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Effect of Mercuric Chloride on Coronary Flow in Perfused Rat Heart

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There are 106 elements out of which 80 are metals. These range from simple ionic salts to complicated structures such as ligands and organometallic compounds. The use of metals started since the emergence of man from the stone age, and so the human exposure and health hazards increased with the advancement of civilization. Environmental pollution and human exposure to metals may occur naturally, i.e., by erosion of surface deposits of metal minerals and by human activities like mining, smelting, industrial application of metals, and fossil fuel combustion. The commercial and industrial uses of metals are continuously increasing. All these activities increase the discharge of metals into the human environment. It is also transported along aquatic and terrestrial routes, and thus enter the food chain. It causes environmental disease e.g. minamata disease caused after eating methylmercury contaminated fish.

Metallic elements are found in all living organisms as structural element, components of control mechanism, stabilizer of biological structure, and as activators of enzymes. Therefore, some are essential elements and its deficiency causes impairment of biological function. When present in excess quantity in the living system, it becomes toxic.

Mercury is a heavy metal of group II elements of the periodic table. It is widely distributed in the earth's crust, sea, ground and rain water; all phyla and species naturally contain traces which varies with localities. Extensive agricultural and industrial usages can significantly affect its redistribution in specific regions. Burning of fossil fuels generate environmental mercury in amounts comparable to that from industrial processes.

Mercurial compounds exert interesting effects on contractile system (Ferri et al. 1972). Production of vasoconstriction in in vitro by mercuric ions was demonstrated earlier (Mitchell 1963). The effect of mercuric chioride on arterial

blood pressure of dog was studied by Worowski et al. (1969). Leszkovszky & Szantho (1970) did the experiments on animals regarding selective uptake of mercury by myocardial infarcts in the rat. Solomon et al. (1975) did the work on the mechanism of mercuric ion (Hg^{++}) induced contraction of vascular smooth muscle. He and co-workers found that in vivo, injection of $HgCl_2$ into the renal artery induced a dose-related reduction in the renal blood flow. The infusion of phenozybenzamine intra-arterially reduced the vascular response to $HgCl_2$ significantly. The coronary flow was also determined in fibrillating heart with and without perfusion with mercuric chloride.

The present study was conducted to investigate the effect of mercuric chloride on the coronary flow of perfused rat heart and the role of phenoxybenzamine in protection against $HgCl_2$. It was found that the reduction in coronary flow was according to dose-response. Alpha adrenergic blockade i.e. phenoxybenzamine partially prevented mercury induced reduction in coronary flow in in vitro condition.

MATERIALS AND METHODS

The method of perfusing the isolated rat heart was the classical Langendorff preparation in which the aorta was cannulated and the coronary vessels were prefused by introducing perfusate into the aorta. Ringer-Locke solution was used as perfusion medium at pH 7.4 equilibrated with pure 0_2 at 37°C. The solution was made each day. The final concentration of salts in the buffer (g/L) were: NaCl 9; $NaHCO_3$ 0.5; on the day of use the buffer was prepared by mixing these stock solutions in the ratio KCl 10% solution (4.2 mL); Calcium chloride molar (1.08 mL) and finally glucose was added in concentration(lg/L).

Male wistar rats weighing between 150 - 300 g were used. The preparation of the heart for perfusion involves in sequence the rapid removal of the heart from the animal by a blow at the base of skull with an iron rod mid sternal incision and opening of the pleural cavity, slitting the pericardial membrane and rapid cutting of the vena cava, pulmonary arteries, veins and aorta. The heart was removed from the pericardial sac and placed in a dish of warmed perfusion solution.

The perfusion cannula was tied into the root of aorta pointing to the aortic valve. The heart was perfused at a pressure of 50 mm of Hg. The perfusing fluid was warmed to 37°C by a water bath held at this temperature. The aorta was tied on to

the terminal of the cannula. Bubbles should not enter the cannula. A thread was sewn through the apex of the ventricles and attached to the linear motion transducer for recording of inotropic and chronotropic properties by the polygraph 7754 D system of Hewlett Packard.

