Ambient Particulate Matter Poisons Cardiac Ion Channels

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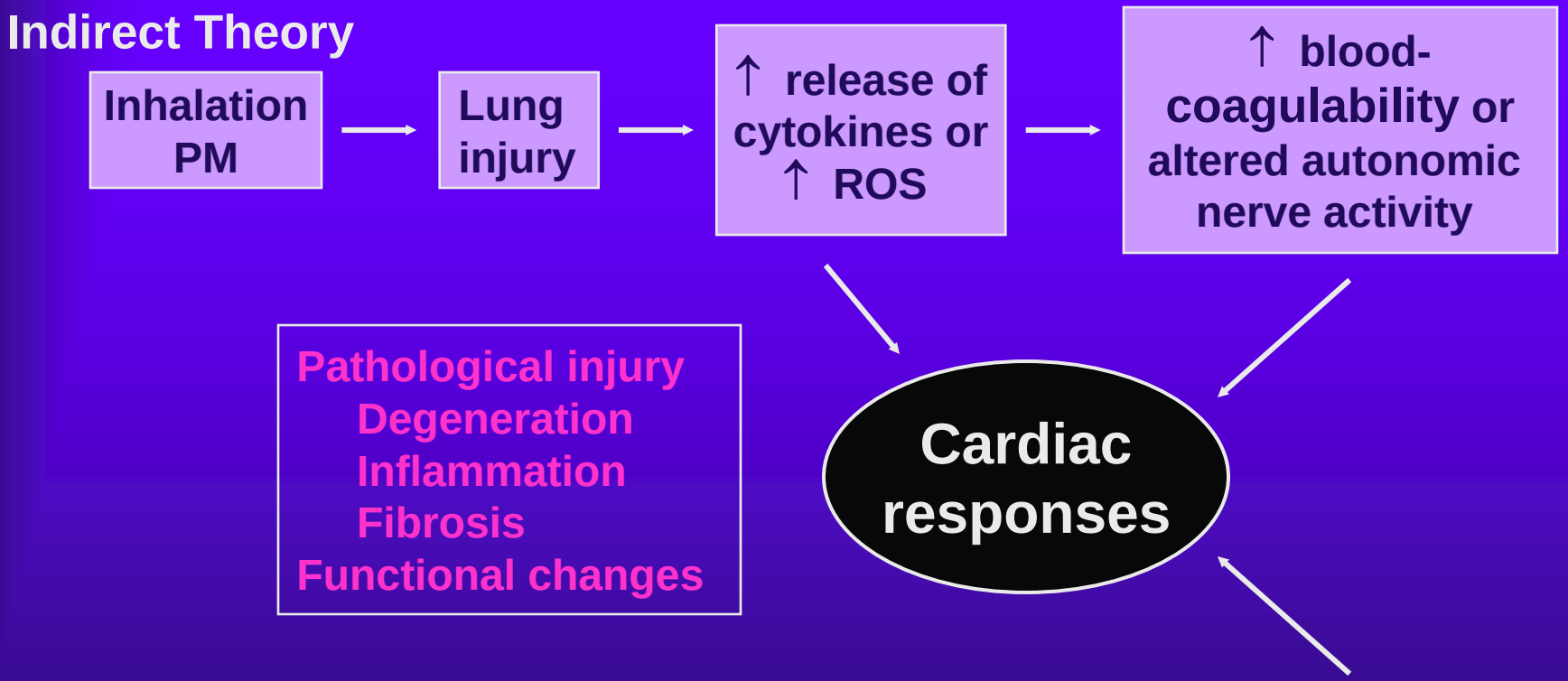
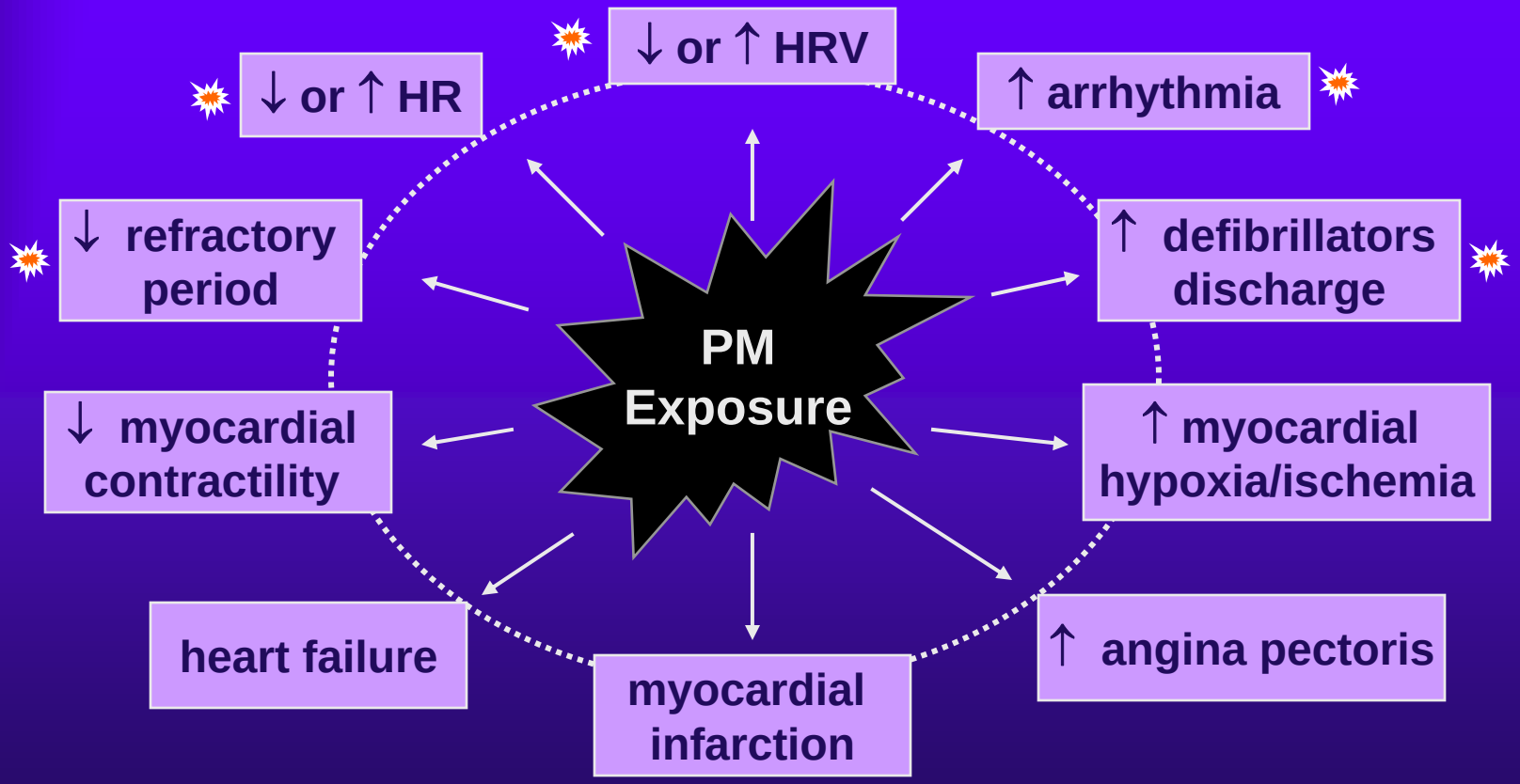
