

Ivabradine reduces heart rate while preserving metabolic fluxes and energy status of healthy normoxic working hearts

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Abstract

Heart rate reduction (HRR) is an important target in the management of patients with chronic stable angina. Most available drugs for HRR, such as β -blockers, have adverse effects, including on cardiac energy substrate metabolism, a well-recognized determinant of cardiac homeostasis. This study aimed at 1) testing whether HRR by ivabradine (IVA) alters substrate metabolism in the healthy normoxic working heart and 2) comparing the effect of IVA with that of the β -blocker metoprolol (METO). This was assessed using our well-established model of ex vivo mouse heart perfusion in the working mode, which enables concomitant evaluation of myocardial contractility and metabolic fluxes using ¹³C-labeled substrates. Hearts were perfused in the absence (controls; $n = 10$) or presence of IVA ($n = 10$, 3 μ M) with or without atrial pacing to abolish HRR in the IVA group. IVA significantly reduced HR ($35 \pm 5\%$) and increased stroke volume ($39 \pm 9\%$) while maintaining similar cardiac output, contractility, power, and efficiency. Effects of IVA on HR and stroke volume were reversed by atrial pacing. At the metabolic level, IVA did not impact on substrate selection to citrate formation, rates of glycolysis, or tissue levels of high-energy phosphates. In contrast, METO, at concentrations up to 40 μ M, decreased markedly cardiac function (flow: $25 \pm 6\%$; stroke volume: $30 \pm 10\%$; contractility: $31 \pm 9\%$) as well as glycolysis (2.9-fold) but marginally affected HR. Collectively, these results demonstrate that IVA selectively reduces HR while preserving energy substrate metabolism of normoxic healthy working mouse hearts perfused ex vivo, a model that mimics to some extent the denervated transplanted heart. Our results provide the impetus for testing selective HRR by IVA on cardiac substrate metabolism in pathological models.

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DISCLOSURES

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Keywords

substrate metabolism; isolated working heart; ivabradine; β -blockers

Heart rate reduction (HRR) is an important target in the management of patients with ischemic heart disease, a major cause of morbidity and mortality in developed countries (for reviews, see Refs. 6 and 45). Given their well-described beneficial effects on cardiovascular outcomes after myocardial infarction and in heart failure, β -blockers remain the first line treatment for many patients with ischemic heart disease (5, 12, 28). However, even new classes of β -blockers exhibit undesirable side effects on cardiac energy substrate metabolism (4, 28), which is now a well-recognized determinant of energy production, redox status, contractile function, ion fluxes, and oxygen consumption as well as hypertrophy development and progression to heart failure (33, 41).

Specifically, acute administration of β -blockers to the isolated perfused heart, which is devoid of neuronal influences, has been shown to affect substrate fluxes through major energy-yielding pathways. The effects include 1) decreased cytosolic glycolytic flux, a process that has been linked to optimal ion pump function, and 2) a shift in mitochondrial substrate selection from long-chain fatty acids (LCFAs) to carbohydrates (CHOs) for oxidation in the Krebs cycle, most likely due to reduction of lipoprotein lipase and/or carnitine palmitoyltransferase I activity (3, 28, 37). Such a shift from LCFA to CHO oxidation may be considered beneficial in the short term, particularly for the ischemic heart due to the greater yield of ATP per unit of O₂ consumed from CHO oxidation (i.e., 11–12%); however, potential long-term consequences include lipotoxicity and energy deprivation (33, 41). In addition, long-term β -blocker therapy may also have unwanted systemic metabolic effects, such as worsening of whole body glycemic control (18). These deleterious metabolic effects are of particular concern given that HRR is used for the management of patients with both ischemic heart disease and diabetes (7, 39). In this regard, ivabradine (IVA), which is the focus of the present study, may represent a good alternative for the management of these patients.

Indeed, IVA belongs to a new class of drugs that selectively inhibits the pacemaker current in the sinoatrial node (15), thereby leading to specific HRR (46), without hemodynamic effects on blood pressure, vascular resistance, and cardiac output. Acute IVA administration confers significant benefits in reducing infarct size after ischemia-reperfusion as well as preserving the metabolic energy and redox status in the ex vivo perfused heart subjected to ischemia-reperfusion. In addition, its long-term administration in vivo in pig or rat models of myocardial infarction prevented postmyocardial infarction adverse structural remodeling while preserving contractile function and high-energy phosphate status (22, 30, 32). In an in vivo dog model of exercise, Colin et al. (10) demonstrated a linear relationship between myocardial oxygen consumption (M \dot{V} O₂) and the extent of HRR by acute intravenous IVA. Under pacing, all effects were abolished (8, 9, 11), thereby demonstrating that they are due to IVA's selective modulation of HR, although this concept has recently been challenged (20, 21).

