

## Cardioprotective Effect of Diazoxide Is Mediated by Activation of Sarcolemmal but not Mitochondrial ATP-Sensitive Potassium Channels in Mice

To the Editor:

Recently, Suzuki et al<sup>1</sup> reported that attenuation of myocardial stunning by diazoxide in mice was mediated through activation of sarcolemmal rather than mitochondrial K<sup>+</sup><sub>ATP</sub> channels. Although the authors used an elegant approach by combining both pharmacological tools (utilizing the mitochondrial K<sup>+</sup><sub>ATP</sub>-channel inhibitor 5-hydroxydecanoate and the sarcolemmal K<sup>+</sup><sub>ATP</sub> channel inhibitor HMR-1098) and molecular tools (sarcolemmal K<sup>+</sup><sub>ATP</sub> channel [Kir6.2]-deficient mice), interpretation of the results is difficult.

Thus, the authors claim to have investigated the protective effects of pretreatment with diazoxide against myocardial stunning, produced by 20 minutes of no-flow global ischemia in isolated buffer-perfused hearts. However, stunning is defined as reversible myocardial dysfunction that persists after a brief period of ischemia despite full reperfusion. In isolated buffer-perfused rodent hearts, 20 minutes of ischemia already results in significant cardiac necrosis. For instance, 20 minutes of global cardiac ischemia in mice resulted in 24±4% necrosis of the left ventricle.<sup>2</sup> As an unfortunate consequence of this, the diazoxide-induced improvement in recovery of left ventricular contractile function (during the subsequent 60 minutes of reperfusion) in the wild-type control mice cannot simply be ascribed to attenuation of stunning, because it could also have resulted from limitation of myocardial infarct size.<sup>3,4</sup> The authors should therefore have chosen a brief period of ischemia (<10 minutes) that results in pure stunning without infarction.<sup>5</sup> In such a protocol, an additional group that received diazoxide just before reperfusion would have allowed further delineation between anti-ischemic and possible reperfusion injury-limiting effects of diazoxide in stunning.

Alternatively, the authors could have supported their conclusions by demonstrating a lack of effect of diazoxide on infarct size. This is, however, unlikely in view of the reported infarct size-limiting effect of diazoxide in mice.<sup>4</sup> Rather, studying additional groups subjected to a long period of ischemia (≥20 minutes) would have allowed assessment of the involvement of mitochondrial versus sarcolemmal K<sup>+</sup><sub>ATP</sub> channels in the infarct size-limiting effects of diazoxide.<sup>3,4</sup>

Finally, diazoxide was administered in a concentration of 100 μmol/L. A concentration of 30 μmol/L may already produce a maximal effect, and a further increase to 100 μmol/L may be associated with loss of mitochondrial K<sup>+</sup><sub>ATP</sub> channel selectivity.<sup>3,4</sup> Hence, studying doses of 30 and 100 μmol/L in both brief and long ischemia protocols would have provided valuable additional information on the role of mitochondrial versus sarcolemmal K<sup>+</sup><sub>ATP</sub> channels in the protection by diazoxide against reversible and irreversible myocardial damage in mice.

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### Response

We thank Drs Duncker and Verdouw for their interest in our article<sup>1</sup> and their comments with respect to the interpretation of the results.

Our study was conducted to determine whether diazoxide-induced protective effect on the ischemia-induced contractile dysfunction could be observed in the heart of sarcolemmal K<sub>ATP</sub> channel-deficient mice. The findings of the study suggested that activation of sarcolemmal K<sub>ATP</sub> channels rather than mitochondrial K<sub>ATP</sub> channels is important for the cardioprotective effect of diazoxide in mouse hearts.<sup>1</sup> We measured the left ventricular function during 20-minute ischemia followed by 60-minute reperfusion and found that diazoxide improved the contractile dysfunction in wild-type but not Kir6.2 knockout hearts. The stunned myocardium is defined as “prolonged postischemic contractile dysfunction of myocardium salvaged by reperfusion.”<sup>2</sup> As pointed out by Duncker and Verdouw, the 20-minute global ischemia might have produced irreversible as well as reversible myocardial damage in isolated mouse hearts. The discrimination between reversible and irreversible injuries could not be determined with certainty because we did not conduct histopathological evaluation in the study.<sup>1</sup> However, our study was conducted to determine whether diazoxide could produce cardioprotection in sarcolemmal K<sub>ATP</sub> channel-deficient hearts rather than to evaluate the effects of diazoxide on purely reversible myocardial damage.

We believe that the concentration of diazoxide used in our study (100 μmol/L) might not be too high. Liu et al<sup>3</sup> reported that diazoxide induced reversible oxidation of flavoprotein, an index of mitochondrial K<sub>ATP</sub> channel activation, with an EC<sub>50</sub> of 27 μmol/L in rabbit ventricular cells, and the maximum response could be obtained with 100 μmol/L. They showed that diazoxide at this concentration failed to activate sarcolemmal K<sub>ATP</sub> channels in physiological conditions.<sup>3</sup> Our study,<sup>1</sup> however, showed that diazoxide could activate sarcolemmal K<sub>ATP</sub> channels in a diseased state, possibly with increased ADP level in the cytosol, indicating that the beneficial effects of diazoxide on ischemic myocardium must be interpreted with caution, at least with regard to mouse hearts.

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