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Cardioprotection of the enkephalin analog Eribis peptide 94 in a rat model of ischemia and reperfusion is highly dependent on dosing regimen and timing of administration

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ABSTRACT

Eribis Peptide 94 (EP94) is an enkephalin analog with cardioprotective properties in ischemia and reperfusion. The aim of the present study was to define the optimal timing and dosing of the administration of EP94 during ischemia and reperfusion in a rat model. 172 anesthetized and mechanically ventilated male Sprague–Dawley rats were randomly assigned to different administration protocols of EP94 and subjected to 30 or 40 min of coronary artery occlusion followed by 2 h of reperfusion. EP94 was administered intravenously at different doses and time intervals. Area at risk (AAR) and infarct size (IS) were determined by staining with Evans Blue (EB) and Triphenyl tetrazolium chloride (TTC), respectively. EP94 reduced IS/AAR when administered as a double bolus (0.5 μg/kg per dose), whereas single (1 μg/kg) or triple boluses (0.5 μg/kg per dose) did not confer any protection. Reduction of IS/AAR was of highest magnitude if EP94 was administered 5 and 0 min before the 30 min ischemic period (47% reduction, $P < 0.05$), with declining cardioprotective effect with later administration during ischemia. When EP94 was administered after 15 and 20 min of a 40-min ischemic period, reduction of IS/AAR was of the same magnitude as when given after 5 and 10 min of a 30-min ischemic period. It is concluded that EP94 confers cardioprotection after double bolus administration. The effects are highly dependent on the timing of administration in relation to ischemia and reperfusion. Time of reperfusion from drug administration seems to be more critical than the total duration of ischemia.

1. Introduction

Myocardial ischemia induces tissue injury in a time-dependent manner (DeBoer et al., 1983; Reimer and Jennings, 1979). In the clinical setting, an acute myocardial infarction is caused by an occlusive thrombotic lesion in a coronary artery. Although reperfusion therapy reduces the size of the infarction and subsequent mortality, it may induce cardiomyocyte death. This phenomenon, often referred to as myocardial reperfusion injury, can paradoxically reduce the beneficial effect of myocardial reperfusion (The GUSTO investigators, 1993; Braunwald and Kloner, 1985). Several studies of myocardial ischemia and reperfusion in animal models have demonstrated cardioprotection by different pharmacological compounds (Gross et al., 2004; Louttit et al., 1999; Park et al., 2006). However, these findings have not been unequivocally reproduced in the clinical setting (Yellon and Hausenloy, 2007). In a majority of the studies with pharmacological intervention the drug has been administered intravenously or intracoronary only minutes before, at, or immediately after reperfusion (Atar et al., 2009; Bates et al., 2008; Piot et al., 2008). However, the optimal regimen in terms of timing of pharmacological intervention has not yet been defined.

Opioids have been demonstrated to exert cardioprotection in animal models. Schultz et al. (1996) demonstrated in a rat model of myocardial ischemia and reperfusion that morphine reduced the size of the infarction. Furthermore, Gross et al. (2012) have demonstrated similar cardioprotective effects of the enkephalin analog EP94, an endogenous opioid peptide. We have previously shown a similar cardioprotective effect of EP94, on top of morphine, in a porcine model (Karlsson et al., 2012). In these studies, EP94 was administrated intracoronary just before and during the early phase of reperfusion, or intravenously with repetitive boluses during the early or late phases of the ischemic period. The positive effects of EP94 in terms of reduced

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infarct size have been consistent in different protocols but the magnitude of the effects have varied, raising the question of optimal timing and dosing regimen of EP94, an issue that has not been properly resolved.

The aim of the present study was to define the optimal timing of the administration and dosage regimen of EP94 in a rat model of myocardial ischemia and reperfusion.

2. Materials and methods

The study was carried out at Experimental Bio Medicine at the University of Gothenburg, Sweden. The local ethical committee on animal research approved the studies and animal experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No. 123, Strasbourg 1985).

