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Myocardial necroses produced by intravenous infusion of isoprenaline into rats

R. J. GRYGLEWSKI, A. KULIG AND E. KOSTKA-TRABKA

Department of Pharmacology Medical Academy in Cracow, Department of Pathology WAM in Lodz, Poland

Intravenous infusion of isoprenaline in doses of 2, 20, 200, 2000 μg/kg per min over 20 min into heparinized Wistar rats results in myocardial necroses and a rise in SGPT activity, erythrocyte sedimentation rate and leucocytosis. A quantitative method for estimation of intensity and distribution of myocardial necrotic foci is described. The intensity of myocardial lesions is observed linearly related to the infused isoprenaline at 2, 20 and 200 μg/kg per min. Damaged areas are mainly found in subendocardial and apical regions of left ventricle.

Isoprenaline produces myocardial necroses in rats, but its dosage, routes of injection and the time of observed effects differ among reports. Isoprenaline has been injected subcutaneously (Moudgil, 1960) or intraperitoneally (Sorokina & Altshuler, 1966) in doses ranging from 5 μg/kg (Dorigotti, Gaetani & others, 1969) up to 500 mg/kg or higher (Wexler, Kittinger & Judd, 1967; Leszkovszky & Gal, 1967) in a single (Arigoni, 1969) or repeated doses (Judd & Wexler, 1969). The heart was examined within 15 min (Korb, 1965) and up to 14 days from the moment of the injection (Wenzel, Raymond & Chou, 1966).

We describe a model of myocardial necroses produced by continuous intravenous infusion of isoprenaline. In rats so treated, the distribution and the intensity of myocardial necroses were investigated. The correlation between morphological and biochemical disturbances were also observed. The proposed model may be used for the quantitative evaluation of cardioprotective properties of β-adrenoceptor blocking drugs.

METHODS

Male Wistar rats, 200–220 g, were injected intraperitoneally with heparin (1000 units/kg), narcotized with sodium amylobarbitone (80 mg/kg) and infused intravenously (0·1 ml/rat per min) during a period of 20 min with a solution of (±)-isoprenaline hydrochloride or with saline. Four dose-levels of isoprenaline 2, 20, 200 or 2000 μg/kg per min were administered into four groups of animals. Twenty h later, rats were again given the barbiturate, the animals were bled and hearts excised.

Blood was used to estimate the activity of serum glutaminate-pyruvate-transaminase (SGPT) according to Reitmann & Frenkel (1957), leucocytosis and erythrocytes sedimentation rate (ESR).

Hearts were fixed in 10% neutral formalin and then dissected transversely into four symmetrical thick slices (see Fig. 1), each used to prepare histological sections 5 μm thick. These were stained with hematoxylin and eosin or with oil-red, trichrom according to Masson, PAS according to McManus and acidic fuchsine according to Selye. Test cards with the standardized outlines of four cardiac slices were also prepared. Histological sections of each heart were examined under the microscope

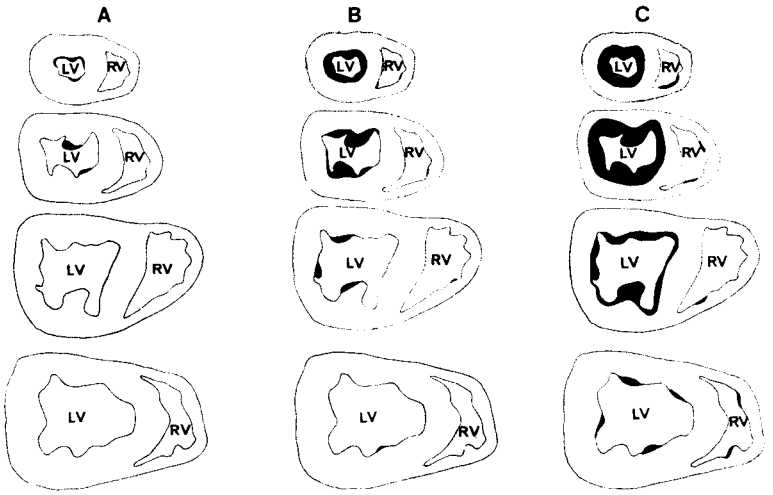

FIG. 1. Test-cards containing the standardized outlines of four cardiac slices. The lesioned areas are marked black. LV—left ventricle, RV—right ventricle, a, b, c, are typical examples of test-cards from rats infused intravenously with isoprenaline 2, 20 and 200 μg/kg per min during 20 min.

