DISCUSSION
DISCUSSION

VENTRICLE

The original subclassification of beta-adrenoceptors into beta₁ in the heart and beta₂ in vascular and bronchial smooth muscles presumes a high degree of organ specificity of the beta-adrenoceptor subtypes (Lands et al., 1967, a, b). The hypothesis has now evolved (mainly based on data derived from radioligand-binding studies) into the concept that in a variety of organs, including heart, both beta₁ and beta₂ receptors coexist, although often one subtype predominates. This was first suggested in 1972 by Carlsson et al., (1972) who showed that equal submaximal chronotropic responses of cat heart to beta-adrenoceptor agonists were antagonised to different extents by the beta₁ adrenoceptor selective antagonist practolol and by the beta₂ adrenoceptor selective antagonist H 35/25.

Similar effects have been reported in numerous in vivo as well as in vitro experiments in cat, dog and guinea pig hearts as well as in guinea pig and rabbit trachea, thus supporting the co-existence of beta₁ and beta₂ adrenoceptors in a single organ (Daly and Levy 1979; Minneman et al., 1981; Stiles et al., 1984; Brodde .1989).

The co-existence of beta₁ and beta₂ adrenoceptors has been demonstrated with the technique of radioligand binding in hearts of rats, cats, guinea pigs, dogs and rabbits.
The present study with rat isolated ventricle using NA and TA has demonstrated [Fig. 15 & 34] the co-existence of \( \beta_1 \) and \( \beta_2 \) adrenoceptors. The data also strongly support the view that in the right ventricular tissues of rats as in human hearts \( \beta_2 \) adrenoceptors can mediate a positive inotropic effect, but these effects are submaximal when compared with \( \beta_1 \) adrenoceptor mediated effects (Brodde 1991).

Studies have demonstrated that chronic treatment with calcium antagonists can alter beta - adrenoceptor. Chronic administration of nifedipine with or without beta - receptor antagonist to patients with heart disease prior to cardiac by-pass surgery results in increased beta - adrenoceptor numbers in atrial tissue (Hedberg et al., 1985). Conversely in rats treated with nifedipine, decreased beta - adrenoceptor numbers were observed in the heart and brain (Gengo et al., 1988). There was no change in beta - adrenoceptor density in rats given verapamil for six weeks (Nayler et al., 1988). However, there was a reduction in cardiac NA levels. The present study with the right ventricles from rats chronically treated with verapamil or nifedipine suggests decrease in the density of beta - adrenoceptors as indicated by the rightward shifts of NA concentration-response curves [Fig. 19 & 20].
Diltiazem suppresses contraction in isolated atrial and ventricular tissues derived from laboratory animals including the frog, rat, dog and guinea pig (Boudot et al., 1978; Greenspan and Morad, 1980; Himori et al., 1975; Morad et al., 1982, Nabata, 1977; Nakajima et al., 1975, 1976). In a comparative study by Perez et al., (1982), diltiazem decreased dF/dtmax (an index of inotropy) in the guinea pig atrium by only 8 % whereas equimolar concentrations of perhexilene, lidoflazine, verapamil and nifedipine decreased this variable by 12, 44, 50 and 65% respectively. Although diltiazem has a negative inotropic action in vitro, its strong vasodilating activity can lead to a net increase in cardiac output in vivo (Narita et al., 1981). In contrast to the results with verapamil and nifedipine, shifts of NA concentration -response curves in diltiazem or nimodipine -treated preparations were leftward [Fig. 21 & 22] suggesting increased density of receptors. The maxima were reduced with all the four calcium channel blockers suggesting a reduced availability of calcium (Kasuya and Goto, 1968).