2.1. Animal preparation

Male Sprague–Dawley (SD) rats, weighing between 250–350 g (Taconic, Denmark) were used in the study protocols. All the rats were housed in cages, four in each, for at least one week before the experiments. During the acclimatization period the rats were provided food and water ad libitum and maintained on a 12:12 h

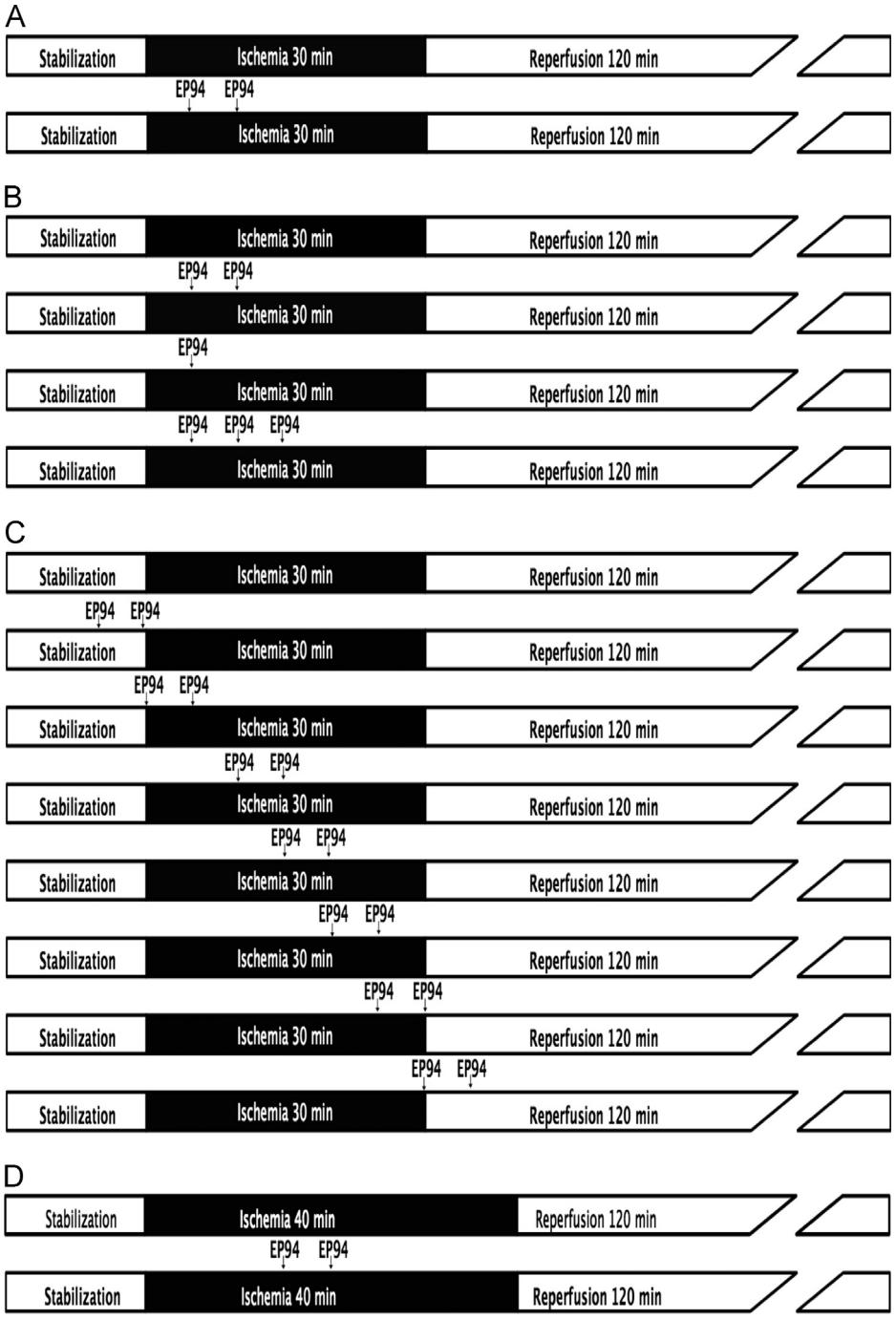

Fig. 1. (A) Protocol I. EP94 0.5 μg/kg given in two doses 5 and 10 min into the ischemia period versus control. (B) Protocol II. EP94 given at different dose and time intervals into the ischemia period versus control. (C) Protocol III. EP94 0.5 μg/kg given in two doses at different time intervals before, during and after 30 min of ischemia period versus control. (D) Protocol IV. EP94 0.5 μg/kg given 15 and 20 min into a prolonged 40-min ischemia period versus control.