CHAPTER-VI

DISCUSSION

In an attempt to gain an insight into the mechanism of action of any new hypotensive agent, it is necessary to investigate its action on cardiovascular haemodynamics (Campbell and Moore, 1981).

Indapamide appears to offer a suitable alternative to more established drugs as a first line treatment in patients of mild to moderate hypertension. Whether it differs significantly from other diuretics when used as antihypertensive therapy either in its mode of action or its side effect profile needs further clarification (Chaffman et al., 1984).

In vivo and in vitro experiments were designed to study the mechanism of antihypertensive action of indapamide and to find if there is any pharmacological evidence for calcium interfering property of indapamide. In the present study, the antihypertensive action of indapamide was not observed in normotensive rats but the drug was found to reduce the blood pressure of DOCA/salt hypertensive rats, suggesting that the drug is only effective in hypertension. The present results are in agreement with those of others. For example, Moore et al.(1977) reported that in normal rats oral doses up to 100 mg/kg failed to change the blood

pressure over a 96 hour measurement period, but had potent hypotensive effect in hypertensive rats. Indapamide (10 mg/kg) administered orally for 10 days in DOCA/saline hypertensive rats with unilateral nephrectomy produces a marked fall in blood pressure of 45 mmHg measured using the indirect tail/cuff method (Finch et al.,1977a,b). Similar results are reported from DOCA/salt rats without nephrectomy (Kyncl et al., 1975).

Since indapamide is a diuretic it was thought that like frusemide it might lower blood pressure through increase in the synthesis of vasodilator prostaglandin (PG). Patak et al.(1975) reported that the loop diuretic frusemide decreases hypertensive blood pressure and increases salt and H_2O excretion as do saluretic agents generally. PG synthetase inhibitor indomethacin is capable of obtunding or preventing the hypotensive effect of frusemide when they were administered together. The natriuretic effect of diuretic agent was decreased; likewise indomethacin per se had slight effect on blood pressure but no effect on sodium excretion in this small group of normotensive and hypertensive patients. This would seem to tie the antihypertensive effect of potent diuretic agent frusemide to the PGs.

Watkins et al.(1980) also suggested that products formed by the arachidonic acid cycloxygenase contribute to the regulation of blood pressure during treatment with both propranolol and thiazide diuretics. Inhibition of cycloxygenase with indomethacin partially antagonizes the hypotensive effect of these drugs. Therefore, the present experiments with indomethacin were designed to study its effect on the hypotensive property of indapamide. Indomethacin treatment (in the doses which block cycloxygenase) had no effect on the blood pressure of normotensive, hypertensive and indapamide treated normotensive rats. Also indomethacin treatment did not result in abrogation of the antihypertensive effects of indapamide suggesting that PGs are not directly involved in the antihypertensive effect of indapamide in DOCA/salt hypertensive rats. Indapamide has been shown to be a potent stimulator of the vasodepressor PGI_2 in vitro (Gbessor et al.,1982). It is possible that there is no direct correlation between the blood pressure level and vascular PGI_2 biosynthesis. Fridjat et al.(1984) have also shown that captopril and dihydrallazine induce significant fall in blood pressure but there is no direct correlation between blood pressure level and vascular PGI_2 biosynthesis. The changes of PGI_2 liberation observed in the presence of arachidonic acid in vitro following dihydrallazine and diltiazem treatment do not seem to be associated with the antihypertensive effect.

It is also possible that indapamide might have failed to release PG in DOCA/salt hypertensive rats. In anaesthetized animals the vascular response to frusemide is dependent on the state of sodium balance (Herbert et al.,1985). In sodium loaded animals there is no frusemide induced increase in renal blood flow whereas in sodium-depleted animals there is a substantial increase which could be inhibited by indomethacin suggesting that PG release may also depend on the state of sodium balance.

Indapamide appears capable of decreasing the inward calcium currents and clonic contractions in in vitro smooth muscle preparations and has gained a provisional classification as a calcium antagonist (Opie, 1980). It was therefore planned to compare the antihypertensive action of indapamide with verapamil (calcium antagonist). Verapamil did not modify the blood pressure in normotensive rats and in indapamide treated normotensive rats. However, verapamil lowered the blood pressure of hypertensive rats and indapamide further lowered the blood pressure in the chronic verapamil-treated rats.