To the best of our knowledge, the effect of IVA-induced HRR on cardiac substrate selection for energy production has not yet been directly assessed. In fact, although both heart rate (HR) and contractility are considered to contribute to $\dot{M}V\text{O}_2$, little is known about the impact of HRR per se, independent of contractility, on cardiac energy substrate metabolism (17, 26, 29, 43). Admittedly, this is difficult to assess in vivo due to the existence of complex regulatory mechanisms extrinsic to the heart. Hence, in this study, we have used our well-established model of ex vivo heart perfusion in the working mode, with concomitant evaluation of myocardial contractility and metabolic flux parameters using ^{13}C -labeled CHO (glucose) and a LCFA (oleate) (25) to test the impact of HRR using IVA on cardiac energy substrate metabolism, in the absence of neuronal influences and at fixed values of arterial compliance (i.e., peripheral resistance), preload, and afterload pressures. As a secondary objective, we compared the effect of IVA with that of the β -blocker metoprolol (METO).

MATERIALS AND METHODS

Chemicals

The sources of chemicals, biological products, and ^{13}C -labeled substrates as well as the procedure for the dialysis of albumin have been described previously (48).

Heart Perfusions in Semirecirculating Working Mode

Animal experiments were approved by the local animal care committee in compliance with the guidelines of the Canadian Council on Animal Care. Male C57Bl/6 mice (3 mo old; Charles River Laboratories) were provided with food and water ad libitum. The procedures for heart isolation and its ex vivo perfusion in the working mode as well as measurements of the various functional parameters have been described previously (25).

Perfusion protocols: effect of IVA—The concentrations of IVA have been selected on the basis of previous studies (8, 34) and orientation experiments in which we evaluated the concentration-dependent HRR effects of IVA in our working mouse heart model ($n = 10$ perfused hearts/dose). Working mouse hearts were perfused for 40 min with a semirecirculating modified Krebs-Henseleit buffer containing physiological concentrations of substrates and hormones (11 mM glucose, 0.8 nM insulin, 50 μM carnitine, 5 nM epinephrine, 1.5 mM lactate, 0.2 mM pyruvate, and 0.4 mM oleate bound to 3% albumin) in the absence or in the presence of 3 μM IVA, with or without atrial pacing to match the HR of controls ($n = 10$ perfused hearts/group). IVA was added after 10 min of perfusion to enable measurements of functional parameters under basal conditions, prior to IVA addition, in each heart. For any given perfusion, one of the unlabeled substrates was replaced by its corresponding labeled substrate, either $[\text{U-}^{13}\text{C}_6]\text{glucose}$ [molar percent enrichment (MPE): 50%; $n = 5$] or $[\text{U-}^{13}\text{C}_{18}]\text{oleate}$ (MPE: 35%; $n = 5$), to probe both CHO and LCFA metabolism, respectively. As described previously (25), atrial influent and coronary effluent perfusate samples were collected to assess 1) lactate dehydrogenase release as an index of membrane integrity, 2) PO_2 , PCO_2 , pH, and Ca^{2+} , and 3) lactate and pyruvate release rates. At the end of the perfusion, hearts were freeze-clamped with metal tongs cooled in liquid nitrogen, weighed, and stored at -80°C for subsequent analyses.

Effect of METO—In a separate group of ex vivo heart perfusion experiments, we attempted to match the HRR obtained with IVA using the β -blocker METO at concentrations ranging from 4 to 40 μM based on previously published data (31, 38). However, this turned out to be impossible because METO markedly reduced cardiac flows and contractility prior to HR. Consequently, because cardiac function affects substrate metabolism and the specific characterization of the effect of METO on metabolic fluxes, independent of function, was beyond the scope of this study, we conducted only selected metabolic flux measurements in working hearts perfused with 4 ($n = 10$) or 40 μM METO ($n = 3$) using [$^{13}\text{C}_6$]glucose or [U- $^{13}\text{C}_{18}$]oleate.

Metabolic Flux Measurements

Our previously published studies (25, 48) provide definitions of the ^{13}C terminology and descriptions for the measurements by gas chromatography-mass spectrometry (GC-MS; Agilent 6890N GC coupled to a 5973N MS) and equations for the calculations of 1) flux ratios relevant to substrate selection for energy production through mitochondrial citrate synthesis from the ^{13}C enrichment of the acetyl (carbons 4 and 5) and oxaloacetate (carbons 1, 2, 3, and 6) moiety of citrate and 2) efflux rates of unlabeled lactate and pyruvate reflecting cytosolic glycolysis from exogenous glucose (for perfusions with [U- $^{13}\text{C}_6$]glucose).

Myocardial Metabolites

Adenine nucleotides and creatine phosphate were determined in perchloric acid extracts of frozen heart tissue by high-performance liquid chromatography (2).

Statistical Analysis

Data are expressed as means \pm SE. Statistical significance was reached at $P < 0.05$ using a t -test for myocardial high-energy phosphates and ANOVA (1-way or 2-way ANOVA for repeated measurements), followed by the Bonferroni multiple comparison posttest.

