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Laboratory of Cardiovascular Clinical Pharmacology
Istituto di Ricerche Farmacologiche Mario Negri
Via Eritrea 62
20157 Milano, Italy

CHIARA CERLETTI
SALVADOR MINOLDO
FRANCESCA BUCCHI
ALDO DEL MASCHIO
GIOVANNI DE GAETANO

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Effect of doxorubicin on calcium binding sites in guinea-pig heart

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There is strong evidence that the acute cardiotoxic effect of Doxorubicin (DXR) is mediated by an inhibitory effect on cell calcium flux. In fact, kinetic studies demonstrated that the cardiotoxic activity of DXR and of its 4′-epi- and 4′-deoxy-analogues is linearly correlated with their capacity to inhibit the fast-exchanging calcium compartment [1].

It was also demonstrated that DXR acutely inhibits calcium influx and the associated magnitude and duration of the slow action potential [2, 3], and moreover inhibits the Na^+/Ca^{2+}-exchange in the sarcolemma [4].

The present investigations were undertaken in order to get a further insight in the DXR–Ca interaction in the myocardiac cell.

Materials and methods

Since previous experiments demonstrated that up to 360/ μM DXR does not modify the heart rate in guinea pig, the present investigations were performed in spontaneously beating isolated guinea-pig atria. Incubation was made in Tyrode solution of the following composition: 11.1 mM glucose, 136.8 mM NaCl, 5.37 mM KCl, 051 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.46 mM NaH_2PO_4, 1.87 mM $CaCl_2$; the medium was gassed with a 95% O_2 + 5% CO_2 mixture to maintain a pH value of 7.4. The organs were loaded with 1.0 g tension and allowed equilibrating for 60 min.

The contractile responses developed by the organs were recorded by means of an isometric tension recording system; dF/dt was used as a contractility index.

A preliminary set of trials indicated a calcium concentration of 5×10^{-5} M as the maximum calcium concentration resulting in a non-working heart. Starting from this concentration, curves of increasing contractile force were obtained by a stepwise increase of calcium levels up to a final cumulative concentration of 1.2×10^{-2} M. For each calcium concentration, the preparations were allowed to stabilize until a new dF/dt value was obtained.

Calcium titration curves were also obtained in the presence of 25–200 μM DXR.

Results and discussion

The stepwise addition of calcium to non-working organs equilibrated with 5×10^{-5} M Ca yields a sigmoid-shaped curve of increasing contractile force reaching its maximum at about 9×10^{-3} M Ca. Different concentrations of DXR bring about a moderate shift of this curve to the right and a dose-dependent depression of the maximal contractile response (Fig. 1). This behaviour is different from that of the typical Ca-antagonist, verapamil, which brings about a competitive calcium antagonism: in fact, the depression of contractile force induced by this drug can be reversed by

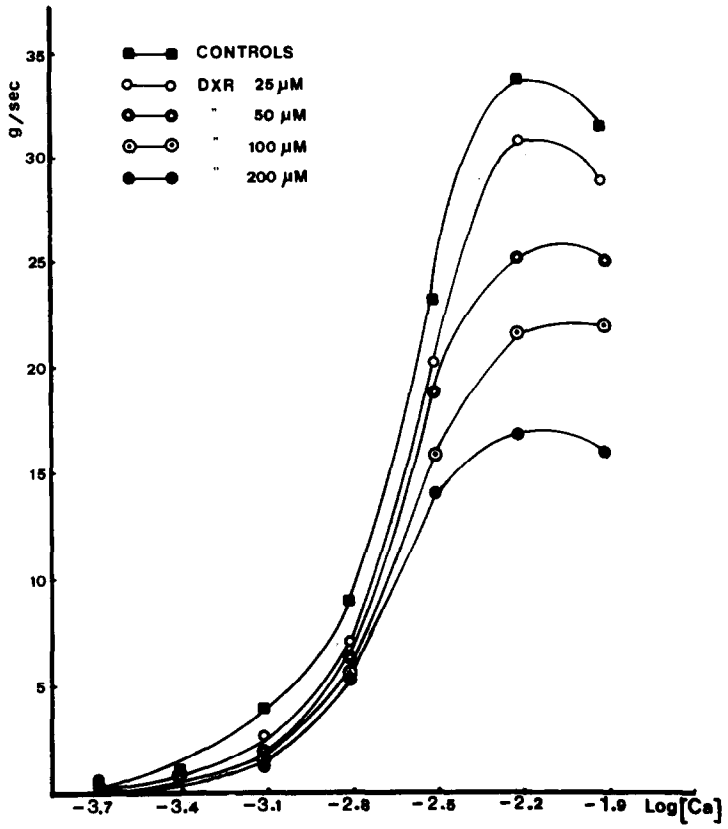

Fig. 1. Effect of different concentrations of DXR on the contractile force developed by increasing Ca concentrations in guinea-pig atria.

increasing the calcium concentration in the incubation fluid, thus allowing contraction to reach a maximal value as high as that of controls. Hence, the antagonism between calcium and DXR is of a non-competitive type. IC_{50} value was calculated to be 208 μM.

The experimental results suggest that DXR does not specifically act on the calcium slow-channels, which are believed to be the target of the Ca-antagonists. This is in agreement with studies performed in isolated guinea-pig atria in which the calcium slow-channels were specifically activated with the method of Adams [5]; in this experimental model DXR did not show any significant effect [6]. Since an inhibition of the rapidly exchanging pool of cellular calcium is involved in the development of the acute cardiotoxicity of DXR, the present experiments suggest that calcium receptors of this kinetically identified pool, other than the Ca slow-channels, are involved. However, a more precise definition of the site of action of DXR remains to be demonstrated.

In summary, the effect of Doxorubicin on calcium binding sites in guinea-pig heart was examined. Curves of contractile force obtained in the presence of different concentrations of Doxorubicin (25–200 μM) indicate that the antagonism between calcium and Doxorubicin is of a non-competitive type, while verapamil develops a typical competitive antagonism. It was suggested that Doxorubicin does not act specifically on the slow-channels.

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FABRIZIO VILLANI*
ELENA MONTI†
FRANCESCO PICCININI†
LUIGIA FAVALLI‡
ANNALINDA ROZZA DIONIGI‡
ENRICA LANZA‡

* *Servizio di Cardiologia—Istituto Nazionale per lo Studio e la Cura dei Tumori di Milano Via Venezian, 1 20133 Milano, Italy*
† *Cattedra di Farmacologia Applicata–Universià di Milano, Italy*
‡ *Cattedra di Saggi e Dosaggi Farmacologici–Università di Pavia, Italy*

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