Sovensen et al.(1985) reported that verapamil reduces blood pressure by vasodilatation without activation of counter-balancing mechanism commonly seen after treatment with vasodilating drugs i.e. tachycardia, activation of renin aldosterone system, water and salt retention and

without affecting renal haemodynamics. Van Nueten and Vanhoutte (1981) reported that verapamil might act as an antihypertensive agent by inhibiting the myogenic tone of the precapillary vessels and decreasing their responsiveness to vasoconstrictor substances, which results in reducing the systemic peripheral resistance and in turn the afterload of left ventricle. Also, all the calcium blockers were originally thought to act similarly; however, it has become apparent that verapamil exerts its effects primarily upon heart whereas other agents (perhexiline, nifedipine and diltiazem) act mainly upon vascular smooth muscle (Steven et al., 1982). In the present study, indapamide further lowered the blood pressure in the presence of verapamil suggesting that it might be having some other action in addition to verapamil like. Also it might be acting on both veins and arteries. It has not been possible to demonstrate any calcium antagonist effect on the heart with indapamide (Mironneau and Gargouil, 1979). Perhaps simultaneous treatment with both the drugs resulted in greater lowering of blood pressure by combined action on heart and blood vessels.

Indapamide has been claimed to have direct vascular actions (Finch et al., 1977; Gargouil and Mironneau, 1977); therefore, it was planned to compare its antihypertensive activity with directly acting agents on vascular smooth

muscle. Moreover, Mclean et al.(1978) have reported that hydrallazine and its derivatives reduce effects on vascular smooth muscle both by interaction with the fluxes of Ca^{2+} from the extracellular space and effects on release from the cell stores. In the present experiments, hydrallazine reduced the blood pressure of hypertensive rats and in hydrallazine treated rats indapamide did not further lower the blood pressure. Frank et al.(1983) suggested that a nitrogen-to-nitrogen moiety in the structure of indapamide shows a possibly similarity to hydrallazine which has direct vascular effect. It is possible that indapamide has direct vascular action much in the manner of hydrallazine. Alternatively, it is also possible that hydrallazine may have produced maximum vasodilatation so that no further dilatation was possible.

The effects of indapamide on heart rate are variable. Dunn et al.(1981) have shown that after 8 week's treatment there was a significant rise in heart rate in both supine and erect positions as measured indirectly by echo and systemic time intervals but not significant when measured by pulse rate. In the present study, there was increase in the heart rate with indapamide in hypertensive rats while in normotensives, there was no effect. The increase in heart rate may be due to reflex sympathetic stimulation and/or stimulation of cardio-accelerator centres in medulla.

Other workers have shown no significant change in pulse rate (Fernandes et al.,1977; Velasco et al.,1980; Bowker and Murphy, 1981) or decrease (Kelly and Hamilton, 1977; Turner et al., 1977; Kubik and Coote, 1981) or a non-significant increase (Anavekar et al., 1980; Weidmann et al.,1980). The variable response observed with heart rate may be indicative of indapamide exerting an action on both arteries and veins.

Indomethacin treatment did not affect heart rate of normotensive, indapamide-treated normotensive rats and hypertensive rats while it prevented the increase in heart rate observed after indapamide treatment in hypertensive rats. In recent studies, it was observed that inhibitors of PG synthesis also block the release of renin and the tachycardia which followed hydrallazine administration (Campbell et al., 1979).

Verapamil treatment was without any effect on heart rate of normotensive rats but there was significant increase when both verapamil and indapamide were given together. Similarly, in hypertensive rats, verapamil alone was without any effect on the heart rate, but it prevented the increase in heart rate observed after indapamide treatment like that observed with indomethacin. Recently verapamil has been shown to be PG antagonist (Das and Bhattacharya, 1980). This might explain that like indomethacin it

prevented the increase in heart rate by indapamide. It is also possible that verapamil has some action on reflex activity as reported by Heesch et al.(1982), as well as Goldman and Saum (1981) that verapamil attenuates afferent baro-receptor nervous discharge evoked in the carotid sinus and the aortic depressor nerves.

Vascular reactivity to different pressor agents has been extensively studied in different types of experimental hypertension (Folkow et al., 1970). The underlying mechanism for the hyper-responsiveness and its role in the development of hypertension is not clear. However, it has been suggested that elevated blood pressure may induce adaptive structural changes in these vessel walls resulting in an increased wall/lumen ratio. This might be responsible for increased vascular reactivity to vasoconstrictor stimuli (Folkow et al., 1958). Many investigators indicated hyper-reactivity of isolated perfused vessels or vascular beds of deoxycorticosterone (DOCA) sodium chloride, renal and genetically hypertensive rats (Mcqueen, 1956; Folkow et al., 1970; Nosaka et al., 1972).

