Lasting alterations of the sodium current by short-term hyperlipidemia as a mechanism for initiation of cardiac remodeling

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Submitted 18 September 2013; accepted in final form 9 November 2013

The conventional wisdom holds that cardiac dysfunction ultimately leading to CHF is primarily caused by coronary (ischemic) heart disease (CHD) and hypertension (14). However, in many cases, such as diabetes, patients remain at increased risk of heart failure even after adjusting for comorbid risk and in absence of hypertension or CHD. These observations indicate that ventricular dysfunction may develop in absence of hemodynamic impairments (11, 31, 32) and suggest that other mechanisms than cardiovascular dysfunction are involved in the development of CHF. Impaired dietary fatty acid uptake in adipose tissues leading to increased cardiac fatty acid uptake is associated with early myocardial contractile dysfunction (5, 19, 25). In the settings of insulin resistance and diabetes, this process ultimately results in a decrease of contractility, slowing of diastole, ventricular hypertrophy, and in later stages by systolic dysfunctions that progress to decompensate heart failure (1). The mechanisms underlying lipid-induced cardiac dysfunction is poorly understood (3), but the observations suggest that early remodeling of cardiac electrical currents and changes in regulation of intracellular calcium in cardiomyocyte are involved.

The cardiac sodium current (\(I_{\text{Na}}\)) is an interesting candidate to explain both the electrical and calcium disturbances caused by exposure of the heart to high fatty acid concentration. \(I_{\text{Na}}\) controls heart excitability and modulates ventricular repolarization (8). Moreover, \(I_{\text{Na}}\) "window current" regulates intracellular sodium concentration and will directly influence the rate of exchange of the sodium-calcium exchanger and diastolic relaxation. In this study we tested the hypothesis that changes in \(I_{\text{Na}}\) are part of the early cardiac remodeling occurring during increased fatty acid delivery to lean tissues and potentially contribute to the initial increase in cellular calcium.

To characterize the earliest changes in \(I_{\text{Na}}\) remodeling, we elevated plasma concentration of nonesterified fatty acids (NEFAs) using intravenous lipid infusion in dog (6, 10), a model very close to human cardiac electrophysiology. Our results indicate that an acute (8 h) Intralipid and heparin (IH) infusion was sufficient to induce long-lasting, electrical remodeling of \(I_{\text{Na}}\), consistent with an increase in intracellular calcium and alteration of cardiac excitability.

METHODS

All animal procedures conformed to the Canadian Institutes of Research, Guide for the Care and Use of Laboratory Animals (No. 036-05), the principles of laboratory animal care (National Institutes of Health Publication No. 85-23, Revised 1985), and were approved by the Institutional Animal Ethics Review Committee of the University of Sherbrooke.

Animal preparation. Female mongrel dogs weighing between 25 and 35 kg and at least 1 yr old were used in this study. Dogs were neither pregnant nor lactating. Only dogs considered healthy after physical examination, cardiopulmonary auscultation, blood analysis, and standard biochemistry profiling were used. The animals were housed in individual kennels (4 × 3 m) under controlled conditions of temperature (21°C) and photoperiod (12-h:12-h light-dark cycles) and supervised by a veterinarian.

Dogs were fed the Purina dog chow real chicken Pro-plan at the same time once a day for a period of 2 to 3 wk before any experiments to ensure weight stabilization. Purina Pro-plan (Nestlé, Mississauga, Ontario, Canada) is a standard diet comparable with other previously reported (17). The Pro-plan was served according the National Research Council recommendation for canine maintenance. Dogs had free access to water.

