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Acute Alcohol-induced Protection against Infarction in Rabbit Hearts: Differences from and Similarities to Ischemic Preconditioning

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Departments of [1] Physiology and [2] Medicine, University of South Alabama, Mobile, AL, USA and [3] Department of Pathophysiology, University of Essen Medical School, Essen, Germany

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M. Krenz, C. P. Baines, G. Heusch, J. M. Downey and M. V. Cohen. Acute Alcohol-induced Protection against Infarction in Rabbit Hearts: Differences from and Similarities to Ischemic Preconditioning. *Journal of Molecular and Cellular Cardiology* (2001) **33**, 2015–2022. Recent studies reveal that brief ethanol exposure induces cardioprotection against simulated ischemia in cardiomyocytes by the activation of protein kinase C-ε. The present study tests the ability of ethanol to induce protection in rabbit hearts in which infarct size was the end-point and explores the signal transduction pathways involved. In isolated rabbit hearts, 50 mM ethanol infused for 5 min with 10 min of washout prior to 30 min of regional ischemia reduced infarct size (triphenyltetrazolium chloride staining) by 49%. Neither adenosine receptor blockade with 8-(*p*-sulfophenyl) theophylline nor the free radical scavenger N-2-mercaptopropionyl glycine inhibited the protection triggered by ethanol. In contrast, protein kinase C inhibition with chelerythrine, protein tyrosine kinase inhibition with genistein, and blockade of ATP-sensitive potassium channels (K_{ATP}) with either 5-hydroxydecanoate or glibenclamide did abolish protection. Thus, transient ethanol exposure followed by washout prior to ischemia elicits a preconditioning-like effect involving protein kinase C, at least one protein tyrosine kinase, and K_{ATP} channels, but neither adenosine nor free radicals.

Key Words: Ethanol; Ischemic preconditioning; Protein kinase C; Signal transduction.

Introduction

Acute ethanol exposure was recently reported to protect isolated rat cardiomyocytes and isolated rat hearts.[1] Cardiomyocytes were exposed to 10–50 mM ethanol either with or without washout and then subjected to simulated ischemia by spinning them into a pellet and covering them with microballoons to exclude oxygen. Ethanol exposure with and without washout significantly decreased osmotic fragility in this model. In isolated rat hearts, exposure to 10 mM ethanol before and throughout ischemia was also protective, as assessed by creatine kinase release during reperfusion. The protection induced by ethanol in rat cardiomyocytes could be blocked by the protein kinase C inhibitor chelerythrine and by an ε isoform-specific peptide inhibitor of protein kinase C. These characteristics resemble those of ischemic preconditioning (IPC). Isoform-selective translocation and activation of PKCε is thought to be a critical step in the signaling pathway of both classical[2] and delayed[3] IPC in rabbits. However, it is not known whether cardioprotection induced by ethanol is the result of the same signaling cascade

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as that of IPC or whether activation of PKCε is the only characteristic shared by IPC and ethanol-induced protection.

Recently we tested the ability of ethanol to protect the isolated rabbit heart from infarction and found that transient exposure conferred protection of similar magnitude to that of IPC.[4] Therefore, the present study was designed to further explore the mechanism of the protection triggered by ethanol in an isolated rabbit heart model in which infarct size was the end-point. We tested for the involvement of protein kinase C, protein tyrosine kinases, ATP-sensitive potassium channels (K_{ATP}), free radicals and adenosine, not because any is required for the known biological effects of ethanol, but because all have been implicated in IPC's mechanism.

Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with recommendations published in the *Guide for the Care and Use of Laboratory Animals*, National Academic Press, Washington, DC, 1996.

Isolated hearts

Eighty-six New Zealand White rabbits (1.5–2.6 kg) were anesthetized and prepared with a snare around a major branch of the left coronary artery as described before.[5] The excised heart was then mounted on a Langendorff apparatus and perfused with Krebs–Henseleit buffer ($CaCl_2$ 2.5, NaCl 118.5, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.8, and glucose 10 mM, 37°C). Perfusion pressure was set at 75 mmHg. A saline-filled latex balloon connected to a pressure transducer (Maxxim Medical, Athens, TX, USA) was inserted into the left ventricle and baseline end-diastolic pressure set at 5 mmHg. The hearts were paced at 200 beats per minute if the spontaneous rate was slower.

Risk zone and infarct size

At the end of the experiment the coronary snare of the isolated hearts was retightened and 1–10 μm zinc cadmium sulfide fluorescent particles (Duke Scientific Co., Palo Alto, CA, USA) were infused for delineation of the area at risk. The hearts were frozen, cut into 2-mm transverse slices, incubated for 20 min in 1% triphenyltetrazolium chloride in 100 mM phosphate buffer (pH 7.4, 37°C), and then immersed in 10% formalin. The borders between fluorescent and non-fluorescent areas were marked under ultraviolet light to identify the risk zone. Infarct and risk zone areas were determined by planimetry.

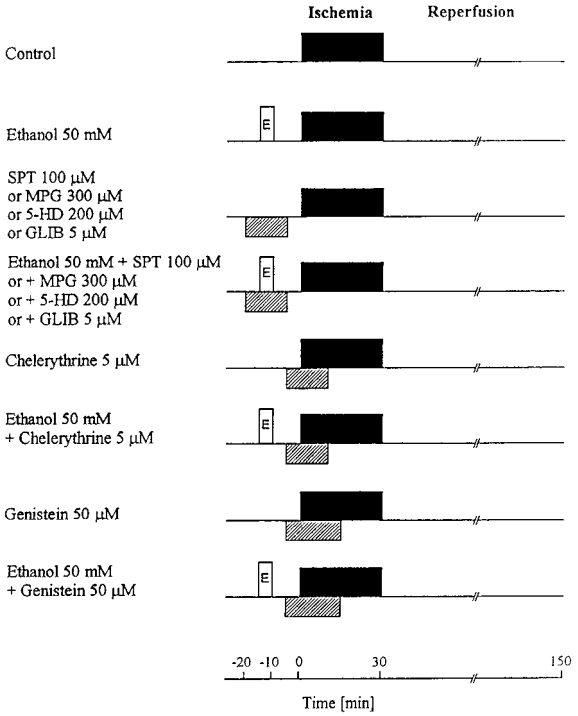

Figure 1 Experimental protocols for the isolated hearts. Timing of the different interventions is shown in relation to the index ischemia. Abbreviations: SPT, 8-(*p*-sulfophenyl) theophylline; MPG, N-2-mercaptopropionyl glycine; 5-HD, 5-hydroxydecanoate; GLIB, glibenclamide.

Experimental protocols

Protocols are depicted in Figure 1. In the control group ($n=6$), hearts experienced only 30 min of regional ischemia followed by 120 min of reperfusion. In the ethanol group, hearts received a 5-min infusion of 50 mM ethanol ($n=6$) followed by a 10-min washout period prior to the 30-min ischemia. This plasma concentration of alcohol is achievable in a human through drinking, and is about three times the legal limit for driving in most states. The SPT group ($n=6$) received the non-selective adenosine receptor antagonist 8-(*p*-sulfophenyl) theophylline (SPT, 100 μM) for 15 min, starting 20 min before the 30-min ischemia so as to bracket the infusion of ethanol. Identical protocols were used in the MPG ($n=6$), 5-HD ($n=6$), and