

CHAPTER - 5

DISCUSSION

Acute intravenous administration of CdCl_2 produced a short-lived depressor effect followed by a persistent pressor effect (Fig. 1A and 2A). The magnitude of the responses observed with 0.1, 0.5 and 1 mg/kg of CdCl_2 was similar to that reported by Fadloun and Leach (1981a). These authors used 0.1, 0.5 and 1 μM of Cd^+ as chloride in their study. Perry et al. (1970) observed lesser degree of depressor response with 0.35 μ moles of Cd after intravenous injection in rats. However, the degree of pressor response was similar to that observed in the present study. The depressor effects of intravenous Cd have been observed in other species but have not been extensively studied. Similarly, the pressor effect following the depressor effect, observed in the present study has not attracted much attention earlier. In dogs, progressive hypotension following weekly intravenous injection of progressively increasing doses of Cd has been shown (Fatkhullaev, 1959). In cat and rabbit, a single similar intravenous injection of Cd produces immediate but transient hypotension (Delham and Friberg, 1954). In rats, intra-arterial administration of smaller doses of Cd (10 μg and 40 $\mu\text{g}/100$ g body weight) produces a pressor response and higher doses (80 μg and 330 $\mu\text{g}/100$ g) produce only a depressor response (Perry and Yunice, 1965; Perry et al., 1970).

In the present study intraperitoneal administration of CdCl_2 (0.5 and 1 mg/kg) produced a pressor response (Fig. 1B, 2B). These results are in line with those of earlier workers' (Schroeder et al., 1970; Perry and Erlanger, 1971b; Perry and Erlanger, 1975; Hall and Nasseeth, 1980a, b; Hall and Hungerford, 1982). Marked persistent pressor effect was promptly induced in anaesthetized rats by administering Cd (0.44 μ moles) intraperitoneally (Perry and Erlanger, 1971b). These authors showed an average maximum increase in diastolic pressure of 33 mm Hg. Schroeder et al. (1970) have shown that intraperitoneal injection of 1.0 mg of cadmium as the acetate was followed by a rise of mean blood pressure of 15 to 27 mm Hg within five min in all the older rats and by 8 to 36 mm Hg in the younger rats. The acute pressor response to intraperitoneal administration of Cd was observed with as low as 0.031 mg/kg in anaesthetized rats and the maximum response was obtained at 0.25 mg/kg (Hall and Hungerford, 1982). In the present study, a rise of about 15-20 mm Hg of blood pressure was obtained after intraperitoneal injection of CdCl_2 (1 mg/kg) in female rats. Doses lower than 0.5 mg/kg did not produce any significant pressor effect. Other investigators have shown that the pressor response to Cd begins within a min of its injection (Perry and Yunice, 1965; Perry et al., 1971; Hall and Nasseeth, 1980a) a finding with which the present study concurs. In the present study the pressor

effect lasted for about 25-30 min, whereas other investigators (Perry et al., 1971; Hall and Nasseeth, 1980a, b) Hall and Hungerford, 1982) have shown that the pressor effect persisted for a few hours. This variation may be due to factors such as difference in dosage, level of consciousness, age, and strain of the animals. It was found that when 5 week old male rats of Sprague-Dawley, Fischer 344, Wistar-Kyoto and Wistar-SH strains were given 2 mg/kg of CdCl_2 intraperitoneally, a pressor response did not occur (Hall and Nasseeth, 1980a). It was further suggested that conscious Wistar rats were better able to maintain normal blood pressure after Cd injection than either Sprague-Dawley or Fischer 344 rats. In the conscious state, the pressor reactions to Cd in the group as a whole, were never reliably obtained but were always observed under anaesthesia. Fischer 344, and Sprague-Dawley rats 6 to 10 weeks old usually gave pressor response regardless of any state of consciousness (Hall and Nasseeth, 1980). In a recent report by Hall and Hungerford (1982) it was shown that in Sprague-Dawley rats, the pressor response was obtained in the conscious animal after reaching a dose of 1 mg/kg but not lower than this dose. It appears that acute pressor response to Cd injection is strongly influenced by strain, age and level of consciousness. One group of investigators studying the conditions have reported it to be both sex and strain dependant (Ohanian et al., 1976) whereas

another equally steadfastly contended that neither factor is important (Perry et al., 1977, 1979).

The mechanism of acute pressor effect as well as the hypertension due to chronic administration of Cd have been the subject of considerable debate and controversy.

The neurotransmitters Adr and NA both regulate local arterial blood pressure and have been associated with hypertension (De Champlain, 1972). The release of NA appears to be accompanied by the release of soluble part of DBH (Viveros et al., 1968). For this reason it has been proposed that plasma DBH activity may serve as an index of the sympathetic system (Wienshilboum et al., 1971).

Plasma DBH activity was significantly increased after acute injection of Cd (Fadloun and Leach, 1981a). If the acute pressor response is due to the release of NA from adrenergic nerve ending or catecholamines from adrenal medulla, as reflected by the increased plasma DBH level, the pressor response should be blocked by adrenoceptor blocking agent. In the present study, acute pressor responses to both intravenous or intraperitoneal administration of CdCl_2 were not prevented by the prior administration of phentolamine (Fig. 6A and 7A). Moreover, acute reserpini- zation though completely blocking the acute pressor response

to tyramine, failed to block the responses to CdCl_2 administered intravenously or intraperitoneally (Fig. 6,C,D, and 7C).

When nicotine is administered to the dog, it produces an increase in the heart rate and blood pressure. The latter is more of a sustained response. In general, the cardiovascular responses to nicotine are due to the stimulation of sympathetic ganglia and the adrenal medulla together with the discharge of catecholamines from sympathetic nerve endings (Gebber, 1969). In this study hexamethonium completely blocked the pressor response to the ganglionic stimulant DMPP, but did not block that to CdCl_2 (i.v. or i.p.). These results suggest that the pressor response to CdCl_2 was not due to ganglionic stimulation or the release of catecholamines from the adrenal medulla (Fig. 6B and 7B). This was further strengthened by the finding that in adrenalectomized animals, the acute pressor response to CdCl_2 was not abolished (Fig. 12A, B). These results are contrary to those of Hart and Borowitz (1974) who observed that Cd increased the release of catecholamines from the bovine adrenal medulla. Though NA has been implicated to be the neurotransmitter taking part in the elevation of blood pressure, it seems possible that the acute pressor response to CdCl_2 (i.v. or i.p.) may be due to a mechanism other than the involvement of the noradrenergic system.