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Surgical procedures. Presurgical setup, anesthesia, and surgical procedures were conducted under aseptic conditions to prevent infections. Dogs were fasted 16 h before the beginning of surgery. On the day of the surgery, dogs were premedicated with subcutaneous acepromazine maleate (0.1 mg/kg), as a sedative to reduce apprehension and atropine sulfate (0.02 mg/kg) as an anticholinergic agent used to prevent throat secretions. General anesthesia was induced with thiopental sodium (15 mg/kg iv) and maintained throughout surgery with 1–2.5% isoflurane and pure oxygen. Arterial O₂ saturation from pulse oximetry, heart rate, and ECG were monitored during anesthesia. Fluid hydration was maintained with Ringer lactate solution (30 drops/min). Once dogs were anesthetized, they were placed on the heated table (37°C), and the anterior portion of the neck was opened by median incision (7 cm), and conjunctive, adipose, and muscular tissues were dissected. Two premounted PRN catheters (Silastic catheter) were prepared. PRN is an injection port adapter (BD luer-lok adapter with short 0.75-in. offset flow path) that serves as line of access to vascular catheters kept internalized under the skin. One adapter with short 0.75-in. offset flow path) served as line of access to vascular catheters kept internalized under the skin. One sterile Silastic catheter (0.03-in. inner diameter, 0.065-in. outer diameter, Down Corning, Midland, MIID) was inserted into the isolated jugular vein and advanced up to the right atrium for continuous peripheral infusions (33). In addition, one sterile Silastic catheter (0.04-in. inner diameter × 0.085-in. outer diameter) was inserted into the left carotid artery and advanced into the aortic arch for arterial sampling. After completion of surgery, dogs were equipped with an Elizabethan collar and an adapted jacket (Lomir, Canada) to protect catheters and PRN from any damages. Fluxes with saline and heparin solutions were performed twice a day until the day of use for blood collection.

Buprenorphine (0.02 mg/kg sc) and Longisil antibiotics (penicillin 10,000 U/lbs im) were given immediately after surgery to prevent pain and septicemia. Local infection at the sites of catheters implantation was controlled by Flamazine applied every day topically. All dogs completely recovered within 7 days as established from full return to normal values of renal, hematology and metabolic parameters.

After surgery (7–10 days), dogs (control and experimental groups, respectively) received saline or Intralipid as 20% triglyceride emulsion (0.02 ml·kg⁻¹·min⁻¹) plus heparin (0.5 U·kg⁻¹·min⁻¹) for 8 h (from time = −510 min to −30 min). The latter was given to stimulate lipoprotein lipase activity to hydrolyze the triglycerides that are infused. At time 0, dogs were fed with an isocaloric meal. During the three experimental periods (saline or Intralipid infusion (−510 to −30 min), basal period (−30 to 0 min), and the postprandial period (0–540 min)), blood was collected and plasma samples were prepared for biochemical and metabolic analysis. The total volume of blood withdrawn did not exceed 20% of total blood volume of the animal.

Plasma cortisol and adrenocorticotropic hormone concentrations were determined using a human antibody (MP, Biomedicals)-based radioimmunoassay method and a commercial RIA kit (Immunocorp, MP, Biomedicals), respectively, adapted for dog. Total NEFAs in plasma were quantified using a commercially available colorimetric assay (NEFA C kit; Wako Chemicals). Plasma triglyceride concentration was determined using a colorimetric assay (Trig/GB, Boehringer Mannheim/Roche Diagnostics).

Samples for determination of glucose and insulin were collected into tubes containing Na₂ EDTA and trazoyl (7,700 KIU/ml, calbiochem) to inhibit proteolysis. Plasma glucose concentration was determined by the glucose oxide method (540 nm). Plasma insulin was measured using a specific canine insulin enzyme-linked immunonassay kit (Cedarlane, Burlington, CA).

Heart excision and cardiomyocyte dissociation. Immediately after the last blood sampling (~12 h after IH treatment), animals were sedated with a mix of Atetrav (0.25 mg/kg im) and heparin (5,000 U) for 30 min to avoid blood coagulation and then anesthetized with pentobarbital sodium (25 mg/kg iv). The beating heart was quickly removed by an incision at the fifth intercostal space causing the euthanasia of the animal. Necropsy was systematically performed to check the position of implanted vascular catheters and to collect organs and tissues.

Myocytes were obtained by enzymatic dissociation as previously described (9). Briefly, a left ventricular wedge was cut and perfused at 35°C through a coronary artery for 10 min with Ca-free Tyrode solution, supplemented with 2 mmol/l EGTA and 0.1% BSA. Perfusion was switched to Tyrode solution containing 0.1 mM Ca and 230 U/ml collagenase (CLS 2, Worthington, Freehold, NJ) and recirculated for 10–20 min until the tissue became discolored and mushy. The wedge was then removed and minced, and tissues were gently stirred in beakers containing the enzymatic solution. The supernatant containing dissociated cells was kept in 10-ml tubes and stored in Krebs solution containing (in mmol/l) 100 potassium glutamate, 10 potassium aspartate, 25 KCl, 10 KH₂PO₄, 2 MgSO₄, 20 taurine, 5 creatine, 0.5 EGTA, 20 glucose, 10 HEPES, and 2% BSA, supplemented with 0.2 mM CaCl₂.

