J. Pharm. Pharmac., 1969, **21**, 188–191 *Received July* 22, 1968

Competitive antagonism of isoprenaline-induced cardiac necroses by β-adrenoreceptor blocking agents

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Histological methods show that low doses of (±)-isoprenaline may produce cardiac necroses in the rat. The percentage of animals with focal necroses was related to the dose within the range 0·005 to 0·5 mg/kg administered subcutaneously. Therefore the heart-damaging effect of isoprenaline as well as the antagonistic effect of β-adrenoreceptor blocking agents could be evaluated quantitatively. Pre-treatment of animals with dichloroisoprenaline, pronethalol and propranolol produced a parallel displacement to the right of the dose-response line for isoprenaline. However, when lesions were already in progress, these agents were without protecting effect.

Isoprenaline and other sympathomimetic drugs produce myocardial necroses in the heart of the rat. Isoprenaline-induced necroses are reproducible although the severity of lesions depends on many factors, such as weight, sex, age, and strain of animals.

The criteria used to assess the extent of cardiac necroses, and also the doses used, have differed among authors. According to Rona (1967) high and repeated doses of isoprenaline are needed to obtain consistent lesions.

We show that a single low dose of isoprenaline produces dose-dependent lesions which allow for an easy quantitative evaluation of β-adrenergic blocking activity.

EXPERIMENTAL

Methods

Groups of male Sprague-Dawley rats, 200–220 g, were injected subcutaneously with increasing doses of (±)-isoprenaline hydrochloride in a volume of 0·2 ml/100 g of body weight. At least 10 animals were used for each dose. The antagonist drugs were injected subcutaneously either 15 or 60 min before isoprenaline or 4 h after.

The animals were decapitated 24 h after isoprenaline administration. Hearts, fixed for 24 h in 6·25% phosphate buffered (pH 7·2) glutaraldehyde solution, were embedded in jelly. Cryostatic frontal sections (10 μm) including the ventricles and the septum were stained with sudan-black.

Two contiguous sections of each heart were examined by two different observers and the hearts were considered lesioned when at least one focus of necrosis was found by both observers. No attempt was otherwise made to grade the severity of the lesions.

The percentage of animals with lesions was plotted against the dose on probit-log paper. Statistical analysis of results was according to the method of probits analysis of Finney (1952).

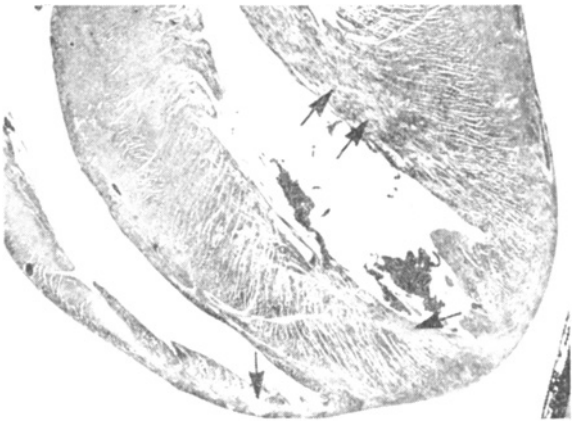

FIG. 1. Frontal section of the heart of the rat. Several cardiac necroses, indicated by arrows, after isoprenaline (0·180 mg/kg s.c.) (×4).

RESULTS

Administration of a single dose of isoprenaline to rats caused necrotic lesions in the cardiac ventricles (Fig. 1). The morphological picture 24 h after isoprenaline injection was typical of focal necrosis. Loss of cell structure and a decrease of sudanophilic droplets at the muscle fibre were associated with infiltration of leucocytes (sometimes rich in lipidic material) in the affected areas (Fig. 2).

The dose of isoprenaline active in 10% of animals was as low as 0·005 mg/kg subcutaneously, while at 0·5 mg/kg, 100% of animals showed numerous focal lesions in each histological section.

The percentage of animals with lesions was related to the dose of isoprenaline, as illustrated in Fig. 3, where satisfactory dose-response curves for isoprenaline, alone and in the presence of different doses of dichloroisoprenaline, pronethalol, and propranolol, are shown. The dose-response lines for animals treated with different doses of β-adrenoreceptor blocking agents were parallel and displaced to the right.

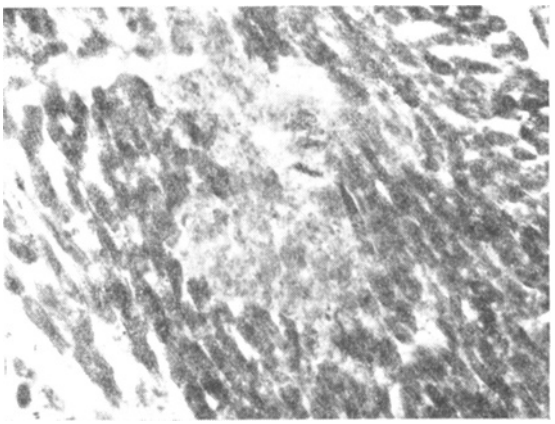

FIG. 2. A focus of cardiac necrosis. Hearts showing at least one of this kind of focus were considered to have lesions. (×250).

