases in [Ca\(^{2+}\)]\(_{\text{cyt}}\), [Ca\(^{2+}\)]\(_{\text{cyt}}\) increased from a baseline of 138 mM to 250 mM and 343 mM after 30- and 60-min exposure to HIF, respectively (Fig. 3). As with Na\(^+\) pump inhibition, HIF-induced increase in steady-state [Ca\(^{2+}\)]\(_{\text{cyt}}\) concentrations was dose-related (Table 1). The sensitivity of the myocytes to relatively low concentrations of HIF is again illustrated with HIF at 0.5 unit/ml, where [Ca\(^{2+}\)]\(_{\text{cyt}}\) was elevated to a level comparable to that produced by 1 \(\mu\)M ouabain (Table 1).

Inhibition of Na\(^+\) pump activity and increases in [Ca\(^{2+}\)]\(_{\text{cyt}}\) in the myocytes were accompanied by enhanced contractility. By use of cell monolayers identical to those used for measuring [Ca\(^{2+}\)]\(_{\text{cyt}}\), the contraction amplitude was measured in single beating myocytes as the ASM by using a phase-contrast video motion-detection system. Effects of HIF were compared with those of ouabain. HIF at 1 unit/ml caused a 37 \(\pm\) 3% increase in ASM, an augmentation in contractility equal to that of the maximal nontoxic dose of ouabain for these cells (5 \(\times\) 10\(^{-7}\) M, producing 41 \(\pm\) 3% increase in ASM, Fig. 4). At these doses of HIF and ouabain no manifestations of toxicity occurred in the cells. Cells treated with 1 \(\mu\)M ouabain, however, showed marked toxic effects, indicated by decrease in ASM, increase in the position of maximal relaxation, and increase in beating frequency (Fig. 4C). Increase in ASM caused by HIF showed a dose-dependent relationship, with maximal effects occurring at an HIF concentration of 0.5 unit/ml (Table 2). Positive inotropic effects of HIF were accompanied by a dose-related decrease in beating frequency. The mechanism for the decrease in rate caused by HIF is unknown. Our findings with HIF would support those of Shimoni and coworkers (32), who found partially purified extracts of sheep brain to enhance contractile tension in isolated sheep ventricular trabeculae. Effects on Na\(^+\) pump activity and Ca\(^{2+}\) metabolism in the tissue were not reported.

The biochemical events underlying cardiac glycoside toxicity and the narrow therapeutic index characteristic of these drugs is incompletely understood, although excessive [Ca\(^{2+}\)]\(_{\text{cyt}}\) secondary to persistently elevated intracellular Na\(^+\) associated with tonic pump inhibition has been postulated as having a central role (33). The work reported here does not address the question of the mechanism by which HIF elevates [Ca\(^{2+}\)]\(_{\text{cyt}}\).
in the myocytes. But the results of our studies suggest that the toxicity induced by ouabain in the neonatal rat myocytes is not simply due to progressive elevation of total [Ca\(^{2+}\)], at least in the pool measured by fura-2 fluorescence. Ouabain at 1 \(\mu M\) in these cells raised [Ca\(^{2+}\)] to 287 \(\pm\) 15 nM (Table 1) and was clearly toxic (Fig. 4C), confirming toxic effects at these levels of [Ca\(^{2+}\)]. caused by 1 \(\mu M\) ouabain found in previous studies (26). HIF at 1 unit/ml raised [Ca\(^{2+}\)] in the same cells to 432 \(\pm\) 18 nM but was accompanied by a stable maximal inotropic effect with no sign of toxicity (Fig. 4E). These findings suggest that the toxicity caused by ouabain in the myocytes must involve drug interactions with the cell that are more complex than simply inhibition of the Na\(^+\) pump or that abnormalities in Ca\(^{2+}\) metabolism within the cell are occurring in compartments not selectively discernible by the fura-2 method. In any case, HIF, at a dose producing similar pump inhibition (1 \(\mu M\) ouabain inhibits the Na\(^+\) pump by 30% during a 20-min incubation in these cells (24)) and greater elevations in [Ca\(^{2+}\)], than the toxic dose of 1 \(\mu M\) ouabain, did not produce this toxicity.

The positive inotropic effect induced by HIF was readily reversible. A 1-min perfusion with HIF-free buffer sufficed to completely reverse HIF-induced steady-state augmentation of contractility, returning the cell to baseline ASM and beating frequency (Fig. 4E). HIF effects appear to be more readily reversible than those of ouabain because a 5-min washout period in ouabain-treated neonatal rat myocytes is required to return enhanced contractility to control levels (34). The ready reversibility of HIF effects on the contractile response in the cardiac myocytes recalls the rapid reversibility in HIF-induced Na\(^{+}/K^{+}\)-ATPase inhibition that occurs in sized (intact cell) preparations (19) but not isolated enzyme (18), and argues for the possibility that, in contrast to the cardiac glycosides, HIF could be a physiologic regulator of Na\(^{+}/K^{+}\)-ATPase activity in vivo.

In summary, HIF potently inhibits the Na\(^+\) pump in cultured neonatal rat myocytes. The Na\(^+\) pump inhibition induced by HIF in these cells is dose-dependent and associated with augmentation of [Ca\(^{2+}\)] content and an increase in contractile force equivalent to that caused by maximal nontoxic doses of the cardiac glycoside ouabain. Comparative studies with HIF and ouabain of pump inhibition and calcium content parameters in treated myocytes suggest that glycoside-mediated cardiac toxicity is more complex than simple elevation of fura-2-measurable increases in [Ca\(^{2+}\)]. Further studies of the mechanism by which HIF increases myocyte contractility should provide new insight into the biochemical events associated with positive inotropy and the role of specific endogenous Na\(^{+}/K^{+}\)-ATPase inhibition in the physiologic regulation of cardiac function.

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