Perry et al. (1967) have shown that the increase in

diastolic pressure following intra-arterial administration of Cd was accompanied by an increase in cardiac output. Propranolol, a beta-adrenoceptor blocking agent, has been shown to reduce the cardiac output (Ulrych, 1968). In the present study it was found that the pressor response to CdCl_2 (i.v. or i.p.) was not blocked by propranolol suggesting lack of stimulation of beta-receptor of the heart by Cd directly or indirectly through the release of catecholamines (Fig. 6E and 7D). Kopp et al. (1978) suggested that Cd may potentially depress the excitability of atrioventricular nodal cell and may also interfere with ventricular cell to cell conduction in the chronically treated rats. Toda (1973a, 1973c) has shown that in isolated rabbit atria Cd (0.02-0.5 mM) caused a marked depression of the action potential and a slight decrease in the resting potential. The results of these in vitro experiments are contradictory to the report of Perry et al. (1967) who have shown an increased cardiac activity with Cd in the intact animals.

Recently, Caprino et al. (1982) determined a prostaglandin effect of Cd in the rabbit. Administration of 0.25-0.5 mg/kg Cd (i.v. or i.p.) gave a dose dependant increase in thromboxane B_2 with inhibition of prostacycline at 4 days. The effect of Cd on prostacycline was biphasic. The metal in doses of 0.25 and 0.5 mg/kg inhibited prostacycline production.

At a higher dose Cd caused an increase in prostacycline formation. The authors concluded that Cd activates the arachidonic pathway. Prostaglandin endoperoxides have variable effect in vascular beds ranging from vasodilatation to vasoconstriction and sometimes they induce vasoconstriction followed by vasodilatation (Dusting et al., 1978). Since the endoperoxides are the substrates for the conversion to other potent prostaglandins, their effects are a result of intrinsic vasoconstrictor coupled with some vasodilator action due to rapid conversion to a prostaglandin that is vasodilator (most probably prostacycline (PGI_2)). PGI_2 is a vasodilator when given intra-arterially or intravenously (Moncada and Vane, 1978; Szezeklik et al., 1978). It is tempting to speculate that the biphasic effects on prostaglandin may in part explain the analogous biphasic effects of Cd on vascular tissue seen by some workers and also in the present study. An attempt has been made to investigate the role of prostacycline in the production of acute depressor and pressor response to Cd^{+} . Indomethacin, a PG synthetase inhibitor was administered in doses (5 mg/kg to 30 mg/kg, i.p. or s.c.), known to inhibit PG synthesis in rats (Fitzpatrick and Wynalda, 1976; Vargafting and Lefort, 1977; Abdel-Halim et al., 1978; Wallenstein and Maurs, 1984). The depressor or the pressor effects of intravenous administration of CdCl_2 were not blocked when it was given one h after intra-

peritoneal pretreatment with indomethacin (20 mg/kg) (Fig. 6G). Similarly the acute pressor response to intraperitoneal administration of CdCl_2 was also not modified after pretreatment with indomethacin (Fig. 7E). The inability of indomethacin to block the blood pressure responses to CdCl_2 suggests that the cardiovascular effects may not be due to the involvement of prostacycline or other prostaglandins as suggested by Caprino et al. (1982).

Calcium and magnesium are essential elements for the normal functioning of vascular smooth muscle. The transmembrane influx of calcium and other divalent cations under the influence of excitatory agent has been demonstrated with electrophysiological ion flux and electronmicroscopic techniques. NA and KCl depolarize rabbit main pulmonary artery smooth muscle in sodium-free solution in which calcium is the major extracellular cation (Somlyo and Somlyo, 1971b). Since under these conditions calcium is the carrier of inward current during depolarization, these findings indicate that both the excitatory agents increase the permeability of the cell membrane to calcium. The magnitude of the change in membrane permeability is proportional to the (unequal) magnitude of the contraction produced by different drugs (Somlyo and Somlyo, 1971a).

Increased ^{45}Ca influx has been observed under the

influence of various excitatory agents including high K depolarizing solution (Goodman et al., 1972), acetylcholine (Potter et al., 1970), NA and prostaglandin F₂ alpha (Van Breeman et al., 1973; Greenberg et al., 1974). These effects are more readily demonstrated in low ⁴⁰Ca mixture (Hudgins and Weiss, 1969; Potter et al., 1970). Various drugs and ions inhibit both the influx of extracellular calcium and contractile response to excitatory agents.

From the foregoing discussion, it seems that fluxes of calcium ion are involved in the excitatory actions of different agents in all the smooth muscles. Kondo et al. (1980) studied the effect of calcium channel blockers such as nifedipine, diltiazem and verapamil on the vasoconstrictor responses to NA and KCl in perfused rat mesenteric vascular bed. It was shown that all these drugs attenuated the vascular responses to KCl in a dose-dependant manner. NA responses were effectively blocked by verapamil but only slightly by nifedipine and diltiazim. The direct vascular contraction induced by KCl is mainly due to the inward movement of the calcium from the extracellular space (Van Breeman and McNaughton, 1970). In the present study the acute pressor responses to CdCl₂ (i.p. or i.v.) were significantly blocked after intravenous pretreatment with verapamil or nifedipine (Fig. 6 I,J,K,L,M and 7 F,G). It

may therefore, be concluded that the acute pressor response to Cd may be due to its calcium like effect or mediated through the influx of calcium ion. Hall and Hungerford (1982) have recently shown that pretreatment with nifedipine completely prevented the acute pressor response to Cd in conscious animals.

