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Protection from reperfusion-induced arrhythmias by polyethylene glycol 600

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Abstract—The effects of polyethylene glycol (PEG) 600 on cardiovascular parameters and reperfusion-induced arrhythmias were investigated using a 5-min period of ligation of the left anterior descending coronary artery followed by reperfusion in an anaesthetized open-chest rat model. PEG 600 was effective in reducing reperfusion arrhythmias, such as ventricular fibrillation and ventricular tachycardia. Mortality was decreased from 29·4% (5/17) in the saline-control to 0% (0/14) in the PEG-treated group ($P < 0·05$). Biochemical investigations during the ischaemia/reperfusion period revealed that PEG infusion resulted in a reduction of cardiac lactate as well as a striking maintenance of the glutathione content of the heart.

Polyethylene glycols (PEGs) of different molecular weights are widely used as solvents for the oral and parenteral administration of drugs. Van Stee (1982) concluded that PEG 200 up to PEG 4000 by themselves do not have any remarkable effects on the cardiovascular system, but can modify responses to cardiovascular drugs. Sakuragawa et al (1986) reported a long circulating life of methoxypolyethylene glycol-modified urokinase, which resulted in a sustained activation of fibrinolysis. Following successful thrombolysis in coronary arteries, arrhythmias such as ventricular fibrillation (VF) may occur (Mathey et al 1981). Oxygen-derived free radicals may be involved in these reperfusion-induced arrhythmias (Woodward & Zakaria 1985). We were interested in investigating the effects of PEG 600 on reperfusion-induced arrhythmias using a coronary occlusion/reperfusion rat model.

Materials and methods

Experimental design. Male Wistar rats, 300 ± 10 g, were housed eight to a cage with a bedding of wood shavings. The animals had free access to purchased rat chow and tap water. The experimental conditions were: room temperature 22 ± 2°C, relative humidity 55 ± 10%, and a natural light cycle. We used a modified technique, described originally by Lepran et al (1983) and Hoffmann (1987). The rats were anaesthetized with urethane (0·9 g kg^{-1}). Arterial blood pressure was recorded from the left femoral artery and a catheter was placed into the right jugular vein for the intravenous administration of the substances. Lead II electrocardiogram was recorded throughout the experiments. Following intubation the rats were ventilated with room air (20 mL kg^{-1}, 54 strokes min^{-1}). Arterial pO_2 was measured repeatedly in order to control the ventilation. The chest was opened by a median thoracotomy and the heart was gently exteriorized. A loose ligature was placed around the left anterior descending coronary artery (LAD), near the origin. The heart was replaced and both ends of the ligature were passed through a small plastic tube, and the infusion was started immediately.

Administration of the substances. The first group of rats received a solution of distilled water containing PEG 600 (50% v/v, PEG group). The infusion rate was adjusted to 3 mL kg^{-1} h^{-1}. The second group received the same volume of 0·9% NaCl (saline control).

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Experimental protocol. After a stabilization period of 30 min, regional myocardial ischaemia was produced for 5 min by LAD occlusion. The regional ischaemia was followed by reperfusion, which was performed by reopening the LAD for 10 min.

Evaluation of rhythm disturbances. The ECG was analysed according to the Lambeth Conventions (Walker et al 1988) for: incidence and number of premature ventricular extrasystoles (pVES); incidence of ventricular fibrillation (VF); incidence of irreversible ventricular fibrillation (iVF); incidence of ventricular tachycardia (VT), defined as 4 or more consecutive pVES; and incidence and number of atrioventricular blocks (AV blocks).

Biochemical determinations. After ischaemia and reperfusion, respectively, the hearts were quickly excised by the freeze-clamp technique (Wollenberger et al 1960). The frozen tissue was pulverized under liquid nitrogen using a mortar, and acidified with perchloric acid (0·66 M), homogenized using a Potter-Elvehjem tissue grinder with a Teflon pestle, and neutralized with K_2CO_3 (5 M). Aliquots of the neutral supernatant were withdrawn for analysis. Lactate was determined using lactate dehydrogenase according to Hohorst (1970). Glutathione was extracted and assayed as described by Ellman (1959).

Statistical evaluation. Data were expressed as mean ± s.e.m. The survival rate and occurrence of arrhythmias were analysed by the χ^2-method. Student's *t*-test was used to test whether there were significant differences in blood pressure, heart rate, and the ECG parameters. The biochemical parameters were compared, using the U-test. $P < 0·05$ was considered to show significance.