RESULTS

Ivabradine Reduces Heart Rate While Increasing Stroke Volume of Perfused Healthy Normoxic Working Mouse Hearts

Values for physiologically relevant parameters assessed after 40 min of perfusion of normoxic healthy working mouse hearts in the absence or presence of 3 μM IVA with or without pacing are reported in Fig. 1 and Table 1. Compared with controls, hearts perfused with IVA displayed a reduction in HR of $\sim 35 \pm 5\%$ ($P < 0.001$ at 40 min; Fig. 1A) and an increase in stroke volume (39%; Fig. 1B), whereas coronary flow, developed pressure, and contractility ($+dP/dt_{\text{max}}$) did not differ significantly (Table 1 and Fig. 1C). When expressed per beat, coronary flow was significantly increased in the IVA-treated group ($P < 0.001$ vs. controls; Fig. 1D). It is noteworthy that in our study model, which is devoid of neuronal influences, and in which compliance, preload, and afterload pressures are fixed, $\dot{M}\dot{V}\text{O}_2$ expressed per beat is increased significantly in the IVA group (Table 1), concurring with the increased stroke volume (+13.2%) and increased coronary flow (+48%, expressed in ml/beat) and with effective matching of energy demand and supply.

Pacing of working hearts in the absence of IVA at a physiological rate of 407 ± 13 beats/min, which was slightly but not significantly different from controls (368 ± 19 beats/min), had a marginal impact on the physiological parameters measured ($n = 10$; data not shown). However, all the aforementioned physiological effects of IVA were reversed by the application of atrial pacing to hearts perfused with IVA to match HR of controls, indicating that any functional changes induced by IVA were a direct consequence of HRR (Fig. 1 and Table 1).

Ivabradine Preserves Energy Substrate Metabolism and Status in Perfused Healthy Normoxic Working Mouse Hearts

Figure 2 depicts metabolic fluxes relevant to energy production, which were assessed in perfused healthy normoxic working mouse hearts. We assessed the effects of IVA on the contributions of oleate and glucose to the generation of mitochondrial acetyl-CoA for citrate synthesis. Acute administration of IVA had no significant impact on the relative contribution of oleate ($19.7 \pm 1.2\%$ with IVA vs. $19.9 \pm 2.3\%$ in controls) or glucose ($28.3 \pm 3.9\%$ IVA vs. $30.2 \pm 1.9\%$ in controls) to acetyl-CoA formation for citrate synthesis. In addition, we found no significant differences between groups for the absolute flux through glycolysis (0.63 ± 0.04 IVA vs. 0.67 ± 0.16 $\mu\text{mol}/\text{min}$ in controls). Pacing of working hearts in the presence of IVA (Fig. 2) or in its absence (data not shown) had no significant effects on the contribution of glucose or oleate to acetyl-CoA formation.

One can estimate the ATP production rate in 1) the mitochondria from the measured $\text{M}\dot{\text{V}}\text{O}_2$ assuming a theoretical ATP/O of 2.83 (25) and 2) the cytosol from the measured glycolytic rates (1 ATP per lactate produced, which typically represents 5–10% of total cellular ATP production). As shown in Fig. 3, mitochondrial (19 ± 2 vs. 28 ± 2 nmol ATP/beat, $P < 0.05$; Fig. 3, A and C) and cytosolic (1.25 ± 0.16 vs. 2.30 ± 0.30 nmol ATP/beat, $P < 0.05$; Fig. 3, B and D) ATP production is not modified per minute (Fig. 3, A and B), whereas it is increased per beat (Fig. 3, C and D) in the IVA treated-group, an effect that was abolished by the application of pacing. Substantiating the notion that myocardial energy status is well preserved in working hearts perfused with IVA, we found no difference in the tissue levels of creatine phosphate, ATP, ADP, or AMP compared with the control group (Table 2).

Altogether, these results demonstrate that in healthy normoxic working hearts the addition of IVA did not affect myocardial substrate selection for energy production or the overall energy status of the heart.

Metoprolol Decreases Contractility, But Not HR, of Perfused Healthy Normoxic Working Mouse Hearts

Because IVA belongs to a new class of drugs, its effect was compared with a β -blocker (METO), which is the first-line choice for HRR in the clinic. A comparison of the direct effects of these two drugs on the heart also appeared to be valuable from a mechanistic point of view. We attempted to match HRR induced by IVA at $3 \mu\text{M}$ with the addition of METO. However, at concentrations up to $40 \mu\text{M}$, METO had little impact on HR ($\sim 19\%$; not significant), yet it dramatically reduced contractility, cardiac output, and stroke volume (by 36, 49, and 35% respectively; Fig. 4). At $40 \mu\text{M}$ METO, hearts displayed unstable function

with irregular rate and force of contraction. At the metabolic level, the absolute flux through glycolysis was significantly reduced (3.9-fold; Table 3), an effect that may be a consequence of METO's effects on cardiac function (38). At 4 μM , a concentration at which METO had only marginal effects on functional parameters compared with controls (data not shown), we observed a small, albeit nonsignificant, decrease in the contribution of exogenous oleate to acetyl-CoA formation or glycolytic rate, whereas the contribution of glucose to pyruvate and acetyl-CoA was unchanged (Table 3).

