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Observations on isoprenaline-induced myocardial necroses

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Isoprenaline hydrochloride in subcutaneous doses of 2 × 10 to 2 × 680 mg/kg caused histologically detectable, characteristic heart necroses. Pathological changes were dose-dependent. The degree of necrosis was assessed by a quantitative histological method based on counting the changed morphological elements. The heart necroses due to 2 × 40 mg/kg of isoprenaline were significantly inhibited by pretreatment with different monoamine oxidase inhibitors (iproniazid, a derivative of hydrazine and pargyline and E-250 of the non-hydrazine type, all at 7 × 50 mg/kg intraperitoneally); their severity was significantly increased by reserpine (7·5 mg/kg/48 hr subcutaneously) and guanethidine (2 × 30 mg/kg subcutaneously). In these effects an important role is attributed to respective changes in catecholamine levels.

REPEATED injections of isoprenaline are claimed to produce characteristic myocardial necroses in rats (Chappel, Rona, Balázs & Gaudry, 1959a; Rona, Chappel, Balázs & Gaudry, 1959; Rona, Kahn & Chappel, 1963). The same effect has also been demonstrated in golden hamsters and cats, but not in dogs or domestic pigs (Rosenblum, Wohl & Stein, 1965a). Rats with isoprenaline-induced heart damage have been the subject of several experimental studies (Selye, Veilleux & Grasso, 1960; Wexler & Kittinger, 1963, 1965; Beznák & Hacker, 1964; Strubelt & Breining, 1964; Kako, 1965). Drugs of different kinds, like thyreostatics (Chappel, Rona & Gaudry, 1959b), monoamine oxidase inhibitors (Zbinden, 1962; Zbinden & Bagdon, 1963), β-adrenergic blocking agents (Ehringer & Gögl, 1963) and, recently, a trinitro-compound (Scriabine & Stebbins, 1966) have all been reported to mitigate the myocardial changes caused by isoprenaline.

We have made a quantitative histopathological evaluation of isoprenaline-induced cardiac necroses. We have also examined whether monoamine oxidase inhibitors not derived from hydrazine afforded protection against isoprenaline-induced heart necroses, as has been reported of monoamine oxidase inhibitors of the hydrazine type (Zbinden, 1962). Finally, reserpine and guanethidine were also used in our experiments, as they have some effects contrary to those of monoamine oxidase inhibitors.

Material and methods

Male albino rats, 150–250 g, from an inbred colony were given doses of isoprenaline subcutaneously on two occasions at intervals of 24 hr. The drug was always freshly dissolved in isotonic saline and the volume adjusted to 0·2 ml/100 g weight. The animals were killed by ether anaesthesia 24 hr after the second injection. The hearts were fixed in formalin and paraffin sections stained with haematoxylin and eosin.

The following drugs were used: (±)-isoprenaline hydrochloride,

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iproniazid hydrochloride, pargyline hydrochloride, E-250 (phenylisopropylmethylpropinylamine hydrochloride; Knoll, Ecsery, Kelemen & others, 1965), guanethidine sulphate, reserpine (Rausedyl, 2·5%; Richter).

Guanethidine was injected twice, in doses of 30 mg/kg subcutaneously, always 6 hr before the injections of isoprenaline. Reserpine, 5 and 2·5 mg/kg, was given subcutaneously 48 and 24 hr before the beginning of the isoprenaline treatment. The monoamine oxidase inhibitors, 50 mg/kg, were given daily intraperitoneally for 7 days; the administration of isoprenaline began 1 day after the last injection. The doses of all drugs except reserpine refer to the salts.

Histopathological changes were evaluated by a quantitative histological method (Gál, Leszkovszky & Lendvai, 1966) in which a numerical index is made of smaller or greater areas of necrosis, interstitial loosening or cellular infiltration; the index of severity indicating the grade of changes in the heart is obtained by converting the counted numerical values in 1 cm^2 of a section of myocardium. In each experimental group the mean value and standard error of the single animal's index numbers were calculated. Different groups were compared by means of Student's *t*-test.

Results

MYOCARDIAL LESIONS AFTER ISOPRENALINE

Gross anatomical necrosis was not seen after doses ranging from 2 × 10 to 2 × 680 mg/kg of isoprenaline in the hearts of some 200 rats.

Microscopically detectable lesions were found and were similar to those described by others in that in addition to the necrotic changes a great number of round cells and fibroblasts representing reactive-reparative processes were found in the myocardium. No pathological changes of the coronary vessels were seen. There was a well-defined dose-response myocardial pathology (Table 1).

TABLE 1. QUANTITATIVE HISTOLOGICAL EVALUATION OF HEART NECROSES INDUCED BY ISOPRENALINE

No.	Pretreatment			Isoprenaline mg/kg s.c.	Number of animals	Index number of severity	
	Drug	Dose mg/kg	Route			mean value	s.e.
1	—			2 × 10	10	2·86	0·47
2	—			2 × 40	41	16·15	1·84
3	—			2 × 160	10	21·47	3·46
4	—			2 × 680	12	31·72	4·74
5	Iproniazid	7 × 50	i.p.	2 × 40	9	9·87	1·72*
6	Pargyline	7 × 50	i.p.	2 × 40	9	6·80	1·99*
7	E-250	7 × 50	i.p.	2 × 40	9	5·94	1·28*
8	Guanethidine	2 × 30	s.c.	2 × 40	20	26·82	4·46†
9	Reserpine	5 + 2·5	s.c.	2 × 40	7	28·80	4·46†

* Differs significantly from group No. 2. $P < 0.001$.
† Differs significantly from group No. 2. $P < 0.02$.