It is a general observation that long-term application of agonists leads to a desensitization and finally to a down-regulation of beta - adrenoceptors (Harden, 1983; Stiles et al., 1984; Hertel and Perkins 1984; Lefkowitz and Caron, 1985; Hausdroff et al., 1990). In the present study also there was down-regulation of the beta - adrenoceptor in ventricle preparations from rats chronically treated with isoprenaline [Fig. 19].
In recent years evidence has accumulated that in the heart the number of beta-adrenoceptors and the number of L-type calcium channels may be coregulated. In patients with hypertrophic obstructive cardiomyopathy, ventricular beta-adrenoceptors density and the density of the calcium channels (determined by \(^3\)H nimodipine binding) was significantly correlated (Ferry and Kaumann 1987). In cultured chick embryo ventricular cells, a 4 hour exposure of the cells to 1 μM isoprenaline caused a decrease in both number of beta-adrenoceptors and calcium channels (assessed by \((H)\) (+) -PN 200-110 binding; Marsh, 1989). Moreover, the numbers of both beta-adrenoceptors and calcium channels in the rat heart were found to be increased following 6-hydroxy-dopamine treatment (Skattebol and Triggle, 1986). In addition calcium entry blockers may modulate the number of beta-adrenoceptors. Incubation of cultured cardiac myocytes isolated from the neonatal rat ventricle with different calcium entry blockers (verapamil, diltiazem and nicardipine) produced a time and concentration-dependent increase in the number of beta-adrenoceptors (Yonemochi et al., 1990).

Chronic treatment with different calcium entry blockers along with isoprenaline may have produced increase in the number of beta-adrenoceptors as deduced from the leftward shifts of concentration-response curves [Fig. 19, 20, 21 & 22].
The cardioselective action of atenolol has also been confirmed in comparative studies with nonselective beta-antagonists by using peripheral vascular response to adrenaline infusion (Hiatt et al., 1985) or the response of diastolic blood pressure or specific airway conductance to isoprenaline infusion (Klausner et al., 1988). These studies have shown that at doses which block beta\textsubscript{1} adrenergic activity, atenolol has virtually no beta\textsubscript{2} antagonist effects. Additionally, while propranolol blocked the effect of beta\textsubscript{2}-adrenoceptor agonist salbutamol (Lawerence et al., 1983), the effect of TA (Strauss et al., 1986) and salbutamol (Lawrence et al., 1983) were not inhibited by atenolol at dosages that decreased resting heart rate and systolic blood pressure and increased peripheral resistance.

The cardioselectivity of atenolol is inversely related to dose (Klausner et al., 1984; Lipworth et al., 1989b, 1991). However, while higher doses have some beta\textsubscript{2} receptor action, single oral doses of atenolol 200 mg have markedly less beta\textsubscript{2} receptor action than propranolol 40 mg (Lipworth et al., 1989 a,b). The present study with the right ventricle preparations treated with atenolol has demonstrated a decrease in response to NA and an increase in the responsiveness to TA [Fig. 28 & 39]. This is against the expected up-regulation of beta\textsubscript{1} adrenoceptors by chronic atenolol treatment. However, several alternate hypotheses for the action of atenolol have been put forth. These are discussed below;
Some beta-blockers act by blocking presynaptic beta2-adrenoceptors, preventing the facilitation of NA release by adrenaline (Frishman and Silverman, 1984). However, this seems unlikely in view of the lack of beta2 action of atenolol (Gothert and Heinrich, 1986). Another possibility is that atenolol causes depletion of NA at the sympathetic terminal by interfering with transmitter turnover (Alexandre and Chevillard, 1980) and uptake (Street and Walsh, 1985) or both. A possible third mechanism of action is that atenolol reduces or reverses the process of arterial hypertrophy or hyperplasia associated with hypertension (Draper et al., 1992).

In 1966, Wenzel and Su were the first to report a positive inotropic effect of an alpha1-adrenoceptor agonist, phenylephrine in rat ventricular strips. Soon thereafter, other investigators observed the positive inotropic effect of various alpha1-adrenoceptor agonists in rabbit and guinea-pig atria (Benfey and Verma, 1967; Govier, 1968).