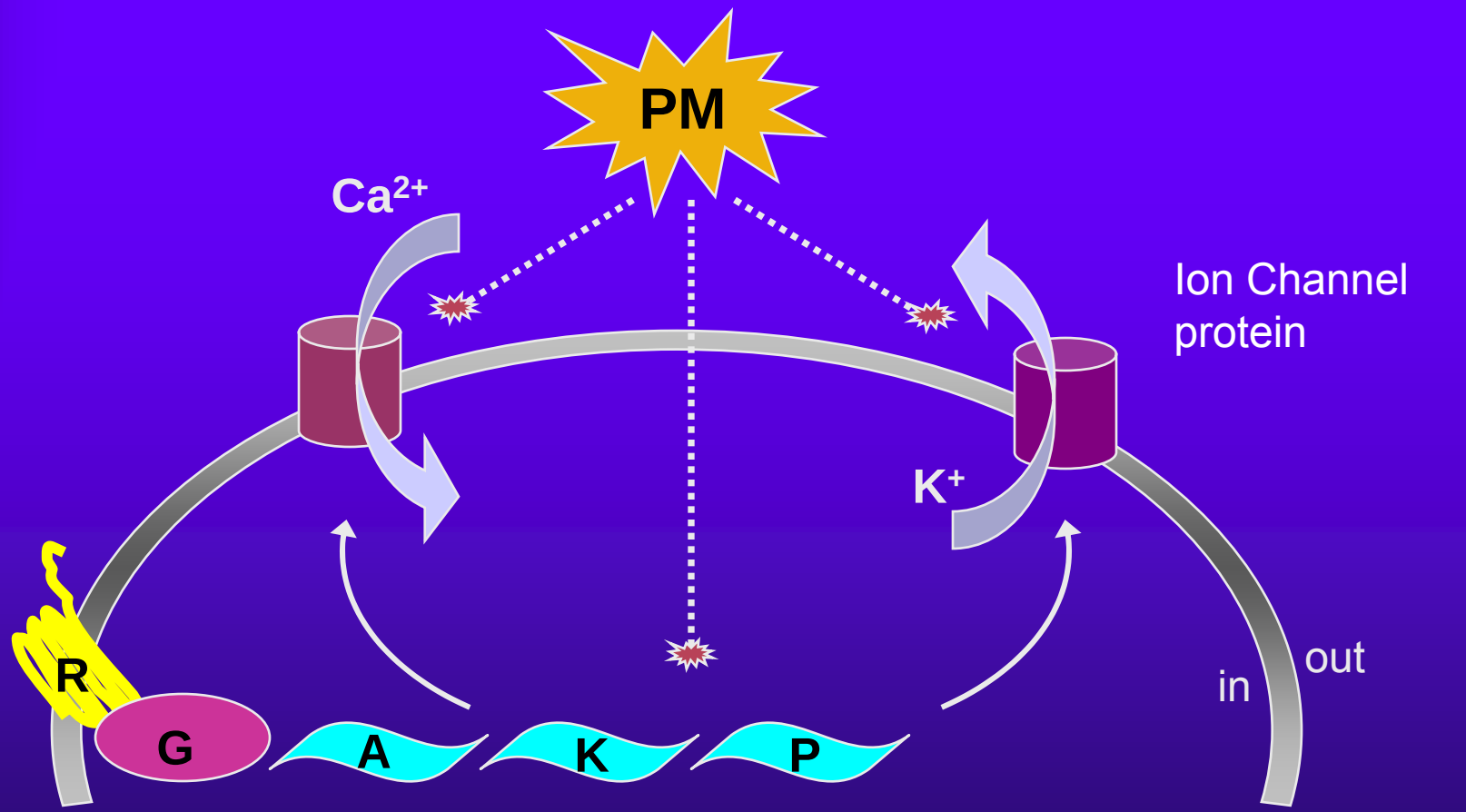
Heart is a target organ of PM exposure