DRUG EFFECTS ON THE NECROSES

The influences of drugs on the effect of 2 × 40 mg/kg isoprenaline were examined, since the same dose had been chosen for various experi-

ments by others (Beznák & Hacker, 1964; Kako, 1965). The results of the quantitative histological evaluation are listed in Table 1.

The index numbers of severity in the three groups pretreated with 7×50 mg/kg doses of the different monoamine oxidase inhibitors are less than in the control group and these differences are statistically significant ($P < 0{\cdot}001$).

The results obtained in the rat groups pretreated with $5 + 2{\cdot}5$ mg/kg reserpine and 2×30 mg/kg guanethidine, respectively, showed aggravation of the histopathological changes. The difference from controls for both drugs is statistically significant ($P < 0{\cdot}02$).

Discussion

Of the numerous substances known to induce myocardial necroses in experimental animals, such as digitalis (Dearing, Barnes & Essex, 1943), vasopressin (Dearing, Barnes & Essex, 1944), amphetamine (Halpern, Morard & Drudi-Baracco, 1962), acetylcholine, adrenaline, noradrenaline, methoxamine or plasmocid (Bajusz & Jasmin, 1963; Rona & others, 1963; Rosenblum, Wohl & Stein, 1965a), isoprenaline seems to have aroused the greatest interest. This can be understood, because cardiac necroses after isoprenaline can be produced quickly and simply in the rat.

This pathology has been proposed (Rona & others, 1959, 1963; Zbinden & Bagdon, 1963) as a model of myocardial infarction. On the other hand, the analogy of isoprenaline-induced necroses with human diseases and the usefulness of such experiments have been denied by Strubelt & Breining (1964). Clementi, Nidiry & Peracchia (1964) suggested that the action of isoprenaline was not mediated by any anoxic state but was exerted directly on contractile elements of the heart. Nevertheless, because isoprenaline is probably the most potent of the catecholamines in increasing the metabolism of the heart (Hornbrook & Brody, 1963; Winterscheid, Bruce, Blumberg & Merendino, 1963) and thus the demands on oxygen, the relationships of relative hypoxia and isoprenaline-induced myocardial necroses cannot be overlooked (Rona & others, 1963; Rosenblum & others, 1965b). The catecholamine-induced increase in heart metabolism is, in the view of Raab (1963), of decisive importance in the pathogenesis of human degenerative heart changes. We therefore believe that this type of experiment may be worth attention.

The necroses could be inhibited in our experiments by pretreatment with three different monoamine oxidase inhibitors. Our iproniazid experiment confirms those of Zbinden (1962) and contrasts with the results of Strubelt & Breining (1964). One question to be considered is whether the protection afforded by the monoamine oxidase inhibitors depends on the inhibition of this enzyme or whether it is due to a pharmacological action of the molecule independent from interference with monoamine oxidase (Timsit, 1965). Our results show both pargyline and E-250 to afford significant protection against the action of isoprenaline, contrary to the observation mentioned by Zbinden & Bagdon (1963), that isoprenaline-induced heart necroses could not be inhibited

by pargyline. Thus we believe that the protective action of different monoamine oxidase inhibitors may be interpreted as a consequence of changes in the monoamine metabolism due to the inhibition of the enzyme. This may lead to such an effect, e.g. by improvement of myocardial energy production, as has recently been proposed for the mechanism of the anti-anginal action of monoamine oxidase inhibitors by Pletscher (1966).

Reserpine and guanethidine increased the severity of myocardial necroses. These two drugs are known to release the catecholamines from various stores including those in myocardium (Krayer, Alper & Paasonen, 1962). According to our view the aggravation of isoprenaline-induced necroses is to be explained by the supersensitivity of the catecholamine-depleted tissues which has been demonstrated for catecholamines in general (Burn & Rand, 1958; Maxwell, Povalski & Plummer, 1959; Holtz, Osswald & Stock, 1960) and isoprenaline in particular (Schmitt & Schmitt, 1960; Lee & Yoo, 1964). Thus, after these pretreatments isoprenaline makes even larger demands on metabolism and consequently, pathological changes will become more serious. Though the heart-depressing action of reserpine (Withrington & Zaimis, 1961; Nayler, 1963) may be considered as a potential co-factor in the aggravation of the necroses, we would attribute the major role to the above changes related to catecholamines.

In a similar way, it is known that heart necroses induced by amphetamine are not aggravated but abolished by reserpine (Halpern, & others, 1962). This observation supports our view, because amphetamine, differing from isoprenaline, is considered to belong to the group of indirectly acting sympathomimetic agents (Trendelenburg, Gomez Alonso de la Sierra & Muskus, 1963). Thus, after depletion of catecholamine stores by reserpine, amphetamine's cardiovascular actions are abolished and consequently, no necroses can be produced. Isoprenaline, on the other hand, is a directly acting catecholamine, sensitivity to which is not diminished but increased by depletion of tissue catecholamine stores.

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