Perfusate which passed through the coronary circulation dripped through the funnel to a beaker and was measured in a graduated tube. Thus coronary flow was measured by timed collection of total heart drainage. Mercuric chloride ($HgCl_2$) solution of concentration 1 ug/mL (1 ppm) was prepared from the stock solution of 1,000 ppm. $HgCl_2$ was mixed directly in the Ringer-Locke solution and poured into Marritor bottle and bubbled with oxygen. Coronary perfusion pressure was reduced by clamping the rubber tube above the camula. Coronary flow decreased linearly with decrease in perfusion pressure i.e. flow of fluid into coronary circulation. Pressure was measured by a manometer attached to cannula with a rubber tube. Different concentrations of mercuric chloride were used by removing the previous solution from Marritor bottle and was washed properly. Heart was fibrilated with the help of oscillator by passing 10 volts to it.

RESULTS AND DISCUSSION

The pressure-flow relationship in perfused rat's heart is linear (The L.B. et al. 1930). It means there is no change in the resistance when pressure is increased from 20 mm Hg to 50 mm Hg. While in the case of dog heart the flow against perfusion pressure increases in exponential fashion. In the case of isolated rabbit heart the pressure-flow relationship is curvilinear with the curve convex to the pressure axis. This difference in the pressure-flow relationship may be due spectes difference the reason for which is not known. It may be due to difference in the coronary circuit.

In figure 1, coronary flow is plotted against time in log value at different pressures (20, 30, 40 & 50 mm Hg) when heart is prefused with 1 ppm (1 ug/mL) of mercuric chloride. The result shows that at the lowest pressure, i.e., ischemic condition, it takes more time for the flow to become zero or no flow. There is parallel shift in flow at higher pressure than at lower pressure.

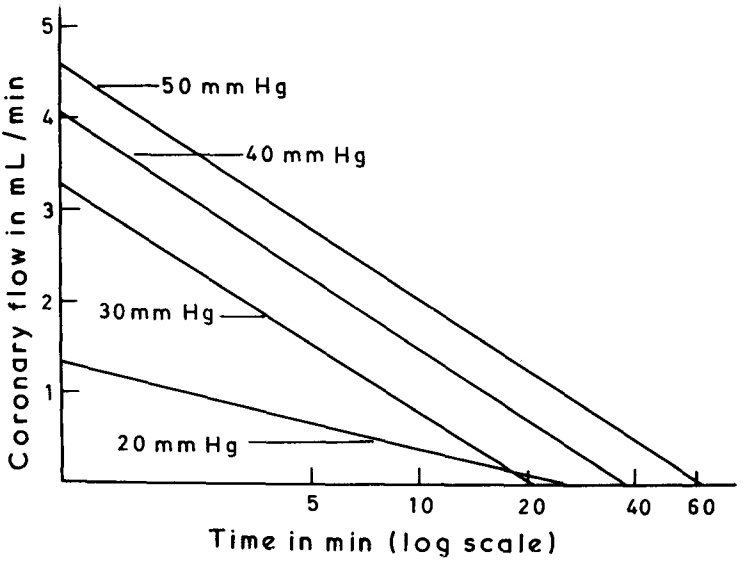

Figure 1: Coronary flow against time in log scale at different pressure perfused with 1 ug/mL of $HgCl_2$.

It might be possible that mercuric chloride has a favourable effect on the components of energy metabolism and contributes to reestablishment of ionic balance across the cell membrane in ischemic foci in the myocardium and improve energy metabolism by increasing the concentration of ATP and the balance between ATP and creatine phosphate (CP) is also maintained (Mrhova et al. 1975). The present result is in agreement with the findings of these authors. It can be said that the enzymes of the basic metabolic cycles which decreases in ischemic condition is improved by mercuric chloride. The effect of mercuric chloride and lead salts on coronary was studied by Jha et al. (1980).