In retrospect, it has been shown that cadmium-induced acute pressor response was blocked by a chelate of zinc (Na_2Zn CDTA, cyclohexane-1, 2-diamine N N N' N' tetra acetate) (Schroeder et al., 1970). Zinc has also been shown to block the acute pressor response to cadmium (Schroeder and Buckman, 1967). Perry and Erlanger (1975) have observed that the acute pressor response to intraperitoneal administration of Cd was significantly blocked by pretreatment with zinc, selenium, hydrallazine, diazoxide, and minoxidil. These authors have also shown that the pressor response to acute intraperitoneal administration of Cd was not blocked after tying renal vessels of both kidneys. Youkilis et al. (1971) observed that the intraperitoneal injection of 1 mg/kg of Cd produced slight constriction of small arteries of bat-wing while 3 mg/kg caused significant dilatation of both arteries and veins. Scot and Haddy (1963) reported that small vessel dilatation resulted from infusing between 0.03 to 5 mg of Cd/minute into the foreleg of 17 kg dog in a system which

maintained the constant brachial flow rate. These authors concluded that the local effect of Cd was vasodilatory and the pressor effect resulted from remote area. Perry and Erlanger (1975) suggested that the pressor effect induced by intraperitoneal administration of Cd results from direct effect on vascular smooth muscle since this effect was blocked by directly acting vasodilators such as minoxidil, diazoxide and hydralazine. However, there are reports of additional calcium antagonistic effect of diazoxide (Wohl et al., 1967) and hydralazine (Mc Lean, 1978). Therefore, it seems likely that the acute pressor response to CdCl_2 observed in the present study, may be either due to its calcium-like action thereby causing direct contractile effect on vascular smooth muscle or due to its effects on fluxes of calcium, since the acute pressor effects are blocked by calcium channel blockers.

The induction of hypertension in rats by feeding Cd chronically was first reported by Schroeder and Vinton (1962). During the next twelve years, Schroeder extended his initial observation in numerous reports (Schroeder, 1964; Schroeder et al., 1968a, 1968b; 1970; Kanisawa and Schroeder, 1969a). Perry et al. (1977, 1979) used groups of 16 female long Evans rats which received 0.1, 2.5, 5, 10, 25 and 50 mg of Cd/litre in drinking water from the time they were weaned until they

were 30 months old. A modest but statistically significant elevation in systolic blood pressure was observed continuously at 6, 12, 18, 24 and 30 months in those groups of rats that had a life-long exposure to drinking water containing 2.5 and 5 ppm Cd. Such a hypertensive state has been produced in rats by various workers (Petering et al., 1979; Fadloun and Leach, 1980; Kopp et al., 1982).

In the present study initial experiments were done on female albino rats by administering CdCl_2 (0, 5, 25 or 100 ppm) in deionized water for 4 weeks or 8 weeks. The dose and duration of treatment were selected on the basis of the report of Fadloun and Leach (1980). They have shown marked hypertension when Cd was given in drinking water in the dose of 12.5 ppm or 25 ppm for 4 weeks. But in the present study, there was no significant difference in systolic blood pressure of rats treated with Cd in all the doses administered for 4 weeks or 8 weeks (Table IX). Such negative observations have been made by others after oral treatment with Cd (Lener and Bibr, 1970; Friberg et al., 1971; Doyle et al., 1975). Various reasons have been put forward by different workers for their inability to induce hypertension after chronic oral treatment with Cd in rats. It was found that when rats were given rye-based diet, Cd (0.1 or 1 ppm) regularly induced elevation of systolic blood pressure. For those rats, given

stock diet instead of rye-based diet, the increase in pressure induced by 0.1 to 1 ppm of Cd was reduced (Perry and Erlanger, 1982). Doyle et al. (1975) found that 5 ppm Cd in water did not induce hypertension in female Sprague-Dawley rats given a diet based on glucose and egg white. Eakin et al. (1980) also found that 10 and 20 ppm Cd failed to induce hypertension in male OSU Brown rats given glucose casein diet. Lener and Bibr (1970) reported that Cd did not induce hypertension in female Wistar strain rats fed a commercial diet. Frickenhaus et al. (1976) reported that 20 or 40 ppm Cd added to food did not induce hypertension. Petering et al. (1979) and Boscolo et al. (1981) found that 17.2 ppm Cd for 29 to 39 weeks and 10 or 20 ppm Cd for 5 months induced hypertension, in Sprague-Dawley rats fed Purina Rodent Lab. Chow. Both Boscolo's and Petering's groups used male rats whereas Perry and Erlanger (1979) have shown that Cd induces comparable hypertension in both male and female rats. Ohanian and Iwai (1979) found that 1 ppm Cd induced hypertension in hypertensive - sensitive Dahl rats fed Agway diet. From the foregoing discussion, it is pertinent to note that hypertension due to chronic oral administration of Cd is controlled by diet, sex, strain, and duration of treatment. The inability to induce hypertension after oral exposure to Cd in the present study may be due to differences in diet or strain or duration of treatment.

Chronic hypertension was induced in rats after intraperitoneal injection of cadmium acetate (2 mg/kg) 2 or 3 times in three weeks (Schroeder et al., 1966; Hall and Hungerford, 1980b). Fadloun and Leach (1981a) observed that intraperitoneal administration of Cd (0.5 and 1 μ M) for 12 days in female rats produced severe hypertension. Puri and Sur (1983) have shown that chronic hypertension was induced by administering cadmium acetate (1 mg/kg) intraperitoneally for one week. In the present study 0.5 or 1 mg/kg of CdCl₂ was injected intraperitoneally to female rats for 2 weeks and significant elevation of blood pressure was recorded. The dose and the duration of treatment used in the present study were approximately similar to those used in the study of Fadloun and Leach (1981a). In successful experiments there is disagreement as to whether male (rats) are more (Petering et al., 1979), less (Ohanian et al., 1979), or equally (Perry et al., 1979) as susceptible as female in developing hypertension by Cd. Data from literature have shown that females are more susceptible to develop hypertension (Altura, 1975; Greenberg et al., 1973). Therefore, female rats were chosen in the present study for the development of hypertension.

Hypertension in female rats after chronic CdCl₂ injection was accompanied by significant reduction in body

weight (Table XV). Similar effects were also observed by other workers (Perry et al., 1977; 1979; Fadloun and Leach, 1981a). These authors have not mentioned any specific reason for the reduction in the body weight. However, it was shown that chronic administration of Cd inhibits the growth of the animals (Flick et al., 1971).

