INFLUENCE OF DIVALENT CATIONS ON RABBIT ATRIA AND MODIFICATION OF CATECHOLAMINE RESPONSE ON PACEMAKER CELLS*

R. D. SRIVASTAVA

Department of Physiology,
Kasturba Medical College,
Manipal - 576 119 (Karnataka)

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Summary : Spontaneously beating isolated atria of rabbits responded to Mn^{++} and Co^{++} with a progressively increasing negative chronotropism and inotropism, which was reversible by washout and by elevating the bath concentration of Ca^{++}. The cumulative dose response curve for adrenaline for chronotropic response was markedly shifted to the right in the presence of Mn^{++} or Co^{++}. This effect was also reversible. Verapamil produced only a moderate decline in spontaneous rate and contractility and did not block the chronotropic response to adrenaline. It is concluded that Mn^{++} and Co^{++} block the action of catecholamine on the pacemaker cells and they differ atleast in part from organic calcium channel antagonists in their mechanism of action.

Key words : adrenaline divalent cations pacemaker

INTRODUCTION

Manganese (Mn^{++}) is accumulated in the mitochondria and seems to be essential for their function as well as for the function of many enzymes (1). Cobalt (Co^{++}) poisoning has been described after the consumption of beer containing the ion and often culminates in death due to cardiac failure (1).

More recently these divalent cations have been used in physio-pharmacological studies for blockade of slow calcium channels (9, 11, 12) and in this respect they resemble "calcium antagonosts" like verapamil, nifedipine and diltiazem.

Although depressiom of myocardial contractility has been reported with these ions (2,3,11,16) no study on their effect on spontaneous rate and contracticility is easily available. Further, interaction of these divalent cations with effect of catecholamines on pacemaker activity has also been investigated only occasionally (8). The present report concerns itself to the effect of Co^{++} and Mn^{++} on spontaneously beating rabbit atria.

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MATERIAL AND METHODS

Albino rabbits of either sex (1–2 *kg*) were stunned by a blow on the neck and exsanguinated. Their hearts were rapidly removed, atria dissected free in cold, oxygenated Tyrode solution and suspended in an organ bath (40 *ml*) filled with Tyrode solution (composition : NaCl 136 mM, KCl 2.6 mM, $CaCl_2$ 1.8 *mM*, $MgCl_2$ 1 *mM*, NaH_2PO_4 0.4 *mM*, $NaHCO_3$ 12 *mM*, Glucose 5.5 *mM*, pH 7.4) maintained at 37 ± 1°C and gassed constantly with 5% CO in O

Spontaneous atrial contractions were recorded by an atrial myograph transducer (Type A, E & M Inst. Company) on a four channel Physiograph (E & M. Inst. Company) at 0.5 *g* tension. The preparations were allowed to equilibrate for 1 hr with frequent change of bathing fluid. A cumulative dose response curve was obtained with Mn or Co^{++} ions (0.25-4.0 *mM*) for chronotropic and inotropic effects, with stepwise increment in concentrations. The preparations were then washed several times, which restored their spontaneous rate and contractility to original values. A cumulative dose response curve was then obtained with adrenaline (10^{-9}–10^{-4}M) for chronotropic response. The preparations were again washed several times and the procedure was repeated in the presence of 1 mM Mn^{++} or Co^{++} ions, which were allowed to act for 3 mins before the adrenaline challenge. Adrenaline dose response curves were also obtained with or without exposure to verapamil (10^{-6}M) for 15 mins.

The maximum chronotropic response to adrenaline (10^{-4}M) was taken as 100% and other responses were expressed as percentage of maximum.

Drug used were Epinephrine chloride (Bengal Immunity) ; $MnCl_24H_2O$; $CoCl_2$ 6H O and Verapamil hydrochloride (Boehringer Knoll). All the chemicals were dissolved in 0.9% W/V NaCl before the experiment and the volume of drug added was <0.5 *ml*.

RESULTS

Both Mn^{++} and Co^{++} ions produced progressively increasing negative chronotropic and inotropic responses over a concentration range of 0.25-4.0 *mM*. The depression of rate and contractility at 4.0 mM was to the magnitude of 55±6.1% and 76±5.5% respectively with Mn and 56±5.4 and 87±3.0% respectively with Co_2 (Fig. 1). These depressant effects on atria were, however reversible on repeated washes. Cation–induced negative inotropism was reversed by increasing the concentration of Ca^{++} ions in the bath (Fig. 2).