Stimulation of myocardial alpha1-adrenoceptors produces a positive inotropic effect in different cardiac preparations (whole hearts, papillary muscles, ventricular strips, atria, isolated cardiomyocytes) from several species (rat, rabbit, guinea-pig, cat, lamb, cow, dog, monkey) (Wagner and Brodde, 1978; Shibata et al., 1980; Skomedal et al., 1983; Terzic and Vogel, 1990; Bruckner et al., 1985; Osnes et al., 1985; Endoh, 1986, 1991; Scholz et al., 1986; Benfey, 1987;
Nawrath, 1989; Puceat et al., 1992). Skomedal et al (1988, 1990) demonstrated a definite contribution of the alpha\textsubscript{1}-adrenoceptor to the inotropic response of heart muscle to endogenous catecholamines in the presence of unopposed beta-adrenoceptors stimulation. These investigators estimated that about 75% of the response to NA is mediated through beta-adrenoceptors and 25% via alpha\textsubscript{1}-adrenoceptor in rat cardiac tissue. Concomitant muscarinic receptor stimulation increases the alpha\textsubscript{1}-adrenoceptor component of the overall inotropic effect of NA (Christiansen et al., 1987). The present results with chronic atropine treatment which produced rightward shift of the NA concentration - response curve and decreased the maxima are in line with this observation [Fig. 23].

It was demonstrated, using alpha\textsubscript{1}-subtype - selective radioligands (e.g., \textsuperscript{3}H prazosin; \textsuperscript{125}I IBE2254), that cardiac alpha-adrenergic binding sites belong to the alpha\textsubscript{1}-type (Steinberg and Bilezikian, 1982; Mukerjee et al., 1983). The density of alpha\textsubscript{1} - adrenoceptor - binding sites varies with species. Rat and rabbit myocardia possess a high density of alpha\textsubscript{1}-adrenoceptor - binding sites when compared with other species. The density of binding sites in sarcolemma enriched membrane fractions of rat, rabbit, dog and feline hearts is 167, 191, 55, and 15 fmol/mg proteins, respectively (Mukerjee et al., 1983). Buxton and Brunton (1986), using \textsuperscript{3}H prazosin as a ligand, estimated that an adult rat ventricular cardiac cell possesses \(8 \times 10^{-4}\) alpha\textsubscript{1} adrenoceptors, or 13 alpha\textsubscript{1}-receptors/um\textsuperscript{2}. This number of
alpha₁-adrenoceptors is comparable to the density of beta₁-
adrenoceptors (33/um²) on rat cardiomyocytes (Buxton and
Brunton, 1985b). In addition, Endoh et al. (1991) showed that
the ratio of alpha₁ to beta-receptors was on average 5-fold
larger in the rat than in the rabbit or dog. Although the
number of alpha₁-adrenoceptors varies between species, no
significant difference in the density of [³H] prazosin-
binding sites was found between the left ventricular
subepicardium or subendocardium and the right ventricle of
the rat heart (Muntz et al., 1985). However, in several
species ventricular tissue possesses a higher density of
alpha₁-adrenoceptors than does the atrium (Steinfath et al.,
1992a).

Prazosin, a selective alpha₁-blocker antihypertensive
drug has been known to cause orthostatic hypotension during
its initial therapy for hypertension. The orthostatic
hypotensive side-effect associated with the first dose of
prazosin is greatly diminished upon subsequent medication
with the drug (Graham et al., 1976; Turner 1976;) suggesting
that upon continued alpha₁-receptor blockade by repeated
prazosin therapy the orthostatic condition of the
cardiovascular system may be normalized via another receptor
mechanism(s) to maintain the sympathetic venous tone. Recent
studies (Smyth, Unemara and Pettinger, 1986; Jeffreis et al.,
1987) have shown that upon repeated prazosin treatment in
normotensive rats, the alpha₂-receptor density was
increased in the kidney and the renal sympathetic nerve stimulation-induced sodium water retention was mediated through activation of alpha\textsubscript{2} adrenoceptors.

In the present study there was a shift of the concentration-response curve to the right in ventricle preparations chronically treated with prazosin; there was no effect on the maximal responses. It is likely that there is a down-regulation of alpha\textsubscript{1} receptor with prazosin treatment [Fig. 26] or another receptor mechanism(s) plays some role as suggested by Graham et al., (1976) and Turner, (1976). No change in maximal responses could be attributed to no change in calcium fluxes. This is in contrast to the reports of Brown (1989) who has shown that prolonged alpha\textsubscript{1}-blockade (oral prazosin) in the rat produces supersensitivity of inotropic responses to alpha\textsubscript{1}-stimulation in isolated papillary muscles, whereas beta-mediated responses are unaffected and beta-mediated chronotropic responses of right atria are similarly unchanged. Chronically treated yohimbine preparations exhibit rightward shift of the concentration-response curve together with decrease of the maxima [Fig. 27] implying downregulation of alpha\textsubscript{2} receptor and decreased availability of calcium. However, our results have shown that prolonged alpha blockade produced upregulation of beta-receptors (leftward shifts of concentration-response curves of TA) [Fig. 38, 40 & 41].