Several workers have studied changes in vascular reactivity after treatment with indapamide. Finch et al. (1977a,b) have reported that pressor responses to intravenous NA or TYR or electrical stimulation of the

sympathetic outflow in the pithed rat preparation are much reduced by pre-treatment with indapamide. Grimm et al.(1981) reported that indapamide may decrease blood pressure in essential hypertension at least partly by lowering an abnormally high cardiovascular NA reactivity without causing an equivalent increase in adrenergic nervous activity.

In the present study, pressor responses to different adrenergic agonists were not affected by indapamide treatment in normotensive or hypertensive rats. Probable reasons may be as follows -

1. Interpretation of these results can often be made difficult by reflex homeostatic mechanism and multiple actions of drugs. Bing et al. (1945) reported that studies in intact animals are complicated by the presence of other compensatory mechanisms which may mask changes initiated at the alpha-adrenoceptor. In particular, changes in arterial pressure will influence the aortic and carotid baro-receptors causing changes in sympathetic nervous activity, NA release and heart rate.
2. Phelan (1966) reported that pre-treatment allowed reactivity to be studied in animals in which blood pressure was not significantly different

at the beginning of the experiments. This is an essential point because the initial blood pressure level can modify vascular responses to pressor agents. Thus, a direct and precise evaluation of vascular reactivity might be expected. Such a discrepancy could probably arise from the nature of strains used and more probably from widely different experimental designs.

3. DOCA by itself induces renal kallikrein and increases the urinary excretion of PG of the E series, events which may be causally related (Nasjlett et al., 1978) suggesting increased PG metabolism in DOCA/salt hypertensive rats. Also, Herbert et al.(1985) reported that chronic salt-loading in various species including man was shown to be associated with decreased, increased or unaltered urinary excretion of PGE which probably reflects PG metabolism. Zimmerman et al.(1973) found that inhibitors of PG synthesis, indomethacin and eicosa 5,8,11,14-tetraynoic acid potentiated the vasoconstrictor responses to adrenergic stimuli. It was suggested that PGs of E series, synthesized in the cutaneous vasculature may play an important role in the modulation of vascular reactivity to adrenergic stimuli.

4. It is also possible that in DOCA/salt hypertensive model increased sympathetic activity might result in increased PG release which in turn would inhibit responses to catecholamines. Some biochemical evidence in favour of increased activity of peripheral sympathetic fibres and of adrenal medulla has been obtained in rats made hypertensive with DOCA and sodium. It is possible that activation of the sympathetic system is primary rather than secondary to elevation of blood pressure in these animals (De Champlain et al., 1968).

To exclude the role of PGs, pressor reactivity to adrenergic agonists was studied after indomethacin treatment. Indomethacin did not modify vascular reactivity in normotensive rats while surprisingly it potentiated the responses to NA in indapamide treated normotensive rats. It is possible that indapamide might have stimulated PG release in normotensive rats but not in DOCA/salt hypertensive rats and it may not be directly responsible for antihypertensive activity. As Herbert et al.(1985) reported that in anaesthetized animals the vascular response to frusemide is dependent on the state of sodium balance. In sodium loaded animals, there is no frusemide-induced increase in renal blood flow whereas in sodium-depleted animals there is a substantial increase which

could be inhibited by indomethacin suggesting that PG release may also depend on the state of sodium balance and LeBel et al.(1983) have reported that indapamide increases urinary excretion of PGE_2 via stimulation of renal production. Blockade of PG release (which is known to inhibit pressor responses to NA) after indomethacin treatment might have resulted in potentiation of responses to NA.

