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A possible mechanism of halocarbon-induced cardiac sensitization arrhythmias

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Abstract

Cardiac sensitization is the term used for malignant ventricular arrhythmias associated with exposure to inhaled halocarbons in the presence of catecholamines. We investigated the electrophysiological changes associated with cardiomyocyte exposure to epinephrine and a halocarbon known to be associated with cardiac sensitization (halon 1301, CF₃Br). Cardiomyocytes (CMs) were isolated from neonatal rats and grown on multielectrode arrays (MEAs). Upon exposure to epinephrine, the CM inter-spike interval (ISI) was decreased 14% at 10 µg/L ($P < 0.05$) and 27% at 100 µg/L ($P < 0.05$) as compared to baseline. Halon alone (50 mg/L) mildly prolonged the field potential (FP) duration (7%). CMs exposed to combinations of epinephrine (100 µg/L) and halon (50 mg/L) for 15 min showed a blunted increase in the ISI (35±12%) and a 38% decrease in conduction velocity ($P < 0.05$) when compared to epinephrine alone. There was no change in field potential properties, but dephosphorylated connexin 43 (Cx43) was increased 60±16% with the combination as compared to epinephrine alone ($P < 0.05$). Treatment with okadaic acid, a phosphatase inhibitor, prevented the Cx43 dephosphorylation and the reduction in conduction velocity upon exposure to halon and epinephrine. Moreover, the electrophysiological changes induced by epinephrine and halon were indistinguishable from those seen with the gap junction inhibitor heptanol. In conclusion, the combination of a halocarbon and epinephrine results in a unique electrophysiological signature including slow conduction that may explain, in part, the basis for cardiac sensitization. The slowing of conduction is most likely related to changes in the phosphorylation state of Cx43.

Keywords

Cardiomyocyte; Epinephrine; Halocarbon; Phosphatase; Gap junction; Electrophysiology; Multielectrodes array; Mapping; Protein phosphorylation; Connexin 43

1. Introduction

Halogenated chemicals are used in many medical and industrial applications, including fire protection, refrigeration and general anesthesia. When inhaled, especially in the presence of catecholamines, these compounds can cause sudden death from ventricular arrhythmias [1]. This effect is known as cardiac sensitization and has also been reported with recreational glue sniffing [2]. Cardiac sensitization is an important limitation in the use of halocarbons since the levels at which a compound causes arrhythmias are similar to their effective concentrations.

Despite its importance, the mechanism of cardiac sensitization is poorly understood. Therefore, we investigated the molecular basis of cardiac sensitization. Since cardiac sensitization is associated with the combination of catecholamines and halocarbon exposure, we set out to compare the electrophysiological effects of these agents, looking for responses unique to the combination of agents that might explain the basis for enhanced arrhythmogenesis.

Halon 1301 (CF₃Br) is a widely employed halocarbon fire suppressant. As with other halocarbons, halon exposure can produce cardiac sensitization [3–6]. It is used by the military as a flooding agent for extinguishing fires in normally occupied, confined spaces [7]. Design of fire suppression systems is made more complicated because of the need to avoid toxic concentration. Here, we use halon 1301 as a representative arrhythmogenic halocarbon and show that the combined exposure of cardiac myocytes to halon and epinephrine has a unique electrophysiological signature that may explain the arrhythmic risk seen with the combination.

2. Materials and methods

2.1. Neonatal rat cardiomyocytes isolation and culture

Ventricular cardiomyocytes were isolated from 2- to 3-day-old Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) using an isolation system from Worthington Biochemical Corp. (Lakewood, NJ). Briefly, ventricles were isolated and minced in sterile calcium- and magnesium-free Hank's Balanced Salt Solution (pH 7.4) and incubated with trypsin (50 µg/mL) overnight at 4 °C. Then, the tissue was treated with a trypsin inhibitor at 37 °C for 30 min followed by collagenase for 45 min at 37 °C. Tissue was triturated, and the supernatant was filtered by a cell strainer. The cell suspension was centrifuged at 1000 rpm for 3 min, and the cell pellet was re-suspended in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (10%), penicillin (100 U/mL) and streptomycin (100 µg/mL). After cell yield and viability were measured, cells were plated on a tissue culture dish for 1.5 h to allow the attachment of non-myocytes. The remaining suspended cells were collected by centrifugation and counted on a hemacytometer. Cells were plated in a dish with a multielectrode array embedded in the floor of the dish (MEA), and the cultures were maintained in a humidified incubator (95% air/5% CO₂) at 37 °C. Cardiomyocytes were grown in the MEA at seed density of 2×10^6 cells per MEA. Unattached cells were removed by washing after 24 h, and spontaneously beating cardiomyocytes were identified as a criterion for a successful isolation. The medium was replaced on alternating days, and all experiments were performed within 1 week after cell plating.