Chronic treatment with nifedipine, diltiazem or nimodipine increased the contractile responses to TA
(leftward shifts of concentration-response curves) suggesting an increase in the beta\textsubscript{2} adrenoceptors. Chronic treatment with all the four calcium channel blockers increased the maximal responses to TA [Fig. 34, 35, 36 & 37]. This should be contrasted with rightward shifts and depressed maximal responses to NA (which principally activates beta\textsubscript{1}-receptors) on chronic treatment with verapamil or nifedipine. It is possible that downregulation of beta\textsubscript{1}-receptors on chronic treatment with calcium channel blockers leads to a compensatory upregulation of beta\textsubscript{2}-receptors accounting for the leftward shifts and increased maximal responses observed with TA. Further work with specific radioligands would be necessary to confirm the above hypothesis.

**Diseased State**

Chronic treatment with DOCA - saline results in the development of hypertension (Selye et al., 1943) and a greatly enhanced susceptibility to isoprenaline - induced mortality. (Guideri et al., 1974; Green et al., 1980; Gunther et al., 1984). There are conflicting data on changes in beta-adrenoceptor density in spontaneously hypertensive (SHR) hearts. A decreased (Baker and Katovich, 1982; Upsher and Khairallah, 1985), unchanged (Blumenthal et al., 1982) and even increased (Mochizuki and Ogawa, 1984) beta-adrenoceptor density in hearts of SHR rats have all been observed. Only data on differences in total beta-adrenoceptor density in SHR
and normotensive rats have been obtained whereas nothing is known about the possible differential changes in beta$_1$ and beta$_2$-adrenoceptor in the development of hypertension (Michel et al., 1987). The present study has shown that in hypertensive rats there was a leftward shift of NA concentration - response curve implying increased beta-adrenoceptor density. There was no change in the maximal response to NA [Fig. 18, 24, 25, 26, 27 & 28]. However with TA there was a significant leftward shift of the concentration - response curve together with increase in the maxima [Fig. 34, 35, 38 & 39], suggesting an increase in the beta$_2$ receptors and increased calcium influx.

Changes in 1,4-dihydropyridines binding sites occur in hypertensive rats which has led some workers to suggest that alterations in VDCCs might reflect functional changes in these animals and these may play a role in the etiology of hypertension. Sharma et al. (1986) found an increase in cardiac sites in older SHRs; however no changes were reported in 1,4-DHP binding in heart membranes from young (10 weeks old) SHRs (Ishii et al., 1983, 1986). In contrast to these findings, in the brain a selective decrease in the $B_{max}$ for nitrendipine binding in membranes from brain stem, but not cerebral cortex or heart, was observed in DOCA/NaCl hypertensive rats (Lee et al., 1985). Further studies are clearly necessary to document the relevance of any such changes in hypertension (Ferrante and Triggle, 1990).
In the present study with hypertensive rats given nifedipine/nimodipine there were significant rightward shifts of the concentration - response curves of NA [Fig. 24 & 25] and TA [Fig. 34 & 35] implying reduction in the cardiac beta-adrenoceptor sites. There was a greater reduction in the beta_2 adrenoceptors than beta_1 adrenoceptors. In line with these results are the microscopic observations in ventricles of hypertensive rats treated with nifedipine which suggested a restoration of the tissue damage to normal.

Chronic treatment of hypertensive rats with atenolol produced rightward shifts of concentration - response curves of NA and TA implying reduction in the beta_1 and beta_2 adrenoceptors respectively. There was more reduction in beta_1 stimulated adrenoceptor as compared to beta_2. Chronic treatment with alpha blockers also shifted the concentration - response curves of NA and DA to the right [Fig. 26, 27 & 28]. It is possible that there may be a presynaptic alpha_2 and beta_2 action with chronic atenolol treatment. This suggestion needs further exploration. The tissue damage detected histologically was restored to normal by atenolol treatment of hypertensive rats only in some preparations. This difference from the effect of calcium channel blockers (nifedipine) would suggest that the latter are superior to beta blockers in normalising the cardiac damage produced in hypertension.