Indomethacin potentiated responses to NA, ANG, ADR in hypertensive rats; however, potentiation was not observed with NA and ANG in indapamide treated hypertensive rats. In SH rats, enhanced formation of PGI_2 by the aortic wall has been observed by several researchers (Pace-Asisk et al., 1978; Botha, 1980). Chronic salt loading in various species was shown to be associated with increased, decreased or unaltered urinary excretion of PGE which probably reflects PG metabolism (Herbert et al., 1985). Ferreira et al.(1971) showed that administration of an inhibitor of PG synthesis, indomethacin caused augmentation of the effect of ADR infusions in the dog spleen. Similarly in the cat spleen augmentation of the responses to NA, ANG II and nerve stimulation was observed after the administration of indomethacin. These observations support the hypothesis that antagonism by endogenous PGs of the effect of NA on smooth muscle may

be important homeostatic mechanism in addition to reduction in NA release. Inhibition of PG synthesis also affected responses of the various beds to adrenergic nerve stimulation and/or to administered catecholamines. Indomethacin or ETA potentiated the vasoconstrictor response of perfused dog paw vasculature to adrenergic nerve stimulation as well as to administered NA (Zimmerman et al., 1973).

Lack of potentiation of pressor responses to NA and ANG in indomethacin plus indapamide treated rats suggests that vascular reactivity to NA and ANG may have been decreased by indapamide.

Decreawe in vascular reactivity to pressor agents by indapamide is proposed to be through decrease in inward calcium flow. Vascular reactivity to exogenous pressor agents by indapamide was therefore studied in chronic verapamil treated rats. Verapamil did not modify pressor responses to the agonists in normotensive rats while it potentiated pressor responses to NA in indapamide treated rats. Surprisingly, verapamil also potentiated the responses to NA, ADR, PE and TYR in hypertensive rats; however, potentiation of NA was significantly less and there was no potentiation of PE responses in indapamide plus verapamil treated hypertensive rats suggesting that

vascular reactivity to NA and PE was decreased by indapamide. Verapamil did not affect ANG responses.

Verapamil enhances NA transmission by blocking the alpha$_2$-adrenoceptors that modulate transmitter release through a negative feedback mechanism. This was proved in the study by Van Meel et al.(1981) and Cavero et al. (1982) who had demonstrated that vascular smooth muscle contains not only alpha$_1$ but also alpha$_2$-adrenoceptors; both mediating contractile responses. The responses to alpha$_2$-adrenoceptors agonists are inhibited by both verapamil and diltiazem whilst responses to alpha$_1$-adrenoceptor agonists are relatively resistant to these calcium antagonists. The apparent verapamil catecholamine interaction probably resulted from release of catecholamines as a reflex response to the verapamil induced decrease in blood pressure. The tachycardia as well as decrease in blood pressure both were abolished by pretreatment with propranolol which by itself decreases arterial pressure and sinus rate (Steven et al., 1982).

Verapamil has been reported to increase transmitter release from motor nerves at neuromuscular junction in frog and in rat (Nishimura et al.,1982). Steinsland (1981) showed that verapamil increased electrical induced overflow of NA from perfused rabbit ear artery. Also verapamil has been found to cause increased NA

overflow in atria from neuronal sites independent of extracellular calcium concentration without involving exocytosis. Intra-neuronally there is reserpine like effect on sympathetic nerves in addition to calcium channel blockade. This might explain partially super-sensitivity to agonists after verapamil treatment. It is also possible that this effect was observed due to PG antagonist action of verapamil. Das and Bhattacharya (1980) recently reported that verapamil is a PG antagonist which might suggest its ability as an anti-arrhythmic agent in which PGs are believed to be involved.

Uotila and Dahl (1984) also reported that thrombo-xane formation during blood clotting is decreased by verapamil. During blood clotting arachidonic acid is released from platelet phospholipids and immediately metabolized to different metabolites including the aggregatory thromboxane A_2. The inhibition of arachido-nate release and that of the metabolism of free arachi-donate through the cycloxygenase or thromboxane synthetase will result in the decreased formation of TXA_2. Both indomethacin, an inhibitor of cycloxygenase and OKY-1581, an inhibitor of thromboxane synthetase readily prevented the formation of thromboxane during clotting; verapamil a calcium channel blocker caused a dose dependent

decrease in the formation of TXA_2. A rather similar inhibition was seen with sulphinpyrazone a platelet active drug. Verapamil inhibits thromboxane formation during spontaneous blood clotting. This inhibition is obviously due to the decreased release of arachidonic acid from platelet phospholipids. This PG antagonist action of verapamil will also explain the potentiation of NA responses by verapamil in normotensive indapamide treated rats similar to indomethacin.

Indapamide treatment reduced vascular reactivity to NA even in the presence of verapamil which suggests that it acts on both potential and receptor operated calcium channels of calcium or the drug might have depleted calcium stores after treatment.

