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INFLUENCE OF ADRIAMYCIN ON CALCIUM EXCHANGEABILITY IN CARDIAC MUSCLE AND ITS MODIFICATION BY OUABAIN

FABRIZIO VILLANI, FRANCESCO PICCININI,* PAOLA MERELLI* and LUIGIA FAVALLI*

Instituto Nazionale per lo Studio e la Cura dei Tumori Via Venezian 1, 20133 Milan, Italy and *Cattedra di Saggi e Dosaggi Farmacologici dell'Università degli Studi di Pavia, Italy

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Abstract—Ten μg/ml of adriamycin produces a significant decrease in peak tension of isolated guinea pig atria without modification of cellular Ca content. The analysis of the curve of Ca exchanges shows that adriamycin significantly reduces the calcium exchangeable fraction, whereby the Ca fast exchanging compartment is suppressed and the slow exchanging one is slightly increased. Ouabain prevents the negative inotropic effect of adriamycin and restores the rapid Ca compartment without significantly modifying either the cellular Ca content or the amount of calcium exchangeable fraction. These results demonstrate that calcium plays a role in the negative inotropic effect of adriamycin in cardiac muscle and confirm the importance of the fast exchanging fraction in regulating the contractile force of the heart.

A cumulative dose-dependent cardiomyopathy is the major factor that limits the use of adriamycin for long-term maintenance treatment. The mechanism by which this compound induces cardiac toxicity is not completely understood. A few years ago, it was demonstrated that ouabain, when administered before adriamycin, prevented the negative inotropic effect produced by the antibiotic [1–3]. Since in experimental animals ouabain is known to exert positive inotropic effects by increasing Ca availability in cardiac muscle [4–6], and since specific Ca pools are thought to be associated not only with excitation–contraction coupling but also with the regulation of the contractile force in the heart [7–10], it could be suggested that adriamycin might decrease myocardial contractility by reducing calcium availability in the cell. The involvement of Ca in the cardiotoxic effect of adriamycin has also been suggested by experiments on isolated dog heart [11] and isolated guinea pig atria [12].

The present investigations were undertaken in order to evaluate the influence of adriamycin on Ca turnover in cardiac muscle and the effect of pretreatment with ouabain on this process. For these reasons, we investigated the tissue Ca content, the amount of tissue Ca exchangeable fraction, and the Ca exchanging rate in isolated guinea pig atria. This animal species was selected because it is very sensitive to the cardiotoxic effects of adriamycin; on the other hand, the effect of ouabain on Ca turnover has been widely studied in guinea pig atria.

MATERIALS AND METHODS

Experiments were conducted on isolated spontaneously beating atria of guinea pig, prepared according to the method of Hoditz and Lüllmann [13]. Preparations were incubated in a Tyrode solution (136.8 mM NaCl, 2.68 mM KCl, 1.36 mM $CaCl_2$, 0.59 mM $MgCl_2$, 8.92 mM $NaHCO_3$, 0.46 mM NaH_2PO_4 and 5.50 mM glucose) that was maintained at 37° and aerated with 5% CO_2 in O_2, thus yielding a pH of 7.3. Contractile force was recorded by means of an isometric tension recording system.

Extracellular spaces were determined by use of [^{3}H]inuline and found to be 25.18 ± 2.20 in control preparations, 26.52 ± 2.70 in adriamycin treated organs, and 26.12 ± 2.30 in adriamycin-ouabain treated preparations. Since no statistical difference was found among the three groups, a size of 26 per cent for extracellular spaces was used for the correction of the amount of ^{40}Ca and ^{45}Ca present in the extracellular spaces.

After 60 min equilibration, adriamycin was added to the final concentration of 10 μg/ml, and $^{45}CaCl_2$, at the concentration of 0.1 μCi/ml. In ouabain-treated preparations, this drug was added to the bathing solution 30 min before adriamycin. After 5, 15, 30, 45, and 60 min of incubation, atria were removed, dipped in a cold bathing fluid, blotted on filter paper, weighed, and subjected to a wet ashing procedure with 1:1 HNO_3–$HClO_4$ at 200°. The residue was dissolved in 0.1 *N* HCl. Aliquots were taken for the spectrofluorometric assay of Ca [14], and for the ^{45}Ca radioassay by liquid scintillation counting. Efficiency was checked by external standardization.