Results

Blood pressure and ECG parameters. Compared with mean arterial pressure values measured before thoracotomy (107 ± 6 mmHg in the PEG group and 106 ± 4 mmHg in the saline control), surgery and the start of the artificial ventilation resulted in a significant reduction in blood pressure of 18 and 23%, respectively (Fig. 1). As the data show, PEG 600 exerted a

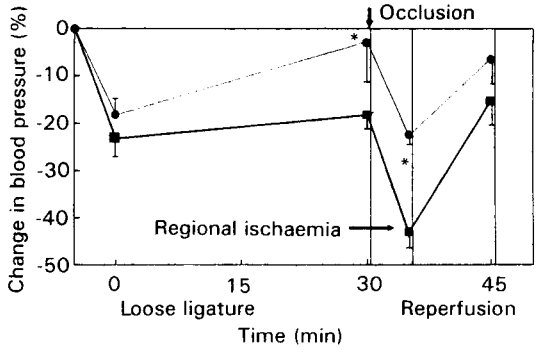

FIG. 1. Effects of PEG 600 on the mean arterial blood pressure. *$P < 0·05$ compared with the saline control. -● PEG group (n = 12), -■- saline control (n = 12–17).

Table 1. Effect of PEG 600 on mortality and incidence of arrhythmias during 10 min reperfusion of the left anterior descending coronary artery.

	Control n = 17	PEG 600 n = 14
Mortality	5	0*
Ventricular fibrillation	11	3*
Ventricular tachycardia	15	8*
Premature ventricular extrasystoles	17	14
Atrioventricular blocks	2	1

*$P < 0.05$ compared with the saline control.

stabilizing effect on the blood pressure. In this way the preligation arterial blood pressure was increased in rats of the PEG group (98 ± 4 mmHg) compared with the saline control (83 ± 3 mmHg). Blood pressure was significantly reduced after coronary occlusion by 20–25% in both groups and returned to pre-ischaemic values after 10 min of reperfusion.

Heart rate declined as a consequence of the thoracotomy from 364 ± 15 to 304 ± 12 beats min^{-1} in the PEG group and from 390 ± 14 to 326 ± 12 beats min^{-1} in the saline control ($P < 0.05$ in both groups). PEG 600 itself had no effect on the heart rate. Table 1 reveals that PEG 600 exerted an antiarrhythmic effect. In comparison with the saline control, mortality (incidence of iVF) was abolished. In the PEG group the incidences of VF and VT were also reduced ($P < 0.05$). The incidence and the number of pVES and AV-blocks were not influenced by PEG ($P > 0.05$). Arrhythmias began after 19 ± 4 and 25 ± 8 s in the PEG group and the saline control, respectively ($P > 0.05$).

The influence of PEG on the PR-interval (atrioventricular conduction) was minimal and there were no significant differences between the groups during the course of the experiment (data not shown).

The initial values of the $R_\tau T$-time (index for intraventricular depolarization/repolarization process) before thoracotomy were 32·5 ± 0·6 ms (PEG group) and 33·0 ± 0·7 ms (saline control, $P > 0.05$). A prolongation of the $R_\tau T$-time was observed in the PEG group before the coronary occlusion (Fig. 2). Myocardial ischaemia was associated with a widening of the $R_\tau T$-interval in the saline control ($P < 0.05$) but not in the PEG group. Ischaemia induced alterations of $R_\tau T$-interval in the saline control were removed after 10 min of reperfusion.

Biochemical parameters. Lactate and glutathione content in the rat hearts are shown in Table 2. The myocardial lactate in untreated anaesthetized rats amounted to 4·14 ± 0·56 μmol (g wet w)$^{-1}$ (n = 10). Myocardial ischaemia resulted in higher lactate in both groups without significant differences between

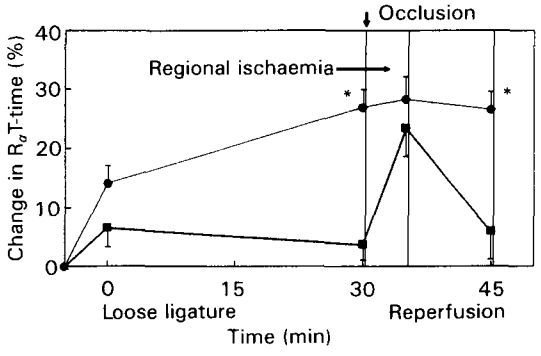

FIG. 2. Effects of PEG 600 on the $R_\tau T$-time. *$P < 0.05$ compared with the saline control. -●- PEG group (n = 14), -■- saline control (n = 12–17).