Cardiovascular dysfunction accounts for most of the significant rise in the death rate associated with elevated ambient PM levels



HYPOTHESIS


PM
Ca^{2+}
Ion Channel protein
K^+
R
G
A
K
P
in
out
signaling pathways
Receptors
G-proteins,
Kinases…
cell membrane

Specific Aims

Aim 1. **To study the effects of PM components on cardiac electrical activity in isolated perfused hearts**

Measurements: HR; HRV; AV conduction
Conditions: spontaneous; compromised; modulation

Aim 2. **To study the effects of acute exposure to PM on electrical excitability of single cardiac myocytes**

Measurements: AP waveforms; ERP; trigered activities
Conditions: control; modulation

Aim 3. **To identify the ion channel proteins responsible for PM components-induced alteration of electrical activity**

Measurements: I_{Ca}; $I_{Ca,T}$; I_{kd} (I_{Kur}, I_{Kr}, I_{Ks}); $I_{K,ACh}$; $I_{K,ATP}$
Conditions: control; modulation
Mechanism(s): blockade; signaling pathways; ROS

The long-term goal is to define the cellular mechanisms that underlie PM-induced cardiac toxicity and to establish a profile of the vulnerable cardiac ion channels that respond to exposure to PM constituents.

Experimental Plan

Animal: adult male Wistar-Kyoto (WKY) rats
PM samples: ambient PM (SRM-2738, NIST) and ROFA PM (Niagara Power Plant)
***ex vivo* perfused lung extraction**
liquid solvent extraction (PM:sovent = 100 mg/10 ml; sonicated, centrifuged at 40,000 x g, supernatant will be used)

Table 1. Five types of PM extracts

Name	Solvent	Ratio (mg/ml)	Dilution factor
Lung Extract	K-H buffer	0.001~0.08	1
Water-soluble	H_2O	10	$10^{-5, -4, -3, -2}$
Acid-soluble	1 *N* HCl	10	$10^{-5, -4, -3, -2}$
DMSO-soluble	DMSO	50	$10^{-6, -5, -4, -3}$
Total DMSO	DMSO	50	$10^{-6, -5, -4, -3}$

Experimental Plan (continued)

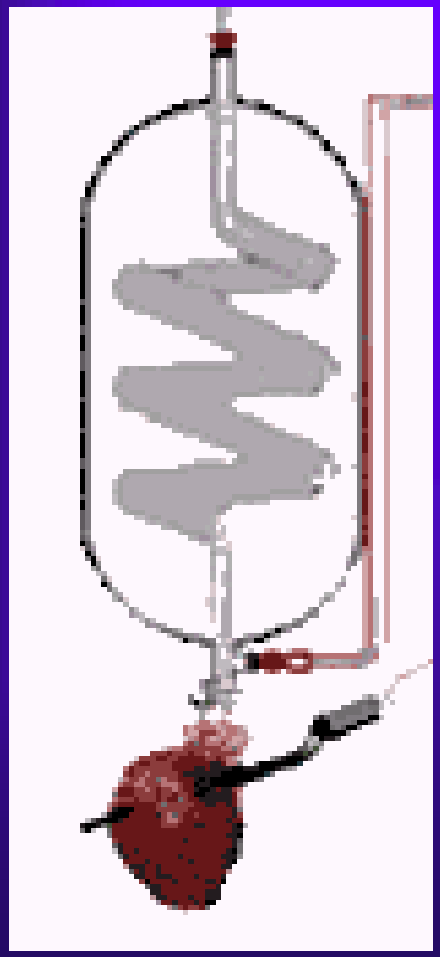
Extracting the toxic components from PM using the *ex vivo* perfused lung preparation

PM (0.3, 2, 8 mg) suspended in 0.5 ml of saline or saline alone will be instilled into the lung to extract dissolvable and penetratable components. The collected eluting perfusate will be applied to cardiac preparations to study the impact on cardiac electrical and ion channel functions

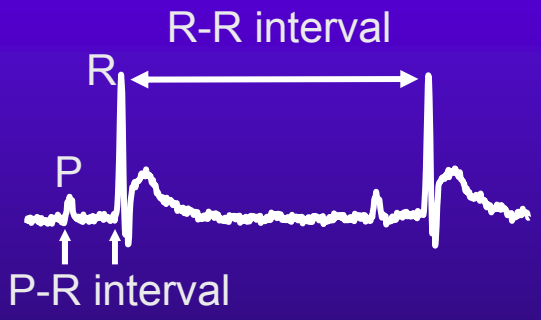
Advantages: 1) mimics the entrance of PM into the lungs from inhalation, therefore the effects of the extract on the heart and single myocyts are relevant to the in vivo inhalation exposure; 2) the integrity of the organ is maintained; and 3) the animals do not suffer from long experimental procedures.

