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Influence of Pre-, Post-, and Simultaneous Perfusion of Elevated Calcium on the Effect of Ascending Concentrations of Lead on Digoxin-Induced Cardiac Arrest in Isolated Frog Heart

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Cardiotoxicity of lead, a ubiquitous environmental pollutant, has already been documented as a potentially lethal, although rarely recognized, complication of lead intoxication (Silver and Rodriguez - Torres 1968; Khan et al. 1967; Williams et al. 1983). Further, it has already been reported from this laboratory (Krishnamoorthy et al. 1987; Muthu and Krishnamoorthy 1992) that lead acetate (LA) preperfusion potentiated cardiotoxicity of digoxin (DGN) in isolated frog heart preparation and that exposure to elevated calcium (elev. Ca^{2+}) prior to, and simultaneously with LA at 10^{-7} M concentration, attenuated this potentiation. As an extension of this work, it was considered of interest to study the effect of perfusion of elev. Ca^{2+} (6.5 mM) prior to, after and simultaneously with ascending concentrations of lead (10^{-9}, 10^{-7} and 10^{-5}M) on DGN induced cardiac arrest (CA) in isolated frog heart, since Pb^{2+} and Ca^{2+} ions are known to compete with each other for the same target sites at the cellular level, an instance of competitive mass action effect (Pounds 1984).

MATERIALS AND METHODS

Digoxin (Sigma Chemical Co., St. Louis, Mo, U.S.A.) and lead acetate GR neutral (Sarabhai M. Chemicals, Baroda, India) were used in this study. All other chemicals used were of analytical grade.

In this study, frogs (Rana hexadactyla) weighing 45-55g were employed to set up isolated frog heart preparation according to Burn (1952). The basic procedural details already reported (Muthu et al. 1993) were followed in this study. The following drug/chemicals were added

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either separately or in combination with normal perfusion fluid (NPF) i.e. frog Ringer's solution in specific concentrations. DGN - 1.92 x 10^{-6} M; LA-10^{-9}/ 10^{-7}/10^{-5}M; and elev. Ca^{2+} - 6.5 mM. The DGN perfusion time (sec), total volume of DGN perfused (ml) for CA (cardiac standstill in contracture) and the heart weight (ht.wt) in mg were noted at the end of each experiment. The DGN exposure was calculated and expressed as µg DGN 10 mg ht. wt.

In another series of experiments, the frog heart was exposed *in situ* and was cannulated for perfusion as in the isolated heart preparation. The procedures followed for recording cardiac contractions and for computing their rate and amplitude were as already described (Krishnamoorthy et al. 1992). In all the experimental groups, after CA, the whole heart was dissected off and processed immediately for the assay of myocardial lead (µg/g wet tissue) and/or DGN (ng/g wet tissue) levels. Assay of myocardial calcium (µg/g wet tissue) was carried out in groups that received perfusion of elev. Ca^{2+}. Six observations were made for each of the three assays, except for myocardial DGN in DGN alone group where 8 observations were made. The lead and calcium assays were carried out employing the Inductively Coupled Plasma Emission Spectrophotometer, ARL - 3410 (Applied Research Laboratories, USA) and digoxin was estimated using ELISA kit supplied by Boehringer Knoll Ltd, India.

The actual data/differences between the individual test and control data were analysed by one way ANOVA followed by *post hoc* analysis employing the Tukey test (Zar 1984).

RESULTS AND DISCUSSION

In the experimental series with LA 10^{-9}M, it was clear that perfusion of elev. Ca^{2+} prior to, after and simultaneously with LA did not exert any significant influence both on DGN perfusion time (1188.22 ± 17.06, 1217.89 ± 15.05 and 1188.89 ± 23.65 sec respectively) and DGN exposure (15.05 ± 0.21 and 15.35 ± 0.24 and 15.11 ± 0.28 µg/10mg ht.wt respectively) compared to the control group (LA-DGN) (1227.43 ± 20.28 sec and 15.71 ± 0.12 µg/10mg ht.wt. respectively). Perfusion of LA 10^{-9} M alone prior to DGN has already been shown not to alter significantly DGN exposure for CA (Krishnamoorthy et al. 1992), presumably because this LA concentration was subminimal. Thus, it is quite conceivable that pre-, post- and simultaneous perfusion of elev. Ca^{2+} was also bereft of any influence over LA - DGN cardiotoxicity.

