The Role of Calcium in the Negative Inotropic Effect of Lanthanum

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Summary. Lanthanum reduces the rate of calcium fast exchanging processes, whereas the slowly exchanging phase is not modified. Experimental data also confirm the importance of calcium fast exchanging fraction in regulating contractile force of cardiac muscle.

The negative inotropic effect brought about by lanthanum on guinea-pig and rat cardiac muscle has been related to the capacity of the cation to affect the release of contractile dependent calcium[1–3]. The analysis of the quantities of ^{45}Ca released from preloaded organs following exposure to lanthanum has suggested that the displaced calcium derives from superficially located sites[2]. These results, however, should be interpreted with caution because lanthanum seems to have a less specific mechanism of action than that of a mere calcium antagonism at the membrane surface[1,4]. In fact, the above-mentioned experiments were carried out by wash-out techniques using brief times of exposure to lanthanum; hence they do not rule out the possibility that lanthanum could also influence the intracellular pool of exchangeable calcium.

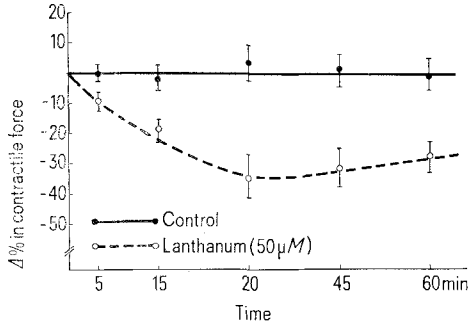

Fig. 1. Time course of changes in contractile force produced by 50 μM lanthanum on spontaneously beating guinea-pig atria isometrically recorded. Mean ± SE.

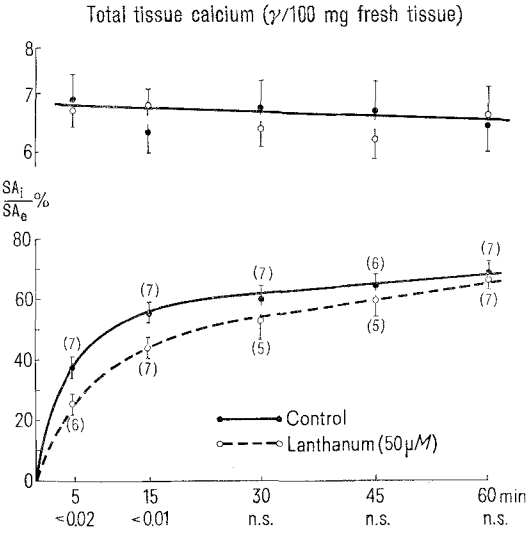

Fig. 2. Effect of 50 μM lanthanum on total tissue Ca content and Ca exchangeable fraction in guinea-pig atria.
Ca exchangeable fraction is given as ratio between the intracellular specific activity (SA_i) and the extracellular specific activity (SA_e). Mean ± SE. Number of determinations is given in brackets.

The present investigations were undertaken in order to investigate the influence of lanthanum on Ca tissue content and on Ca turnover by determining the time course of Ca exchangeable fraction which allows detection of changes in the fast-exchanging superficial calcium and in the pool of slow exchangeable intracellular calcium[5].

Methods. Atria of guinea-pig were prepared according to the method of Hoditz and Lüllmann[6]. The isolated spontaneously beating atria were incubated in Tyrode solution composed of: 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; 5.5 mM glucose. The incubation medium was maintained at a constant temperature of 37 °C and aerated with 5% CO_2 in O_2. Contractile force was recorded by means of an isometric tension recording system.

The atria were equilibrated for 60 min before the experiment was started. Lanthanum was added to give a final concentration of 50 μM; $^{45}CaCl_2$ at the concentration of 0.1 μCi/ml. Bathing medium was changed every 30 min. After different times of incubation (5, 15, 30, 45 and 60 min) atria were removed, dipped in cold bathing fluid, blotted on filter paper, weighed, and finally subjected to a wet ashing procedure at 200 °C with 1:1 HNO_3–$HClO_4$.

For each individual preparation, both the tissue calcium content and the ^{45}Ca exchange were measured simultaneously. The residue was dissolved in 0.1 N HCl: aliquots were taken for the spectrofluorometric assay of Ca[7] and for the ^{45}Ca radioassay by liquid scintillation counting.

The calcium exchangeable fraction was calculated as the ratio between the specific radioactivity (cpm/μEq Ca) of the tissue and that of the incubation medium, taking into account the amount of calcium and radioactivity present in the extracellular spaces. A size of 26% for the extracellular space was used for the calculations both in control and in treated preparations, since extracellular spaces determined by ^3H-inuline were not found to be different in control and lanthanum treated atria (25.18 ± 2.2 and 26.77 ± 3.7 respectively). Oxygen consumption was measured in a Warburg apparatus using a 1:4 suspension of mechanically minced atria.