Electrophysiology. Dissociated myocytes were placed in a chamber mounted on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan) and superfused with solution containing (in mmol/l) 120 NaCl, 10 Na₂SO₄, 2.8 Na acetate, 4 KOH, 0.5 CaCl₂, 1.5 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4 with NaOH). Tetraethyl ammonium (5 mmol/l), CoCl₂ (1 mmol/l), and BaCl₂ (5 mmol/l) were used to block transient outward (Iₜₒ), L-type calcium (I_{Ca,L}), and inward rectifier (I_{K₁}) currents, respectively. Membrane currents were measured in the whole cell configuration of the patch-clamp technique as previously described (13). All recordings were obtained at room temperature (22°C) using an Axopatch 200B amplifier (Axon Instruments, Union City, CA), equipped with a CV-201A head stage (Axon Instruments, Foster City, CA). Patch pipette had electrical resistance between 1 and 3 MΩ when filled with a solution containing (in mmol/l) 15 NaCl, 5 KCl, 120 CsF, 1.0 MgCl₂, 4 Na₂-ATP, 10 EGTA, and 10 HEPES (pH 7.2 with CsOH). All the solutions were adjusted at 300 mosmol/l with sucrose. Currents were filtered at 5 kHz and digitized at 10 kHz. Data acquisition and analysis were performed using pCLAMP programs V9.2 (Axon Instruments), EXCEL (Microsoft), and ORIGIN 7.0 (Microcal Software, Northampton, MA) softwares. Whole cell capacitance and series resistance compensation (85%) were optimized to minimize the capacitive artifact and reduce voltage errors. Sodium window current was calculated using the classical Hodgkins and Huxley model based on the overlap of the steady-state inactivation and activation curves using the equation $I_{Na} = G_{Na} m^3 h (V_m - E_{Na})$ where $G_{Na}$, $m$, $h$, $V_m$ and $E_{Na}$ represent the maximum conductance, the fraction of current activated and inactivated, the membrane potential, and the sodium reversal potential respectively, as described in Figs. 3–5.

Statistical analysis. Data are expressed as means ± SE. Comparison between saline-infused dogs and IH-treated dogs was performed using a two-way ANOVA.

RESULTS

Infusion of IH (0.5 U·kg⁻¹·min⁻¹) for 8 h significantly increased the levels of triglycerides and NEFAs for the whole duration of the infusion and resumed to normal levels thereafter (Fig. 1A). Corticotrophin (adrenocorticotropic hormone) and cortisol were measured during and after IH treatment to eliminate the possibility of stress-related artefacts in our measurements. Figure 1B shows that plasma concentrations of both hormones remained stable and comparable between sham- and IH-treated animals. Moreover, the increase in circulating NEFAs during infusion did not change the levels of insulin or glucose neither during IH nor infusion in the following postprandial period (Fig. 1C), indicating that our treatment did not induce diabetes in these animals.

Chronic exposure to high serum concentrations of lipids could lead to onset of type 2 diabetes and eventually triggers
remodeling of the heart, leading to diabetic cardiomyopathy. Polyunsaturated fatty acids applied in vitro are known to alter the kinetic and to reduce expression of the cardiac $I_{Na}$ heterologously expressed in HEK cells and in isolated cardiomyocytes (2, 16, 21, 22, 30, 34–36). We therefore tested whether early electrophysiological remodeling occurs following IH treatment by measuring the amplitude of the cardiac $I_{Na}$. Figure 2A shows that IH infusion slightly increased the amplitude of the maximum peak current from $-41.4 \pm 1.9$ to $-58.4 \pm 7.4$ pA/pF. Current-voltage analysis of the recordings showed a shift from $-30$ to $-35$ mV of the maximum current voltage (Fig. 2B). The threshold for activation of the current was similarly shifted by $-5$ mV, indicating changes in the voltage dependence of activation of the channels. Interestingly, maximum amplitude remained larger in IH-treated cells for the 3 days, during which we were able to keep dissociated myocytes alive and reliably measure $I_{Na}$ (Fig. 2C). Analysis of $I_{Na}$ activation revealed that IH treatment shifted the voltage dependence of opening of the channels by $-10$ mV (Fig. 2D). Maximum conductance obtained as the slope of the linear portion of the $I$–$V$ relationship was similar in sham- and IH-treated animals (Fig. 2E), thus indicating that the lipid infusion mainly affected the gating of the current rather than expression of new channels. To determine if a change in the availability of the sodium channels participates to the voltage shift in maximum current, we next assessed $I_{Na}$ voltage dependence of inactivation. Fig. 3, A and B, shows that IH treatment induced a small, albeit significant, $-5$ mV shift in midinactivation potential, indicating that availability of the channels will be more importantly reduced at depolarized potentials in IH-treated animals. This effect is not consistent with the increased $I_{Na}$ observed on the $I$–$V$ relationship (Fig. 2B) and indicates that the increase in maximal $I_{Na}$ amplitude is mostly due to a change in channels activation gating.