Experimental Plan (continued)

Electrophysiological Studies of Langendorff Hearts



R-R and P-R intervals

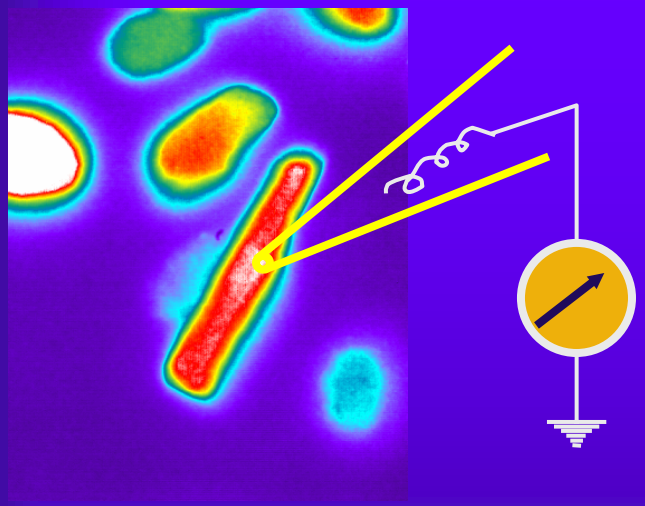


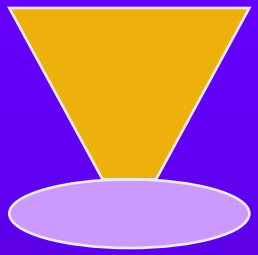
HR
HRV
AV conduction

Pacing/drug modulation

Experimental Plan (continued)