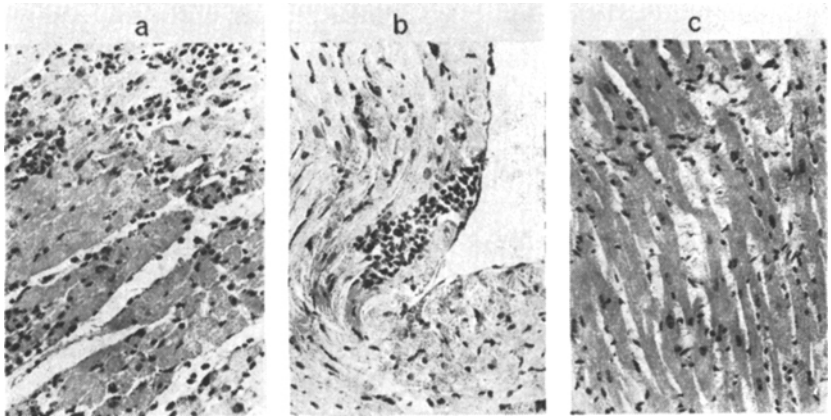

FIG. 2. Histological sections of rat myocardium stained with hematoxylin and eosin, magnification 180× after a 20 min infusion.

a. Subendocardial section of left ventricle of a rat infused with isoprenaline (200 μg/kg per min). Typical mosaic area contains few necrotic or degenerated muscle fibres and leucocytes. Proliferative swelling of stroma is composed of fibroblasts and histiocytes.

b. Endocardial surface of right ventricle of a rat treated with isoprenaline (200 μg/kg per min). Subendocardial necrotic focus is closely packed with granulocytes and histiocytes and surrounded by a monolayer of degenerating muscle fibres.

c. Section of left ventricle of a rat treated with isoprenaline (2000 μg/kg per min). Myocardial fibres have lost their cell structure. Sarcoplasma is swollen and vacuolized. Nuclei are also affected.

and affected areas were drawn into the standardized outlines of corresponding cardiac slices (Fig. 2). Then lesioned areas were cut out and weighed. The intensity of cardiac damage was expressed as the ratio of weight of the lesioned area to the total area in each slice. This gave a quantitative measure and a pattern of distribution of the cardiac lesions induced by different doses of isoprenaline.

The lesions were considered as mosaic areas surrounded by healthy myocardium: they usually contained few necrotic disintegrated muscle fibres but were abundantly

infiltrated with granulocytes and histocytes. In these areas the degenerated muscle fibres could be visualized by trichrom or by PAS staining. Some fibres was oil-red positive. The borderline between healthy and lesioned myocardium were not distinct but the approximate size and shape of the affected area was obvious.

RESULTS

Intravenous infusions of isoprenaline in doses 2, 20 and 200 μg/kg per min for 20 min cause myocardial lesions in rats, and 20 h after the infusion the lesioned area has a mosaic appearance because it consists of necrotized, degenerated and disintegrated muscle fibres mixed with healthy fibres that are embedded in a fibroblastic swelling and infiltration composed of granulocytes and histiocytes (Fig. 2a, b). Isoprenaline, 2 mg/kg per min results in a 50% death rate, the animals showing myocardiocytolysis (Fig. 2c).

The intensity of myocardial lesions is linearly dose dependent over the range 2–200 μg/kg min^{-1}.

The distribution of cardiac lesions is characteristic. All major myocardial lesions are found in the subendocardial area of the left ventricle. The septum is less affected and the right ventricle is almost free, except after 200 μg/kg per min (Fig. 1). About 80% of lesions are in the lower apical half of the hearts. At the 200 μg/kg per min dose some necrotic lesions are noted towards the base of the heart.

Biochemical and haematological changes accompanying heart damage are given in Table 1. The most sensitive but the least specific test for detection of myocardial micronecroses is the estimation of erythrocyte sedimentation rate (ESR). The least sensitive test is SGPT activity. Determination of leucocytosis seems to be fairly reliable as a measure of the severity of morphological disturbances in myocardium.

