Glucagon-Like Peptide-1 Increases cAMP but Fails to Augment Contraction in Adult Rat Cardiac Myocytes

Martín G. Vila Petroff, Josephine M. Egan, Xiaolin Wang, Steven J. Sollott

Abstract—The gut hormone, glucagon-like peptide-1 (GLP-1), which is secreted in nanomolar amounts in response to nutrients in the intestinal lumen, exerts cAMP/protein kinase A–mediated insulinotropic actions in target endocrine tissues, but its actions in heart cells are unknown. GLP-1 (10 nmol/L) increased intracellular cAMP (from 5.7±0.5 to 13.1±0.12 pmol/mg protein) in rat cardiac myocytes. The effects of cAMP-doubling concentrations of both GLP-1 and isoproterenol (ISO, 10 nmol/L) on contraction amplitude, intracellular Ca$^{2+}$ transient (CaT), and pH$i$ in indo-1 and seminaphthorodanfluor (SNARF)–1 loaded myocytes were compared. Whereas ISO caused a characteristic increase (above baseline) in contraction amplitude (160±34%) and CaT (70±5%), GLP-1 induced a significant decrease in contraction amplitude (−27±5%) with no change in the CaT after 20 minutes. Neither pertussis toxin treatment nor exposure to the cGMP-stimulated phosphodiesterase (PDE2) inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine or the nonselective PDE inhibitor 3-isobutyl-1-methylxanthine nor the phosphatase inhibitors okadaic acid or calyculin A unmasked an ISO-mimicking response of GLP-1. In SNARF-1–loaded myocytes, however, both ISO and GLP-1 caused an intracellular acidosis (ΔpH$i$ = −0.09±0.02 and −0.08±0.03, respectively). The specific GLP-1 antagonist exendin 9-39 and the cAMP inhibitory analog Rp-8CPT-cAMPS inhibited both the GLP-1–induced intracellular acidosis and the negative contractile effect. We conclude that in contrast to β-adrenergic signaling, GLP-1 increases cAMP but fails to augment contraction, suggesting the existence of functionally distinct adenylyl cyclase/cAMP/protein kinase A compartments, possibly determined by unique receptor signaling microdomains that are not controlled by pertussis toxin–sensitive G proteins or by enhanced local PDE or phosphatase activation. Furthermore, GLP-1 elicits a cAMP-dependent modest negative inotropic effect produced by a decrease in myofilament-Ca$^{2+}$ responsiveness probably resulting from intracellular acidification. (Circ Res. 2001;89:445-452.)

Key Words: cardiac myocytes  ■  glucagon-like peptide-1  ■  cAMP  ■  calcium

Glucagon-like peptide-1 (GLP-1) is an intestinally derived hormone processed from proglucagon by L cells of the gut in response to luminal nutrients and secreted into the circulation, where it reaches target tissues. GLP-1 exerts insulinotropic actions, which are preserved in patients with type 2 diabetes mellitus. Furthermore, GLP-1 inhibits glucagon secretion and can effectively lower blood glucose levels. It has recently been demonstrated that GLP-1 administered intravenously can completely normalize blood glucose1 and is being tested as a potential treatment for diabetes. Thus, understanding the biological effects of GLP-1 in extrapancreatic tissues becomes essential. Although receptors for GLP-1 have been found in a variety of tissues including the heart,2 currently, evidence exists mainly supporting the role of GLP-1 as a modulator of pancreatic hormone release (eg, insulin, glucagon, and somatostatin). The most prominent property of GLP-1 is its potentiation of glucose-induced insulin release from pancreatic β cells, attributed to the binding of GLP-1 to specific G protein–coupled receptors and activation of adenylyl cyclase (AC) resulting in an increase in cAMP, activation of protein kinase A (PKA), and an increase in intracellular calcium.3–5

In the heart, the effects of GLP-1 on cAMP production and contractility are essentially unknown. However, other interventions that increase cAMP and activate AC and PKA, such as β-adrenergic stimulation, increase cardiac contractility and enhance relaxation through a mechanism involving PKA-dependent phosphorylation of several proteins that promote Ca$^{2+}$ entry, sarcoplasmic reticulum Ca$^{2+}$ uptake, and reduced myofilament Ca$^{2+}$ responsiveness.6–8 The aim of the present study was to examine whether GLP-1 increases CAMP production in intact cardiac myocytes and to characterize its functional implications.

