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Absence of potentiation of phenylephrine-induced cardiac necroses by theophylline. Selective inhibition by dihydroergocryptine and nicergoline

In rats the cardiac necrotizing activity of low doses of isoprenaline is competitively inhibited by β-adrenoceptor blocking agents (Dorigotti, Gaetani & others, 1969) and is potentiated by theophylline as shown by Martorana (1971) who has suggested a mediatory role for cyclic AMP in the isoprenaline-induced cardiac necroses.

We now report that focal necroses induced by a pure α-adrenoceptor agonist phenylephrine, are not potentiated by theophylline but inhibited selectively by the α-adrenoceptor blocking agents dihydroergocryptine and nicergoline.

The experimental procedure was as described by Dorigotti & others (1969) and by Martorana (1971).

Twenty-four h after administration of phenylephrine the hearts of the treated rats showed focal isoprenaline-like necroses consisting of loss of cross-striation, fragmentation and marked vacuolization of the muscle fibres, infiltration of mononuclear inflammatory cells and of leucocytes.

The percentage of animals with lesions was related to the dose of phenylephrine at least in the range from 1 to 6 mg/kg (s.c.).

The two α-adrenoceptor blocking agents dihydroergocryptine and nicergoline (Arcari, Dorigotti & others, 1968), when administered shortly before the agonist, reduced the incidence of lesions in a dose-dependent way. In the presence of antagonists there was a shift to the right of the dose-response curves and the ED50 of phenylephrine increased many times (Table 1).

In contrast to these findings, high doses of propranolol had no antagonizing action to phenylephrine, while neither dihydroergocryptine nor nicergoline protected the rats from isoprenaline, in accordance with our previous results.

Theophylline, administered as aminophylline, was tested in several groups of rats according to Martorana (1971) but failed to potentiate the responses to 0·5 to 2·5 mg/kg of phenylephrine even at high doses (75 and 150 mg/kg) which themselves induced necroses in the same animals. In control experiments, isoprenaline was fully potentiated as found by Martorana (1971).

Table 1. *Dose of phenylephrine producing cardiac lesions in 50% of the animals (ED50) in the presence of α-adrenoceptor blocking agents administered s.c., 20 min before the agonist.*

Antagonist and dose	ED50 (mg/kg)	Straight line equation
Phenyleprhine alone	2·45 (1·8– 3·48)*	y = 3·31 + 4·25 x
Dihydroergocryptine 1 mg/kg	20·25 (10·82–37·91)*	y = 3·01 + 1·52 x
Dihydroergocryptine 5 mg/kg	31·30 (18·79–52·13)*	y = 1·09 + 2·61 x
Nicergoline 2·5 mg/kg	7·13 (3·15–16·15)*	y = 0·52 + 5·25 x
Nicergoline 25 mg/kg	52·36 (25·85–103·60)*	y = 5·44 + 6·07 x

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

Thus our present and previous results show that stimulation of cardiac adrenoceptors by pure α- and β-stimulants induces myocardial focal necroses which, although morphologically similar, can be distinguished by the use of theophylline and by selective α- and β-adrenoceptor blockers.

Farmitalia, Istituto Ricerche,
20146–*Milan, Italy.*

O. BELLINI
A. H. GLÄSSER

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Phosphatase activity of guinea-pig tissues on creatinol *O*-phosphate *in vitro*

Creatinol *O*-phosphate (COP; Aplodan) is a new drug synthesized by Ferrari & Casagrande (1965). It has a positive inotropic effect on the isolated rabbit atrium, and an ability to antagonize the toxic action of digitalis on the isolated atrium of rabbits previously treated with EDTA (Ferrini, unpublished observations). COP also increased the contractile force in the rat isolated heart, in the rabbit isolated and hypoxic heart and in rabbit hearts with experimental infarction *in situ* (Marchetti, Merlo & Noseda, 1971). Musso, De Ambroggi & Taccardi (1971) obtained a decrease in the duration of atrio-ventricular block and a rapid return to normal of reduced coronary flow in guinea-pig isolated hearts treated with high doses of (−)-adrenaline. These responses are the result of the action of whole COP molecule. In man, COP achieved good results in congestive heart failure, in atrio-ventricular conduction disturbances, in improving tolerance to digitalis (Melloni, Camerini & others, 1968; Bianchi, Guzalian & others, 1970) and in coronary insufficiency (Natale, 1968).

We have investigated *in vitro* the kinetics of dephosphorylation of COP incubated with blood and some tissue extracts. In effect, the first catabolic stage of COP is its dephosphorylation to give creatinol, a reaction catalysed by phosphatases.

When COP was incubated with alkaline phosphatase, the rate of COP dephosphorylation was about 10 times higher than with acid phosphatase. In addition, experiments on COP dephosphorylation at pH 4, 5·6, 8·1, 9·3, 10·5, 11·3 demonstrated that the optimum pH in this reaction is 10·5. All our experiments were therefore made at pH 10·5 in 0·1 M glycine buffer.

Skeletal muscle, heart muscle, liver, the small intestine and kidney were homogenized in glycine buffer (100 mg of tissue/ml of buffer). The homogenate was centrifuged and the supernatant incubated at 37° with 7 μmol/ml of COP. The phosphate released was determined quantitatively (*a*) (Fiske & Subbarow, 1925) at 30 min, 1, 2 and 3 h. The same determinations of phosphate released were made on