Pretreatment of rats with heparin is essential to avoid inducing disseminated lung infarcts, which strongly interfere with the development of myocardial lesions.

DISCUSSION

We introduced the intravenous route of administration of isoprenaline because only this route assures the steady concentration of isoprenaline in blood that reaches myocardium and a known duration of drug action.

The period of 20 min of an excessive β-adrenoceptor stimulation proved to be sufficient to induce the damage of myocardium in 100% of animals, even at 2 μg/kg per min of isoprenaline.

We consider that the small scatter of intensity of myocardial lesions within groups of rats and the good linear relation between dose and necrotic response are due to standardized conditions of β-adrenoceptor stimulation that were achieved by use of the intravenous route.

Hearts examined 20 h after an infusion showed the morphological alterations in myocardium precisely the changes correlated well with biochemical and haematological changes in blood (Kowalczykowa, Gryglewski & others, 1971; Gryglewski, Kowalczykowa & others, 1971).

The distribution of cardiac lesions in subendocardial areas of apical region of left ventricle is the most striking observation. The lack of myocardial damage in right ventricle proves that isoprenaline does not pass through the endocardium, but has to be transferred via the coronary circulation to the myocardium. The abundance

Table 1. *The effect of the 20 min intravenous infusion of isoprenaline on serum glutaminate-pyruvate transaminase (SGPT), leucocytosis, sedimentation rate of erythrocytes (ESR) and myocardial lesions in rats after the relapse of 20 h from the moment of isoprenaline infusion.* Figures represent mean value ±s.e. for n animals in a group.

Treatment μg/kg per min	SGPT Wróblewski U	Leucocytosis $\times 10^{-3}$ mm³	ESR mm 2h	Cardiac damage % of affected area
Control ..	22·4 ± 1·13 n = 10	27·3 ± 1·12 n = 80	4·16 ± 0·16 n = 40	0 n = 10
Isoprenaline 2 ..	20·9 ± 2·2 n = 6	31·9 ± 5·4 n = 7	16·0 ± 3·4+ n = 6	6·22 ± 3·58+ n = 6
Isoprenaline 20 ..	24·7 ± 1·51 n = 12	40·5 ± 6·6+ n = 8	13·0 ± 2·6+ n = 8	27·57 ± 5·5+ n = 12
Isoprenaline 200	32·25 ± 3·8+ n = 9	52·2 ± 7·5+ n = 8	11·0 ± 2·4+ n = 8	46·23 ± 1·98+ n = 12
Isoprenaline 2000	18·10 ± 1·6 n = 16	26·6 ± 2·5 n = 8	13·0 ± 2·6+ n = 11	15·69 ± 3·38+ n = 12

+ statistically significant at $P < 0{\cdot}01$.

of coronary blood supply to left ventricle makes the isoprenaline more accessible than right ventricle is.

Subendocardial distribution of micronecroses in the apical region suggests that metabolic and mechanical components are involved in the necrotizing activity of isoprenaline on the myocardium. Isoprenaline results in an "oxygen-wasting effect" in the whole heart (Raab, 1963) but it increases the pressure mostly inside the left ventricle (Boerth, Covell & others, 1969). Jedeikin (1964) found the highest concentration of glycogen and phosphorylase at the endocardial surface of the left ventricle. Therefore it may be assumed that this region of the myocardium is biochemically prepared to be the most reactive to β-adrenoceptor stimulation by isoprenaline. The endocardial surface of the left ventricle reacts most to isoprenaline, but at the same time this region becomes deprived of macroenergic phosphates and oxygen (Boerth & others, 1969), moreover tissue perfusion is decreased and mechanical pressure increased to much higher extent than at the epicardial surface of both ventricles (Myers & Honig, 1966; Hefner, Sheffield & others, 1962). The above facts explain our observation on the susceptibility of endocardial layers to damaging effect of isoprenaline.

There is a practical significance of the data. The prevention of isoprenaline-induced myocardial micronecroses by cardioprotective drugs is usually evaluated semi-quantitatively (Dorigotti & others, 1969; Fleckenstein, 1970). Our method offers a quantitative approach. For such purposes the method can be simplified by using only one slice for histological examination, the second slice from the apex (Fig. 1) being fairly representative for the evaluation of the intensity of cardiac damage.

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