Materials and Methods

Cardiac Myocyte Isolation

Single cardiac myocytes were isolated from hearts of adult male Sprague Dawley rats by the standard enzymatic digestion.9 All
experiments were performed in accordance with the NIH guidelines for animal care and use of laboratory animals.

[Ca^{2+}], pH, and Contraction Measurements

Standard epifluorimetric techniques were used to record [Ca^{2+}], and pH in single myocytes loaded with either the Ca^{2+} sensor indo-1 or the pH sensor seminaphthorhodafluor-1. Cell length was monitored simultaneously using the bright-field image of the cell projected onto a photodiode array.\textsuperscript{10,11}

Determination of cAMP

Cellular cAMP was assayed using a standard RIA technique using a cAMP [\textsuperscript{3}H] assay kit (Amersham).

Statistics

All data are mean±SEM. Comparisons within groups were made by an appropriate paired or unpaired Student \( t \) test, and \( P<0.05 \) was taken to indicate statistical significance.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results

Effect of GLP-1 and Isoproterenol (ISO) on cAMP Production

The effects of GLP-1 and ISO on the production of cAMP were measured in freshly isolated cardiac myocytes, demonstrating similar dose-response curves with an EC\(_{50} \approx 10\) nmol/L and achieving saturation at 0.1 \( \mu \)mol/L with cAMP production increasing \( \approx 3 \)-fold from basal levels (Figure 1A, inset). Figure 1A shows that the effects of half-maximally activating concentrations (10 nmol/L) of GLP-1 and ISO (cAMP increasing from a basal value of 5.7±0.5 to 13.12±0.12 pmol/mg protein for GLP-1, and to 12.7±0.6 pmol/mg protein for ISO) were substantially enhanced by 1 mmol/L 3-isobutyl-1-methylxanthine (IBMX), and that the GLP-1–induced cAMP increase is abolished by its competitive antagonist exendin (Ex) 9-39 (0.1 \( \mu \)mol/L) (5.21±0.04 pmol/mg protein). Forskolin (1 \( \mu \)mol/L) caused effectively maximal AC activation achieving >150 to 200 pmol cAMP/mg protein (not shown). It is noteworthy that the combination of GLP-1 and ISO (at either 1 or 10 nmol/L each) does not produce an additive effect, increasing cAMP only to levels comparable with that achieved by either GLP-1 or ISO alone. Figure 1B shows that GLP-1 and ISO display similar time courses of cAMP production.

Effect of ISO and GLP-1 on Contraction and Intracellular Ca\(^{2+}\) Transient (CaT)

We next compared the effects of ISO and GLP-1 concentrations that produced comparable increases in cAMP, on the
electrically stimulated contraction and CaT in indo-1–loaded cardiac myocytes. As expected, ISO (10 nmol/L) induced a positive contractile effect with an increase in CaT and accelerated relaxation of both CaT and contraction (Figure 2A). In contrast, GLP-1 (10 nmol/L) induced a slight decrease in contraction, rather than the increase anticipated by its effect on cAMP production. Furthermore, the GLP-1–mediated progressive decrease in contraction with time was not associated with a decrease of the CaT, suggesting that GLP-1 reduced the myofilament Ca^{2+} responsiveness (Figure 2B). Figure 2C shows that in the continued presence of GLP-1, ISO is still competent to induce a positive inotropic and lusitropic effect. Figure 2D depicts the contrasting effects of ISO and GLP-1 on myocyte contraction amplitude. After 15 minutes of exposure, ISO increased contraction amplitude by 100±25% whereas GLP-1 reduced it by 25±5%. Furthermore, the negative inotropic effect of GLP-1 was completely reversed by its competitive antagonist Ex 9-39. The effects of GLP-1 and ISO were fully reversible on washout (not shown).