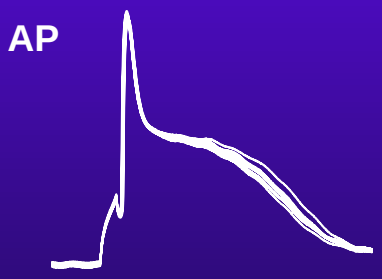
Patch-Clamp Recording

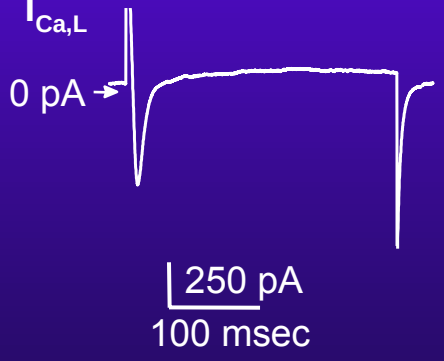


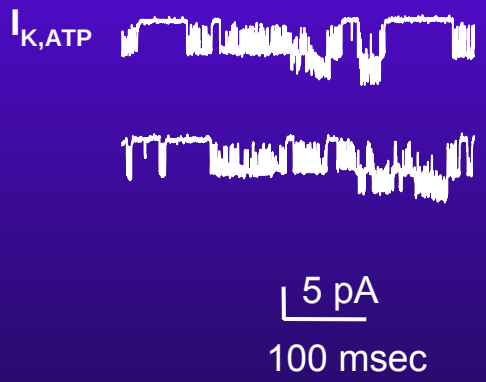


Whole-cell or
Cell-attached

Inside-out



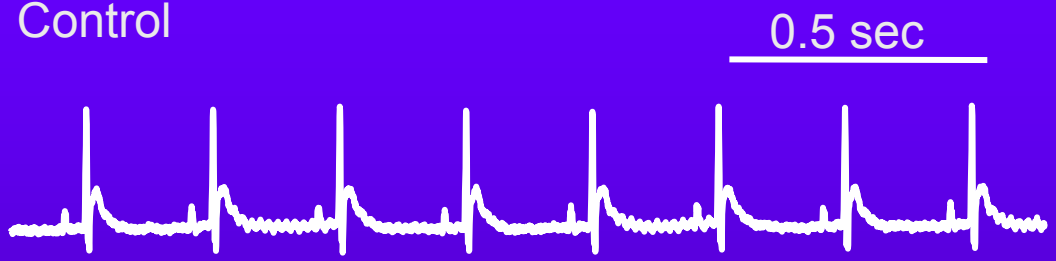




Preliminary studies

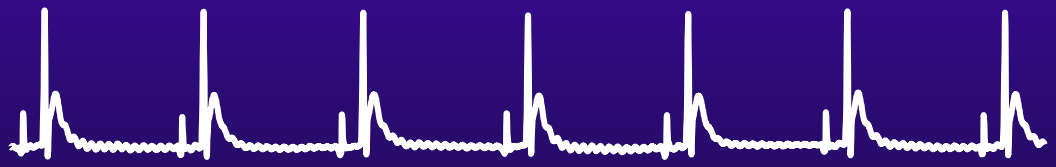
Water-leachable ROFA components slowed siaoatrial beating rate and prolonged AV nodal conduction.

A. Control



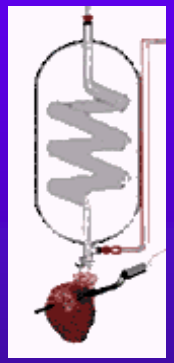
B. 1% water-leachable ROFA-PM

C. Washout



Preliminary studies (continued)

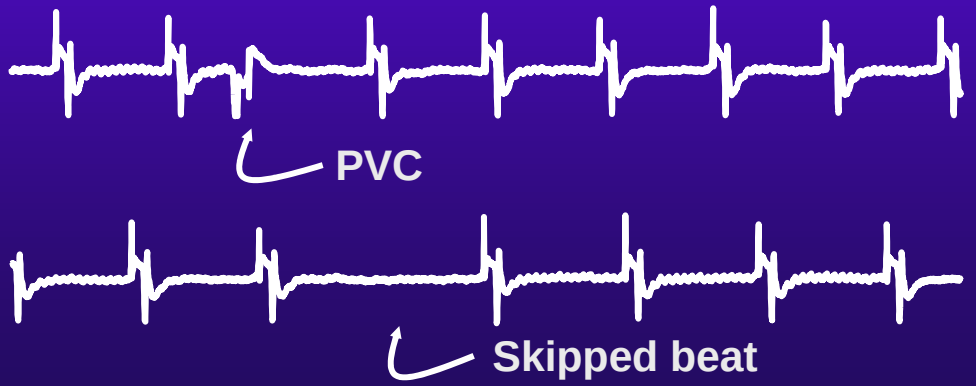
ROFA extract obtained from lung extraction caused a premature ventricular contraction and a skipped sinoatrial beating