Alternatively, verapamil induced change in pressor responsiveness to catecholamines in hypertensive rats might be a non-specific consequence of the decrease in basal (pre-infusion) blood pressure.

Chronic hydrallazine treatment also resulted in decrease in reactivity to NA and indapamide potentiated this action by inhibiting responses to even higher dose of NA suggesting that indapamide decreases reactivity to NA. Worcel (1978) reported that hydrallazine exerts significant additional inhibitory effect at sympathetic

nerve terminal by preventing release of NA in vitro. Hicks et al.(1981) further reported that hydrallazine is similarly effective in antagonizing the vasoconstrictor responses to exogenous NA and ANG and those following sympathetic nerve stimulation in pithed rats. This might explain hydrallazine-induced inhibition of NA responses. Also Mclean et al.(1978) reported that hydrallazine and its derivatives produce effects on vascular smooth muscle both by interaction with the fluxes of Ca^{2+} from extracellular space and effects on release from cell stores.

Impaired baro-reflex responsiveness was first demonstrated in established hypertension and ascribed to structural changes in the arterial wall in baro-receptor regions (Bristow et al., 1969). Therefore, the effect of indapamide on reflex was studied. Heart rate with different i.v. pressor agents was compared before and after indapamide treatment in normotensive and hypertensive rats. It was found that in hypertensive rats treated with indapamide bradycardia was greater following i.v. pressor agents compared to that in untreated hypertensive rats suggesting that indapamide might be having some action on vagal tone or sympathetic reflex. Caretta et al.(1983) have reported that chronic treatment with indapamide enhances baro-receptor sensitivity and resets the reflex in essential hypertension. The mechanisms by which indapamide

interferes with the baro-receptor reflex require further investigations.

Weinstock and Schorer-Apelbaum (1985) observed genetic differences in the baro-receptor control of heart rate in normotensive rabbits in response to a pressor stimulus and further reported that this difference may represent (1) difference in receptor sensitivity (2) difference in sensitivity to component of reflex in CNS or (3) difference in end-organ sensitivity. Indapamide induced effect on baro-receptor sensitivity may be due to its effect on any one or more of these components of baro-receptor control of heart rate.

Following indomethacin treatment reflex bradycardia to pressor agents was greater compared to that in untreated normotensive and hypertensive rats. This suggests that blockade of PG synthesis does modify baro-receptor reflex control of heart rate. Change in baro-receptor reflex activity observed after indapamide alone was not observed in indapamide and indomethacin treatment together.

Taylor and Kowalski (1984) reported that some but not all of the calcium channel inhibitors interfere with the activation of the baro-receptor vagal reflex arc. In the present study, NA and ADR produced bradycardia following verapamil treatment. Potentiation of baro-receptor reflex

activity by indapamide was not observed when indapamide and verapamil were given together in normotensive and hypertensive rats suggesting that both might be interfering at the same site of reflex bradycardia. Millard et al.(1982) have reported that diltiazem and verapamil reduce the bradycardiac responses evoked by pressor responses to the alpha-adrenoceptor agonist methoxamine in conscious dogs.

The in vivo results strongly suggest that indapamide has direct action on blood vessels. However, interpretation of in vivo results is often difficult because of reflex homeostatic mechanisms and multiple actions of drugs. Therefore, the effects of indapamide were investigated in vitro. Three tissues namely arterial, venous and vas deferens a non-vascular smooth muscle were selected.

Many studies have shown that like chlorthalidone, indapamide has minimal diuretic activity at low doses. It has been proposed that indapamide might exert its hypotensive action by decreasing vascular reactivity to endogenous pressor agents through inhibition of the net inward flow of calcium (Mironneau and Gargouil, 1979; Schleiffer et al., 1980; Campbell and Moore, 1981; Mironneau et al., 1981).

Moreover, it is reported that in DOCA-saline treated uninephrectomized dogs, the dogs that developed hypertension were found to contain more calcium in their arterial tissues than those dogs similarly treated which fail to develop hypertension. The arteries of hypertensive animals show increased excitability to a number of vasoconstrictors; this could well be due to the increase in calcium content (Seidel and Bohr, 1971). However, if this increase in calcium is intracellular, it has been very difficult to determine how much of it is sequestered and how much is free inside the cell, a fact that would be important in determining the role of this increased calcium in the maintenance of hypertension. Tobian and Chesley (1966) have indeed shown that arterial calcium is raised during experimental arterial hypertension including that of renal origin. Calcium ions play an important role in the mechanism of contractile proteins of vascular smooth muscle and condition peripheral vascular resistance influencing blood pressure (Jones and Hart, 1975).