Cellular ^{40}Ca and ^{45}Ca were calculated after correction for the amount present in the extracellular spaces. The ratio between the specific activity in the cellular compartment and that in the medium corresponds to the amount of cellular Ca exchanged after different periods (5, 15, 30, 45, and 60 min) of exposure to ^{45}Ca. The curve obtained from these values depicts the kinetics of ^{45}Ca exchanges. The steady state value, reached after 60 min of exposure to ^{45}Ca, represents the absolute amount of cellular calcium exchangeable fraction.

RESULTS AND DISCUSSION

Figure 1 shows that adriamycin, at the tested dose, produces a significant decrease in peak tension of

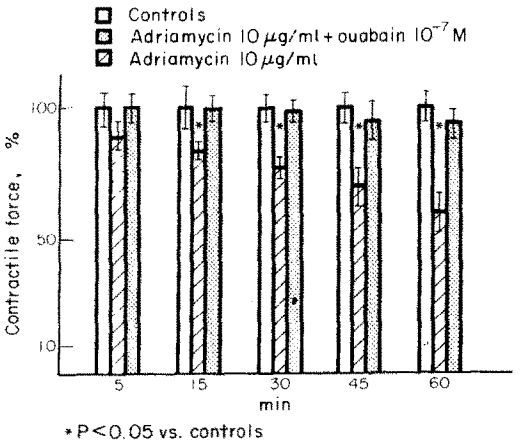

Fig. 1. Effect of ouabain pretreatment on the negative inotropic action of adriamycin in isolated guinea pig atria. M ± S.E. of 8–12 experiments.

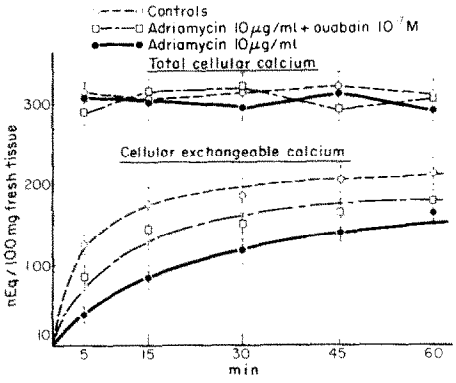

Fig. 2. Influence of Adriamycin (Adr) with and without ouabain on total calcium content and calcium exchangeable fraction of isolated guinea pig atria.

freshly dissected guinea pig atria, while pretreatment with ouabain prevents this effect. These results are in agreement with those reported by Arena *et al.* [1] on isolated rabbit heart and more recently by Necco *et al.* [15] on cultured cardiac cells and by Villani *et al.* [3] on humans. In fact, it has been shown that left ventricular performance, evaluated by the measurement of systolic time intervals, is acutely reduced by adriamycin, and that the pretreatment of patients with cardioactive glycosides prevents the cardiotoxic effect of the antibiotic [3]. On the basis of these considerations, it can be suggested that investigations conducted using isolated guinea pig atria can give valuable information on the mechanism of the acute adriamycin-induced cardiotoxicity. According to our working hypothesis that the negative inotropic effect induced by adriamycin and the antagonistic effect of ouabain might be supported by a modification of Ca turnover in the myocardial cell, the effect of adriamycin on tissue calcium content and on the rate of Ca exchange in control and ouabain-pretreated atria was studied.

Figure 2 shows that adriamycin, at the tested dose, does not significantly influence the total tissue calcium content (323.5 ± 15.8 nequiv/100 mg of fresh tissue in controls and 304.5 ± 17.2 nequiv/100 mg in adriamycin-treated atria); nor does adriamycin affect the Ca content in ouabain-pretreated preparations (314 ± 12.30 nequiv/100 mg fresh tissue). These results are in agreement with the fact that drugs that have cardioactive effects do not influence the total amount of Ca in the heart within the usual range of therapeutic doses. Instead, adriamycin significantly reduces the amount of Ca exchangeable fraction (168 ± 18 nequiv/100 mg fresh tissue in adriamycin-treated atria *vs.* 211.50 ± 19.1 nequiv/100 mg in control preparations).