In hyperthyroidism the density of alpha-adrenoceptors is unchanged (Ciaraldi and Marinetti, 1978) or decreased.
(Ciaraldi and Marinetti, 1977; Sharma and Banerjee, 1978; Williams and Lefkowitz, 1979; McConnaughey et al., 1979) and the number of beta-adrenoceptors and the responses to beta-agonists are increased (Williams et al., 1977; Tse et al., 1980; Scarpace and Abrass, 1981; Stiles and Lefkowitz, 1981; Krawietz et al., 1982; Bilezikian and Loeb, 1983; O'Donnell and Wanstall, 1986; O'Donnell et al., 1987) or unchanged (Banerjee and Kung, 1977). In hypothyroidism the density of alpha-adrenoceptor is increased (Sharma and Banerjee, 1978; Kunos et al., 1980; Chang and Kunos, 1981), unchanged (Ciaraldi and Marinetti, 1978) or decreased (Ciaraldi and Marinetti, 1977; 1978; McConnaughey et al., 1979; Noguchi and Whitsett, 1983) and the beta-adrenoceptor population in the cardiac muscles is decreased (Banerjee and Kung, 1977; Chang and Kunos, 1981; Ciaraldi and Marinetti, 1977; McConnaughey et al., 1979; Kunos et al., 1980; Tse et al., 1980; Stiles and Lefkowitz, 1981; Krawietz et al., 1982; Noguchi and Whitsett, 1983).

The thyroid gland has multiple actions on cardiac ventricular but not atrial muscles, thyroid hormones regulate the actin-activated ATPase and isoenzyme patterns (Banerjee, 1983) and they also accelerate the accumulation of Ca$^{2+}$ into the sarcoplasmic reticulum through activation of Ca-ATPase (Suko, 1973). These actions may be related to an increase in cyclic AMP concentration (Suematsu et al., 1985) by increasing the number of beta-adrenoceptors.
Thyroid hormones also decrease the density of Ca$^{2+}$ channels (Hawthorn et al., 1988). Moreover, additional multiple actions of thyroid hormones on cellular processes and components, including protein synthesis through nuclear transcription, mitochondrial activation and plasma membrane effects are well documented (Sterling, 1979; Oppenheimer, 1979, 1985).

Limas and Limas (1987) showed that the density of beta-adrenoceptor in rat cardiac muscle increased progressively during daily injection of L-thyroxine. In contrast the number of alpha$_2$-adrenoceptor declined rapidly and following cessation of thyroxine administration, the receptor number returned rapidly to normal values within 2 days. Therefore in the present study chronic treatment with calcium channel blockers or alpha- or beta-adrenoceptor antagonists was given simultaneously with L-thyroxine.

It was observed that chronic L-thyroxine treatment produced a leftward shift of NA concentration - response curve and reduced the maximal response [Fig. 29, 30, 31, 32 & 33] implying increased beta-adrenoceptor density and decreased calcium influx. However with TA, there was a significant leftward shift of the concentration - response curve together with increase in the maxima [Fig 40, 41 & 42] suggesting an increase in the beta$_2$-receptors and increased calcium influx. Most of the studies suggest a correlation between increase in beta-receptor density and
responsiveness in various tissues (Williams et al., 1977; Tse et al., 1980; Scarpace and Abrass, 1981; Stiles and Lefkowitz, 1981; Krawietz et al., 1982; Bilezikian and Loeb, 1983; O'Donnell and Wanstall, 1986; O'Donnell et al., 1987; Malbon, 1980; Bilezikian et al., 1979) to beta-agonists. Our results similarly suggest that increase in beta (\(\beta_1\) and \(\beta_2\)) adrenoceptor density may be due to thyroid hormone-induced changes in various receptor-effector coupling factors as speculated by Malbon et al., (1984). As expected nifedipine or nimodipine treatment reduced the number of beta receptors and the calcium influx and atenolol treatment reduced only the number of receptors [Fig. 29, 30 & 33].