In trying to evaluate the mechanism of cadmium-induced hypertension, various experiments have been conducted on female albino rats. The contribution of adrenal medulla to the regulation of blood pressure in normotensive and DOCA-Salt hypertensive rats (De Champlain et al., 1976, 1977) as well as in SHRs (Aoki, 1963; Ozaki, 1966) has been demonstrated. Catecholamine metabolism is disturbed in DOCA-Salt hypertension (De Champlain et al., 1969). Bilateral adrenalectomy produces greater fall in blood pressure in DOCA-Salt hypertensive rats than in normotensive rats, suggesting active but partial role of adrenal medulla (De Champlain, et al., 1977). Also the role of adrenal medulla in rats made hypertensive after the application of clip on the renal artery for 3 weeks (De Champlain and Van Ameringan, 1972), in androgen-induced hypertension (De Champlain, 1977), in Japanese strain of SHR (Ozaki, 1966) and in neurogenic hypertension (De Quattro et al., 1969) has been suggested.

In all the above studies, bilateral adrenalectomy either

produced significant lowering of the blood pressure or prevented the development of hypertension. In such hypertensive model increase in the turnover of catecholamine in adrenal medulla has been reported (Morisawa, 1968; De Champlain et al., 1969; Grobecker et al., 1982). However, in SHR of New Zealand strain bilateral adrenalectomy did not prevent the development or maintenance of hypertension (Nolla-Panades and Smirk, 1964; Phelan, 1968). Bilateral adrenalectomy prevented the development of hypertension induced by bilateral nephrectomy in rats but not if 1% NaCl rather than water was given to drink (Del Greco et al., 1968; Wilson et al., 1971). It was observed that catecholamine was released from isolated bovine adrenal medulla by divalent ions of subgroup IIB of the periodic table (Hart and Borowitz, 1974). Cd is moderately effective in releasing the catecholamine from adrenal medulla whereas mercury is a potent releaser (Hart and Borowitz, 1974). It was suggested by these authors that divalent metallic cations Cd and Hg may mimic calcium action in mediating adrenal catecholamine release. However, surprisingly in the present study, bilateral adrenalectomy in rats did not prevent hypertension produced by chronic CdCl₂ treatment (Fig. 11) though adrenalectomy significantly reduced the blood pressure in untreated animals. Moreover, acute intraperitoneal or intravenous administration of CdCl₂ produced a pressor effect even in the adrenalectomized

animals (Fig. 12). These experiments therefore, suggest that adrenal glands may not play an important role either in producing chronic hypertension or acute pressor response to CdCl_2 .

The role of peripheral sympathetic system has been suggested in experimental renal hypertension (De Champlain, 1977), in New Zealand strain of SHR (Smirk, 1970) and also in neurogenic hypertension (Doba and Reis, 1973). It was found that chemical sympathectomy produced marked lowering of blood pressure in all the above models of hypertension. Previous studies attempting to exclude the peripheral sympathetic system in renal hypertension by immuno-sympathectomy (Dorr and Brody, 1966; Ayitey-Smith and Varma, 1970), or chemical sympathectomy by 6-OHDA (Finch and Leach, 1970) have been equivocal due to inadequate controls and incomplete assessment of the degree of peripheral sympathetic system. Treatment with 6-OHDA and immuno-sympathectomy have been shown to produce only an incomplete sympathectomy particularly with respect to vasculature (Clark et al., 1972; Berkowitz et al., 1972; Finch et al., 1973). The administration of guanethidine to adult rats produces a marked longer-lasting and permanent destruction of peripheral sympathetic nervous system without significant cytotoxic action on the adrenal medulla and central noradrenergic neurone (Johnson and O'Brien, 1976).

Revis and Zinsmeister (1981a) showed that in human subject there is significant association between blood Cd and plasma NA level. Fadloun and Leach (1981b) observed that the NA contents of the heart, kidney, portal vein, vas deferens and anococcygeus muscle were increased in the Cd treated animals. It was suggested by these authors that hypertension following Cd administration is the result of altered sympathetic system. In the present study, chemical sympathectomy produced by guanethidine did not prevent chronic CdCl_2 induced hypertension in rats (Fig. 13). The pressor responses to the acute intravenous or intraperitoneal injection of CdCl_2 were also not abolished in the chemically sympathectomized rats. Therefore, this study failed to establish the probable role of sympathetic system in CdCl_2 induced hypertension.

The vascular lesions in the kidney of rats fed subtoxic levels of Cd which became hypertensive were characteristic of those found in rats (Schroeder, 1942; Schroeder and Newman, 1942) and dogs (Goldblatt, 1937; Goldman et al., 1952; Dammin et al., 1956) when hypertension followed partial constriction of one renal artery and some other procedure to cause chronic elevation of blood pressure. They resemble the renal lesion associated with chronic renal hypertension of benign variety in man (Kanisawa and

Schroeder, 1969). These authors showed typical lesion in hypertensive rats fed Cd. There was a generalized thickening of the renal arterioles. The lumina were narrowed. Kanisawa and Schroeder (1969) were unable to explain the exact pathogenesis of Cd-induced hypertension, except to say that it differs from that of renal ischaemic hypertension but is associated with similar and less severe renal vascular lesion.

Perry and Erlanger (1973) observed that intraperitoneal injection of 1.8 μ moles of Cd produced significant elevation of renin at all time intervals tested from 1 minute to 1 week after injection. 5 ppm of Cd in drinking water produced increased renin activity which was significant after one week and one month. The increase in diastolic blood pressure averaged 22 ± 8 mm Hg of Cd-treated rats. These authors postulated a probable role of renin in Cd-induced hypertension. Dustain et al. (1970) found good correlation between plasma renin activity and the degree of hypertension that occurs in renovascular disease. A role that has often been suggested for renin in renal hypertension is that renin secreted by one kidney or by damaged portion of the kidney can pass into the blood stream to the undamaged renal tissue of the same kidney or of the opposite kidney and cause fluid and salt retention. Such a mechanism would elevate arterial pressure.

The antinatriuritic effect of Cd was first recognized by Vender (1962). Subsequently Lener and Musil (1971), Foulke et al. (1974), Doyle et al. (1975), observed sodium retention following Cd feeding. Recently Perry and Erlanger (1980, 1981) showed that hypertension associated with Cd exposure could result from this antinatriuretic effect. It is not clear whether the antinatriuretic effect of Cd is a direct effect on the kidney or due to the activation of renin-angiotensin system.