Because both ISO and GLP-1 stimulation increase cAMP, we examined why they have such distinct functional behaviors. Whereas both β_{1}- and β_{2}-adrenoceptor stimulation induce similar increases in cAMP in cardiac cells, the contractile response to β_{2}-agonists is blunted with respect to β_{1}. Because inhibition of pertussis toxin (PTX)–sensitive G proteins (G_{i}) by PTX markedly enhances β_{2}-adrenoceptor–stimulated positive inotropy in rat, dog, and murine heart cells (ie, achieving levels similar to those of β_{1}-adrenoceptor stimulation), the functional dissociation has been attributed to coupling of β_{2}-adrenoceptors (but not β_{1}) to G_{i} in addition to G_{s}. In view of the apparent similarity between the β-adrenergic system (β_{1} versus β_{2}) and the one investigated here (ISO versus GLP-1), differential coupling to PTX-sensitive G proteins (G_{i}) seemed a possible mechanism to reconcile the functional dissociation we observed. Thus, cells were treated with PTX and effects of GLP-1 on contraction were studied. We found that G_{i} inhibition by PTX pretreatment did not restore any positive inotropic effect of GLP-1 (Figure 2D), in contrast to that seen during β_{2}-adrenergic stimulation. In parallel experiments, the successful inactivation of PTX-sensitive G proteins was verified by a loss in the ability of acetylcholine to reverse the positive inotropic effect of the β_{1}-adrenoceptor (AR) agonist, ISO (not shown).

The likely explanation for the failure of GLP-1 to induce a positive contractile response despite its ability to increase cAMP levels may relate to some unique compartmentalization of GLP-1 receptor signaling compared with that of the β-adrenergic system. Specifically, increased activity of highly localized phosphodiesterases (PDEs) or phosphatases controlling the “excitation-contraction apparatus” at the junctional diad involved in β-adrenoceptor–mediated positive inotropy could prevent local cAMP increases and/or the activation by PKA phosphorylation of functional targets at these specific sites, eliminating the positive inotropic response (without apparently affecting total cAMP levels). Pharmacological inhibition of PDE and phosphatase activity was utilized to test these scenarios. Cells were treated with either the nonspecific PDE inhibitor IBMX (0.1 mmol/L) or the specific cGMP-stimulated PDE inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA, 30 μmol/L) (which has been shown to be capable of selectively preventing basal activation of the L-type calcium channel) or with specific inhibitors of phosphatase types 1 and 2 (major phosphatase isoforms in cardiac muscle), calyculin A and okadaic acid.

**Figure 2.** Effect of ISO and GLP-1 on the electrically stimulated contraction and CaT in indo-1/acetoxymethyl ester–loaded cardiac myocytes. A, Chart recording and individual contractions and CaT show that ISO increases contraction amplitude and CaT. a, Control; b, 20 minutes after ISO administration (10 nmol/L); c, overlay of data normalized from panels a and b. B and C, With the same presentation scheme as in panel A, effect is shown of GLP-1 and of ISO (10 nmol/L each) in the continued presence of GLP-1, respectively, on contraction and CaT. D, Time course of average contractile effects induced by either ISO (n=6), GLP-1 (n=7), GLP-1 in the continued presence of its competitive antagonist Ex 9-39 (n=7), or GLP-1 in the presence of PTX (n=5). Negative contractile effect of GLP-1 was reversed by Ex 9-39 but not by PTX.
and the effect of GLP-1 on contraction amplitude was studied. Figure 3 shows the failure of either PDE or phosphatase inhibition to unmask GLP-1–induced positive inotropy.