The cumulative dose response curve obtained with adrenaline (10^{-9}–10^{-4}M) for its chronotropic response was markedly shifted to the right in the pessence of 1 *mM* of

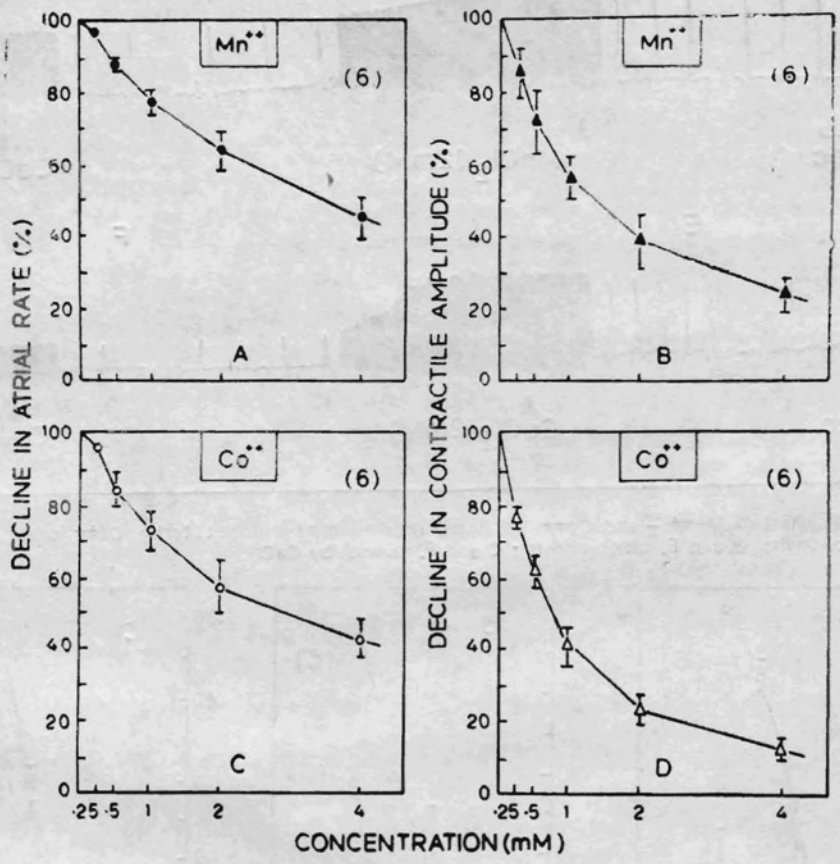

Fig. 1 : Isolated spontaneously beating rabbit atria. Mean effects (±SEM : vertical bars) of Mn^{++} and Co^{++} ions on spontaneous rate (A and C) and contractile amplitude (B and D). in 6 experiments each.

Mn^{++} or Co^{++} ions (Fig 3, A and B). This blockade of chronotropic response of adernaline was also reversible and this was occasionally checked after washout by testing the response to a specific molar concentration of adrenaline which produced approximately same degree of positive chronotropism as obtained in preantagonist dose response curve. Verapamil (10^{-6}M)produced a decrease in spontaneous rate (11.5±2.7%) and contractility (20.0±3.2%) but did not shift the adrenaline dose response curve to the right (Fig. 3, C).

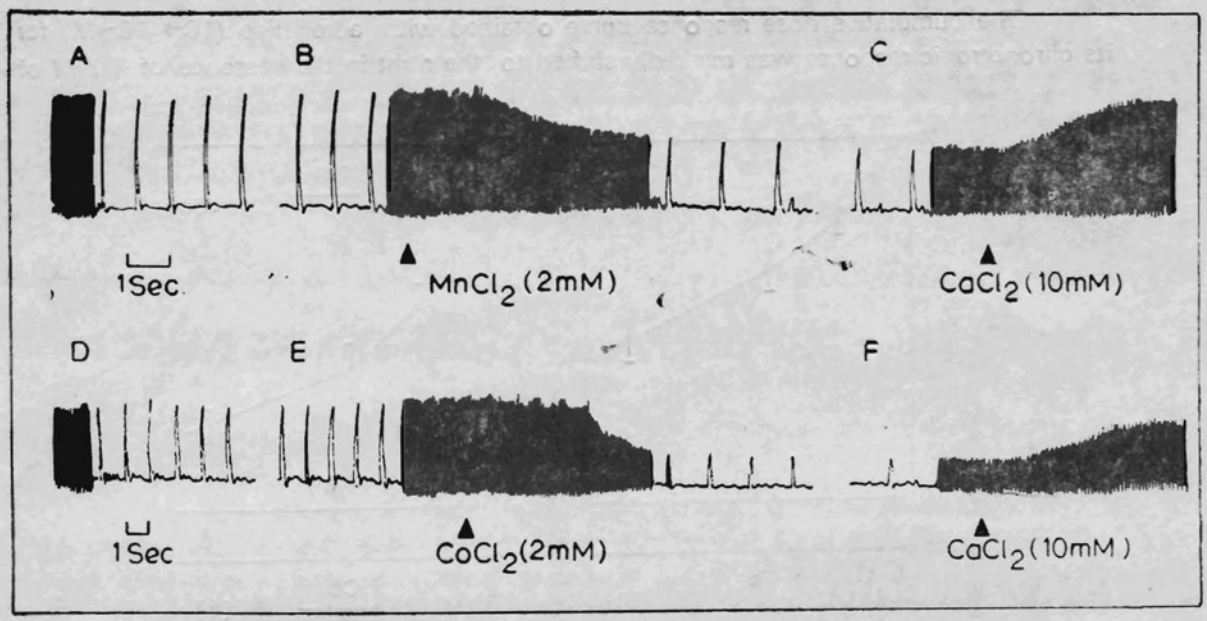

Fig. 2 : Reversal of Mn^{++} – and Co^{++} – induced depression of contractility by Ca^{++} ions. A and D, controls; B and E, cation-effects ; C and F, reversal by $CaCl_2$.