It has been found that captopril, an orally effective inhibitor of angiotensin converting enzyme, reduces the blood pressure in normal as well as high renin animal model of hypertension (Laffan et al., 1978). Rubin et al. (1978) had shown that captopril reduced the blood pressure of two kidney-renal hypertensive rats, previously clipped for 6 weeks. Laffan et al. (1978) observed that captopril (SQ 14225) markedly lowered the blood pressure of the renin dependant aortic ligated and two kidney-Goldblatt hypertensive rats and failed to reduce the blood pressure in the one-kidney Goldblatt hypertensive rats. Oral doses of captopril moderately reduced the blood pressure of Wistar-Kyoto spontaneously hypertensive rats but not that of normotensive Wistar-Kyoto rats. These results suggest that captopril acts primarily by inhibiting the renin-angiotensin system to reduce the elevated blood pressure

especially in renin-dependant model of hypertension. From the foregoing discussion it seems that renin may be one of the factors responsible for different forms of hypertension including the one which is produced by Cd.

In the present study, captopril did not prevent the hypertension due to chronic administration of CdCl_2 (Fig. 16). The acute pressor response to intravenous or intraperitoneal administration of CdCl_2 was also not abolished in the captopril pretreated animals (Fig. 17). The relationship of Cd to the renin-angiotensin system has not been completely delineated. Even though Perry and Erlanger (1973) showed an increase in plasma renin activity after oral and parenteral administration of Cd, such observations have not been confirmed in similar experiments in rabbits (Wilson et al., 1974). Similarly hypertension has not been observed in factory workers even when the exposures to Cd were sufficient to cause renal damage and proteinuria (Friberg, 1971). Dammin et al. (1956) suggested that lesions in the glomeruli might result from nephrotoxic substance such as Cd and they can be associated with chronic non-renal hypertension. The present study suggests that the rats chronically treated with CdCl_2 exhibit few changes in kidney such as focal area of cloudy swelling in the tubules (Fig. 50A). Conceivably renin or the tubular damage may not play much role in

causing Cd induced hypertension since captopril did not block this effect.

It is generally recognized that most forms of hypertension are associated with increased peripheral resistance due to maintained constriction of the small resistance vessels (Folkow and Neil, 1971; Blaustein, 1977). Thickening of vessel walls and consequent narrowing of lumina may contribute to the maintenance of the chronic hypertensive state. The most straightforward assumption is that change in tone reflects change in intracellular calcium and that rise in the mean intracellular calcium may be the final common path by which most, if not all, hypertension is produced (Blaustein, 1977). This assumption is consistent with the conclusion of Greenberg and Bohr (1971), that altered reactivity of the smooth muscle fibres in the veins of spontaneously hypertensive rats is the consequence of elevated calcium.

Although a large number of studies have been conducted on arterial tissue of normotensive animals to determine the importance of calcium in the contractile process, surprisingly little work has been done in hypertensive animals. In one kidney Goldblatt hypertensive rats there was an increase in calcium content of mesenteric arterioles (Tobian and Chesley, 1961), rat tail artery (Rorive and Vancauwenberg, 1973) and

other arterial tissues (Constantopoulos et al., 1975). In DOCA-salt treated, unineph^rectomized dogs, the dogs that developed hypertension were found to contain more calcium in their arterial tissue than those similarly treated which failed to develop hypertension (Constantopoulos et al., 1975). Lederbelle Pederson et al. (1978) have observed that dependency of extracellular calcium is increased in aorta of SHR. Furthermore, it was demonstrated that chronic oral treatment with the calcium blocking agent, verapamil reduced the blood pressure in SHRs and probably also modified the increased calcium dependency (Lederbelle Pederson, 1979). The tension development of the SHR strips was greatly inhibited by calcium antagonists (Aoki et al., 1982). The DOCA-Salt hypertensive rat has been reported to be most sensitive model of antihypertensive effect of nifedipine (Iriuchijima, 1980; Ishii et al., 1980). Aguas and Nickerson (1983) have shown that verapamil prevented the DOCA-induced hypertension in rats and ameliorated the incidence of severity of cardiovascular lesion.

In the present study it was observed that hypertension was not developed when verapamil or nifedipine was administered simultaneously with CdCl_2 (Fig. 18 and 20). It was further found that acute injection of CdCl_2 did not produce significant pressor effect when it was given intraperitoneally

or intravenously to the verapamil or nifedipine pretreated animals (Fig. 19 and 21). The ability of calcium channel blockers to block the chronic hypertensive effect and the acute pressor responses to CdCl_2 , shows that this metal may either produce a calcium-like action thereby causing a direct contractile effect of vascular smooth muscle or promote the fluxes of calcium ion for causing an acute pressor effect or chronic hypertension.

To study the effects of Cd and its interaction with various agonists, several in vitro smooth muscle preparations such as rat hindquarter, rat isolated aorta, rat isolated portal vein, rat vas deferens and rat anococcygeus muscle were used. Perfused hindquarter of rat is one of the most commonly used models to investigate the vascular reactivity in various forms of hypertensive animals (Chang and Shibata, 1980). Perry et al. (1967) had observed that there was significant increase in the resistance of large arteries when rat isolated hindquarter was perfused with $1.6 \times 10^{-5}\text{M}$ of Cd ion. In the present study, it was observed that intra-arterial administration of CdCl_2 in rat hindquarter preparation caused increase in perfusion pressure suggesting vascular constriction (Fig. 22, 23). Furthermore, the basal perfusion pressure was increased when Cd (1 ug/ml or 3 ug/ml) was infused continuously in the rat hindquarter preparation.

(Fig. 24). It was observed that phentolamine or pretreatment with reserpine could not prevent the acute increase in perfusion pressure after intra-arterial CdCl_2 . However, this effect was significantly blocked by verapamil (Fig. 25). These results are in line with other data of the present study i.e. prevention of acute pressor effect and chronic hypertension due to CdCl_2 by calcium channel blockers such as verapamil or nifedipine.

Rat aortic strip is extensively used for studying sensitivity to different agonists because it is thin and allows rapid drug diffusion. Moreover, it lacks sympathetic innervation (Berkowitz et al., 1971; Patil et al., 1972). This nerve free preparation is sensitive to catecholamines (Maling et al., 1971).