DISCUSSION

This study was undertaken to evaluate the metabolic impact of IVA, the first clinical available drug that selectively inhibits the major pacemaker current I_f in the sinoatrial node and leads to specific HRR. Specifically, we examined substrate fluxes through major energy-producing pathways as well as the energy status using our well-established model of healthy normoxic working mouse hearts perfused ex vivo with ^{13}C -labeled substrates with continuous monitoring of functional parameters (25, 48). It mimics to some extent the conditions prevailing in patients following heart transplant for which IVA was recently proposed to be of some benefit (16). With this model, we assessed the effects of IVA on heart function and metabolism in the absence of confounding external neuronal or hormonal influences and with no change in peripheral resistance (which is fixed by the buffer volume of the arterial compliance chamber). Healthy mouse hearts were perfused under physiologically relevant conditions with respect to substrate supply (mixtures of CHOs and a LCFA bound to albumin) and energy demand (fixed preload of 15 mmHg and afterload of 50 mmHg).

The concentrations of IVA that have been used in many animal investigations in vivo (0.6 mg/kg) and in vitro (3 μM) were aimed at achieving the clinical target of 20% HRR (8, 20). In our ex vivo heart model, we have used 3 μM IVA and achieved a 35% HRR. As a whole, our data concur with previous studies in demonstrating that IVA increases stroke volume, most likely due to prolonged diastole and increased left ventricular filling. Other measured functional parameters were not significantly affected by IVA. The observed effects of IVA on stroke volume and myocardial perfusion were apparently the direct consequence of the effect on HRR, since they were reversed to normal by pacing. The effect of IVA on coronary perfusion may explain some of the benefits of IVA under ischemic conditions (8, 47), since improving cardiomyocyte oxygenation may reduce the risk of functional electrophysiological heterogeneity, a predictor of channel dysfunction and action potential duration (23, 42).

Consistent with the increased stroke volume and myocardial perfusion when expressed per beat, IVA also increased $\text{M}\dot{\text{V}}\text{O}_2$ per beat, whereas it had no impact on $\text{M}\dot{\text{V}}\text{O}_2$ per minute. This is most likely because of the increase in left ventricular cavity volume, due to the prolonged diastole, which, according to the Frank-Starling law, would increase left ventricular wall stress and hence $\text{M}\dot{\text{V}}\text{O}_2$. In contrast, HRR is known to reduce $\text{M}\dot{\text{V}}\text{O}_2$ in heart failure (35). It is important to consider that the isolated denervated heart is perfused with a crystalline buffer, which has a lower oxygen carrying capacity than blood, at fixed pre- and afterload pressures. In this ex vivo model, coronary flow and $\text{M}\dot{\text{V}}\text{O}_2$ vary linearly in

response to changes in afterloads. These conditions differ from those that prevail in vivo, where the heart is also controlled by the sympathetic and parasympathetic systems, which play an important role in vivo and might be activated in patients with coronary artery disease and result in enhanced myocardial $\dot{M}V\text{O}_2$ prior to IVA administration.

At the metabolic level, our relative flux data obtained using our ex vivo heart perfusion model demonstrate that, at a dose reducing the heart rate by 35% (3 μM), IVA has no impact on substrate selection for energy production, the rate of glycolysis (expressed per minute), or the myocardial levels of high-energy phosphates. It is noteworthy that the ATP production rates in the cytosol (from the measured glycolytic rates) and in the mitochondria [from the measured $\dot{M}V\text{O}_2$ assuming a theoretical ATP/O of 2.83 (25)] did not differ between groups when expressed per minute. However, when expressed per beat, these rates were significantly higher for the IVA-treated group (Fig. 3), concurring with our finding of an increased stroke volume and supporting an effective matching of energy supply and demand per beat. In our study, the application of pacing to working heart with IVA abolished all functional effects observed with IVA alone. This finding concurs with that of others obtained in various study models both in vivo and ex vivo (8, 9, 11).

Because IVA belongs to a new class of drugs that reduce HR by a direct action on the sinoatrial node, we wanted to compare its effects with that of a β -blocker, which is the first line choice for HRR. We have attempted to match the effects of IVA to that of the widely used β -blocker METO using concentrations ranging from 4 to 40 μM on the basis of previous studies (13, 49). However, we found only marginal, nonsignificant HRR effects under our experimental conditions, i.e., in the isolated healthy working heart perfused under normoxic conditions. In contrast, METO adversely affected cardiac function, including a large reduction of contractility and cardiac output, as well as irregular beating, demonstrating that β -blockers are important regulators of the myocardium even in our explanted heart model. At the metabolic level, our finding of a significantly reduced glycolytic rate with 40 μM METO concurs with that of Sharma et al. (38). As was pointed out by those authors, because cardiac metabolism is driven by cardiac function, one cannot exclude that METO's effects on cardiac substrate metabolism may be a consequence of its effects on cardiac function. In support of this notion, we found only marginal, nonsignificant effects of METO both on cardiac function and on metabolic fluxes, including glycolysis as well as oleate and glucose contribution to acetyl-CoA production, when applied at 4 μM METO.

