Bull. Environ. Contam. Toxicol. (1993) 50:77–84


Environmental Contamination and Toxicology

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

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Lead is a toxic metal that occurs ubiquitously in modern times as an environmental pollutant. It remains a significant health hazard as human exposure to it, has been steadily increasing with progressive industrialization. Cardiotoxicity is already documented as a potentially lethal, although a rarely recognized complication of lead intoxication (Myerson and Eisenhauer 1963; Freeman 1965; Khan et al. 1967; Silver and Rodriguez - Torres 1968; Williams et al. 1983). It has already been reported that lead preperfusion potentiated cardiotoxicity of digoxin (DGN) - a widely prescribed cardiotonic drug in human beings - in isolated frog heart preparation (Krishnamoorthy et al. 1987).

The interaction between lead and calcium is a complex, well recognized, but poorly understood phenomenon and at cellular level, they are known to compete with each other for the same target sites (Pounds 1984). In a previous report (Krishnamoothy et al. 1987), it has been shown that exposure to high $Ca^{2+}$ (6.5 mM) prior to, and simultaneously with lead attenuated the cardiotoxicity of lead - digoxin interaction. Subsequently, it was intriguing to study the converse, the influence of pre -and simultaneous perfusion of lead - in ascending concentrations - on the effect of elevated calcium on DGN - induced cardiac arrest in isolated frog heart.

## MATERIALS AND METHODS

Lanoxin ampules (Burroughs Wellcome, Bombay, India) containing 0.25mg/ml of digoxin and lead acetate GR neutral (Sarabhai M. Chemicals, Baroda, India) were used in this study. All other chemicals used were of analytical grade.

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Frogs (Rana hexadactyla) weighing 45-55g were used. The isolated frog heart - preparation was set up according to the procedure of Burn (1952). In all the experimental groups, a minimum of six isolated hearts were employed; the heart was initially perfused for 30 min with frog Ringer's solution as the normal perfusion fluid (NPF) for equilibration and finally, DGN was perfused until cardiac arrest. The drug/ chemicals were added separately to the NPF in the following concentrations: DGN - $1.92 \times 10^{-6}$ M; lead acetate (LA) - $10^{-9}/10^{-7}$ $/10^{-5}$M; and elevated calcium - 6.5 mM. In this experimental set up, five Mariotte's bottles fixed to adjustable stands were used as reservoirs for NPF, NPF with DGN, NPF with LA, NPF with elevated calcium and NPF with LA and elevated calcium. The perfusion cannula passed into the isolated heart was connected to any one of these Mariotte's bottles for a specific duration and in the sequence according to the design of the various experimental groups.

The DGN perfusion time (sec) and total volume of DGN perfused (ml) for cardiac arrest were recorded. The heart weight in mg was noted in each case. The DGN exposure for cardiac arrest was computed and expressed as Ìg DGN/ 10mg heart weight.

The data were analyzed by one way ANOVA, and in case, F-ratio was found to be significant, post-hoc analysis was carried out by the Tukey test (Zar 1984).

**RESULTS AND DISCUSSION**

The preperfusion of elevated calcium for either 5 or 20 min, has significantly diminished ($P<0.05$) the perfusion time as well as DGN exposure required for cardiac arrest compared to the control (DGN alone group) (Table - 1). However, remarkably, while the difference in perfusion time was not significant, the variation in DGN exposure for cardiac arrest was significant between the second (preperfusion of elevated calcium for 5 min) and the third (preperfusion of elevated calcium for 20 min) groups. This reflects that synergistic cardiac effect of calcium and DGN was greater on prolonging the duration of calcium preperfusion from 5 to 20 min as evidenced by higher diminution in DGN exposure required for cardiac arrest. A remarkable similarity is known to exist between the inotropic and toxic actions of digitalis and those of elevated extracellular calcium (Orrego 1984) and this synergism was observed in the presence of digitalis intoxication both in animals and human beings (Nyiri and Du Bois, 1930; Nalbandian et al. 1957). Thus, the present data are consonant with and reflects this well recognized synergism.

**Table - 1: Effect of preperfusion ( 5 and 20 min) of elevated calcium (elev. $Ca^{2+}$) (6.5 mM) on digoxin (DGN) (1.92 x $10^{-6}$ M) - induced cardiac arrest.**

| Experimental groups | Sequence of perfusions | For cardiac arrest | |
|---|---|---|---|
| | | DGN perfusion time (sec) | DGN exposure (Ìg/10 mg heart weight) |
| I | DGN alone | 1465.67 ± 12.50[b] | 16.69 ± 0.32[a] |
| II | Elev.$Ca^{2+}$ --> DGN (5 min) | 820.67 ± 14.94[a] | 13.56 ± 0.50[b] |
| III | Elev. $Ca^{2+}$ --> DGN (20 min) | 832.17 ± 18.09[a] | 9.22 ± 0.23[c] |

The data represent mean ± S.E. of 6 observations and were analyzed by one way ANOVA: DGN perfusion time, $F_{2,15}$ ratio - 578.35, $P < 0.001$; DGN exposure, $F_{2,15}$ ratio - 103.31, $P<0.001$. The post hoc analysis was carried out by the Tukey test. The minimal significance level was fixed at $P<0.05$. The data with the same superscript do not differ significantly from each other.