Subsequently, the influence of perfusion of elev. Ca^{2+} on the effect of LA at 10^{-7} and 10^{-5}M was compared with that of LA 10^{-9} M for series of pre-, post - and simultaneous perfusion of elev. Ca^{2+} separately. The differences between the values of DGN perfusion time and DGN exposure respectively for CA of various groups and those of groups receiving LA alone at respective concentrations prior to DGN, are depicted in Figure A and B. Perfusion of elev. Ca^{2+} prior to, after and simultaneously with LA 10^{-9}M induced uniformly a decrement in both the parameters, which was not statistically significant as mentioned earlier. However, in similar experimental situations involving LA at 10^{-7} and 10^{-5}M, the results have been broadly comparable to each other with the following general features : prior and simultaneous perfusion of elve. Ca^{2+} with LA at 10^{-7} and 10^{-5} M induced an increase in both the parameters. On the other hand, perfusion of elev. Ca^{2+} after LA 10^{-7} and 10^{-5}M, caused a decrement in both the parameters, the decrement in DGN perfusion time only being significant. Taken together, the data have shown clearly that there was no significant difference in the magnitude of the effect on DGN perfusion time as well as DGN exposure for CA between experimental groups with LA 10^{-7} and 10^{-5}M that received pre-, post- and simultaneous perfusion of elev. Ca^{2+} respectively.

The data regarding rate and amplitude at the end of 10 and 20 min of DGN perfusion in the various experimental groups are shown in Table 1. Scanning the data collectively, in all the experimental groups, there was a reduction in heart rate and an increase in amplitude of cardiac contractions during DGN perfusion. However, it is striking that there was no significant difference in the eventual effects on cardiac rate and amplitude between the three groups that received sequential and simultaneous perfusions of LA and elev. Ca^{2+}.

Thus, in the present study, the significant differences recorded in DGN perfusion time and DGN exposure (Fig A and B) without concomitant differences in the efect on rate and amplitude of cardiac contractions in these three groups, rules out any influence of the latter two parameters in this regard.

The myocardial DGN data at CA revealed that there was significantly no difference between the values of the group that received perfusion of elev. Ca^{2+} after (4.53 ± 0.28 ng/g wet tissue) and simultaneously (4.18 ± 0.26 ng/g wet tissue) with LA and that of the LA-DGN group (4.01 ± 0.17 ng/g wet tissue).

However, it was striking that the myocardial DGN level in the group with preperfusion of elev. Ca^{2+} (6.37 ± 0.22 ng/g wet tissue) was significantly higher ($P<0.05$) than that of the LA-DGN group, and further, interestingly enough, the value was not significantly different from that of the DGN alone group (5.72 ± 0.40 ng/g wet tissue).

The preperfusion of LA (10^{-7}M) has already been reported to potentiate DGN cardiotoxicity as evidenced by a decrement in DGN perfusion time as well as DGN exposure for CA (Krishnamoorthy et al. 1992). A significant increase in myocardial DGN level seen in the group that had elev. Ca^{2+} perfusion prior to LA 10^{-7}M indicated significant reversal of LA-induced potentiation of DGN cardiotoxicity, which confirmed the earlier report (Krishnamoorthy et al. 1987). Additionally, in the present study, it was striking that the reversal of lead effect by elev. Ca^{2+} preperfusion was complete as denoted by the myocardial DGN value being not significantly different from that of the DGN alone group. Thus eventually, variations in myocardial DGN levels could be taken as directly reflective of changes in DGN cardiotoxicity in these groups. However, mycocardial DGN variations did not portray this reversal consequent to simultaneous perfusion of elev. Ca^{2+} with LA contrary to the earlier inference based on augmentation in DGN pefusion time as well as DGN exposure for CA. Thus, in the absence of significant changes in myocardial DGN, reversal of LA - induced potentiation of DGN cardiotoxicity could possibly occur due to several mechanisms other than myocardial DGN uptake such as exacerbated myocardial sensitivity to DGN and other complex mechanisms related to an interference with Ca^{2+} - dependent cellular processes.