In ventricular preparation treated with nimodipine and L-thyroxine there was restoration of tissue damage as observed microscopically in control thyroxine treated preparations suggesting that restriction of calcium supply to the myocardium by calcium channel blockers in hyperthyroidism may be responsible for normalization of the structure. The effects of calcium channel blockers and various antagonists on the somatic parameters (Table 7) further supports this suggestion.

AORTIC STRIP

It is generally assumed that an increase in vascular smooth muscle contractile activity is associated with an increase in intracellular calcium ion concentration over that present in the resting state of the muscle cell. Vascular
Smooth muscle contraction occurs following an influx of extracellular calcium into the cell, raising intracellular concentrations of calcium approximately 100-fold (from 0.1 to 10 μmol/L) (Kuriyama et al., 1983; Ohashi et al., 1983; Opiem 1984; Van Breeman et al., 1981). The calcium then initiates a cascade reaction which results in increased contractile activity in an experimental preparation or an increased vascular tone in an intact circulatory system (Piepho, 1983). Thus the contractile process in vascular smooth muscle is dependent upon the presence of free calcium ion at sufficient intracellular concentrations (Flaim, 1982; Flaim et al., 1982; Zelis and Flaim, 1981).

Receptor-operated channels are likely to be the most responsible in the initiation of vascular smooth muscle contraction. They may be opened by the interaction of a NA molecule with an alpha-adrenoceptor on the cell membrane, or by the binding of other agonist agents (HA, 5-HT, prostaglandins, etc.) to other membrane receptors (Rahwan 1983).

Potential-dependent channels, which open in response to a depolarising stimulus such as electrical impulse or a relatively high concentration of potassium (Rahwan, 1983; Van Nueten, 1982), are present in myogenically active blood vessels such as precapillary arterioles and in the portal mesenteric vasculature but usually not in other veins or larger arteries (Vanhoutte, 1982). In some vascular tissues,
potassium depolarisation may cause release of endogenous NA, thus indirectly activating receptor-operated channels (Van Nueten, 1982).

Therefore in the present study with aortic strip preparations changes in the receptor-operated channels and potential-dependent channels were studied by comparing the responses to different agonists such as NA, DA, phenylephrine, HA, 5-HT, ACh and KCl. In the dog, guinea pig, and rabbit aorta, the predominant receptor is the $\alpha_1$-type (Docherty et al., 1981; Griendling et al., 1984; Polonia et al., 1985; Ruffolo and Waddell, 1982; Ruffolo et al., 1982). The $\alpha_1$-selective agonists (phenylephrine, methoxamine) and antagonist (prazosin) are relatively potent in most species, while the sensitivity to $\alpha_2$-agonists and antagonists differs in different species. The rabbit and guinea pig aortae have been shown to be least sensitive to clonidine and yohimbine, while the hamster, cat and dog aortae have intermediate and the rat aorta is highly sensitive to both the drugs (Doggrell and Paton, 1978; Downing et al., 1981; Ruffolo et al., 1979).

Postjunctinal $\alpha_1$ and $\alpha_2$ adrenoceptors both mediate contraction of vascular smooth muscle (McGrath, 1982; Starke, 1987). Pressor responses mediated by each subtype exhibit differential sensitivity to calcium channel blockers. It has been suggested that $\alpha_1$-adrenoceptors are coupled to both the release of cellular bound calcium and the
opening of calcium channels, while alpha\textsubscript{2}~adrenoceptors are
linked on to the latter (Van Zwieten and Timmermans, 1987). The sustained contraction of rat aorta following alpha\textsubscript{1} adrenoceptor activation is partially inhibited by Ca\textsuperscript{2+} channel blocker (Godfraind et al., 1982; Beckeringh et al., 1984; Chiu et al., 1986; Bognar and Enero, 1988).

In low concentrations DA activates postsynaptic DA-1 receptors mediating vasodilatation and presynaptic DA-2 receptors inhibiting NA release. In higher concentration DA activates beta\textsubscript{1}-adrenoceptors and at very high concentrations it can activate postsynaptic alpha\textsubscript{1} and alpha\textsubscript{2}~adrenoceptors mediating vasoconstriction as well as presynaptic alpha\textsubscript{2}~adrenoceptors inhibiting NA release (Brodde, 1990).