Subsequently, the effect of perfusion of ascending concentrations of LA on the synergistic effect of calcium on DGN - induced cardiac arrest was investigated with the group that received preperfusion of elevated calcium for 5 min serving as the control. It is clearly evident that prior perfusion of LA at $10^{-9}$M has definitely antagonized $Ca^{2+}$ - DGN synergism, as indicated by a significant increase in both perfusion time and DGN exposure needed for cardiac arrest (Figure - 1). On the otherhand, preperfusion of LA at $10^{-7}$ and $10^{-5}$M resulted in a significant decrement in DGN exposure required for cardiac arrest, which may be taken as indicative of potentiation of cardiotoxicity of $Ca^{2+}$-DGN interaction. However, in case of preperfusion of LA $10^{-7}$M, there was an increase in perfusion time for cardiac arrest. While it is known that both DGN perfusion time and DGN exposure needed for cardiac arrest reflected truly the myocardial DGN level, the indicator of variation in DGN cardiotoxicity (Krishnamoorthy et al. 1992), DGN exposure appears to be more reliable in this regard.

In another experimental series, the effect of simultaneous preperfusion of elevated calcium and ascending concentrations of LA - $10^{-9}$, $10^{-7}$ and $10^{-5}$M

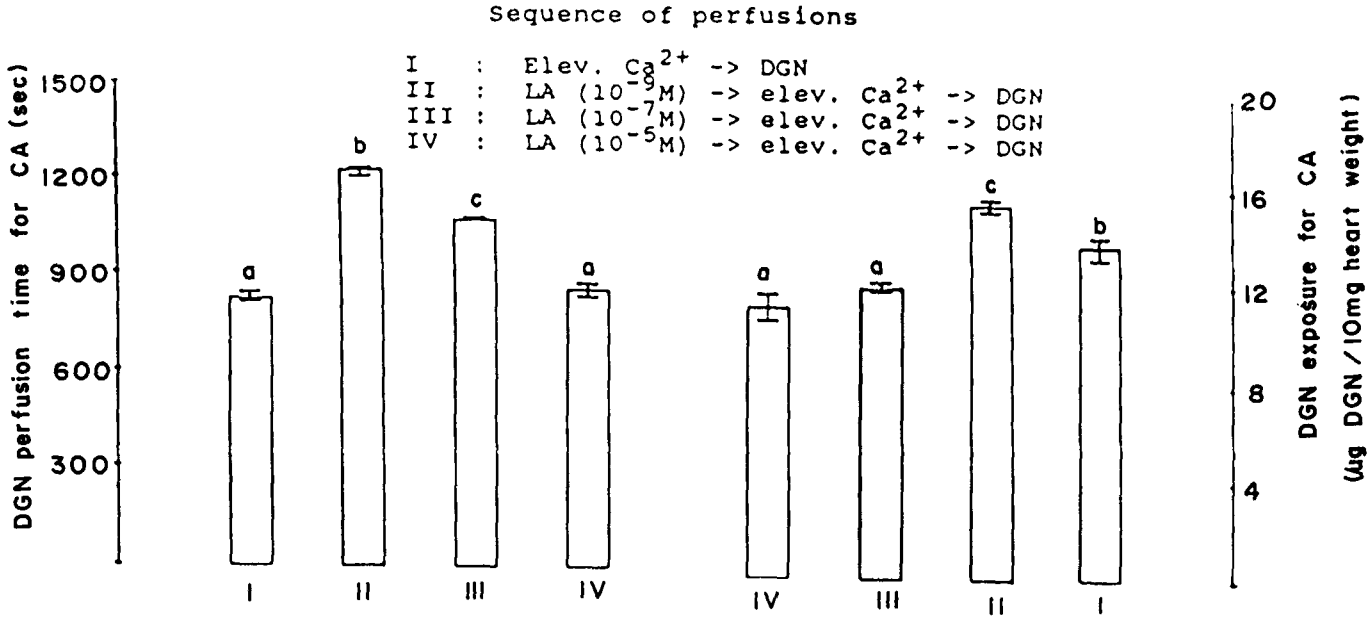


Figure-1; Influence of preperfusion of ascending concentrations of lead acetate (LA) ($10^{-9}$, $10^{-7}$ and $10^{-5}$M, 20 min) on the effect of elevated calcium (elev. $Ca^{2+}$) (6.5 mM, 5 min) on digoxin (DGN) ($1.92 \times 10^{-6}$M) - induced cardiac arrest (CA). The data represent mean ± S.E. of 'n' observations (Groups I, III and IV - 6 each; II-9) and were analyzed by one way ANOVA: DGN perfusion time, $F_{3,23}$ ratio - 149.08, $P<0.001$; DGN exposure, $F_{3,23}$ ratio - 29.32, $P<0.001$. The post-hoc analysis was carried out by the Tukey Test. The minimal significance level was fixed at $P<0.05$. The bars with the same superscript do not differ significantly from each other.