**Effect of GLP-1 on pH$_i$**

The results shown in Figure 2B suggest that GLP-1 diminishes the myofilament Ca$^{2+}$ responsiveness. Because pH$_i$ changes modulate myofilament Ca$^{2+}$ responsiveness, the effect of GLP-1 (10 nmol/L) on pH$_i$ was compared with that of ISO (10 nmol/L) (Figure 4). Both GLP-1 (middle tracing) and ISO (lower tracing) induced a slowly evolving acidification of similar magnitude (0.09 ± 0.02 and 0.08 ± 0.03 pH units, respectively) and time course compared with control buffer (upper tracing). Figure 4B shows the average pH$_i$ changes observed after incubation for 20 minutes in control, ISO, GLP-1, or GLP-1 together with its competitive antagonist, Ex 9-39. ISO and GLP-1 induced acidifications of similar magnitude, and the GLP-1 effect was completely suppressed by Ex 9-39. Blocking the Na$^+$-H$^+$ exchanger (NHE) using HOE642 did not cause a significant acidosis (20.03 ± 0.02 pH units versus basal after 15-minute exposure to 1 mmol/L HOE642), nor did it affect the GLP-1–induced acidification (not shown). If the GLP-1–mediated increase in cAMP activates PKA and in turn causes the observed acidification and consequent reduced myofilament Ca$^{2+}$ responsiveness and contraction amplitude, then using a specific cAMP antagonist should inhibit both of these phenomena. Experiments were performed using the cAMP analog, Rp-8-CPT-cAMPS, which effectively inhibits PKA-dependent processes.$^{13}$ All cells were preincubated with 100 μmol/L Rp-8-CPT-cAMPS (at 37°C) for at least 1 hour before the experiment. Figure 5 shows that in the continued presence of Rp-8-CPT-cAMPS both the GLP-1–induced intracellular acidosis and the negative contractile response were markedly inhibited, suggesting that these GLP-1–induced phenomena are at least in part mediated by increased cAMP/PKA activation. Parallel experiments showed that Rp-8-CPT-cAMPS inhibited the ISO-induced positive inotropic effect and acidosis to a similar degree (not shown).

**Discussion**

The major finding of this study is that although GLP-1 augments total cAMP production to a degree similar to that induced by an equimolar dose of the β-AR agonist ISO, it nevertheless exerts a dramatically opposite effect on myocyte contraction; GLP-1 induces a mildly negative inotropic effect (mediated, at least in part, by a cAMP-dependent acidosis), whereas ISO has robust positive inotropic actions for comparable increases in total cAMP. These results suggest the existence of functionally distinct AC/cAMP/PKA compartments determined by unique receptor signaling microdomains.

Previous investigations provide evidence suggesting potential cardiovascular actions of GLP-1, affecting blood pressure and heart rate.$^{15,16}$ However, because these studies were performed using in vivo models, it is uncertain whether these effects were of systemic origin (e.g., the vasculature) or from...
Buxton and Brunton\textsuperscript{22} ascribed the functional dissociation between PGE\textsubscript{1} and ISO to the capability of ISO to produce cAMP accumulation in both soluble and particulate compartments, whereas PGE\textsubscript{1} activated only the soluble pool and had no effect on particulate cAMP or protein kinase. The similarity between GLP-1 and PGE\textsubscript{1} to accumulate cAMP\textsuperscript{18} and decrease contractility,\textsuperscript{23} in addition to the ability of both these compounds to induce positive chronotropism,\textsuperscript{16,23} suggests that GLP-1 could have a mode of action similar to that of PGE\textsubscript{1}, that is, to selectively accumulate cAMP in certain microdomains, together with its functional exclusion from other microdomains, notably the excitation-contraction coupling apparatus.

Compartmentalization of G protein–coupled signaling has been the subject of numerous recent reports (see Steinberg and Brunton\textsuperscript{24} for recent review), and it is increasingly recognized that spatiotemporal regulation of PKA activity involves regulation of discrete cAMP pools. To activate PKA half-maximally, cAMP levels must achieve $>70$ nmol/L.\textsuperscript{25} Recent elegant micropipette studies concluded that if cAMP was not produced in diffusionally restricted microdomains, cAMP would not achieve levels sufficient to regulate PKA, even near AC.\textsuperscript{26} Furthermore, without such microdomains, cAMP would need to increase globally to activate PKA, even if this signal was only required in a discrete location. All PKA would become activated under these conditions, and discrete functional control would be lost. Finally, all of this cAMP would require hydrolysis (or extrusion) to return to the basal state, which would be both costly and slow compared with that needed under local control schemes.