The coronary flow at a constant pressure of 50 mm Hg and different concentration of mercuric chloride i.e. 0.2, 0.6, 1.0 and 2.0 ug/mL decreases in accordance with the concentration at the fifth minute as shown in figure - 2. The coronary flow depends on the factors like physical, neural, neurohumoral, myogenic and metabolic (Berne 1964).

Reduction in coronary flow seems to be due to myogenic factor - the effects on vascular smooth muscle and myofibrillar proteins where the contraction force is generated. It is known that the coronary flow increases and decreases with increases and decrease of vigor of contraction (Guyton 1976). The amplitude of

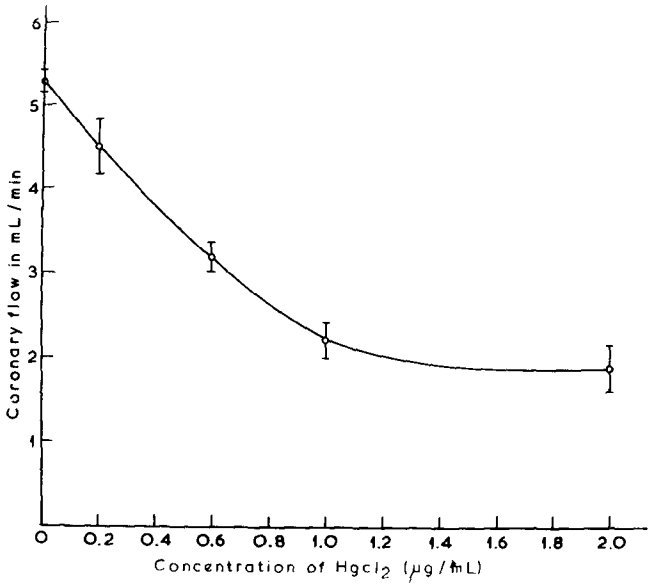

Figure 2: Coronary flow at 50 mm Hg perfused with different concentration of mercuric chloride at fifth minute.

contraction i.e. inotropic property gets reduced which indicates the lessening of the vigor of contraction and thereby affecting the contractile proteins. The actin ATP ase inhibition has been reported in skeletal muscle (Shamoo 1976).

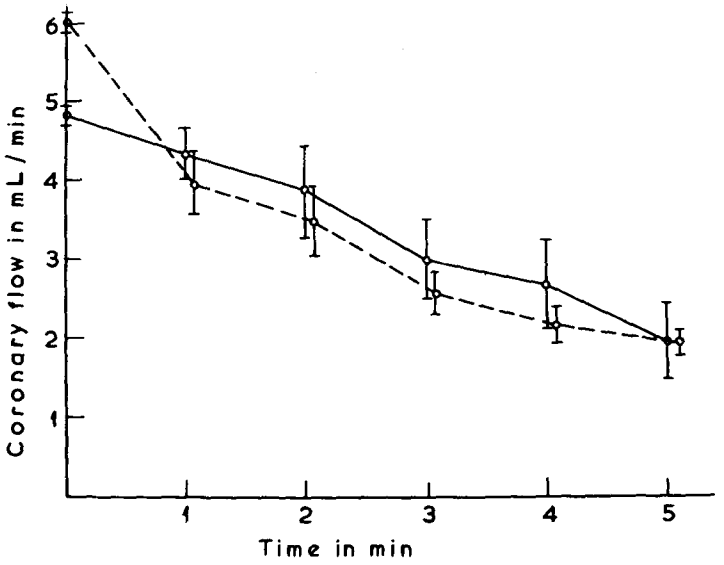

Figure 3: Coronary flow in normal and fibrillating heart perfused with 1 ug/mL of $HgCl_2$ at different timings.