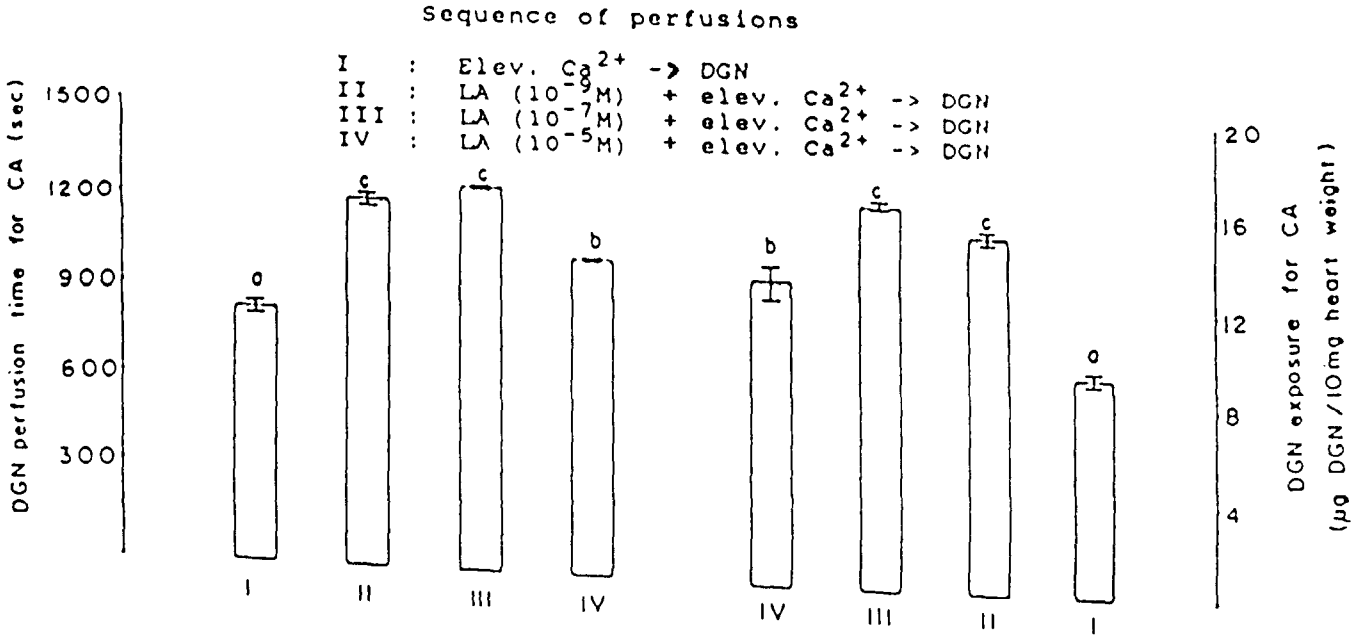


Figure 2: Influence of simultaneous perfusion (20 min) of ascending concentrations of lead acetate (LA) ($10^{-9}$, $10^{-7}$ and $10^{-5}$M) with elevated calcium (elev. $Ca^{2+}$) (6.5mM) on the effect of elev. $Ca^{2+}$ on digoxin (DGN) ($1.92 \times 10^{-6}$M) - induced cardiac arrest (CA). The data represent mean ± S.E. of 'n' observations (Groups I, III and IV - 6 each; II-9) and were analyzed by one way ANOVA: DGN perfusion time, $F_{3,23}$ ratio - 87.47, $P<0.001$; DGN exposure, $F_{3,23}$ ratio - 50.56, $P<0.001$. The post-hoc analysis was carried out by the Tukey Test. The minimal significance level was fixed at $P<0.05$. The bars with the same superscript do not differ significantly from each other.

on DGN - induced cardiac arrest was studied. For this series, the group that received perfusion of elevated calcium for 20 min prior to that of DGN served as the control. Interestingly, in this series, the data representing groups II and III were not significantly different from each other, suggesting that simultaneous perfusion of LA at $10^{-9}$ and $10^{-7}$M along with elevated calcium behaved similarly and at the same magnitude in attenuating cardiotoxicity of $Ca^{2+}$ - DGN interaction as evidenced by a significant increase in DGN perfusion time as well as exposure for cardiac arrest (Figure - 2). In group IV, simultaneous perfusion of LA $10^{-5}$M with elevated calcium also led to decrement in $Ca^{2+}$ - DGN cardiotoxicity, but it was significantly lesser compared to that of groups II and III.