2.2. Recording cardiomyocyte electrical activity

Extracellular recording from rat cardiomyocytes was performed using an MEA data acquisition system (Multi Channel System, Reutlingen, Germany). The MEA consists of a matrix of 60 titanium nitride coated gold electrodes (30-µm diameter) in an 8 × 8 layout grid

with an inter-electrode distance of 200 μm . The MEA was inserted in the amplifier system, which includes a heating device. Simultaneous recordings of bipolar extracellular electrograms or field potentials (FPs) from all electrodes were performed at a sampling frequency of 10 kHz and at 37 °C. One electrode at the border of the array was grounded and used as a reference electrode. Results were identical using an independent ground electrode in the bath. As described previously [8], the data were analyzed off-line with a customized toolbox programmed for MATLAB (Mathworks, Natick, MA). For presentation, FP parameters were averaged for seven predefined locations on the array. The earliest sites of activation were compared at the beginning and end of each recording.

2.3. Cardiomyocyte exposure

At the start of each experiment, the MEA culture dish was sealed with a custom fabricated Teflon top that allowed solution inflow and evacuation. The effects of cardiomyocyte exposure to buffer only, epinephrine, halon 1301, heptanol, or epinephrine plus halon, epinephrine plus halon and okadaic acid were compared. In the buffer control group, cardiomyocytes were perfused constantly with an oxygen saturated HEPES buffer [(in mmol/L) NaCl 140, KCl 5.4, CaCl_2 1.8, MgCl_2 1, HEPES 10, glucose 10 (pH 7.4 by NaOH)] for 30 min (HEPES control group) using a closed, gravity-driven system. For the epinephrine group, separate cultures were exposed to HEPES buffer for 5 min followed by the concentrations of epinephrine at 0.1, 1, 10 or 100 $\mu\text{g/L}$ in HEPES buffer for 25 min. For the halon group, separate cultures were exposed to HEPES buffer for 5 min followed by 25.7 mg/L or 50 mg/L of halon 1301 in buffer for 15 min. To test for cardiac sensitization, we used a protocol mimicking the standard Beagle dog assay for halocarbon-induced arrhythmia [2]. Cardiomyocytes were exposed to HEPES buffer for 5 min followed by epinephrine (100 $\mu\text{g/L}$) for 5 min and finally epinephrine plus halon (50 mg/L) for 15 min (Epi+halon group). For the epinephrine plus halon and okadaic acid group, cardiomyocytes were exposed to HEPES buffer for 5 min followed by epinephrine (100 $\mu\text{g/L}$) for 5 min and finally epinephrine plus halon (50 mg/L) and okadaic acid (OA, 100 nM) for 15 min (Epi+halon+OA group). For the heptanol group, separate cultures were exposed to HEPES buffer for 5 min followed by 0.25 mM of heptanol in buffer for 15 min.

Solutions containing various concentrations of halon (RemTec International, Inc. Holland, Ohio) were made fresh before each experiment by diluting a saturated solution. Saturated solutions of halon in buffer were generated by bubbling the halon gas into buffer for 15 min (0.03% by weight at room temperature).

2.4. Immunoblot analysis for phosphorylated Cx43

Cardiomyocytes were cultured in T-25 flasks for 48 h until they formed spontaneously contractile, confluent monolayers morphologically indistinguishable from those cultures recorded in the MEAs. Cultures were perfused with epinephrine (100 $\mu\text{g/L}$) for 5 min, and then the cultures were exposed subsequently to the combination of epinephrine (100 $\mu\text{g/L}$) and halon 1301 (50 mg/L) for 15 min (Epi+halon group) or epinephrine (100 $\mu\text{g/L}$), halon 1301 (50 mg/L) and okadaic acid (100 nM; Epi+halon+OA group). After their respective treatments, all cultures were washed in ice-cold phosphate-buffered saline, harvested in lysis buffer (Tris 50 mmol/L, NaCl 150 mmol/L, EDTA 1 mmol/L, EGTA 1 mmol/L, sodium deoxycholate 0.25%, Triton X-100 1%, PMSF 1 mmol/L, leupeptin 1 $\mu\text{g/mL}$), and cells were disrupted by sonication. Homogenate protein concentrations were determined by the Lowry protein assay (Bio-Rad, Hercules, CA). Proteins were resolved on 12% SDS-PAGE and probed with total and non-phosphorylated connexin 43 (Cx43) antibodies (1:1000; Zymed Laboratories Inc., South San Francisco, CA). The Cx43 and non-phosphorylated Cx43 levels were determined using an ECL Western blotting kit (Amersham Pharmacia

Biotech, Piscataway, NJ). Protein amounts were analyzed by densitometry using Image-Pro Plus software (Media cybernetics, Silver Spring, MA).

All chemicals were obtained from Sigma Chemicals (St. Louis, MO) unless otherwise noted.

2.5. Data analysis

Data were expressed as mean $\pm$ SE for n experiments. ANOVA or Student's t test was performed using GraphPad Prism 3.0 (GraphPad Software Inc., USA). A value of $P\leq 0.05$ was taken as significant.