The pattern of Ca exchangeable fraction as a function of time (Fig. 2) shows that two components are present in normal heart: an early phase of rapid Ca exchanges and a second phase of slow exchanging processes. The rapid phase is considered to represent Ca located in membrane structures that face the extracellular spaces [9, 10], whereas the second phase of slow exchanging processes probably corresponds to intracellular Ca [16].

Figure 2 shows that adriamycin induces a marked decrease of Ca exchanges; in fact, differences in Ca exchangeable fraction are statistically significant at each experimental time. Combined administration of ouabain counteracts the adriamycin-induced inhibition of Ca exchanges; indeed, the curve is not significantly different from that of controls.

The analysis of the time course of calcium exchanges in normal heart clearly demonstrates that two compartments are present (Fig. 3). In this graph,

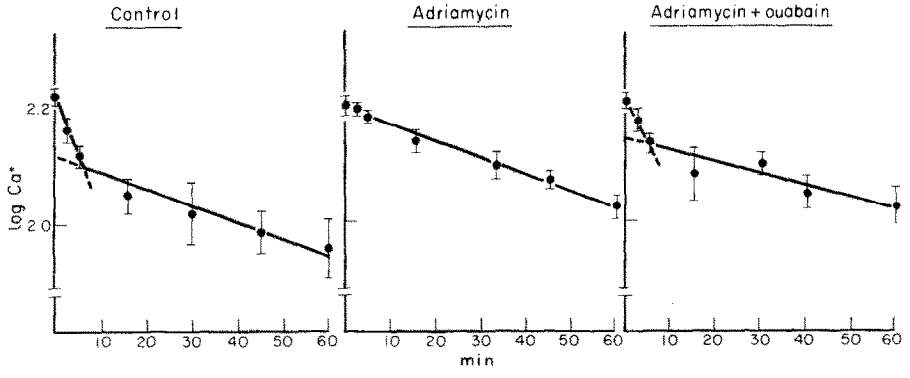

Fig. 3. Influence of Adriamycin (Adr) with and without ouabain on the rapid and slow exchanging calcium compartments. M ± S.E.
*Log Ca = differences between exchanged Ca at $t = \infty$ and Ca exchanged at a given time.

the rate of calcium exchanges was evaluated by calculating on semilogarithmic scale the differences between exchanged Ca at ∞ time and that exchanged at each experimental time, as a function of time. Adriamycin suppresses the early phase of fast exchangeable Ca, whereas ouabain reverses this effect. The curve obtained from preparations treated with combined adriamycin and ouabain indeed shows the presence of the Ca fast exchanging compartment, and its slope is parallel to that of control preparations. Figure 3 also shows that the rate of the slow exchangeable fraction is not influenced by adriamycin or ouabain. In fact, the semilogarithmic curve of treated organs runs parallel to that of control preparations.

These results suggest that adriamycin exerts its negative inotropic effect on guinea pig cardiac muscle mainly by inhibiting the Ca fast exchangeable fraction, i.e., the processes involving the membrane loosely bound Ca. In fact, there is cumulative evidence that in guinea pig atria this cellular fraction is strongly correlated to the contractile state of the muscle [9, 10]. On the contrary, the slow exchangeable Ca fraction and the absolute amount of cellular exchangeable Ca could hardly be related to the contractile state of the muscle, nor do they seem to mediate conditions that cause a rapid change in contractile force [9, 10, 16]. This is confirmed by the results obtained with the combined administration of adriamycin and ouabain. In fact this drug, which prevents the negative inotropic effect developed by adriamycin, restores the rate of Ca exchanges of the early phase without a significant modification of the slow exchangeable fraction. These results confirm the importance of the Ca fast exchanging phase in the regulation of the contractile force of cardiac muscle.

As regards the ouabain-adriamycin interaction in connection with Ca exchanges in cardiac muscle, experimental data suggest that adriamycin enhances the stability of the membrane-calcium complex of the fast exchanging processes. Ouabain could reverse this effect by labilizing the membrane-to-calcium complexes, thereby restoring the rapid Ca exchanging compartment.

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