The cardiac refractory period is highly dependent on recovery of sodium channels from their inactivated state and is involved in many types of arrhythmias such as reentry. To test for changes in recovery from inactivation, we used the double-pulse protocol shown in Fig. 3C. Statistical analysis ($F$-test) of the fit to data (Fig. 3D) did not reveal a significant difference in the kinetic of reactivation of the channels.

The overlap of the activation and inactivation curves creates semistable conditions where a fraction of sodium channels remains in transition between the inactivated (closed) and
activated (open) states and vice versa. As a consequence, a very small but persistent inward “window” current ($I_W$) exists in the voltage range of this overlap. $I_W$ thus contributes to establish the resting membrane potential and modulates the sodium gradient between the intra- and extracellular milieus.

Figure 4 shows that IH infusion shifted the voltage range where $I_W$ is active closer to the normal myocytes resting membrane potential (-80 mV) and increased its maximum amplitude.

**DISCUSSION**

We demonstrated that IH infusion increases plasma free fatty acids and triglycerides concentration without inducing hyperinsulinemia, hyperglycemia, or changes in circulating stress hormones levels. These results confirm that the alterations of $I_{Na}$ are linked to alterations of the lipid content and/or delivery to the heart. We found that IH increased the maximal amplitude of $I_{Na}$ by 41% and shifted the voltage dependence of activation of the channels toward more negative potentials. Physiologically, a negative shift in $I_{Na}$ activation lowers the voltage threshold for activation of sodium channels and is a well-known mechanism to increase cardiac excitability that will be further enhanced by the increase in $I_{Na}$ amplitude. Our results also indicate that IH infusion had smaller effects on inactivation of the $I_{Na}$ that could not account for the observed changes in the $I-V$ relationship.

Alterations of the lipid content in cardiomyocyte plasma membrane are known to modulate the gating of $I_{Na}$. In rat cultured cardiomyocytes and cardiac muscle, eicosapentaenoic acids, docosahexaenoic acids, and other polyunsaturated fatty acids (PUFAs) increased the threshold for action potential firing (67, 68), had inhibitory effect on $I_{Na}$, and enhanced the kinetic of inactivation (69, 66). Similar effects were also
Sham IH

by-products of suggest that other modulatory mechanisms, possibly related to chronic changes in myocardial other in vivo adaptive mechanisms may contribute to modulate exposure to fatty acids were measured. It is also possible that (10 to 50 min) to lipids. Therefore, only acute effects of experiments, myocytes were exposed for short period of time after the dogs were left to recover from treatment. Such early electrophysiological remodeling of the cardiac myocytes is a new finding. Our results therefore indicate that circulating NEFAs can remodel the heart within a time frame as short as a few hours and well before the onset of insulin resistance or detection of type 2 diabetes.

Another interesting finding is the negative voltage shift in sodium window current caused by IH infusion. The consequences of this are twofold. First, it will increase the inward (leak) sodium current close to −80 mV, thus causing a depolarization of the normal resting membrane potential. Combined with our observation of a negative shift in activation and augmented INa amplitude, this will increase cardiac excitability. Second, the augmentation of the window current near the resting membrane potential of the cell will increase the sodium influx and the intracellular sodium concentration, thereby reducing the sodium gradient. The sodium gradient between the intra- and extracellular compartments plays a key role in regulating calcium homeostasis by directly regulating the turnover of the sodium-calcium exchanger. A reduction in the sodium gradient may therefore translate into a diminished extrusion of intracellular calcium. This mechanism is well characterized and contributes to the calcium overload observed during hypertension, cardiac hypertrophy, and heart failure (7, 15, 18, 24, 27).