Table 2. Lactate and glutathione in heart tissue following ischaemia and reperfusion.

	5 min ischaemia			5 min ischaemia followed by 10 min reperfusion		
	n	mean	s.e.m	n	mean	s.e.m.
		Lactate content (μmol (g wet wt)$^{-1}$)				
PEG	11	17·71	1·37	14	5·69*	0·17
Saline	10	16·06	1·43	12	8·82	2·05
		Glutathione content (nmol (g wet wt)$^{-1}$)				
PEG	10	513·0*	42·2	14	180·0*	7·6
Saline	9	183·4	25·7	12	117·4	13·7

*$P < 0.05$ compared with the saline control.

them. After 10 min reperfusion we observed a sharp drop in myocardial lactate content with lower values in the PEG group ($P < 0.05$). In non-ischaemic tissue, glutathione content was 543 ± 69 nmol (g wet w)$^{-1}$ (n = 10). After 5 min of ischaemia the glutathione content decreased in the saline control ($P < 0.05$), whereas it remained unaffected in the PEG group. Reperfusion resulted in a decrease of the glutathione content in both groups ($P < 0.05$), but the values were still higher in the PEG group.

Discussion

The experimental design regarding the duration of ischaemia was chosen from previous experiments and the data available in the literature (Manning & Hearse 1984). The 5-min ischaemia seemed to be most appropriate because the level of mortality (30–40%) and the incidence of VF (60–70%) allowed for changes in either direction to be detected in pharmacological studies.

The present results demonstrate very complex effects of PEG 600 on cardiovascular parameters and reperfusion-induced arrhythmias. Heilman et al (1972) reported that there is no effect of PEG 600 itself on the blood pressure in the anaesthetized dog, but PEG enhanced the blood pressure response to adrenaline, noradrenaline, and acetylcholine. Present results show that there is also stabilization of the blood pressure by PEG under the stress of surgery (see Fig. 1).

The observed fall in blood pressure following ligation confirmed the successful occlusion of the LAD. Reperfusion returned arterial blood pressure to pre-ischaemic values as described by Riva et al (1987). In the present experiments, a less severe arrhythmia in the PEG group was registered as demonstrated by an increased survival rate and reduction of VF and VT. Since PEG reduces blood pressure effects associated with occlusion and reperfusion, homeostasis of blood pressure might be a factor involved in the arrhythmia reduction after reperfusion. Furthermore, there was a diminished lactate production in the hearts of PEG-treated rats. Martorana et al (1987) demonstrated a reduction of cardiac lactate content following ischaemia and reperfusion during administration of the antiarrhythmic substance nicainoprol. They explained these results by the diminished myocardial oxygen consumption due to the decreased occurrence of arrhythmias. An anti-ischaemic PEG effect was also reported by Burke et al (1983), who described a functional protection against acute renal failure by administration of isotonic PEG (300 mOsm L^{-1}).

Regional ischaemia produced by coronary occlusion leads to a prolongation of $R_\tau T$-time as shown in these experiments (saline control, Fig. 2) and previously described by Penny & Sheridan (1983). PEG-infusion prolonged $R_\tau T$-time, also. This effect might be explained by the alteration of membrane fluidity due to PEG (Herrmann et al 1983; Arnold et al 1985) as well as

by the possible Ca^{2+}-chelating action of glycolic and triglycolic metabolites of PEG (Klugmann et al 1981).

There is increasing evidence that ischaemia and reperfusion are related to radical-producing processes (Woodward & Zakaria 1985; Bernier et al 1986) leading to tissue damage (Roth et al 1985). Glutathione acts against oxidative damage by scavenging free radicals. Protection of the glutathione content by PEG 600 may therefore be interpreted as a radical scavenging effect of this substance. In supporting this view Klugmann et al (1981) demonstrated the protection from adriamycin-induced cardiac morphologic alterations in mice by PEG 400. The biochemical mechanism of the cardiotoxic adriamycin action is concerned with the generation of free radicals (Bachur et al 1977; Rajagopalan et al 1988).

In conclusion, our results show that PEG is effective in reducing the severity of reperfusion-induced arrhythmias. The underlying mechanisms are not yet known, but the blood pressure-stabilizing effect, an anti-ischaemic action, a radical mechanism, and the formation of Ca^{2+}-chelating metabolites have to be taken into consideration.

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