There are recent demonstrations of discrete signaling compartmentalization in the cardiac myocyte, for example, comparing $\beta_{1}$ versus $\beta_{2}$-AR signaling,\textsuperscript{27,28} or the underlying nature of PKA signaling compartmentalized to the cardiac ryanodine receptor (RYR) 2.\textsuperscript{29} Regarding $\beta_{1}$-AR signaling, the cAMP that is produced is diffusive through the cytosol and enhances not only local L-type Ca\textsuperscript{2+} channel activity, but also increases phosphorylation of phospholamban, troponin I, and C protein, causing accelerated muscle relaxation and reduced Ca\textsuperscript{2+}-myofilament sensitivity. In contrast, $\beta_{2}$-AR signaling (via cAMP) is localized to the plasmalemma (selectively enhancing local L-type Ca\textsuperscript{2+} channel activity) and thus completely fails to affect phospholamban, troponin I, or C protein, so relaxant effects are not observed. This difference results from differential G protein coupling of these $\beta$-ARs ($\beta_{2}$-AR to G\textsubscript{i}, only, whereas $\beta_{1}$-AR to both G\textsubscript{i} and G\textsubscript{o}),\textsuperscript{30} and from differential $\beta$-adrenoceptor subtype targeting between intracellular and surface membrane compartments as well as between caveolar and noncaveolar membrane compartments.\textsuperscript{28}

We have investigated several mechanisms potentially responsible for different patterns of cAMP compartmentalization that could contribute to the different effects of GLP-1 and ISO. This could be the result of differences in the local control of cAMP microdomains via increased activity of highly localized PDEs and/or phosphatases that may fully consume cAMP or promote dephosphorylation, and prevent PKA activation, respectively, in the immediate vicinity of specific functional targets, such as the L-type calcium chan-
nel and the sarcoplasmic reticulum. PDE2 appears functionally localized to the L-type Ca\textsuperscript{2+} channel and prevents the cAMP/PKA-dependent activation of the basal calcium current in human atrial myocytes.\textsuperscript{34} However, inhibition of PDEs using either the PDE-nonspecific\textsuperscript{31} or PDE2-specific\textsuperscript{32} PDE inhibitors, IBMX or EHNA, respectively, did not unmask an "ISO-like" positive inotropic response to GLP-1. It is also possible that GLP-1 activates a parallel inhibitory pathway, which hinders the translocation of PKA, increases the availability of the heat-stable inhibitor of protein kinase, or promotes dephosphorylation (via increased phosphatase activity) of PKA-target proteins. If any of these mechanisms were true, then GLP-1 might be expected to prevent the contractile effects of ISO. However, GLP-1 does not inhibit any of the contractile effects of ISO (Figure 2C). Notably, a similar finding was obtained by Buxton and Brunton\textsuperscript{22} when comparing PGE\textsubscript{1} and ISO.

Muscarnic M\textsubscript{2} receptors, as well as adenosine A\textsubscript{1} receptors, counteract the effect of PKA, in part via activation of phosphatases.\textsuperscript{33} Protein phosphatases, type 1 (PP1) and type 2 (PP2), regulate PKA-dependent L-type channel activity and are localized in close proximity to the channel.\textsuperscript{34} A similar mechanism limiting the β-adrenergic contractile response has recently been suggested.\textsuperscript{30} Subcellular targeting of PKA and PP2B regulates GLP-1-mediated insulin secretion in pancreatic β cells.\textsuperscript{35} Thus, effectors of the excitation-contraction apparatus might be guarded against GLP-1–induced PKA phosphorylation and activation by increased phosphatase activity. However, inhibition of phosphatases using either of the PP1- and PP2-specific inhibitors, calcycin A or okadaic acid, did not unmask a positive inotropic effect of GLP-1 (Figure 3).