To understand the marked difference between the observed effects of IVA and METO on HR, contractility, and cardiac flow in our ex vivo heart model, it is important to consider that these effects are exerted under basal conditions specifically on the healthy normoxic mouse heart, which is devoid of any external neuronal influences, except for a local release of norepinephrine from endogenous neuronal stores. Although epinephrine is routinely added to the perfusate buffer at a physiological concentration [i.e., 5 nM (25)], it is unlikely that the parasympathetic system, which appears to be relevant for HR control in mice (44), would be optimally active. Nevertheless, IVA remains effective in this ex vivo condition because it exerts its HRR effects by directly acting on the pacemaker current in the sinoatrial node, and more importantly, this occurs without affecting contractility or any of the

measured metabolic parameters *ex vivo*. It is noteworthy that results obtained with IVA represent the first demonstration that pure HRR, *i.e.*, independent of inotropy, does not impact on cardiac energy substrate metabolism under basal conditions.

As for β -blockers, to the best of our knowledge, the dose-dependent acute effects of these drugs on cardiac flows and contractility (decreased) vs. HR (unaffected) in isolated healthy hearts perfused *ex vivo* at physiological pre- and afterload under normoxic conditions have not previously been reported. Previous studies have examined the effect of a selected dose of β -blocker in nonworking Langendorff-perfused hearts under stress conditions (31, 40), except for that of Sharma et al. (38), which was conducted in the working rat heart, but functional parameters were not reported. METO, which was selected because it is one of the most commonly used β -blocker in clinical practice, is a specific β_1 -adrenergic receptor antagonist. Given the higher ratio of β_1 - to β_2 -receptors in the myocardium (~ 4) than in the sinus node (36), admittedly, one could have probably anticipated that inhibition of β_1 -receptors, with consequent lowering of cAMP production, would have a greater impact on contractility than HR. Nevertheless, we believe that our observations using METO are valuable from a mechanistic point of view in the specific context of the present study, where its effects are compared with those of IVA. Collectively, our results concur with *in vivo* studies where IVA, but not β -blockers used at clinically relevant doses, was found to be effective for HRR under basal conditions in healthy animals (1, 10, 19). However, it is noteworthy that both classes of drugs have been shown to be effective at reducing HR *in vivo* under conditions of increased HR. These include, for example, sympathetic stimulation and exercise as well as pathological conditions under which these drugs are normally prescribed in the clinic (1, 14, 19, 24).

With regard to the potential clinical relevance of this study, the lack of information in the literature about the impact of HRR *per se* on cardiac energy substrate metabolism created the impetus to characterize the acute effect of pure HRR by IVA in the isolated normoxic working healthy heart prior to its chronic effect being evaluated in a clinically relevant disease model for which potentially confounding factors such as cardiac gene remodeling will need to be considered (33, 41). Despite the limitations of our study model, such as the basal conditions, absence of neuronal influence, and fixed compliance, we believe that this experimental model mimics to some extent the human transplanted heart, a condition for which IVA has been shown to be beneficial for the control of HR (16, 27).

Collectively, our results demonstrate that acute administration of IVA to healthy normoxic working hearts perfused *ex vivo* with a mixture of substrates mimicking the *in situ* milieu induces selective HRR, while preserving cardiac substrate selection for energy production, as well as optimal matching of energy supply and demand. Indeed, beyond HRR the only functional changes that were observed following IVA addition (increased stroke volume and coronary flow when expressed per beat) were reversed by pacing, thereby providing evidence that these effects were a direct consequence of HRR. Our results provide the impetus for testing the impact of IVA on energy substrate metabolism in pathological animal models of atherosclerosis or diabetic cardiomyopathy that display metabolic alterations, such as a shift in substrate selection, impaired glycolysis, and/or decreased tissue levels of high-energy phosphates (33, 41).