3. Results

The purpose of these experiments was to determine whether conditions associated with cardiac sensitization resulted in a unique electrophysiological response that might explain the increased risk of arrhythmia. Cardiomyocyte electrophysiological behavior was measured from a two-dimensional syncytium of cells grown on an MEA. Using a sealed culture chamber allowed sampling of the extracellular FPs of spontaneously active cardiomyocytes under a variety of experimental conditions including exposure to the gaseous halogenated hydrocarbon halon 1301. Previously, the FPs have been shown to correlate well with the underlying cellular electrophysiology [8], and this arrangement allowed for evaluation of changes in the dominant pacemaker, a surrogate for ectopic activity, and of conduction velocity [9,10]. Mapping of two-dimensional cardiomyocytes cultures has proven an efficient methodology for exploring arrhythmic risk [9–16].

Control cultures between 2 and 7 days of age exposed only to the recording solution showed stable electrophysiological characteristics over the time course of these experiments and were unaffected by perfusion alone. Fig. 1 shows a representative FP (panel A) and a conduction map demonstrating uniform conduction across the MEA (panel B). The average amplitude (693 ± 158 μ V at 5 min, 746 ± 149 μ V at 30 min), the maximum rate of FP change ($-dV/dt_{\max}$; 3.95 ± 0.87 mV/ms at 5 min, 4.62 ± 0.96 mV/ms at 30 min) and the FP duration (53 ± 6 ms at 5 min, 49 ± 1 ms at 30 min) of myocytes were not affected by 30 min of perfusion in control solution, and the inter-spike intervals (ISI; 180 ± 27 ms at 5 min, 198 ± 38 ms at 30 min) and the conduction velocity (28 ± 7 cm/s at 5 min, 31 ± 8 cm/s at 30 min) were unchanged (panel C).

3.1. Epinephrine alone affected the inter-spike interval only

As expected, epinephrine exposure resulted in an increased spontaneous beating rate measured as a reduced ISI and, at the highest doses, altered the site of earliest activation, consistent with the known tendency for catecholamines to enhance automaticity and increase ectopy [17,18]. The ISI was shortened significantly by $14\pm 5\%$ and $27\pm 6\%$ after treatment with 10 and 100 μ g/L epinephrine, respectively (Fig. 2; $P<0.05$ for both). The average FP amplitude, $-dV/dt_{\max}$ and FP duration and conduction velocity were unchanged with exposure to 0.1, 1, 10 and 100 μ g/L epinephrine for 25 min (data not shown).

3.2. Halon exposure alone had little effect on cardiomyocyte electrophysiology

Two doses of halon were used, corresponding to blood levels calculated in man [7]. Exposure to halon alone at either dose resulted in minimal changes in the FP properties. The FP amplitude, $-dV/dt_{\max}$, FP duration, ISI and conduction velocity were unaltered when cardiomyocytes were treated with 25.7 mg/L halon 1301, ($n=5$, data not shown). At the higher dose (50 mg/L), only the FP duration was mildly prolonged at 15 min of treatment ($7\pm 3\%$, $P<0.05$, Fig. 3).

3.3. Halon and epinephrine reduce conduction velocity

The electrophysiological response of cardiomyocytes exposed to the combination of epinephrine and halon was unique when compared to the response to either agent alone. In order to mimic the currently accepted standard test of cardiac sensitization [2], cardiomyocytes were exposed to epinephrine followed by the combination of epinephrine and halon. Consistent with its expected effect and compared to control data, 100 $\mu\text{g/L}$ of epinephrine for 5 min shortened ISI $25\pm6\%$. Perfusion with epinephrine and halon 1301 (50 mg/L) did not change the FP parameters (Fig. 4A). Nevertheless, epinephrine+halon opposed the shortened ISI seen with epinephrine alone. In the Epi+halon group ISI was prolonged by $35.1\pm12.3\%$ at 15 min of treatment compared to the Epi group ($P<0.05$, Fig. 4A). Additionally, conduction velocity was decreased by $38.4\pm9.8\%$ ($P<0.05$ compared epinephrine, Fig. 4A). Representative conduction maps from the Epi or Epi+halon groups are compared in Figs. 4B and C.

Cardiac conduction velocity is a function of Na^+ channel availability and gap junctional resistance. Since FP parameters, including $-\text{dV}/\text{dt}_{\text{max}}$ that is a marker of Na^+ channel availability [19], were unchanged with epinephrine and halon, it seemed plausible that the observed changes could be explained by changes in gap junctional resistance. Therefore, we compared the electrophysiological effects of the gap junctional inhibitor heptanol to those seen in cardiomyocytes exposed to Epi+halon. The FP amplitude, $-\text{dV}/\text{dt}_{\text{max}}$ and the FP duration were unaltered when cardiomyocytes were treated with 0.25 mM heptanol for 15 min. On the other hand, heptanol prolonged the ISI and slowed conduction velocity in a manner similar to that of epinephrine+halon. With heptanol, the ISI was prolonged $31.8\pm7.0\%$ at 15 min of treatment ($P<0.05$ compared to control), and the conduction velocity was decreased by $39.4\pm10.0\%$ ($P<0.05$ compared to control, Fig. 4D).