A. Control

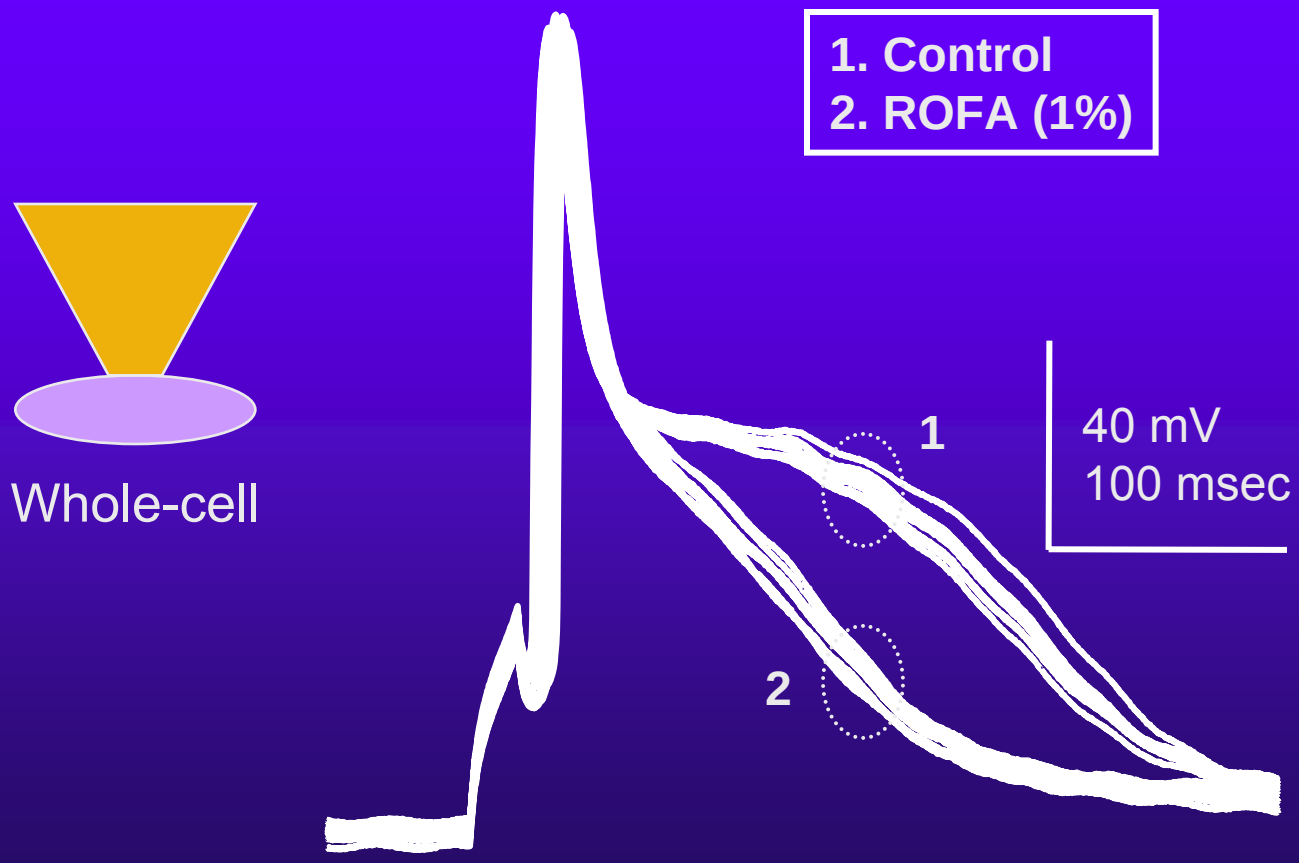
B. Lung extract after saline instillation

C. Lung extract after ROFA instillation



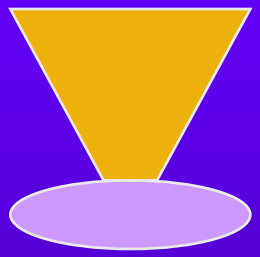
Preliminary studies (continued)