Depolarization of the cell by high extracellular K^+ opens the potential sensitive calcium channels (POC) which are very sensitive to inhibition by calcium entry blockers such as verapamil (Van Breemen et al.,1982).Indapamide inhibited responses to K^+ in all the 3 tissues studied except that in vas deferens higher dose of indapamide had to be used to

produce inhibition. This suggests selectivity of indapamide for vascular smooth muscle. In support of this, it has been shown that the concentrations of indapamide are approximately 9 times higher in vascular smooth muscle than in the perfusate (Campbell et al., 1977). Gross (1977) also reported that lipophilic mature of indapamide permits it to accumulate in vascular smooth muscle at a concentration 10 times higher than that of protein free perfusate. The drug binds to elastin a major polymer of vascular smooth muscle.

Dick and Feddo (1984) failed to report any relaxation effect towards high K^+ by indapamide in rabbit aortic strip. Probably their exposure time for indapamide (20 minutes) might have been insufficient to reduce the transmembrane influx of calcium or to deplete calcium stores as has been proposed by Mironneau et al.(1979, 1981). Or it may simply be a case of species variation.

Stimulation of receptors by agonists such as NA appears to have releasing effect upon intracellular stored calcium inducing immediate contraction and is able to open receptor operated calcium channels (ROC) to allow calcium influx (Bolton, 1979; Meisheri et al., 1981; Van Breemen et al., 1982). In the present study, indapamide inhibited responses to NA in aorta and portal vein in lower dose and

in vas deferens in higher dose suggesting selectivity of indapamide for vascular smooth muscle.

Similar results of inhibition of NA responses by indapamide have been reported by many workers (Kyncl et al., 1975 and Moore et al., 1977 in rat and rabbit aortae and on rabbit inferior venae cavae; Uhlich et al., 1977 in rat aortic strip; Kraetz et al., 1978 in rat mesenteric artery; Borkowski et al., 1981 in portal vein). However, Dick and Feddo (1984) failed to observe any effect in rabbit aorta.

Noveck et al.(1983) reported that in association with ionic current, two components of contraction were identified a rapid 'phasic' component followed by a slow 'tonic' component. Indapamide inhibited the former component while second tonic contraction occured either in the presence of NA or under prolonged depolarization. This part of the overall contraction is probably due to release of intracellular stores of calcium from microvesicles on the surface membrane and from the sarcoplasmic reticulum itself. Although the NA-induced contraction was not immediately inhibited by indapamide, in longer lasting experiments, indapamide could reduce the amplitude of the contraction. Based on this observation, Mironneau and Gargouil (1979) suggested that depletion of the internal

stores of calcium occurs when the inward calcium current is inhibited by prolonged treatment with indapamide.

An artery depolarized and contracted by Ca^{2+} can be chosen as model for investigation of the effects of the drugs on contraction because this type of contraction is dependent upon extracellular calcium (Van Breemen and McNaughton, 1970). If preparations are first incubated in Ca^{2+}-free depolarizing solution and the extracellular concentration of the ion is then increased progressively the resulting concentration response curve provides an indirect means to determine the sensitivity of Ca^{2+} channels (Godfraind and Kaba, 1972). Indapamide inhibited $CaCl_2$ responses in rat aorta and portal vein in lower dose and in vas deferens in higher dose. This observation again supports indapamide's action on transcellular calcium influx.

The electrophysiological and mechanical properties of vascular and cardiac muscle have been studied to elucidate the mechanism of action of indapamide at the level of the muscle cell membrane (Campbell, 1983). Mironneau and Gargouil (1979) studied action potentials, transmembrane currents and muscle contraction in longitudinal strips of rat or rabbit portal vein: short lasting depolarizing (50 msec) caused an inward flow of calcium ions which

triggered the phasic component of muscle contraction in the portal vein. This calcium current was unaffected by NA but was increased by ANG. Incubation with indapamide 0.3 mmol/l for only 3-5 minutes reduced the action potential amplitude (50%), the inward (27%) and outward (19%) transmembrane currents and the phasic contraction (29%). Recent work has shown that contractions of this tissue are reduced even at 3 umol/l (Krikorian, 1980). The increase in calcium current and the phasic contraction to ANG were abolished in the presence of 0.3 mmol/l indapamide. Mironneau and Gargouil (1979) have shown that indapamide is 10-200 times more potent in abolishing the ANG induced increase in calcium current and phasic contractions than hydrochlorothiazide. Chlorthalidone on the other hand has no effect on the electrophysiological parameters. Similarly at 0.3 mmol/l indapamide reduced the amplitude of the action potential, the inward calcium current and the outward potassium current but hydrochlorothiazide and chlorthalidone at the same concentration were without significant effect.