The major ventricular gap junction protein is Cx43, and downregulation of this connexin has been associated with arrhythmic risk [20–22]. This protein is regulated by phosphorylation, with the unphosphorylated version of the channel generally showing a reduced open probability that results in reduced gap junction conductance [23]. Because of the rapidity of the Epi+halon effect and its similarity to that of heptanol, we hypothesized that Cx43 dephosphorylation may have contributed to the changes in conduction velocity. Dephosphorylation of Cx43 can be mediated by protein phosphatase such as PP1 and PP2A [24]. In order to further test the idea that the conduction slowing uniquely mediated by halon and epinephrine might be dependent upon the status of phosphorylated Cx43, cardiomyocytes were exposed to epinephrine followed by the combination of epinephrine, halon and okadaic acid, a phosphatase inhibitor. Inclusion of okadaic acid (100 nM) in the perfusate prevented the Epi+halon-mediated changes in ISI and conduction velocity (Figs. 5A, B). Control experiments showed that total and non-phosphorylated Cx43 levels were unchanged by perfusion itself and that okadaic acid alone for 15 min had no effect on ISI and CV when compared to baseline (ISI $104.7\pm2.8\%$, CV $110.3\pm15.2\%$ vs. baseline ($P>0.05$, $n=3$)).

Consistent with this idea, exposure to the combination of halon and epinephrine was associated with a $60\pm16\%$ increase in non-phosphorylated Cx43 without a change in total Cx43 protein levels ($P<0.05$ compared epinephrine, Figs. 5C and D). Additionally, okadaic acid prevented the dephosphorylation of Cx43 seen in the Epi+halon group without altering total Cx43 protein levels. Based on studies in nonischemic cardiomyopathy, this magnitude of change in Cx43 phosphorylation is consistent with the conduction slowing observed [25,26].

4. Discussion

Cardiac sensitization is a condition where tachyarrhythmic risk is enhanced by the combination of adrenergic activation and exposure to halogenated halocarbons as compared to that risk incurred as the result of exposure to either agent alone. Our results suggest that the combination of adrenergic activation and halocarbon exposure results in a reduction in conduction velocity that is unique to the combination of agents. This reduction in conduction velocity might help explain the unique electrophysiological behavior of the combination.

4.1. Epinephrine and arrhythmic risk

Epinephrine alone can be arrhythmogenic [27,28]. Epinephrine enhances automaticity, lowers fibrillation thresholds [17,18,27,29,30] and increases extrasystoles [28,31]. The effects on the action potential vary, but epinephrine alters calcium handling and increases the likelihood of delayed after-depolarizations [32–35]. Nevertheless, at doses consistent with blood levels predicted in man [7], epinephrine did not show arrhythmic tendencies based upon the changes observed. At the highest dose used, we did observe an increase in the spontaneous beating rate. The presence of a halocarbon seemed to antagonize the effect of epinephrine on spontaneous beating frequency, however suggesting that the arrhythmic effect of the combination cannot be explained purely by epinephrine-mediated changes.

4.2. Halocarbons and arrhythmic risk

Alone, some halocarbons are arrhythmogenic. The risk varies depending on the compound, time of exposure and concentration [36]. Studies implicate halocarbons in alterations of a number of ion channels, and these changes may contribute to their arrhythmic effect. Halocarbon effects include activation of hyperpolarizing, background potassium channels [37,38], reduction of gap junction conductance between cells [39], alterations of voltage-gated calcium channel activity [40], increases in calcium release from the sarcoplasmic reticulum [41–44] and depression of the sodium current [36,45–47].

Despite these reports, at doses associated with arrhythmia, we observed little effect of halon alone on cardiomyocyte electrophysiological behavior as measured with the MEA. In our experiments, the lower dose of halon, 25.7 mg/L, corresponded to the arterial blood concentration estimated in dogs after a 5 min exposure to halon 1301 at the cardiac sensitization lowest-observed-adverse-effect level [7]. At that level, we observed no change in the electrophysiological parameters measured, suggesting that halon-mediated changes alone are unlikely to explain cardiac sensitization. By increasing the concentration to 50 mg/L, well above the level associated with arrhythmias in the presence of epinephrine, halon alone induced mild FP prolongation. Assuming the same degree of change in the action potential, this prolongation would be unlikely to increase arrhythmic risk substantially.

4.3. The combination of halon and epinephrine reduced conduction velocity

Unique to the combination, conduction velocity across the syncytium was reduced with the combined exposure to epinephrine and halon. Since reduced conduction velocity is associated with arrhythmic risk [48], this effect may help explain cardiac sensitization. Suggesting an effect on gap junctions, the electrophysiological changes observed in the MEA were similar when comparing the effects of heptanol, a gap junction inhibitor, and the combination of epinephrine and halon. Possibly explaining the observed reduced conduction velocity, non-phosphorylated Cx43 was increased in cells exposed to the combination of agents. Moreover, preventing Cx43 dephosphorylation with okadaic acid prevented the electrophysiological changes seen with epinephrine and halon.