Water-leachable ROFA-PM components on action potential waveforms

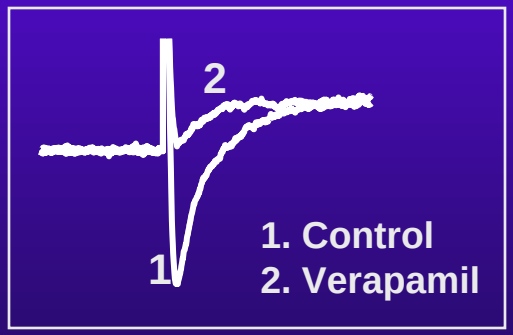


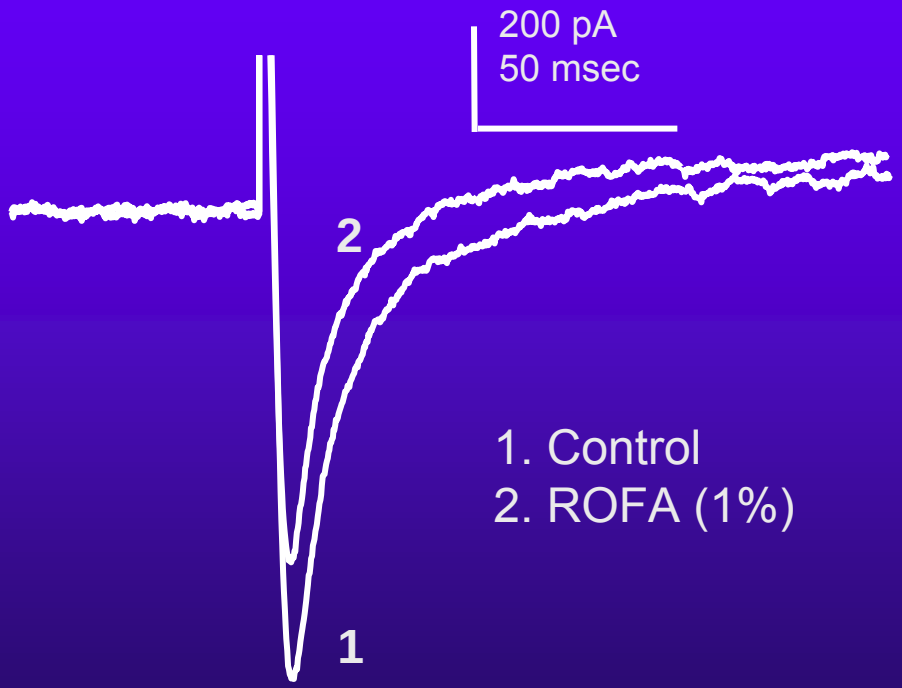
Preliminary studies (continued)

Effect of water-leachable ROFA PM components on L-type Ca^{2+} current ($I_{Ca,L}$) in rat ventricular cells



Whole-cell





College of Pharmacy University of South Carolina

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Water-leachable components of ambient particulate matter alters regulation of cardiac electrical activity by adrenergic and cholinergic agonists

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Ambient particulate matter (PM) exposure triggers acute cardiac events including cardiac arrhythmias, altered heart rate variability, angina pectoris, and myocardial infarction. Cardiac function is closely regulated by autonomic nervous systems. We assessed the hypothesis that water-leachable components of residual oil fly ash (ROFA, a surrogate for ambient PM) disrupt the regulation of cardiac electrical activity by β-adrenergic and muscarinic agonists. Atrial electrograms in Langendorff-perfused rat hearts, action potentials and L-type Ca^{2+} currents ($I_{Ca.L}$) in isolated rat ventricular myocytes were studied, using electrophysiological and patch-clamp techniques, respectively. The ROFA extraction was conducted using dd-H_2O at a ratio of 4 mg:1 ml. The supernatant (40,000g-centrifuge) was used at a final concentration of 1.0%. In the spontaneously beating hearts, β–adrenergic agonist isoproterenol (ISO, 3 nM) increased the beating rate from 251.4 ± 16.6 to 458.7 ± 9.9 beats/min ($p<0.01$, $n=4$). Addition of ROFA (1.0%) completely reversed ISO-caused rate increase (257.0 ± 42.9 beats/min). ROFA also partially reversed ISO-induced shortening of PR interval. In contrast, when the beating rate was decreased by 28.6 ± 5.9% by muscarinic agonist carbachol (CCh, 30nM; $p<0.05$, $n=4$), ROFA (1.0%) additively reduced the beating rate to 46.9 ± 10.3% of control ($p<0.05$) and markedly prolonged the PR interval (from 41.4 ± 1.9 for CCh alone to 62.0 ± 11.3 msec for CCh plus ROFA, $p<0.05$). Patch-clamp recordings indicated that ROFA shortened ventricular action potential duration (APD_{90}, measured at 90% of repolarization) by 25.9±4.1% ($p<0.01$, $n=6$) and reduced $I_{Ca.L}$ density by 15.0 ± 4.1% ($p<0.05$, $n=4$). In conclusion, water-leachable components of ROFA PM can greatly alter the regulation of cardiac function by autonomic nervous systems and the mechanism for this impact appears to be mediated by attenuating $I_{Ca.L}$, at least in part.

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