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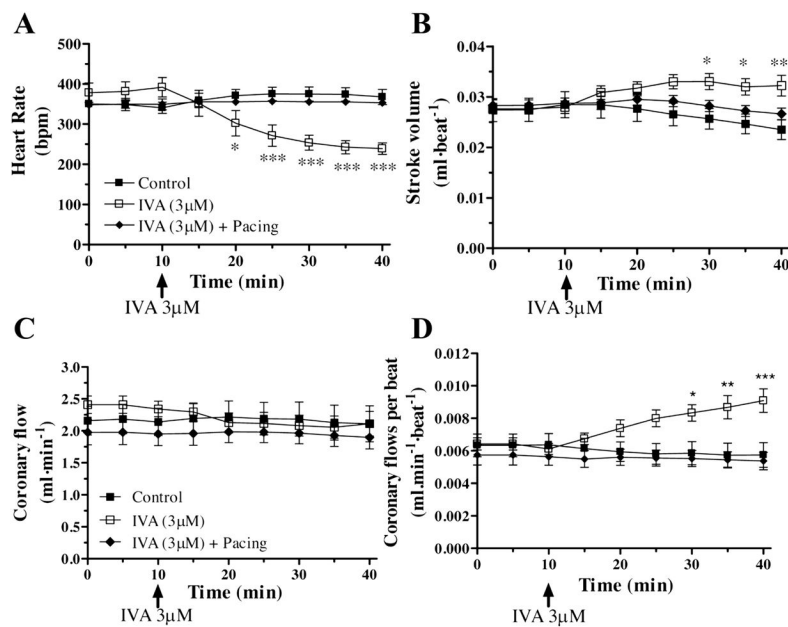
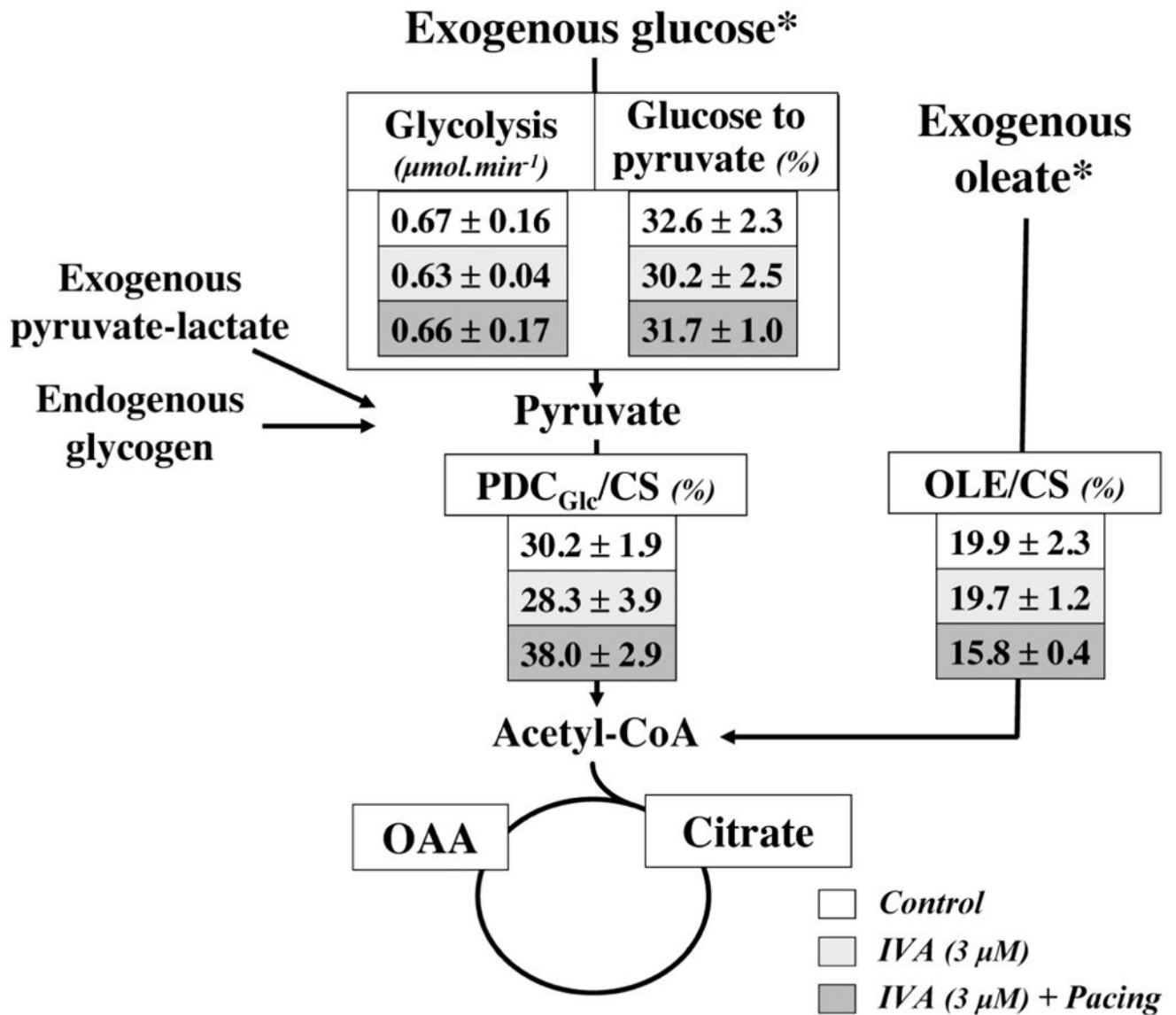


Fig. 1. Impact of ivabradine (IVA) on heart rate (*A*), stroke volume (*B*), coronary flow expressed per minute (*C*) and coronary flow expressed per beat (*D*) of isolated working C57Bl/6 mouse hearts perfused in the absence (control) or in the presence of 3 μM IVA with or without atrial pacing. Data are expressed as means ± SE of 9–12 hearts. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. control group.