Genetic downregulation of Cx43 is associated with slow conduction and arrhythmogenic death [22], and several reports have demonstrated an inverse relationship between conduction velocity and non-phosphorylated Cx43 [25,26]. Therefore, it seems plausible that phosphorylation-dependent changes in Cx43 cause the gap junction uncoupling that may underlie part of the phenomenon of cardiac sensitization. Nevertheless, additional effects of the combination of halocarbons and epinephrine cannot be excluded, including changes in the localization of Cx43 or effects on other connexins.

Halothane (1,1,1-trifluoro-2-bromo-2-chloroethane), a two-carbon halocarbon inhaled anesthetic, is known to block connexins even in the absence of catecholamines [49]. The relationship between this effect and the one that we describe here is not entirely clear. The usual concentration range for the halothane effect is >1 mM [39,50]. There are no reports associating the halothane effect on connexins with channel dephosphorylation, but this remains a possibility. In our experiments, the maximum concentration of halon used was 0.34 mM, a concentration known to cause cardiac sensitization. It is conceivable that halon would also inhibit connexins independently of catecholamines at higher doses. Consistent with this idea, two-carbon halocarbons have shown a greater proclivity toward cardiac sensitization than one-carbon compounds such as halon [51]. Alternatively, the halon and halothane effects could occur by independent mechanisms. More investigation will be required to discriminate among the possibilities.

Limitations of this study include the fact that we cannot directly demonstrate an arrhythmic effect of halon and epinephrine in our recording system. Moreover, while the expected results with the changes in connexins seen, the similarity to genetic models of connexin downregulation, the lack of change in other field potential parameters, the similarity of responses between heptanol and the combination of epinephrine and halon and the correlation of the prevention of the effect with the lack of connexin dephosphorylation suggest that the majority of the effect is secondary to connexins, we cannot exclude effects on other ion channels that may contribute to arrhythmic risk during cardiac sensitization. Other channels not studied include the voltage-gated sodium and calcium channels. Finally, CV measurements were made without controlling for changes in heart rate. CV is inversely dependent on heart rate, and this relationship is known as the CV restitution curve. The shape of this curve has been defined previously for neonatal rat heart cultures and is steepest at high heart rates [52]. In our study, using the maximum and minimum cycle lengths observed under any conditions, the variance in heart rate could only account for a 9% change in CV, insufficient to account for the effect seen with halon and epinephrine. Moreover, this combination of agents increased the ISI, an effect that should have improved CV.

In summary, the combination of a halocarbon and epinephrine results in a reduction in conduction velocity not seen with either agent alone. The reduced conduction velocity could be explained, at least in part, by reduced Cx43 phosphorylation. These changes were unique to the combination of agents and may help explain cardiac sensitization.