In the present study also dose-related contractile responses to ANG were inhibited by indapamide in aorta, portal vein and in vas deferens. Similar results were

reported by Kyncl et al.(1975) in rabbit vena cava, and Uhlich et al.(1977) in rat aortic strip. ANG mainly produces contraction by intracellular release of calcium (Gilles and Manuel, 1982) again suggesting that indapamide might be interfering with the availability of intracellular calcium. In vas deferens indapamide inhibited submaximal response to ANG in lower dose suggesting that ANG-induced increase in calcium movement is very sensitive to indapamide.

TYR is claimed to act directly in rat aorta (Krishnamurty, 1974) and in other tissues (Vane, 1960; Gulati and Kelkar, 1971; Maling et al., 1971). Indapamide shifted the DRC of TYR towards the right with depression of the maximal response. Campbell and Moore (1981) reported that indapamide inhibits responses to electrical stimulation and various agonists including NA, ADR, TYR, ANG and Ba^{2+}. Usui et al.(1978) indicated that the effect of indapamide on contraction may be due to a direct action on vascular smooth muscle. Indapamide also inhibited responses to TYR in vas deferens, which suggests that indapamide might be inhibiting the release of NA by TYR or it might be inhibiting uptake of TYR. TYR an indirectly acting amine is taken up by the adrenergic neurone by neurone uptake mechanism (Fischer et al., 1964) and

releases NA from the neurone (Vanhoutte, 1978). Chaffman et al.(1984) reported that interference with neuronal amine uptake was the apparent cause of the inhibition of nicotine and TYR-induced vasoconstriction by indapamide.

Compared with other vascular smooth muscles of different species thus far studied the reactivity of the rat vascular smooth muscle to NA, 5-HT and KCl seems to be dependent upon effective utilization of extracellular Ca^{2+} which is sensitive to SKF 525-A. 5-HT utilizes mostly membrane bound and/or superficially or loosely bound Ca^{2+} and to some extent intracellular Ca^{2+} (Krishnamurty, 1974).

In this study with rat aorta the DRC of 5-HT was shifted by indapamide to the right with depression of maxima suggesting its action by blocking utilization of Ca^{2+}. In rat vas deferens, indapamide did not modify responses to 5-HT in lower dose. The contractile response to 5-HT is mediated through its direct action on tryptaminergic receptors (Rathod, 1978).

The effect of indapamide on K^{+} induced depolarization was also studied in the presence of verapamil. Indapamide in the presence of verapamil further inhibited responses of aorta and vas deferens to K^{+} suggesting that both by acting at the same site produce greater blockade of

potential sensitive calcium channels. However, in adrenergically innervated blood vessels part of the contractile response to high K^+ level is also due to release of endogenous NA (Vanhoutte, 1976).

There is fairly general agreement that hypertension is accompanied by evidence of increased vascular reactivity to constrictor stimuli and indapamide is proposed to act by decreasing reactivity. The mechanism(s) responsible for increased vascular reactivity are proposed. One of them is local intravascular release of vasoactive substances (particularly PGs) (Doyle, 1982). It is also thought that indapamide might be acting by stimulation of vasodilator PGs. Therefore, effect of indomethacin, a PG antagonist on NA responses were studied.

Indomethacin modified middle portion of the DRC of NA in rat aorta and did not modify NA responses in vas deferens in the doses used. Coupar and Mclennan (1978) reported that PGs are required for the vasoconstrictor action of NA in rat mesenteric blood vessels and that this effect is distal to the drug-receptor interaction. They also discussed the possible involvement of PGs with intracellular calcium ions. In both rat aorta and vas deferens indapamide further inhibited responses to NA in

the presence of indomethacin suggesting that PGs are not directly responsible for indapamide-induced inhibition of NA responses.

Thus, it is concluded that indapamide decreases blood pressure through relaxation of vascular smooth muscle possibly through its ability to decrease calcium inward flow, vascular reactivity and peripheral arterial resistance.