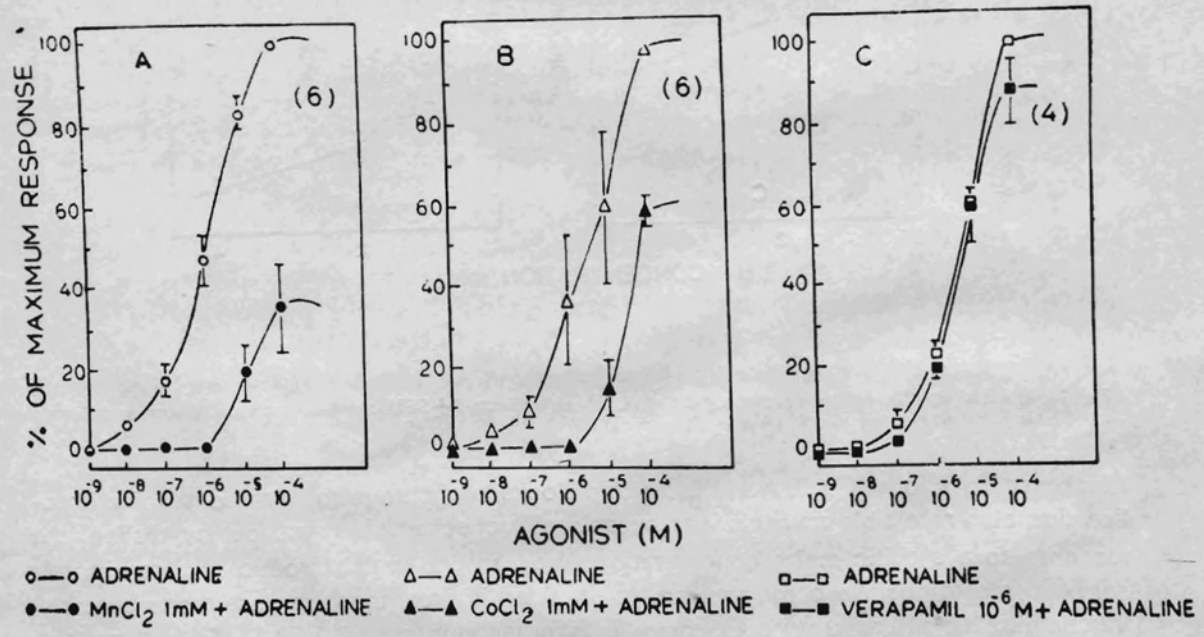

Fig. 3 : Isolated spontaneously beating rabbit atria. Concentration positive chronotropic effect curves for adrenaline before and after exposure to $MnCl_2$ (in A), $CoCl_2$ (in B) and verapamil (in C.). Each point is a mean (±SEM, shown as vertical bars) from 6 or 4 independent experiments.

DISCUSSION

The results of this study demonstrate that both the divalent cations are direct depressants of the mypcardium. However, Co^{++} ions are greater depressant of contractility in comparison to Mn^{++} ions (Fig. 1) and this finding is in conformity with the observation on cardio depressant activity of these ions (potency rating, Ni>Co>Mn; 7). Severe depression of myocardial contractility and pacemaker activity at high concentrations could explain the cardiac failure associated with acute Co poisoning. The reversibility of cardiac dpression by washout suggests that presumably there is no long term conformational change in the calcium binding sites at sarcolemma. Since elevating the bath concentration of calcium reversed the cations effects it would lend support to the view (15) that divalent cations have non-selective ability to substitute for Ca^{++} binding sites including calcium channels (5,6) and plasma membrane (10,14).

The catecholamines are known to exert their effects on pacemaker cells by promoting transfer of Ca^{++} ions through slow inward calcium channels (4) and displacement of calcium ions by divalent cations at these sites would explain the blockade of chronotropic responses to adrenaline by Co^{++} and Mn^{++} ions in the present study. There is no *in vitro* study to compare the present results but in an *in vivo* study on dogs, noradrenaline induced increase in A.V. Node conduction velocity was blocked by Mn^{++} ions (8). It can, therefore, be tentatively concluded that the dialent cations block the catecholamine responses on pacemaker cells.

As verapamil (10^{-6}M) could not block the chronotropic responses to adrenaline in this study and noradrenaline responses in another study (13) it is reasonable to infer that the mechanism of action of divalent cations on heart is in part different from the organic calcium channel antagonists. The severe cardiac depression associated with divalent cations, however is a major limiting factor in their utility as therapeutic agents.

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