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References

1. Katz RL, Epstein RA. The interaction of anesthetic agents and adrenergic drugs to produce cardiac arrhythmias. *Anesthesiology*. 1968; 29:763–784. [PubMed: 4874157]
2. Reinhardt CF, Azar A, Maxfield ME, Smith PE Jr, Mullin LS. Cardiac arrhythmias and aerosol “sniffing”. *Arch Environ Health*. 1971; 22:265–279. [PubMed: 5099701]
3. Azar A, Trochimowicz HJ, Terrill JB, Mullin LS. Blood levels of fluorocarbon related to cardiac sensitization. *Am Ind Hyg Assoc J*. 1973; 34:102–109. [PubMed: 4715924]
4. Trochimowicz HJ, Azar A, Terrill JB, Mullin LS. Blood levels of fluorocarbon related to cardiac sensitization: II. *Am Ind Hyg Assoc J*. 1974; 35:632–639. [PubMed: 4418796]
5. Mullin LS, Reinhardt CF, Hemingway RE. Cardiac arrhythmias and blood levels associated with inhalation of Halon 1301. *Am Ind Hyg Assoc J*. 1979; 40:653–658. [PubMed: 484490]
6. Brock WJ, Rusch GM, Trochimowicz HJ. Cardiac sensitization: methodology and interpretation in risk assessment. *Regul Toxicol Pharmacol*. 2003; 38:78–90. [PubMed: 12878057]
7. Dodd DE, Kinkead ER, Wolfe RE, Leahy HF, English JH, Vinegar A. Acute and subchronic inhalation studies on trifluoriodomethane vapor in Fischer 344 rats. *Fundam Appl Toxicol*. 1997; 35:64–77. [PubMed: 9024674]
8. Halbach M, Ebert U, Hescheler J, Banach K. Estimation of action potential changes from field potential recordings in multicellular mouse cardiac myocyte cultures. *Cell Physiol Biochem*. 2003; 13:271–284. [PubMed: 14586171]
9. Feld Y, Melamed-Frank M, Kehat I, Tal D, Marom S, Gepstein L. Electrophysiological modulation of cardiomyocytic tissue by transfected fibroblasts expressing potassium channels: a novel strategy to manipulate excitability. *Circulation*. 2002; 105:522–529. [PubMed: 11815438]
10. Meiry G, Reisner Y, Feld Y, Goldberg S, Rosen M, Ziv N, et al. Evolution of action potential propagation and repolarization in cultured neonatal rat ventricular myocytes. *J Cardiovasc Electrophysiol*. 2001; 12:1269–1277. [PubMed: 11761415]
11. Stett A, Ebert U, Guenther E, Hofmann F, Meyer T, Nisch W, et al. Biological application of microelectrode arrays in drug discovery and basic research. *Anal Bioanal Chem*. 2003; 377:486–495. [PubMed: 12923608]
12. Meyer T, Boven KH, Gunther E, Fejt M. Micro-electrode arrays in cardiac safety pharmacology: a novel tool to study QT interval prolongation. *Drug Safety*. 2004; 27:763–772. [PubMed: 15350150]
13. Bursac N, Parker KK, Iravanian S, Tung L. Cardiomyocyte cultures with controlled macroscopic anisotropy: a model for functional electrophysiological studies of cardiac muscle. *Circ Res*. 2002; 91:e45–e54. [PubMed: 12480825]
14. Abraham MR, Henrikson CA, Tung L, Chang MG, Aon M, Xue T, et al. Antiarrhythmic engineering of skeletal myoblasts for cardiac transplantation. *Circ Res*. 2005; 97:159–167. [PubMed: 15976318]
15. Kucera JP, Kleber AG, Rohr S. Slow conduction in cardiac tissue: insights from optical mapping at the cellular level. *J Electrocardiol*. 2001; 34 Suppl:57–64. [PubMed: 11781937]
16. Beauchamp P, Choby C, Desplantez T, de Peyer K, Green K, Yamada KA, et al. Electrical propagation in synthetic ventricular myocyte strands from germline connexin43 knockout mice. *Circ Res*. 2004; 95:170–178. [PubMed: 15192022]
17. Hoffman BF, Siebens AA, Cranefield PF, Brooks CM. The effect of epinephrine and norepinephrine on ventricular vulnerability. *Circ Res*. 1955; 3:140–146. [PubMed: 14352395]
18. Papp JG, Szekeres L. Analysis of the mechanism of adrenergic actions on ventricular vulnerability. *Eur J Pharmacol*. 1968; 3:15–26. [PubMed: 5654666]
19. Beeres SL, Atsma DE, van der LA, Pijnappels DA, van Tuyn J, Fibbe WE, et al. Human adult bone marrow mesenchymal stem cells repair experimental conduction block in rat cardiomyocyte cultures. *J Am Coll Cardiol*. 2005; 46:1943–1952. [PubMed: 16286184]
20. Danik SB, Liu F, Zhang J, Suk HJ, Morley GE, Fishman GI, et al. Modulation of cardiac gap junction expression and arrhythmic susceptibility. *Circ Res*. 2004; 95:1035–1041. [PubMed: 15499029]