Perry et al. (1967) have shown that in rabbit isolated aorta Cd ($1 \times 10^{-4}\text{M}$) produces an inhibitory effect on the contractile response to NA. However, the same concentration did not affect the angiotensin response. Toda (1973a) studied the influence of Cd ion on the contractile response of rabbit isolated aorta to various stimulatory agents. It was shown that Cd (0.02 mM) inhibited the contractile response to K^+ ion and with a higher concentration (0.1 mM), the contractile responses to NA and angiotensin or barium

were reduced. However, Thind et al. (1970b) found a different effect in rabbit aorta. Cd was more potent in inhibiting NA response than K^+ . It has been postulated by these authors that cadmium interferes with the movement of calcium ion across the cell membrane in the above-mentioned concentrations. It was further shown that the inhibitory effect of Cd was associated with the reduced binding of drugs in the receptive places on the cell membrane (Toda, 1973a). Hayashi and Toda (1977) had observed that Cd (5-100 μ M) attenuated the calcium-induced response in cerebral and peripheral arteries of the dog.

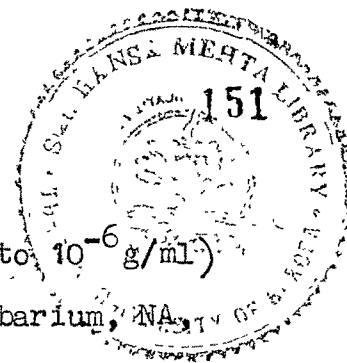
In the present study with the isolated rat aorta, low concentrations of $CdCl_2$ (4.8×10^{-8} M and 4.8×10^{-7} M) caused a significant leftward shift of the dose-response curve of KCl with an increase in maxima (Fig. 26,27). However, increased concentration of $CdCl_2$ (1.44×10^{-5} M) caused significant rightward shift of the dose-response curve of KCl with a depression of maxima implying an inhibitory effect (Fig. 26). The results are in line with the finding of Niwa and Suzuki (1982) who showed that Cd in low concentration produced supersensitivity to KCl, NA and Ba^{++} responses in rat isolated aorta. Moreover, these authors observed an inhibitory effect to the agonist when the Cd concentration was increased.

Like KCl, NA response was also significantly potentiated in the presence of low concentrations of CdCl_2 (Fig. 28) with an increase in maxima. However, in higher concentration, CdCl_2 shifted the dose-response curve of NA to the right as observed with the KCl response (Fig. 28).

Lower doses of CdCl_2 per se caused small contractile responses of the rat aorta. Increased concentration, however, did not produce any stimulant effect at all (Fig. 29). The dose-response curve was bell-shaped (Fig. 30). These contractile effects on rat aorta were abolished in the absence of calcium ion. Besides, phentolamine did not block this effect (Fig. 29).

In guinea pig ileal preparation, Schnieden and Small (1971) observed that Cd ($1 \times 10^{-5}\text{M}$ to $8 \times 10^{-5}\text{M}$) depressed the contractile responses to methacholine, acetylcholine, and histamine. Concentration lower than this did not show any significant effect. A similar effect was also observed by Triggle et al. (1975). Contrary to this, it was shown that Cd ($1 \times 10^{-8}\text{M}$ to $1 \times 10^{-4}\text{M}$) caused a transient contraction of longitudinal ileal smooth muscle of guinea pig (Asai et al., 1982). The dose-response curve was bell-shaped.

In guinea pig vas deferens, Niwa et al. (1981) have



shown that a low concentration of Cd (10^{-9} to 10^{-6} g/ml) enhanced the contractile responses to KCl, barium, NA, and acetylcholine. They have observed that the enhancing effect was more significant for K^+ than for Ba^{++} , Ca^{++} , acetylcholine and NA. In the present study in rat vas deferens, the KCl induced contractile response was potentiated by as low as 1.44×10^{-8} M of $CdCl_2$. However, the dose-response curve of NA was not altered by this low concentration of $CdCl_2$. The concentration of $CdCl_2$ used in the vas deferens to produce an enhancement of KCl response, was three times lesser than the concentration used in the rat aorta to produce a significant potentiation (Fig. 33). In the rat vas deferens even the inhibition of responses to KCl and NA was achieved at a lesser concentration of $CdCl_2$ than used for inhibiting the rat aorta and portal vein. (Fig. 33, 34, 35).

In the rat isolated anococcygeus muscle $CdCl_2$ produced inhibitory effect on responses to KCl and NA. The inhibitory effect was enhanced when the calcium in the perfusion fluid was reduced (Fig. 36, 37, 38, 39). The concentration of $CdCl_2$ used in this study, produced inhibitory effects in other tissues also. However, KCl response was more affected than NA response..

In the rat aorta KCl-induced contractile response is mediated through the influx of extracellular calcium (Briggs, 1962). In Ca^{++} free medium KCl is ineffective in inducing contractile response (Durbin and Jenkinson, 1961; Edman and Schild, 1961; Briggs, 1962; Hinke, 1965a; Hudgin and Weiss, 1968). The enhancement of response to KCl by low doses of CdCl_2 may be due to increased influx of extracellular calcium because KCl also produces its effect mainly through mediation of extracellular calcium as shown by many authors. The role of calcium in the mediation of response to low doses of CdCl_2 is further strengthened by the finding that the contractile response to Cd is abolished in the absence of calcium (Fig. 29).

The ability of low dose of CdCl_2 to enhance the effect of NA may be due to more than one mechanism. It has been shown that low doses of Cd significantly inhibited the MAO and COMT in rat aorta (Revis, 1977). Unlike in the rabbit aorta, the contractile effect of NA in the rat aorta is mainly mediated through extracellular calcium (Goodman and Weiss, 1971; Krishnamurthy, 1974). Therefore, enhancement of response to NA by low doses of CdCl_2 may be due either to an increased influx of calcium as suggested in the case of KCl response in this study, or inhibition of COMT or MAO as suggested by Revis (1977). However, the enhancement of the

KCl response by low doses of CdCl_2 is more significant than the supersensitization to NA. In rat isolated portal vein, KCl response was potentiated by a low dose of CdCl_2 , and a high dose of CdCl_2 inhibited it (Fig. 31). However, the NA responses were not significantly enhanced by the low concentration of Cd and a higher concentration of Cd produced inhibition (Fig. 32). It is logical to conclude that the enhancement of KCl response by low dose of Cd may be a calcium mediated mechanism, as observed in the case of isolated rat aorta. In the rat vascular mesenteric bed unlike KCl, NA produces constriction in calcium free medium, suggesting that NA primarily releases calcium from intracellular stores (Manku and Horrobin, 1976; Kondo et al., 1977). Since low doses of CdCl_2 selectively potentiated the KCl induced response in portal vein of rats without altering the NA response, it is possible that CdCl_2 must be selectively influencing the influx of extracellular calcium. However, higher concentration of CdCl_2 produced an inhibitory effect on responses to NA and KCl. But in portal vein at least three times higher concentration of CdCl_2 was required than in rat aorta.