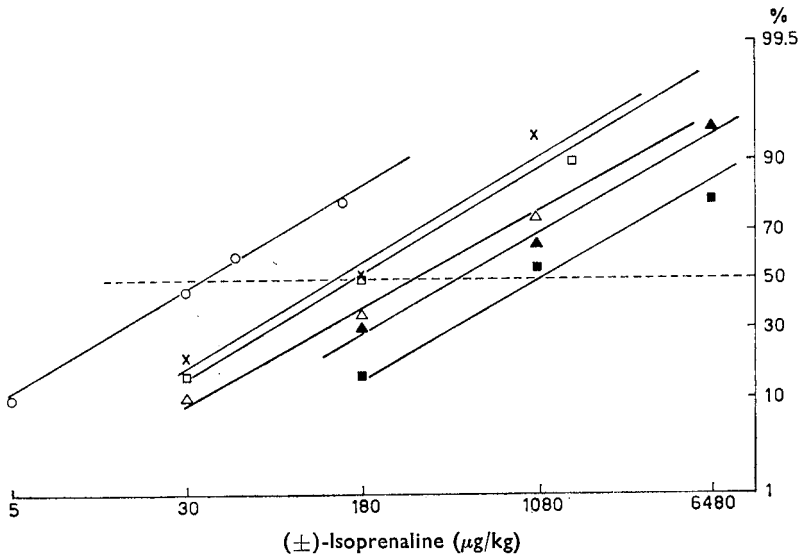

FIG. 3. Dose response curves of isoprenaline (○), isoprenaline after dichloroisoprenaline 5 mg/kg (×), isoprenaline after pronethalol 5 mg/kg (□), isoprenaline after propranolol 2 mg/kg (△), isoprenaline after propranolol 5 mg/kg (▲), and isoprenaline after pronethalol 25 mg/kg (■).

Table 1. *Dose of isoprenaline producing cardiac lesions in* 50% *of the animals* (*ED*50) *in the presence of β-adrenergic blocking agents administered* 15 *min before the agonist*

Antagonist and dose	Isoprenaline ED50 (μg/kg)	Straight line equation
	33·7 (21·7–63·5)*	y = 2·358 + 1·681 x
Dichloroisoprenaline 5 mg/kg ..	143 (72·5–284)*	y = 1·454 + 1·645 x
Propranolol 2 mg/kg	311 (151–638)*	y = 1·376 + 1·454 x
„ 5 mg/kg	487 (250–974)*	y = 0·817 + 1·557 x
Pronethalol 5 mg/kg	166 (83·5–333)*	y = 1·381 + 1·629 x
„ 25 mg/kg	1140 (485–2704)*	y = 0·983 + 1·313 x

* Confidence limits for $P = 95\%$.

Potencies of antagonistic drugs are shown in Table 1.

The pretreatment of the rats with high doses of dihydroergocryptine (20 or 40 mg/kg, s.c., 15 min before 0·5 mg/kg, s.c., of isoprenaline) or of phenoxybenzamine (5 or 25 mg/kg, s.c., 15 and 60 min before isoprenaline) did not reduce the necrotic effects of isoprenaline.

In the presence of β-adrenoreceptor blocking agents, the ED50 for isoprenaline was raised from 33·7 to 1140 μg/kg, for instance, in animals pretreated with 25 mg/kg of pronethalol.

As can be seen from the Table, propranolol was the most active antagonist. On the other hand, α-adrenoreceptor blocking agents were without effect.

Groups of animals treated 4 h after isoprenaline with 5 mg/kg of propranolol, or with 25 mg/kg of pronethalol, showed focal necroses of the type described above 20 h later.

DISCUSSION

From the present study it is evident that myocardial necroses may be produced in the rat by a single administration of isoprenaline and by dosages smaller than those used previously (Leszokovszky, Gal & Tardos, 1967). Hence, isoprenaline may induce severe myocardial lesions even in the range of doses of pharmacological and therapeutic importance.

The percentage of animals with lesions was directly related to the dose of isoprenaline. While the antagonistic effect of β-adrenoreceptor blocking agents could be confirmed by our results (Méhes, Raykovits & Papp, 1966) we showed, in addition, that dichloroisoprenaline, pronethalol, and propranolol caused a parallel displacement of dose response lines for isoprenaline in proportion to the potencies of the blocking agent. Hence, β-blockers seem to act as competitive antagonists not only to the pharmacological but also to the morphological effects elicited by the β-adrenoreceptor stimulant agent, isoprenaline. However this type of antagonism was evident solely in animals pretreated with β-blockers and not when lesions were already in progress (Ferrans, Hibbs & others, 1964), i.e. when the blocking drugs were injected 4 h after isoprenaline administration.

The antagonistic effects of phenoxybenzamine and dihydroergocryptine were also investigated. Neither prevented the development of isoprenaline-induced lesions, although according to Méhes & others (1967), lesions produced by α-receptor stimulants (adrenaline, noradrenaline, phenylephrine, and methoxamine) are inhibited by dibenamine.

In conclusion our results have shown that isoprenaline-induced cardiac necroses may be put on a quantitative basis by plotting numbers of animals with at least one histological lesion against doses of isoprenaline.

Since known β-adrenoreceptor blocking agents were shown to possess specific and dose-related antagonistic properties, the procedure, as modified by us, could be used to study the potency and the type of antagonism induced by other β-blockers.

Acknowledgement

The authors wish to thank Miss O. Bellini for her valuable technical assistance.

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