21. Gutstein DE, Morley GE, Tamaddon H, Vaidya D, Schneider MD, Chen J, et al. Conduction slowing and sudden arrhythmic death in mice with cardiac-restricted inactivation of connexin43. *Circ Res.* 2001; 88:333–339. [PubMed: 11179202]
22. Eckardt D, Theis M, Degen J, Ott T, van Rijen HV, Kirchhoff S, et al. Functional role of connexin43 gap junction channels in adult mouse heart assessed by inducible gene deletion. *J Mol Cell Cardiol.* 2004; 36:101–110. [PubMed: 14734052]
23. Beardslee MA, Lerner DL, Tadros PN, Laing JG, Beyer EC, Yamada KA, et al. Dephosphorylation and intracellular redistribution of ventricular connexin43 during electrical uncoupling induced by ischemia. *Circ Res.* 2000; 87:656–662. [PubMed: 11029400]
24. Duthe F, Plaisance I, Sarrouilhe D, Herve JC. Endogenous protein phosphatase 1 runs down gap junctional communication of rat ventricular myocytes. *Am J Physiol: Cell Physiol.* 2001; 281:C1648–C1656. [PubMed: 11600429]
25. Akar FG, Spragg DD, Tunin RS, Kass DA, Tomaselli GF. Mechanisms underlying conduction slowing and arrhythmogenesis in nonischemic dilated cardiomyopathy. *Circ Res.* 2004; 95:717–725. [PubMed: 15345654]
26. Ai X, Pogwizd SM. Connexin 43 downregulation and dephosphorylation in nonischemic heart failure is associated with enhanced colocalized protein phosphatase type 2A. *Circ Res.* 2005; 96:54–63. [PubMed: 15576650]
27. Bertel O, Buhler FR, Baitsch G, Ritz R, Burkart F. Plasma adrenaline and noradrenaline in patients with acute myocardial infarction. Relationship to ventricular arrhythmias of varying severity. *Chest.* 1982; 82:64–68. [PubMed: 7083938]
28. Tisdale JE, Patel RV, Webb CR, Borzak S, Zarowitz BJ. Proarrhythmic effects of intravenous vasopressors. *Ann Pharmacother.* 1995; 29:269–281. [PubMed: 7606074]
29. Goodford PJ. Metabolic factors and ventricular fibrillation. *Br J Pharmacol.* 1958; 13:144–150.
30. Murnaghan MF. The effect of sympathomimetic amines on the ventricular fibrillation threshold in the rabbit isolated heart. *Br J Pharmacol.* 1975; 53:3–9. [PubMed: 1125490]
31. Gilbert JL, Lange G, Polevoy I, Brooks CM. Effects of vasoconstrictor agents on cardiac irritability. *J Pharmacol Exp Ther.* 1958; 123:9–15. [PubMed: 13539783]
32. El Sherif N, Gough WB, Zeiler RH, Mehra R. Triggered ventricular rhythms in 1-day-old myocardial infarction in the dog. *Circ Res.* 1983; 52:566–579. [PubMed: 6682722]
33. Shimoni Y, Spindler AJ, Noble D. The control of calcium current reactivation by catecholamines and acetylcholine in single guinea-pig ventricular myocytes. *Proc R Soc Lond, B Biol Sci.* 1987; 230:267–278. [PubMed: 2438702]
34. Yatani A, Imoto Y, Goto M. The effects of caffeine on the electrical properties of isolated, single rat ventricular cells. *Jpn J Physiol.* 1984; 34:337–349. [PubMed: 6088873]
35. Haiech J, Demaille JG. Phosphorylation and the control of calcium fluxes. *Philos Trans R Soc Lond, Ser B: Biol Sci.* 1983; 302:91–99. [PubMed: 6137012]
36. Raatikainen MJ, Trankina MF, Morey TE, Dennis DM. Effects of volatile anesthetics on atrial and AV nodal electrophysiological properties in guinea pig isolated perfused heart. *Anesthesiology.* 1998; 89:434–442. [PubMed: 9710402]
37. Talley EM, Bayliss DA. Modulation of TASK-1 (Kcnk3) and TASK-3 (Kcnk9) potassium channels: volatile anesthetics and neurotransmitters share a molecular site of action. *J Biol Chem.* 2002; 277:17733–17742. [PubMed: 11886861]
38. Stadnicka A, Bosnjak ZJ, Kampine JP, Kwok WM. Modulation of cardiac inward rectifier K⁺ current by halothane and isoflurane. *Anesth Analg.* 2000; 90:824–833. [PubMed: 10735783]
39. He DS, Burt JM. Mechanism and selectivity of the effects of halothane on gap junction channel function. *Circ Res.* 2000; 86:E104–E109. [PubMed: 10850971]
40. Kanaya N, Nakayama M, Kobayashi I, Fujita S, Namiki A. Effect of isoflurane on epinephrine-induced arrhythmias in ischemic-reperfused dog hearts. *Res Commun Mol Pathol Pharmacol.* 1998; 100:181–186. [PubMed: 9667072]
41. Vogel SM, Weinberg GL, Djokovic A, Miletich DJ, Albrecht RF. Analysis of halothane effects on myocardial force-interval relationships at anesthetic concentrations depressing twitches but not tetanic contractions. *Anesthesiology.* 1995; 83:1055–1064. [PubMed: 7486156]