In the present study chronic treatment with verapamil, nifedipine or diltiazem produced a partial inhibition of contraction with NA, phenylephrine [Fig. 54, 55, 56, 62, 73, 74 & 78]; and DA [Fig. 67, 68 & 69]. With chronic nimodipine treatment there was potentiation of responses to lower concentration of NA and all concentrations of phenylephrine. These results are opposite to those with other calcium channel blockers and could be related to the occurrence of different isoforms of L-type calcium channels (Godfraind et al., 1992) [Fig. 57, 63, 75 & 79].

The existence of a component in the contraction which is resistant to Ca\textsuperscript{2+} blockers suggests two possibilities (1) this component does not require the enhanced Ca\textsuperscript{2+} entry, or
(2) Ca\textsuperscript{2+} entry through 'receptor-operated' Ca\textsuperscript{2+} channels which are insensitive to organic Ca\textsuperscript{2+} channel blockers (Bolton, 1979; Cauvin et al., 1983) contributes to this contraction. Alternatively there is the possibility of down-regulation of the alpha-adrenoceptors on chronic treatment with nifedipine, verapamil, diltiazem, or nimodipine [Fig.54, 55, 56, 57, 62, 68, 73, 74, & 78].

Finally the inhibitory effect on the contractile response with DA on chronic verapamil treatment may be related to blockade of voltage-operated L-calcium channels as already proposed by Morel and Godfraind (1991) for the NA-evoked contraction of rat aorta.

Chronic prazosin treatment abolished responses to NA, DA and phenylephrine which could simply be due to the persistent alpha\textsubscript{1} blocking action of prazosin. Chronic yohimbine treatment potentiated and chronic atenolol treatment inhibited the responses to lower concentrations of NA [Fig. 59, 60, 65, & 66] and DA [Fig. 70, & 71]. The former could be due to block of presynaptic alpha\textsubscript{2} receptors and the latter due to block of presynaptic beta receptors.

The inhibition of NA & DA responses by chronic atropine treatment may be due to inhibition of phosphoinositide metabolism in the smooth muscle of rat aorta [Fig. 61 & 72] as suggested by Satake et al. (1992) for rabbit aorta.
Previous studies have demonstrated that non-cumulative addition of 5-HT to the rat aorta produces initial fast and secondary slow contractile responses (Hughes and Doggrell, 1985; Nakakj, Roth, Chuang and Costa 1985; Doggrell, 1987). Recently it has been demonstrated that immediate fast and secondary slow responses of the rat aorta are also obtained on cumulative addition of 5-HT (Doggrell, 1992). Furthermore, the magnitude of fast or slow 5-HT responses obtained non-cumulatively and cumulatively were not significantly different and the concentration-response curve of an agonist is obtained in a shorter time using cumulative rather than non-cumulative challenge. Therefore, in the present experiments concentration-response curves of 5-HT were obtained in a cumulative manner.

5-HT is considered to contract the rat aorta by stimulating 5-HT$_2$ receptors only. Alpha-adrenoceptors are not involved as prazosin and idazoxan (selective alpha$_1$ and alpha$_2$ adrenoceptor antagonists, respectively) have no effect on the 5-HT responses (Doggrell, 1987). In the present study chronic treatment with verapamil, nifedipine, diltiazem, nimodipine, prazosin or yohimbine, produced decreased contractions with 5-HT implying decreases in 5-HT receptor density [Fig. 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102 & 103]. The observations with prazosin and yohimbine are in disagreement with those of Doggrell (1987). Interestingly atenolol and atropine also produced a partial block of 5-HT responses.
By current convention the subtypes of muscarinic receptors defined pharmacologically are designated as \( M_1 \), \( M_2 \) and \( M_3 \), while those that have been revealed by molecular cloning are termed \( m_1 \), \( m_2 \), \( m_3 \), \( m_4 \) and \( m_5 \) (Bonner, 1989). Fortunately \( m_1 \), \( m_2 \) and \( m_3 \) appear to correspond to \( M_1 \), \( M_2 \) and \( M_3 \) but there is less information about the nature and cellular location of \( m_4 \) and \( m_5 \) receptors. \( M_1 \) receptors are found in ganglia and various glands, \( M_2 \) receptors predominate in the myocardium and also appear to be found in smooth muscle, \( M_3 \) receptors are located in smooth muscle and secretory glands (Lefkowitz et al., 1991).