Fig. 3. Short-term increase in plasma free fatty acids shifts the availability of sodium channels toward more negative potentials. A: representative current recordings at a test pulse of −10 mV following a series of conditioning pulses (−140 to +40 mV) in increments of 5 mV from a holding potential of −120 mV (inset). B: inactivation curves (availability) were obtained by plotting the ratio of INa to its maximum value against the conditioning pulse voltage. Data were fitted against a Boltzmann distribution function and yielded mid-inactivation potentials (V1/2) of −66.7 ± 0.4 and −70.8 ± 0.2 mV for sham (n = 8) and IH (n = 14) conditions, respectively (P < 0.05). C: recovery from inactivation is not changed by short-term exposure to increased level of NEFAs. Representative INa recordings during application of a standard electrophysiological double pulse protocol (S1-S2, 20 ms) to measure recovery of INa from inactivation (inset). D: recovery from inactivation expressed as the ratio of the initial current (I0) elicited during the second pulse (I2) and plotted against the interpulse interval duration (Δt) in sham (n = 8) and IH (n = 14) conditions. Data are presented as means ± SE and were fitted to a two exponential distribution function (solid line).

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00715.2013 • www.ajpheart.org

observed in Nav1.5 channels expressed in HEK cells (71, 65, 72, 73). Part of these results may be explained by changes in the fluidity of the plasma membrane surrounding the sodium channels (66). Such hypothesis is consistent with specific alterations of the activation gating of the channel since the structures involved in activation are transmembrane segments imbedded in the plasma membrane, whereas the inactivation gate of the channel is in the hydrophilic intracellular milieu. However, the voltage shift in INa gating linked to changes in membrane fluidity are opposite to the effects of in vivo exposure to IH we report here. While species differences might be involved, it is important to note that in previous in vitro experiments, myocytes were exposed for short period of time (10 to 50 min) to lipids. Therefore, only acute effects of exposure to fatty acids were measured. It is also possible that other in vivo adaptive mechanisms may contribute to modulate myocardial INa. The opposite results in our in vivo experiments suggest that other modulatory mechanisms, possibly related to by-products of β-oxidation were activated over the course of 8 h.

Surprisingly, short-term IH infusion was sufficient to induce chronic changes in INa gating that could still be recorded 12 h after the dogs were left to recover from treatment. Such early electrophysiological remodeling of the cardiac myocytes is a
Ih and the ventricular repolarization on the other hand is initiated by activation of Ikr and considered a risk factor to develop insulin resistance and ultimately type 2 diabetes. Interestingly, most studies in nongenetically altered diabetic mice report an increase in intracellular calcium levels without significant changes in the amplitude of ICaL. These findings argue against a role for ICaL in the increase in intracellular calcium observed in type 2 diabetes. Our results on the other hand provide evidence that alteration of INa may significantly contribute to the process. Studies have demonstrated that diabetic hearts are characterized by an increase in cellular calcium, ultimately leading to heart failure. Alterations in the activity of the sarcoplasmic reticulum Ca2+ ATPase pump were proposed as a potential mechanism to explain that cardiomyopathies once type 1 and 2 diabetes is well established. However, these studies did not provide an initiation mechanism leading to impaired Ca2+ handling. Our results suggest a new paradigm by which changes in INa gating modulate the sodium gradient to alter calcium homeostasis as plasma free fatty acids increase and insulin resistance develops. We further demonstrate that cardiac adaptation to elevated plasma NEFAs occurs within a relatively short period. In this context, a few repeated exposures to high-fat plasma concentrations might be sufficient to chronically remodel the heart. It is therefore tempting to speculate that cardiac remodeling is triggered by free fatty acids well before insulin resistance and type 2 diabetes can be clinically detected.

In summary, we show that 8-h infusion of PUFAs in dog increased INa amplitude by 43%, lower its voltage threshold for activation, and increase its window current. These findings are consistent with an increased excitability and intracellular calcium concentration that potentially contribute to ventricular arrhythmias associated to an increase in plasma free fatty acid concentration such as the ones observed during ischemia or diabetes. However, our animal model, while suited to study the effects of acute in vivo exposure to high-fat plasma levels of free fatty acids, does not take into account the chronic hyperlipidemia and hyperglycemia that concomitantly develop during diabetes. Therefore, further studies are warranted to determine if chronic exposure of dogs to fatty acids will result in the development of diabetic cardiomyopathies and if this is accompanied by changes in INa gating.

ACKNOWLEDGMENTS

We thank Jean Philippe Gagné for technical contribution to the surgical procedures.

GRANTS

This work was funded by grants from the Canadian Institute of Health and the Heart and Stroke Foundation of Canada (to R. Dumaine).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

REFERENCES