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Results and discussion. The Table shows that metabolic properties of myocardial tissue were not modified at the lanthanum concentrations tested. Therefore, in all successive experiments using spontaneously beating atria, the highest concentration of lanthanum (50 μM) has been used. In these experimental conditions, lanthanum causes a significant decrease in peak tension: the effect is already evident after 5 min, reaches the maximum (about 30% diminution) after 30 min, and remains practically constant thereafter (Figure 1). Since lanthanum does not modify metabolic properties of cardiac muscle, it can be excluded that the development of the hypodynamic state was due to a lack of energy.

Effect of different concentrations of lanthanum on the oxygen consumption in guinea-pig heart muscle

Treatment		Concentration (μM)	O_2 consumption[a] (μl/min/g tissue)	P
–	(12)	–	2.29 ± 0.06	–
Lanthanum	(12)	5	2.39 ± 0.04	n.s.
Lanthanum	(12)	50	2.28 ± 0.05	n.s.

[a] Mean ± SE.
Number of determinations is given in brackets.

The investigation of calcium distribution in isolated atria after each incubation period has shown that no differences exist between control and lanthanum-treated preparations (Figure 2). The mean total Ca content of control atria was 6.69 ± 0.30 γ/100 mg fresh tissue and 6.59 ± 0.18 in atria exposed to lanthanum. These results demonstrate that lanthanum does not produce any modification of total tissue calcium content; therefore, it can be concluded that changes in Ca content are not responsible for the decline in peak tension. Figure 2 also depicts the ^{45}Ca exchange curves. As shown in this graph, the size of total exchangeable fraction was not significantly different in controls and lanthanum-treated atria (69.9 ± 4.1 and 66.6 ± 1.9 respectively). On the contrary, a considerable difference was found in the rate of exchange between control and treated atria. In fact, in the former group equilibrium was attained after 30 to 45 min, whereas in lanthanum-treated atria calcium exchanges proceeded considerably slower, reaching equilibrium in about 60 min.

For each of the curves obtained it was possible, according to CARRIER et al.[5], to distinguish 2 components: an early phase of fast exchanging processes, and a second phase of slowly exchanging processes. The fast phase of the calcium exchanging curve is considered to represent calcium located in membraneous structures facing the extracellular space[5], whereas the slowly exchanging phase probably represents intracellular membrane-bound calcium[8]. Lanthanum significantly decreases the rate of the former phase, whereas the second one seems not to be affected. Therefore, it can be concluded that lanthanum inhibits transmembrane calcium movements and seems not to affect the intracellular calcium compartment.

This investigation also confirms that the fast exchanging compartment is correlated to the contractile force developed by heart muscle.

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The Effect of Oxine-5-Sulphonic Acid on the Hepatic Drug Metabolism in the Rat

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Summary. Oxine-5-sulphonic acid inhibits the metabolism of aminopyrine in the rat liver in vitro. The characteristics of this inhibition vary according to whether the oxidative *N*-demethylation of the substrate is determined by the formation of the metabolite 4-aminoantipyrine or by the production of formaldehyde.

Recent investigations have indicated that certain quinoline derivates are potent inhibitors of the drug-metabolizing enzyme systems of liver microsomes. Ethoxyquin was shown to be a competitive inhibitor of the rat hepatic microsomal biphenyl-4-hydroxylase and of ethylmorphine-*N*-demethylase in vitro[2]; in addition, the glucuronidation of *p*-nitrophenol and 4-methylumbelliferone was inhibited by oxine (8-hydroxyquinoline) in enzyme preparations also obtained from rat liver microsomes[3].

The present paper concerns the influence of the oxine-5-sulphonic acid (OSA) on the hepatic microsomal drug metabolism in vitro.

Methods. Male Wistar rats, weighing between 180 and 220 g, were used in all the experiments. The animals were exsanguinated, then the livers were perfused with cold 1.15% KCl and removed. The tissue was homogenized in 3 volume of ice-cold 1.15% KCl, using a Potter-Elvehjem homogenizer coupled with a motor-driven pestle. The homogenates were centrifuged at 10,800 *g* for 20 min to remove cell debris, nuclei and mitochondria. After discarding the floating fat layer, the 10,800 *g* supernatant fraction was further centrifuged for 90 min at 105,000 *g* using a Beckman L5-50 ultracentrifuge. The resulting supernatant fraction was collected and the microsomal pellet resuspended in 1.15% KCl. Protein concentration was determined according to the LOWRY[4] procedure using bovine serum albumin as the standard. The microsomes were diluted to a concentration of 3 mg of protein/ml and the microsomal suspension was then recombined

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