There was no difference either in myocardial calcium or lead levels at CA between the experimental groups that received elev. Ca^{2+} prior to, after and simultaneously with LA. This was presumably so since these total cationic levels at CA - the end point of the experiment - were incapable of revealing the subtle modulations in Ca^{2+} - dependent cellular processes that possibly occurred during the entire course of the experiment.

Interaction of Pb^{2+} and Ca^{2+} has been recognized for many years and it is also known that at the cellular level, calcium appeared to be protective against the toxic effects of lead (Silbergeld and Adler 1978). Kopp and Barany (1980) already reported that elevated extracellular calcium partially attenuated lead-induced

Table 1 Effect of interaction of elevated calcium (elev. Ca^{2+}) (6.5 mM), lead acetate (LA) (10^{-7}M) and digoxin (DGN) (1.92 x 10^{-6}M) on the rate and amplitude of contraction of perfused frog heart.

Experimental groups (sequence of perfusions)	Rate (difference from equilibration value) at 10&20 min of DGN perfusion	Amplitude (folds of equilibration value) at 10&20 min of DGN perfusion
	Treatment mean	Treatment mean
LA ->DGN (20 min)	-7.25^{a}	1.15^{a}
elev.Ca^{2+} ->LA ->DGN (5 min) (20 min)	-5.42^{ab}	1.67^{b}
LA -> elev.Ca^{2+} ->DGN (20 min) (5 min)	-5.58^{ab}	1.79^{b}
(elev.Ca^{2+}+ LA) ->DGN (20 min)	-3.17^{b}	1.48^{ab}

The treatment means are computed from 6 observations each of rate and amplitude at 10 and 20 min of DGN perfusion. The data were analysed using two way ANOVA, and if F-values were significant, multiple comparisons were carried out by the Tukey test. The minimal significance level was fixed at $P<0.05$. The data with at least one common superscript do not differ significantly from each other.

cardiotoxicity. It must be emphasized that in the present study, elev. Ca^{2+} preperfusion completely reversed LA (10^{-7}M) - induced potentiation of DGN cardiotoxicity. Pb^{2+} ions possibly impaired intracellular Ca^{2+} utilization through antagonism of transmembrane Ca^{2+} transport processes either directly by displacement of Ca^{2+} from membrane binding sites (Langer et al. 1974) or indirectly by inhibition of phosphorylation of sarcolemmal proteins required for Ca^{2+} influx. Pb^{2+} is known to antagonize the action of Ca^{2+} at many sites and also mimic many of the biological effects of Ca^{2+} (Simons 1986). Additionally, lead possessed many effects entirely unlike those of calcium such as inhibition of (Na^{+}, K^{+}) - ATPase (Raghaven et al. 1981) and reduction in the level of