**Fig. 2.**

Relative contribution of glucose and oleate to mitochondrial acetyl-CoA formation for citrate synthesis and rates of glycolysis in working C57Bl/6 mouse hearts perfused in the absence (control) or in the presence of IVA with or without atrial pacing. Data are expressed as means \pm SE of 4–5 hearts perfused for 40 min with [U- $^{13}\text{C}_{18}$]oleate or [U- $^{13}\text{C}_6$]glucose. The contribution to acetyl-CoA formation of 1) glucose via pyruvate decarboxylation (PDC_{Glc}) and 2) oleate via β -oxidation (OLE) are expressed relative to citrate synthesis (CS). Details about the calculations of these flux ratios and glycolytic rates are in MATERIALS AND METHODS. *Substrates that were labeled with carbon 13. OAA, oxaloacetate.

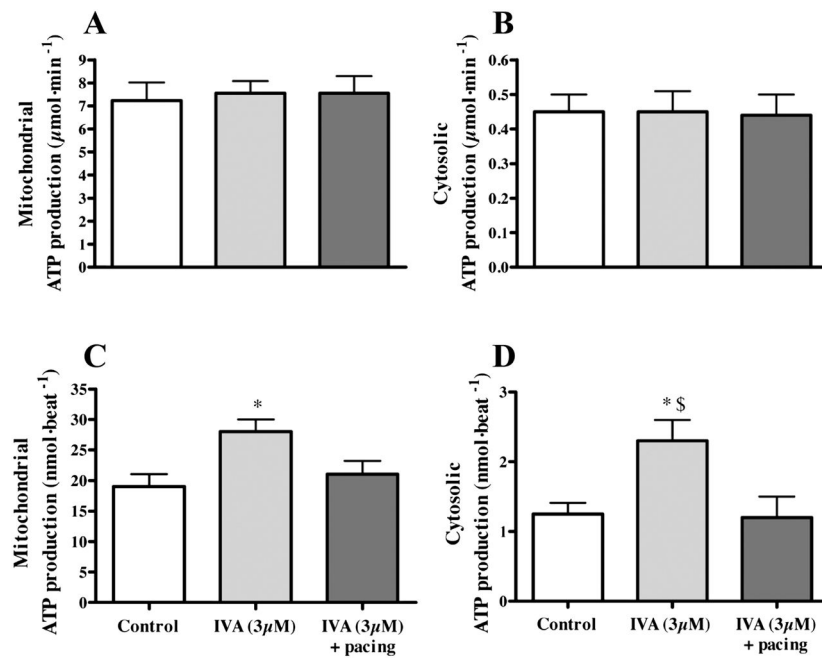


Fig. 3.

Energy production in working C57Bl/6 mouse hearts perfused in the absence (control) or in the presence of IVA with or without atrial pacing. Data are expressed as means \pm SE of 4–5 hearts. Rates of ATP production in cytosol and mitochondria are calculated from the measured rates of 1) glycolysis and 2) $M\dot{V}O_2$, (assuming an ATP/O ratio of 2.83), respectively, and are expressed per minute (A and B) and per beat (C and D). * $P < 0.05$ vs. control group; § $P < 0.05$ IVA vs. IVA + pacing group.

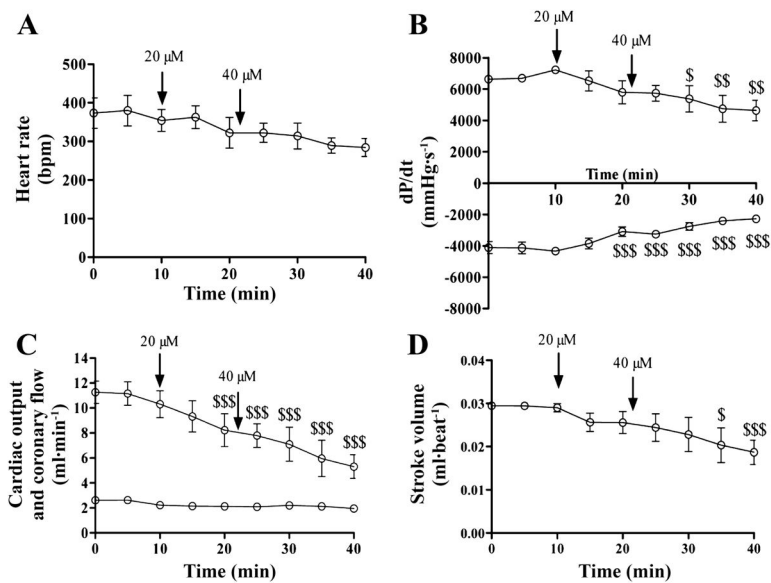


Fig. 4. Impact of 20 and 40 μM metoprolol on heart rate (*A*), contractility (*B*), cardiac output and coronary flow (*C*), and stroke volume (*D*) of isolated working C57Bl/6 mouse hearts. Data are expressed as means \pm SE of 3 hearts. $\$P < 0.05$; $\$\$P < 0.01$; $\$\$\$P < 0.001$ vs. $t = 10$ min.