In vas deferens the enhancement of KCl response by low concentration of CdCl_2 may be due to acceleration of calcium influx by CdCl_2 because KCl induced contraction is maintained

by calcium influx. It has been shown that tonic phase of KCl response in vas deferens is mainly due to the influx of extracellular calcium (Niwa et al., 1981). Furthermore, a low concentration of CdCl_2 enhanced the tonic phase of contractile response to KCl (Fig. 34) suggesting an involvement of extracellular calcium. In guinea pig vas deferens enhancement of response to NA by CdCl_2 is not as significant as that to KCl (Niwa et al., 1981). In line with this there is no potentiation of NA response by low doses of CdCl_2 . This may be due to the utilisation of different pools of calcium by NA besides extracellular calcium (Suzuki and Kinoshita, 1976).

From the foregoing discussion of acute in vitro studies in rats, it seems that in low concentrations Cd enhanced mainly the responses to KCl and partly to NA. In high concentrations it inhibited the response to both agonists. It is possible that in low concentrations Cd might promote the calcium influx and in high concentrations, it might inhibit the influx of calcium ion.

These in vitro results are in line with the other data of the present in vivo studies, i.e., the ability of calcium channel blockers to inhibit the CdCl_2 induced acute pressor effect and chronic hypertension.

Comparing the in vitro vascular reactivity with in vivo studies, a few more correlations can be made. After the i.v. administration of CdCl_2 the blood pressure responses to acute i.v. administration of low doses of NA were reduced (Fig. 3A); however, the responses to angiotensin II , isoprenaline and acetylcholine were not modified. The responses to NA and angiotensin II were not modified after acute i.p. administration of CdCl_2 . Schroeder et al. (1970) have shown that blood pressure responses to acute i.v. administration of NA and angiotensin II were not modified after acute i.p. administration of CdCl_2 . In the present study, the reduction of responses to a low dose of NA after acute i.v. CdCl_2 administration may be due to reduced binding of the drug to the receptor as suggested by Toda (1973a). Such an inhibitory effect on responses to NA was observed in rat isolated aorta and other tissues.

In hypertensive animals the in vivo vascular reactivity to various agonists is a subject of considerable controversy. Schroeder et al. (1970) administered 10 ppm of Cd in drinking water for six months to produce severe hypertension in rats. When NA and angiotensin II were given i.v. to these hypertensive animals, there was significant reduction of the blood pressure responses to these agonists, suggesting a decreased vascular reactivity.

In the present study, there was potentiation of the blood pressure responses to NA (Fig. 9A) in rats treated with chronic low doses of CdCl_2 (0.1 mg/kg/day, i.p., for 2 weeks). But the blood pressure responses to angiotensin acetylcholine and isoprenaline were unaltered. In rats treated chronically with higher doses of Cd (0.5 and 1 mg/kg, i.p., two weeks), there was no change in blood pressure responses to acute i.v. administration of NA, angiotensin, isoprenaline and acetylcholine.

Fadloun and Leach (1981a) have shown that female rats made hypertensive with chronic Cd (1 μM i.p., daily for 12 days) treatment, exhibited an increased sensitivity of the blood pressure to NA. However, though the present study followed approximately a similar dosage schedule, such an effect was not observed. The difference in results may be due to the way in which the blood pressure was measured. These authors (Fadloun and Leach, 1981a) measured the acute intravenous effect of NA in pithed rats where the compensatory mechanisms were not operating. Nachay et al. (1977) have observed that rats treated chronically with low doses of Cd showed potentiation of blood pressure response to NA. Phelan (1966) has studied vascular reactivity in rats with renal hypertension and inherited hypertension and in controls subjected to sham operation using angiotensin

and NA. There was no difference in pressor responses to angiotensin. It was further stated by this author that the pressor response to NA was decreased when the mean blood pressure was greater than 170 mm Hg. In the present study, in rats treated chronically with a hypertensive dose of CdCl_2 (1 mg/kg/day, for two weeks), there was no change in sensitivity of the blood pressure responses to NA and angiotensin.

Thind et al. (1970a) have shown reduced vascular reactivity to angiotensin in rabbits made hypertensive by chronic i.p. administration of Cd^{++} . However, these authors did not observe any change in response to NA when aorta from Cd-induced hypertensive rabbit was tested. Fadloun and Leach (1980) have shown that portal veins of rats made hypertensive by administering 12.5 and 25 ppm Cd orally for one month showed enhanced contractions to KCl and NA. Porter et al. (1975) have shown decreased vascular reactivity of aorta to NA and angiotensin in CdCl_2 treated animals.

In the present study in vitro vascular reactivity to various agonists was tested on the hindquarter preparation, isolated aorta, portal vein, vas deferens and anococcygeus muscle of rats treated chronically with a hypertensive dose

of CdCl_2 (1 mg/kg/day, i.p., for two weeks). Hindquarters of treated rats showed increased sensitivity to NA (Fig.41). In isolated aorta there was leftward shift of the dose-response curve of NA suggesting increased reactivity. But there was no such effect with KCl response in the aorta, (Fig. 42,43). The responses to KCl and NA were not modified in the isolated portal vein, vas deferens and anococcygeus muscle.

Such discrepancies in vascular reactivity to various agonists in CdCl_2 treated animals may be due to differences in dosage, duration of treatment, route of administration and also species.