FIGURE

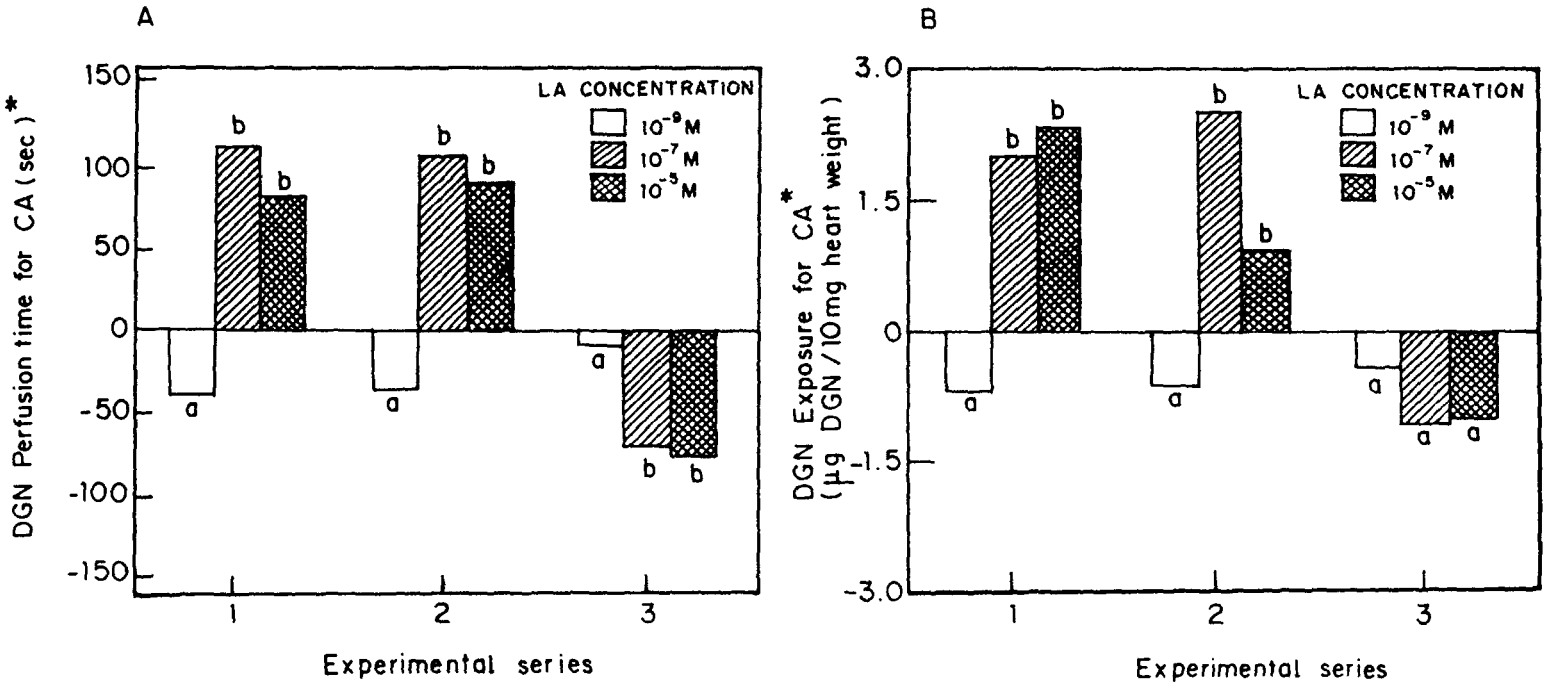

Figure - A & B : Influence of perfusion of elevated calcium (elev. Ca^{2+}) (6.5mM) prior to (5 min), after (5 min) and simultaneously (20 min) with lead acetate (LA) (10^{-9}, 10^{-7} and 10^{-5} M, 20 min) on the effect of LA on digoxin (DGN) (1.92 x 10^{-6} M) - induced cardiac arrest (CA). *Data presented as means of differences in values between the control group (LA-DGN) and the groups receiving perfusion of elev. Ca^{2+} also with LA at the same concentration. Experimental series : 1 - Preperfusion (n=9); 2 - simultaneous perfusion (n=6); 3 - post perfusion (n=6). The data were analysed by one way ANOVA and if F values were significant, multiple comparison were carried out by the Tukey test. The minimal significance level was fixed at $P<0.05$. In each experimental series, the bars with common superscripts do not differ significantly from each other.

digitalis binding to this enzyme (Siegel and Fogt 1979). These mechanisms may be quite pertinent to discuss the cardiotoxicity consequent to LA-DGN interaction. The reversal of the negative inotropic effect of lead by elev. Ca^{2+} was postulated to represent a competitive mass action effect (Kopp and Barany 1980), which might also be applicable for the reversal of LA-induced potentiation of DGN cardiotoxicity by elev. Ca^{2+}. However, in the present study, there was absence of any significant difference in the effects between groups involving perfusion of LA at 10^{-7} and 10^{-5} M with pre- and simultaneous perfusion of elev. Ca^{2+} and between groups involving the ascending concentrations of LA with postperfusion of elev. Ca^{2+}. Thus, the present data indicate that LA - elev. Ca^{2+} -DGN interaction does not represent strictly a competitive mass action effect.

Despite recognition of Pb^{2+} - Ca^{2+} interaction for many years, there exists a paucity of information regarding the apparent antagonistic relationship between them in the heart. Moreover, there are fewer previous reports concerning its impact on DGN cardiotoxicity and the influence of perfusion of ascending concentrations of LA on this interaction. The present findings in isolated frog heart regarding the impact of Pb^{2+} - Ca^{2+} interaction on cardiotoxicity of DGN - a widely prescribed cardiotonic drug in human beings - is considered as a distinct contribution to the existing knowledge in this area.

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