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Isoproterenol Potentiation of Methyl Mercury Effects *In Vivo* on Cardiac ATPases and ^{3}H-dopamine Uptake

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Isoproterenol, a potent B-adrenergic receptor agonist, has been known to produce infarct-like myocardial lesions in rats characterized by swelling of endoplasmic reticulum (Katsuna 1972). The swelling of this system is interpreted as an influx of large amount of extracellular fluid into myocardial cells by disturbances of the electrolyte metabolism (Bloom and Cancilla 1969). Isoproterenol is employed clinically as a bronchodilator in respiratory disorders and as a stimulant in heart block and cardiogenic shocks (Innes and Nicherson 1970). In spite of its clinical use, possible drug-chemical interactions leading to adverse health effects are obvious when individuals on a regular isoproterenol treatment are exposed to an environmental contaminant such as methyl mercury. Consumption of fish and fish products is by far the most significant route of exposure to environmental mercury (Inskip and Piotrowski 1985). In spite of such a possibility, little is known about isoproterenol-methyl mercury interaction. The present study forms the first of this kind to report such interactions and their effects on cardiac membrane bound enzymes such as Na^+-K^+ and Ca^{2+}-ATPases. Since Na^+-K^+ATPase has been implicated in uptake and release processes of catecholamines (Seidler et al. 1977) the effects were also studied on ^{3}H-dopamine uptake by sarcoplasmic reticulum. As a prelude to our proposed long-term chronic studies with non-lethal doses in the present report only single and sub-lethal doses were used for a shorter (48h) duration.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing about 250-300 g each, obtained from the Charles River Breeding Laboratories, Wilmington, MA, were used in the present study. Rats were divided into six groups of three each and treated in the following manner: Group one received corn oil and served as controls. The second group was given 5 mg/kg dose of ISO (i.p.). This ISO dose forms the sublethal dose, since the median lethal dose (LD_{50}) of ISO to rats was reported to be 680 mg/kg (Rona et al. 1959). Although Nagumo et al. (1985) used 10 mg/kg/day dose of CH_3HgCl for 7 consecutive

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days for getting acute intoxication, in the present study only one such dose was administered (10 mg/kg) to the third group for getting consistent and reproducible biochemical changes. Such dose levels of ISO and CH_3HgCl which produce measurable and consistent biochemical alterations were necessary to observe, if any potentiating effects. Both ISO ((5 mg/kg) and CH_3HgCl (10mg/kg) were administered at the same time to the fourth group. Rats in group five were pretreated with ISO (5mg/kg) and 24 hours later the rats were given CH_3HgCl (10mg/kg). the last group of rats was pretreated with CH_3HgCl (10mg/kg) and ISO (5mg/kg) was given after 24 hours. All animals were sacrificed 48 hours after the first treatment or 24 hours after the second treatment, the heart tissues were removed and the membrane vesicles composed primarily of sarcoplasmic reticulum were prepared as described by Jones et al, (1979) and modified by Lindemann et al, (1983).

Ca^{2+}-ATPase activity was determined by measuring the inorganic phosphate liberated during ATP hydrolysis as described earlier (Ahammad Sahib and Desaiah, 1987). The inorganic phosphate was estimated by the method of Lowry and Lopez (1946). Na^+-K^+-ATPase activity was obtained by the difference between the ATPase activity measured in the presence and absence of 1 mM ouabain. Protein was determined by the method of Lowry et al, (1951).The uptake of ^{3}H-dopamine by rat heart SR was determined using slighlty modified methods of Seidler et al, (1977) and Slotkin et al, (1978). The specific uptake was obtained by substracting non-specific uptake from the total radioactivity and expressed as CPM^{-1}, mg $protein^{-1}$. The results were subjected to statistical analysis by computing students' t test. Differences between control and experimental results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The myocardial swelling following ISO treatment has been shown to be due to influx of extracellular fluid into myocardial cells by disturbances of the electrolyte metabolism (Bloom and Cancilla 1969). In the present study the cardiac SR Na^+-K^+-ATPase and ^{3}H-dopamine uptake in rats treated with 5 mg/kg ISO were not altered significantly (Table 1, Figure 1), indicating the absence of consistent ISO effect on cardiac membranes at this dose level.

Though the adverse health effects of ISO are mainly due to high doses (employed accidentally or rarely for suicidal intent), when used in doses recommended for therapeutic use it induces no side effects (Innes and Nicherson 1970). On the other hand methyl mercury, which has no clinical value or therapeutic use, forms the major contaminant entering the human body through consumption of fish and fish products (Inskip and Piotrowski 1985). In individuals who are on a regular ISO treatment this unwarranted intake of CH_3HgCl may lead to the occurrence of drug-chemical interactions. In the present study when the rats were treated alone with a single dose of CH_3HgCl (10 mg/kg), which is known to induce no apparent neurotoxic signs (Taylor and Distefano 1976) significant decrease in cardiac SR Ca^{2+}-ATPase and ^{3}H-dopamine

uptake were observed (Table 1, Figure 1), suggesting that non-lethal doses of CH_3HgCl produce changes in cardiac SR. Although the membrane bound Na^+-K^+-ATPase was implicated in the uptake process of the catecholamines in the heart (Burgen and Iverson 1965) in the present study the decrease in cardiac SR Na^+-K^+-ATPase was not statistically significant.

Table 1. *In vivo* effects of isoproterenol (ISO, 5 mg/kg) and methyl mercury (CH_3HgCl, 10 mg/kg) alone or in combination on Na^+-K^+-ATPase and Ca^{2+}-ATPase of rat cardiac sarcoplasmic reticulum.