Because the GLP-1 receptor, like the β-AR, belongs to the family of G protein–coupled receptors,\textsuperscript{2} we examined whether differences between GLP-1 and ISO could relate to differences in G protein coupling, comparable with that between β\textsubscript{1}- and β\textsubscript{2}-adrenergic receptors. Although native expression of the GLP-1 receptor (in pancreatic β cells) is primarily coupled to the AC pathway via activation of G\textsubscript{\alpha}\textsubscript{s}, receptor overexpression studies in Chinese hamster ovary cells found coupling to G\textsubscript{\alpha}\textsubscript{i} and, to a certain extent, G\textsubscript{\alpha}\textsubscript{o}.\textsuperscript{36} However, the inhibition of G\textsubscript{i} via PTX treatment did not unmask an underlying positive inotropic response of GLP-1 (Figure 2D).

Other scenarios, which we did not probe but which deserve future attention, involve the targeting of GLP-1–activated PKA via A-kinase-anchoring proteins (AKAPs) to sites excluded from the excitation-contraction compartment. AKAPs have been localized to a host of different subcellular compartments, including mitochondria, nuclear matrix, endoplasmic reticulum, and plasma membrane (where it modulates L-type Ca\textsuperscript{2+} channel activity) (reviewed in Schillace and Scott\textsuperscript{37}). Subcellular targeting of PKA via association with AKAPs facilitates GLP-1–mediated insulin secretion from pancreatic islets.\textsuperscript{38} AKAP-mediated signaling compartmentalization has also been recognized in cardiac myocytes. Control of RyR2 by cAMP/PKA signaling is locally restricted to the junctional diad by a macromolecular complex comprising RyR2, FKBP12.6, PKA, PP1 and PP2A, and murine AKAP.\textsuperscript{29} Furthermore, disruption of AKAP-mediated PKA anchoring was found to alter the β-adrenergocor–stimulated contractile response in cardiac myocytes.\textsuperscript{39}

Another important insight from this study is that apparently infinitesimal changes of total cAMP, likely localized to specialized signaling microdomains, seem to be sufficient to elicit the entire functional range of β-adrenergic activation. This can be deduced from the fact that GLP-1, ISO, and GLP-1+ISO produced essentially equivalent increases in whole-cell cAMP, across which the entire contractile range of β-adrenergocor–stimulation could be observed. The absence of any measurable cAMP increase with ISO+GLP-1, versus either agent alone is not a trivial result of having achieved maximal AC activation (Figure 1). Receptor-coupled AC activity was not maximal in the presence of either 10 nmol/L GLP-1 or ISO. Because cAMP production was not additive even at 1 nmol/L ISO+GLP-1 (Figure 1A, inset), we speculate that GLP-1 and β-adrenergocor–receptors couple to the same AC population outside of the junctional diad. However, we assume that the “restored” Ca\textsuperscript{2+} response on ISO addition to GLP-1 (Figure 2) is mediated by cAMP microdomains at the junctional diad 15-nm cleft modulated selectively by β-AR agents, but not by GLP-1. Because this microdomain is extremely small versus the whole-cell domain, cAMP changes in only this compartment would be virtually unsolvable by conventional whole-cell measurements. Because contractile-modulatory effects of GLP-1 could not be unmasked by PDE or phosphatase inhibition, we speculate that the GLP-1 receptors are absent from the junctional diad. These issues remain for future investigations.

In rat cardiac myocytes, GLP-1 increased cAMP and induced a slowly evolving negative inotropic effect. This effect developed without changes in the Ca\textsubscript{T} suggesting a reduction in myofilament Ca\textsuperscript{2+} responsiveness, which could relate to several different mechanisms including altered H\textsubscript{i}, whereby acidosis decreases and alkalosis increases myofilament Ca\textsuperscript{2+} responsiveness.\textsuperscript{40} That the GLP-1–induced negative inotropic effect is associated with an acidification that develops over a parallel time course, and, more importantly, that the inhibitory cAMP-analog, Rp-8-CPT-cAMPS, attenuates both the GLP-1–induced negative inotropic effect and the intracellular acidification to similar proportions, indicates that the GLP-1–induced negative inotropic effect is determined, at least in part, by a cAMP/PKA-dependent acidosis that mediates the reduction in myofilament Ca\textsuperscript{2+} responsiveness. Furthermore, we have shown that a comparable degree of acidosis (produced during the washout of NH\textsubscript{4}Cl) causes a negative contractile effect similar to the present results.\textsuperscript{41} However, our results do not rule out the possibility that the GLP-1–induced reduction in myofilament responsiveness could still be mediated via other mechanisms in addition to pH\textsubscript{i}, such as by PKA-induced changes in myofilament regulation, for example, by troponin I phosphorylation.