Table 1

Functional and physiological parameters of isolated working C57Bl/6 mouse hearts perfused in the absence (control) or in the presence of IVA with or without atrial pacing

Treatment Effect (40 min vs. Control)	Control	IVA, 3 μ M	IVA (3 μ M) + Pacing
HR, beats/min	368 \pm 19	239 \pm 14 ***	368 \pm 19
LVSP, mmHg	95 \pm 4	107 \pm 5	100 \pm 3
Min-P, mmHg	-1.09 \pm 0.02	-3.61 \pm 0.02	-4.52 \pm 0.02
LVDP, mmHg	86 \pm 4	97 \pm 6	92 \pm 4
+dP/dt, mmHg/s	5,168 \pm 381	5,446 \pm 625	5,412 \pm 222
-dP/dt, mmHg/s	-3,672 \pm 324	-4,056 \pm 418	-4,112 \pm 218
Rate pressure product, mmHg \cdot beats ⁻¹ \cdot min ⁻¹	31,242 \pm 2,260	22,884 \pm 2,303	32,432 \pm 1,407
Aortic flow, ml/min	6.32 \pm 0.67	5.44 \pm 0.52	7.24 \pm 0.48
Aortic flow/beat, ml/beat	0.018 \pm 0.002	0.023 \pm 0.001	0.021 \pm 0.001
Coronary flow, ml/min	2.06 \pm 0.26	2.11 \pm 0.19	1.90 \pm 0.18
Coronary flow/beat, ml/beat	0.006 \pm 0.001	0.009 \pm 0.001 ***	0.005 \pm 0.001
Cardiac output, ml/min	8.38 \pm 0.77	7.55 \pm 0.69	9.39 \pm 0.42
Stroke volume, ml/beat	0.023 \pm 0.002	0.032 \pm 0.002 **	0.027 \pm 0.001
Cardiac power, mW/beat	0.005 \pm 0.001	0.007 \pm 0.001	0.005 \pm 0.000
Cardiac efficiency, mW \cdot mmolO ₂ ⁻¹ \cdot min ⁻¹ \cdot beat ⁻¹	0.004 \pm 0.001	0.005 \pm 0.000	0.005 \pm 0.001
M \dot{V} O ₂ , μ mol/min	1.3 \pm 0.2	1.3 \pm 0.1	1.3 \pm 0.1
M \dot{V} O ₂ /beat, μ mol/beat	0.004 \pm 0.000	0.006 \pm 0.000 ***	0.004 \pm 0.000
LDH, mU/min	24.1 \pm 3.5	35.3 \pm 7.3	20.0 \pm 3.4

Values are expressed as means \pm SE of 9–12 hearts for the 35- to 40-min perfusion period. IVA, ivabradine; HR, heart rate; LVSP, left ventricular systolic pressure; min-p, minimum pressure; LVDP, left ventricular developed pressure; M \dot{V} O₂, oxygen consumption; LDH, lactate dehydrogenase.

** $P < 0.01$;

*** $P < 0.001$ vs. controls.

Table 2

Myocardial HEP in working C57Bl/6 mouse hearts perfused for 40 min in the absence (control) or presence of IVA

HEP, $\mu\text{mol/mg prot}$	Control	IVA, 3 μM
Creatine	216 \pm 26	237 \pm 14
Phosphocreatine	63.8 \pm 10.7	80.0 \pm 7.5
AMP	19.6 \pm 4.4	22.7 \pm 3.7
ADP	29.4 \pm 2.3	33.8 \pm 1.4
ATP	60.5 \pm 10.3	71.0 \pm 8.6

Data are expressed in $\mu\text{mol/mg}$ protein and are means \pm SE of 4–5 hearts. HEP, high-energy phosphates.

Table 3

Metabolic flux parameters assessed in working C57Bl/6 mouse hearts perfused in the presence of METO

Metabolic Flux Parameters	METO, 4 μ M	METO, 40 μ M
Glycolysis, μ mol/min	0.45 \pm 0.09	0.17 \pm 0.07 ***
Glucose to acetyl-CoA: PDC _{Glc} /CS (%)	29.2 \pm 4.1	23.9 \pm 8.0
Oleate to acetyl-CoA: OLE/CS (%)	15.8 \pm 2.9	ND

Data are expressed as means \pm SE of 3–5 hearts. METO, metoprolol; PDC_{Glc}, glucose via pyruvate decarboxylation; OLE, oleate via β -oxidation; CS, citrate synthesis; ND, not determined.

*** $P < 0.001$ vs. controls reported in Fig. 2.