42. Harrison SM, Robinson M, Davies LA, Hopkins PM, Boyett MR. Mechanisms underlying the inotropic action of halothane on intact rat ventricular myocytes. *Br J Anaesth.* 1999; 82:609–621. [PubMed: 10472232]
43. Sauviat MP, Frizelle HP, Descorps-Declere A, Mazoit JX. Effects of halothane on the membrane potential in skeletal muscle of the frog. *Br J Pharmacol.* 2000; 130:619–624. [PubMed: 10821790]
44. Chini EN, Walker H. FK506 (tacrolimus) increases halothane-induced Ca^{2+} release from skeletal muscle sarcoplasmic reticulum. *Anesthesiology.* 2000; 92:1361–1365. [PubMed: 10781282]
45. Sirois JE, Pancrazio JJ CL III, Bayliss DA. Multiple ionic mechanisms mediate inhibition of rat motoneurons by inhalation anaesthetics. *J Physiol.* 1998; 512:851–862. [PubMed: 9769427]
46. Weigt HU, Kwok WM, Rehmer GC, Turner LA, Bosnjak ZJ. Voltage-dependent effects of volatile anesthetics on cardiac sodium current. *Anesth Analg.* 1997; 84:285–293. [PubMed: 9024016]
47. Hayes ES, Barrett TD, Burrill DE, Walker MJ. Effects of halothane and isoflurane on rat ventricular action potentials recorded in situ. *Life Sci.* 1996; 58:1375–1385. [PubMed: 8614295]
48. Kleber AG, Rudy Y. Basic mechanisms of cardiac impulse propagation and associated arrhythmias. *Physiol Rev.* 2004; 84:431–488. [PubMed: 15044680]
49. Burt JM, Spray DC. Volatile anesthetics block intercellular communication between neonatal rat myocardial cells. *Circ Res.* 1989; 65:829–837. [PubMed: 2766493]
50. Wentlandt K, Carlen PL, Kushnir M, Naus CC, El Beheiry H. General anesthetics attenuate gap junction coupling in P19 cell line. *J Neurosci Res.* 2005; 81:746–752. [PubMed: 15971264]
51. Subcommittee on Iodotrifluoromethane, Committee on Toxicology, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council of the National Academies. Iodotrifluoromethane: toxicity review. Washington, DC: The National Academies Press; 2004.
52. Entcheva E, Kostov Y, Tchernev E, Tung L. Fluorescence imaging of electrical activity in cardiac cells using an all-solid-state system. *IEEE Trans Biomed Eng.* 2004; 51:333–341. [PubMed: 14765706]

Fig. 1. Electrophysiological parameters measured using the multielectrode array (MEA). (A) A representative field potential (FP) recording with the parameters measured. (B) A representative activation map. The site of the earliest activation is initiated in the upper right hand corner. The black dots indicate the electrode positions. The lines indicate 1 ms isochrones. (C) Electrophysiological parameter stability over time with perfusion. The FP amplitude, FP duration, the maximum rate of change of the FP ($-dV/dt_{max}$), the inter-spike interval (ISI) and the conduction velocity (CV) were constant with 30 min of perfusion.

Fig. 2.

Epinephrine shortened the inter-spike interval (ISI). The ISI as a function of time and epinephrine concentration normalized for the ISI in control conditions. As expected, the ISI was shortened in a concentration-dependent manner by treatment with epinephrine 10 $\mu\text{g/L}$ ($n=7$, filled circles) and 100 $\mu\text{g/L}$ ($n=11$, open circles) (* $P<0.05$ as compared to control; $P<0.05$ for 10 $\mu\text{g/L}$ as compared to 100 $\mu\text{g/L}$).

Fig. 3.

Halon alone minimally altered cardiomyocyte electrophysiological parameters. The FP amplitude, FP duration, the maximum rate of change of the FP ($-dV/dt_{\max}$), the inter-spike interval (ISI) and the conduction velocity (CV) at 15 min are plotted normalized to the values measured at $t=0$ min. The amplitude of the FP, $-dV/dt_{\max}$, ISI and conduction velocity were unchanged over time during cardiomyocyte exposure to 50 mg/L halon ($P>0.05$, $n=7$). The only statistically significant change was in the FP duration after 15 min ($*P<0.05$, $n=7$).

Fig. 4.

Conduction velocity slowing as a result of exposure to epinephrine and halon. Panel A shows that the FP amplitude, FP duration, the maximum rate of change of the FP ($-dV/dt_{\max}$), the inter-spike interval (ISI) and the conduction velocity (CV) at 15 min are normalized to the values measured at $t=0$ min ($*P<0.05$ compared to epinephrine, $n=9$). Panels B and C show activation maps in the Epi and Epi+halon groups, respectively. Panel D shows that the FP amplitude, FP duration, the maximum rate of change of the FP ($-dV/dt_{\max}$), the inter-spike interval (ISI) and the conduction velocity (CV) at 15 min after exposure to 0.25 mM heptanol normalized to the values measured at $t=0$ min ($*P<0.05$ compared to control, $n=8$ in each case).

Fig. 5.

Preventing dephosphorylation of connexin 43 (Cx43) prevented the electrophysiological effects of epinephrine and halon. Inter-spike interval (ISI, A), and conduction velocity (CV, B) are plotted as a function of time normalized to the values measured at $t=0$ min (* $P<0.05$ compared to epinephrine, $n=8$). Okadaic acid prevented the changes in ISI and CV seen with epinephrine and halon alone. Changes in Cx43 phosphorylation state paralleled the changes in CV. Panel C shows representative Western blots and panel D shows grouped densitometry data for non-phospho-Cx43 (open bars) and total Cx43 (black bars) after exposure to HEPES buffer, epinephrine, the combination of epinephrine and halon (Epi+halon) and the combination of epinephrine (Epi), halon and okadaic acid (Epi+halon+OA). The Cx43 phosphorylation state was unchanged by perfusion with buffer or solutions containing epinephrine. The combination of epinephrine and halon increased the amount of non-phosphorylated Cx43, and the okadaic acid could block Cx43 dephosphorylation. Total Cx43 was unchanged among the four conditions. (* $P<0.05$; $n=3$ for each bar).