Increase in coronary flow results from abolition of coordinated cardiac contraction by ventricular fibrilation (Burne 1964). This is understood from figure - 3, where the flow is more than normal value at zero time, when mercuric chloride in concentration of 1 ug/mL is perfused through the coronary circulation. The reduction in the flow starts in the first minute itself. It is clear from this that the decrease in coronary flow is also mainly due to reduction in the force of contraction i.e. inotropic property. The partial protection in the reduction of coronary flow by phenoxybenzamine is seen from figure - 4. Up to the third minute of mercuric chloride (1 ug/mL) perfusion the flow difference from control is significant (P 0.01), whereas at the fourth and fifth minute, the reduction in flow was not significant (P 0.01).

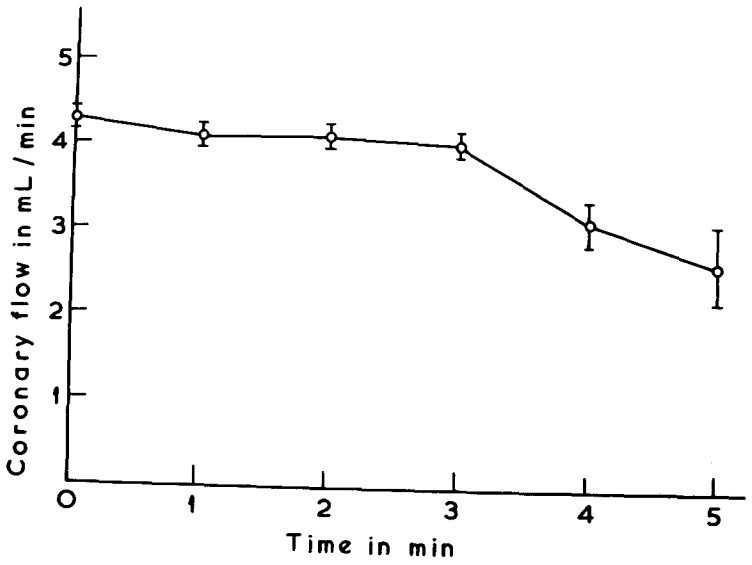

Figure 4: Partial protection in reduction of Coronary flow perfused with 1 ug/mL of $HgCl_2$ and 25 ug/mL of phenoxybenzamine at different time intervals.

Atropine was tried in the concentration 5-25 ug/mL in the perfusion fluid did not show any change in the reduction of coronary flow. Whereas, phenoxybenzamine, 25 ug/mL prevented the reduction of flow to some extent as described above. The concept regarding involvement of receptor protection was first sugges -ted by Furchgott (1954) and later on verified by Solomon et al.

(1975). The mechanism of the protection of receptor from mercuric chloride might be that mercuric ion does not act directly on the alpha adrenergic receptor but by activating the norepinephine receptor and thereby causing the release of catecholamine. The contraction of vascular smooth muscle reduces the lumen of the vessel and therefore, the coronary flow is reduced. But the contraction cannot increase due to the reasons that $HgCl_2$ inhibits Ca^{++} ATP ase as well as Ca^{2+} transport (Shamoo et al. 1976). Therefore, the contraction of vascular smooth muscle as well as cardiac muscle become less. It is known that mercuric chloride inhibits $(Na^{+}+ K^{+})$ ATP ase and so active transport. The non-working of active transport may increase the passive diffusion to keep the balance. But in this case, it is possible that the inhibition of active transport and increase of passive diffusion do not keep the balance due to continuous, perfusion of mercuric chloride. The reason for the passive diffusion not to increase might be due to blockage caused by Hg^{++} with lipid. This will cause depolarization, thereby causing constriction in vascular smooth muscle. The reduction in the vascular smooth muscle and so in coronary flow is due to blockage of gate of Na^{+} i.e., inhibition of K pump. Finally, it can be concluded that the reduction in the coronary flow in rat's heart perfused with different concentration of mercuric chloride is due to the vascular smooth muscle (i.e. myogenic response).

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