Collectively, the data representing the effects of perfusion of LA in ascending concentrations prior to, and simultaneously with elevated calcium on DGN - induced cardiac arrest, have definitely shown that exposure to lead in both the above situations had an impact on $Ca^{2+}$ - DGN cardiotoxicity. Further, simultaneous perfusion of LA at all the ascending concentrations with calcium has definitely brought down the synergistic $Ca^{2+}$-DGN cardiotoxicity. On the other hand, in the experimental series that received perfusion of ascending concentrations of LA prior to elevated $Ca^{2+}$, the antagonism of $Ca^{2+}$-DGN cardiotoxicity was demonstrable only in group II that received preperfusion of LA at $10^{-9}$M. Comparing the results of the above two experimental series, it must be underscored, that the differences in results could be speculated to be due, in a large measure, to the variation in the duration of perfusion of elevated calcium, namely 5 min in the series that received perfusion of LA prior to elevated calcium, and 20 min in the series involving simultaneous perfusion of LA and elevated calcium.

The present findings are concordant with the report that $Pb^{2+}$ antagonizes the action of $Ca^{2+}$ at many sites and also mimic many of the biological effects of $Ca^{2+}$ (Simons 1986). Even though, an antagonism by lead on calcium - stimulated cardiac contractility and myofibrillar protein phosphorylation has been already recorded (Williams et al. 1983), there is hardly any previous report on the impact of this antagonism on DGN - cardiotoxicity. At subcellular levels, lead has been shown to inhibit the uptake of calcium in the mitochondria of cardiac cells of the rat (Parr and Harris 1976). Furthermore, Habermann et al. (1983) reported that lead is a fully potent substitute for calcium with respect to calmodulin - dependent phosphorylation. It has been suggested that lead may

ultimately perturb $Ca^{2+}$ regulated/mediated functions by any one of the following mechanisms: directly by interfering with calcium transport or storage processes, indirectly by altering cell functions required for calcium homeostasis or by substitution of $Pb^{2+}$ for $Ca^{2+}$ at functionally important calcium binding sites (Pounds 1984).

The partial reversal of the negative inotropic effect of lead by elevated extracellular calcium is postulated to represent a competitive mass action effect. The present data led us to infer that antagonism of the effect of elevated calcium by lead on DGN cardiotoxicity could also be explicated on similar lines. Further, the competitive mass action effect of $Pb^{2+}$ - $Ca^{2+}$ interaction could explain the difference in the results between the two experimental series receiving perfusion of calcium for varied durations of 5 and 20 min respectively. In the former series, the potentiation of cardiotoxicity of $Ca^{2+}$-DGN interaction observed with preperfusion of LA at $10^{-7}$ and $10^{-5}$M could be due to the concentration of extracellular lead being much higher than that of calcium. Extending this hypothesis, in the second series receiving simultaneous perfusion of LA and elevated calcium for 20 min, the antagonism of $Ca^{2+}$-DGN cardiotoxicity was noted even with LA at $10^{-7}$ and $10^{-5}$M, presumably consequent to the presence of adequately higher extracellular $Ca^{2+}$ , for the extracellular $Pb^{2+}$ to compete with.

Eventually, the potentiation of $Ca^{2+}$-DGN cardiotoxicity with preperfusion of LA at $10^{-7}$ and $10^{-5}$M could be ascribed to the predominance of toxic effect of lead, that exists in greater concentration, in potentiating DGN cardiotoxicity. This concurs well with the contention that despite many similarities between $Pb^{2+}$ and $Ca^{2+}$, lead posesses many effects entirely unlike those of calcium and hence the toxicology of lead could not be explained solely by its interaction with calcium (Simons 1986). For instance, lead is known to inhibit ($Na^{+}$, $K^{+}$)-ATPase (Raghaven et al. 1981) as well as the level of digitalis binding thereto (Siegel and Fogt 1979). Thus, in experimental situations where the extracellular $Pb^{2+}$ is higher than $Ca^{2+}$, it is presumed that initially, lead competed for calcium -mediated contractility, while latent effects of lead could be attributed solely to the toxic effect of lead (Prentice and Kopp 1983).

Despite the recognition of the protective role of high extracellular calcium against lead toxicity, there is hardly any previous report on the impact of $Pb^{2+}$-$Ca^{2+}$ interaction on DGN cardiotoxicity and hence,the present findings are considered to be of immense pharmaco-toxicological significance.

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Received March 20, 1992; accepted June 30, 1992.