Experimental Condition	Na^+-K^+-ATPase (μ mole Pi/mg protein /h)	Ca^{2+}-ATPase (μ mole Pi/mg protein/h)
Control	12.72 ± 1.49	8.82 ± 0.77
ISO (5 mg/kg)	9.55 ± 0.60 (25)	8.15 ± 0.46 (8)
CH_3HgCl (10 mg/kg)	8.74 ± 0.37 (31)	5.69 ± 0.36*(36)
ISO + CH_3HgCl treated at the same time	7.22 ± 0.43*(43)	3.98 ± 0.20*(55)
ISO followed by CH_3HgCl at the 24th hour	6.74 ± 0.12*(47)	3.79 ± 0.45*(57)
CH_3HgCl followed by ISO at 24th hour	8.65 ± 0.27*(32)	5.90 ± 0.46*(33)

Each value is the mean ± SE of three independent observations and each was done in triplicate. Asterisks denote values significantly ($p < 0.05$) different from controls. Figures in parenthesis indicate percent decrease from control. ISO and CH_3HgCl are adminstered through intraperitoneal injection in all the experimental conditions and the rats were sacrificed after 48 hours of the respective treatments.

Treatment of rats with 5 mg/kg dose of ISO and 10 mg/kg dose of CH_3HgCl (such dose levels of ISO and CH_3HgCl were necessary to obtain measurable and consistent biochemical alterations and to delineate possible potentiating effects) at the same time or administration of CH_3HgCl to ISO pretreated rats resulted in greater inhibition of Na^+-K^+-ATPase and ^3H-dopamine uptake (Table 1, Figure 1), when compared to rats treated with ISO or CH_3HgCl alone indicating that ISO when present increases the sensitivity of cardiac enzymes to CH_3HgCl. Although the exact mechansism for this greater sensitivity is not known, ISO through some unknown mechanism appears to facilitate greater translocation of CH_3HgCl to cardiac SR. Although Ca^{2+} was reported to inhibit

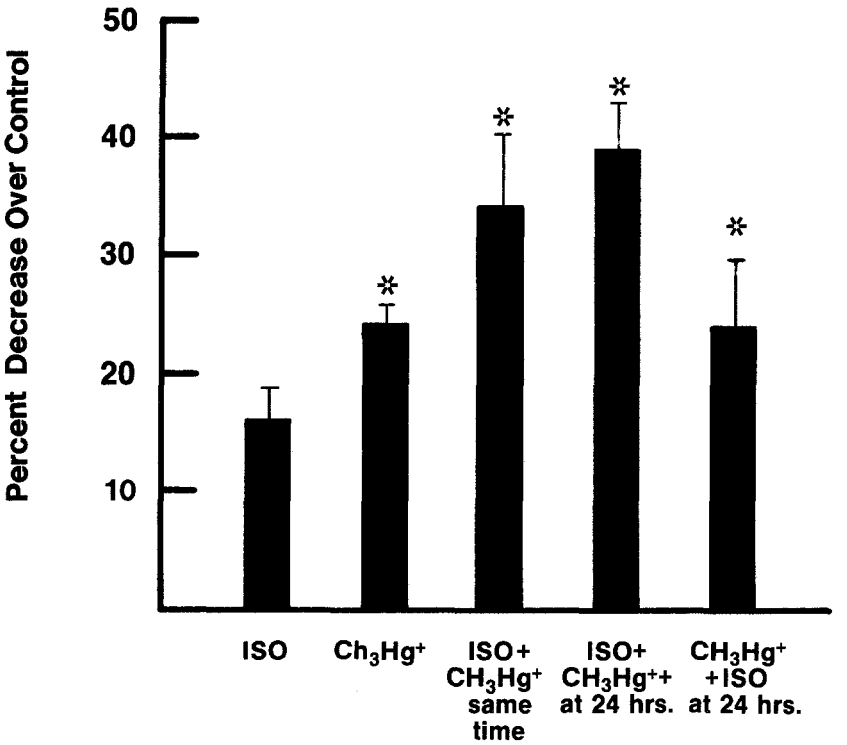

Figure 1. Effects of Isoproterenol and methyl mercury alone or in combination on cardiac sarcoplasmic reticulum ^{3}H-dopamine uptake. Treatment and conditions are as described in 'Material and Methods'.

Na^+-K^+-ATPase (Sulake and Dhalla 1971) which in turn was implicated in uptake and release process of catecholamines in the mammalian heart (Burgen and Iverson 1965) the ISO-CH_3HgCl mediated effects were not the same on all the parameters studied. The decrease observed in Ca^{2+}-ATPase was more than additive suggesting greater impact on the calcium pump. On the other hand, when ISO was given to rats pretreated with CH_3HgCl the extent of inhibition in any of the three parameters was simply the same as in rats treated with CH_3HgCl alone.

In summary, cardiac cation transporting enzymes were greatly reduced in rats treated with ISO and CH_3HgCl at the same time or when CH_3HgCl was administered to ISO-pretreated rats as compared to rats treated with ISO or CH_3HgCl alone. Administration of ISO to CH_3HgCl-pretreated rats resulted in a decrease in the inhibitory potency. The ability of ISO and CH_3HgCl to reduce myocardial ATPases parallels their effects on ^{3}H-dopamine uptake by SR fracton. The ISO-potentiation of CH_3HgCl effects appears to be selectively greater on the calcium pump. The results imply that even non-lethal doses of CH_3HgCl (entering via the food chain) becomes potentially toxic to individuals who are on regular

ISO treatment and cellular injury may occur when energy substrates are (ATP) depleted (as a result of Ca^{2+} accumulation) in the cell to the levels insufficient to maintain the structural integrity of the cells(Ramos et al. 1983).

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