Another interesting finding of this study is that β-adrenergocor–stimulation with ISO induces an acidification with a similar time course and to the same degree as GLP-1. In contrast to GLP-1, however, the coincident small negative inotropic effect resulting from β-induced acidification is more than offset by its overwhelming positive inotropic effects. In the case of both GLP-1 and ISO, cAMP-dependent...
aciddification could result from changes in NHE activity or mitochondrial metabolism, or via enhancement in glycolysis. We are aware of only one other report showing a β$_1$-adrenoceptor–mediated intracellular acidosis. Shida et al demonstrated that β$_1$-stimulated acidification could be blocked by either of the two glycolysis inhibitors, 2-deoxyglucose or iodoacetate, but not by the NHE or anion exchanger blockers, amiloride and DIDS, respectively, concluding that β$_1$-adrenoceptor stimulation causes acidification via enhancement of glycolysis.

In cardiac myocytes pH$_i$ is regulated by four primary membrane transporters, as follows: NHE and Na$^+$HCO$_3^-$ cotransport (NBC) which mediate acid efflux, whereas Cl$^-$HCO$_3^-$ exchange (AE) and Cl$^-$OH$^-$/exchange (CHE) mediate acid influx. Because the present experiments are performed in HEPES buffer, HCO$_3^-$ levels and, hence, NBC and AE activities, are negligible. Not surprisingly, acid influx due to CHE is very low at and below normal resting pH i, levels and is negligible at pH 6.95, so it is unlikely to be responsible for acidosis in the present experiments. Even if CHE were somehow activated during β-AR or GLP-1 receptor stimulation by a previously unknown cAMP/PKA-dependent mechanism, still NHE would be more than capable to substantially compensate and blunt the acidosis. NHE1 is ubiquitously distributed and is the primary NHE subtype found in the mammalian cardiac cell, accounting for ~60% of H$^+$ removal capability. Of the five presently known NHE isoforms found in the plasma membrane of mammalian cells, cardiac cells lack all but NHE1. Intracellular acidosis is the major stimulus for NHE1 activation, although additional regulation occurs largely via phosphorylation reactions in response to hormones, autocrine/paracrine factors, and mechanical stimuli. PKA and protein kinase C stimulate transport by NHE1. Given the activation of NHE1 by PKA, it is even more unlikely that some unforeseen activation of CHE would be able to explain the acidosis in the present experiments. Furthermore, the NHE blocker, HOE642, by itself did not cause any significant acidosis, nor did it block the development of GLP-1-mediated acidosis. Thus, the GLP-1/PKA-mediated acidosis is unlikely to be caused by regulation of the sarcosomal H$^+$ transporters, but rather is more likely due to an underlying change in metabolism (such as by an increased metabolic acid production due to enhanced glycolysis and/or alteration in mitochondrial metabolism).

In summary, the failure of the GLP-1–mediated increase in cAMP to induce a typical β-adrenergic pattern of contractile augmentation suggests the existence of a novel pathway causing both functional localization of cAMP/PKA signaling possibly to the myofibrillar, mitochondrial, and/or nuclear compartments together with its functional exclusion from Ca$^{2+}$–induced Ca$^{2+}$–release apparatus. This novel receptor/AC/cAMP/PKA compartmentalization is not the result of PTX-sensitive G proteins, nor of enhanced local PDE-activation, nor of localized phosphatase activation. Cardiac GLP-1 receptor stimulation also induces a cAMP/PKA-dependent acidification together with a parallel decrease in myofilament responsiveness to Ca$^{2+}$ resulting in a modest negative inotropic effect. Given the roles played by cAMP signaling beyond that involved in contractility, these findings could have significant implications for GLP-1 modulation of cardiac metabolism, growth, and potentially for the regulation of survival and apoptosis, suggesting the need for further investigations.


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