Literature reports on increased (in vitro) vascular sensitivity to vasopressor agents in experimental hypertension are not unequivocal. For example, McGregor and Smirk (1970), and Haeusler and Finch (1972) reported that the mesenteric arterial blood vessel bed from genetically spontaneously hypertensive rats showed greater sensitivity than from the normal rats. On the other hand, Clineschmidt et al. (1970) found no change in sensitivity to NA. The tail artery and aorta from renal hypertensive rats showed enhanced response to NA and posterior pituitary hormone (Gordon and Norgueira, 1962 and Hinke, 1965b). In aortic strip from DOCA-salt treated hypertensive rats or chronic renal hyper-

tensive rats, Readleaf and Tobian (1958) and Mallow (1959) failed to obtain any supersensitivity to NA or Adr. Kalsner et al. (1971) also found no change in apparent sensitivity to NA in aortic strip from DOCA-salt hypertensive rabbit; they found greater reactivity to KCl. Bohr (1961) observed lower sensitivity to Adr, NA and angiotensin in aorta and arterioles from renal hypertensive rats. Bhatt (1984) has shown that aorta from the estrogen-treated hypertensive rats showed no significant change in response to NA. Sun and Haing (1983) have shown that the contractile responses of aortic preparation to NA and KCl were significantly lower in SHR than in Wistar-Kyoto rats. It has been shown that hypertensive vascular smooth muscle contains more sodium or sodium and calcium than normal rats (Bohr, 1964; Hinke, 1966 and Tobian and Chesley, 1966) and increased reactivity may be due to increased efficiency in the utilization of calcium (Hinke, 1966). A possible explanation for the diminished contractile response may be due to morphological changes in the wall of the aorta caused by an increased formation of collagen and reduced distensibility of vessel walls which was reported to occur in SHR (Iwatsuki et al., 1977 and Andresen et al., 1978).

In the present study, with isolated aorta from CdCl₂-induced hypertensive rats, the increased responsiveness to

NA and lack of any change in the sensitivity to KCl is not well understood. Similarly, in portal vein and other smooth muscle preparations, there was lack of change in the sensitivity to NA and KCl. Such discrepancies are also reported in literature for SHR, DOCA-salt treated and CdCl_2 -induced hypertensive rats and are discussed earlier. One possible explanation for the increased vascular reactivity in rat aorta may be due to the inhibition of MAO and COMT by Cd (Revis, 1977).

It seems that Cd may be having a dual action as observed in the present study and the studies conducted by several workers. Cd has been shown to produce excitatory or inhibitory or both effects in the vascular smooth muscle. For example, intra-arterial administration of Cd produced a pressor effect in low dose and a depressor effect in high dose (Perry et al., 1967). Intravenous administration produced a depressor effect followed by a pressor effect.

Perry et al. (1970) have shown that administration of very small doses of Cd i.v. produced only a pressor effect. In the present study, i.p. administration of CdCl_2 (1 mg/kg) produced only a pressor effect. Very high i.p. dose produce arteriolar and venular dilatation (Perry et al., 1970). In rat aorta, a low concentration produced a small contractile effect and this response was not observed with a higher

concentration. Furthermore, lower concentrations of Cd potentiated KCl and NA responses in rat aorta and KCl responses in portal vein and vas deferens. Other workers have also found similar increase in the response of rabbit ear artery (Williams, 1978) and rat aorta (Niwa et al., 1982) to various agonists by low doses of Cd.

From the foregoing discussion it is evident that the acute and chronic cardiovascular effects are variable. Lack of general agreement at dose level properly termed "low" or "high", makes it difficult to compare results reported in the literature. The terms are defined differently by investigators who in general, employ them to distinguish between levels arbitrarily set in a particular study but unrelated to any fixed reference point.

Several possible mechanisms can be put forward for the pharmacological effects of cadmium observed in the present study.

In vitro studies have shown that low concentrations of Cd produced contractile effect on the rat aorta. This effect may be due to the increased influx of calcium ion by Cd since the contractile effect on aorta by low concentration of Cd was abolished in the absence of Ca^{++} ion. Similar effect was also reported by Asai et al. (1982) who have

shown that low concentrations of Cd produced contractile effect in guinea pig ileum and this effect was abolished by the complete removal of Ca^{++} ion from the perfusion medium. Moreover, in the present study with rat aorta, portal vein and vas deferens KCl responses were enhanced by a low concentration of Cd. Possibly the lower concentrations of Cd seem to increase the availability of Ca ion for the contractile machinery. This mechanism may also contribute to the chronic hypertensive effect of Cd observed in the present study and also reported by others. However, the ultimate effect of Cd may be on the membrane Ca. One of the difficulties in estimating the transmembrane Ca movement is the fact that Ca regulates the permeability of the membrane not only to other ions (Jones, 1974; and Jones and Hart, 1975) but also to itself (Somlyo and Somlyo, 1968 and Collins et al., 1972). Experiments in the spontaneously hypertensive rats carried out by Aoki et al. (1974) and by Jones and Hart (1975) on DOCA-salt hypertensive rats showed that there is a reduction in the Ca binding capacity on the arterial subcellular membrane. Aoki et al. (1976; 1982) and Moore et al. (1978) have suggested the possibility of disorder of Ca channel, and Ca uptake and binding by the membrane may increase the level of intracellular Ca in arterial smooth muscle and cause hypertension. Conceivably Cd in low concentration might affect the Ca binding or

uptake by the membrane leading to increase in the influx of extracellular Ca and other ions causing hypertension.

In vivo studies have shown that Cd produced depressor followed by a pressor response on i.v. administration and a pressor effect on i.p. administration and these pressor effects were blocked by calcium channel blockers such as verapamil or nifedipine. Hall and Hungerford (1982) have observed similar effect on i.p. administration of Cd and these authors suggested that Cd might have a partial agonistic effect to Ca ion. Therefore, the other possibility is that Cd might mimic Ca ion and produce a direct contractile effect on vascular smooth muscle. In such a case verapamil and nifedipine by virtue of their Ca antagonistic effect might block the entry of Cd into the cell and antagonize the pressor response. However, it is not clear whether the direct effect on the vascular smooth muscle or increased influx of Ca ion is possible that both the mechanisms might be operating for chronic hypertension due to Cd. It is concluded that Cd might produce its effect either by direct action on vascular smooth muscle and/or by altering the fluxes of Ca ion.