Muscarinic \( M_3 \) receptors appear to activate an as yet unidentified G protein that is responsible for stimulation of phospholipase C activity, the immediate result is hydrolysis of phosphatidylinositol polyphosphates (which are components of the plasma membrane) to form inositol phosphate isomers (chiefly inositol-1, 4, 5- triphosphate) causing release of intracellular \( \text{Ca}^{2+} \) from stores in the endoplasmic reticulum and leading to the contraction of smooth muscle and secretion (Berridge, 1988). The second product of the phospholipase C reaction, diacylglycerol activates protein kinase C (in conjunction with \( \text{Ca}^{2+} \)). This arm of the pathway plays a role in modulation of function or in the latter phases of the function responses (Nishizuka, 1986). Nifedipine has been shown to inhibit airway smooth muscle constriction induced by cholinergic agents both in vitro and in vivo in man (Baronti et al., 1984; Horio et al., 1984; Henderson et al., 1983;
Kneussl et al., 1983; Malik et al., 1982). In the present study with aortic strip preparations chronically treated with nifedipine, diltiazem, nimodipine or verapamil there was complete inhibition of contractile responses induced by ACh, suggesting a powerful inhibition of the pathway outlined above.

In preparations obtained from rats chronically treated with prazosin, yohimbine, atenolol or atropine, there was a partial or complete inhibition of contractions implying a decrease in the density of muscarinic receptors [Fig. 104, 105, 106 & 111].

HA-stimulated inositol phospholipid hydrolysis has been demonstrated in a number of peripheral tissues including canine and bovine trachea (Grandory et al., 1987; Hall and Hill, 1988; Hall et al., 1989. Madison and Brown, 1988), bovine adrenal chromaffin cells (Nobel et al., 1986), human umbilical endothelial cells (Lo and Fan, 1987; Resink et al., 1987; Pollock et al., 1988), guinea-pig ileum (Donaldson and Hill, 1985; Best et al., 1985, Mallows and Bolton, 1987; Bielkiewicz-Vollrath et al., 1987), guinea-pig bladder (Iacovou et al., 1988) and guinea pig aorta (Lonchampt et al., 1988). In most of these tissues, pharmacological characterisations of the inositol phospholipid response to histamine is consistent with an H₁ receptor-mediated response except in the longitudinal smooth muscle myenteric plexus preparation of guinea-pig ileum, where the situation is more complex (Donaldson and Hill, 1985, 1986c).
HA can initiate smooth muscle contraction by releasing calcium from intracellular stores or by opening either receptor operated or voltage-operated calcium channels (Bolton, 1979). Calcium, from intracellular stores released by $H_1$ receptor stimulation has been demonstrated in endothelial cells (Pollock et al., 1988, Jacob et al., 1988) airway smooth muscle (Kotlikoff et al., 1987; Takuwa et al., 1987), rat aorta (Matsumoto et al., 1986), BC$_3$H$_1$ cells (Brown et al., 1986b) and 132 1N1 astrocytoma cells (McDonough et al., 1988), although in most cases there is also an appreciable stimulation of transmembrane calcium influx (Hill, 1990).

In the present study with aortic strip preparations treated with verapamil there was a marginal decrease in the density of HA receptors [Fig. 83] as evidenced by rightward shift of concentration-response curve. This is similar to the finding of Morel et al., (1987) who have shown that the contractile response to $H_1$ receptor stimulation in the intestinal smooth muscle is largely sensitive to inhibition by dihydropyridine-based antagonists of voltage-dependent calcium channels.

The tension developed at high concentrations of HA in intestinal smooth muscle is partly resistant to nifedipine, indicating that a component of the contractile response may be mediated by inositol 1,4,5-triphosphate-induced release of calcium from intracellular stores (Morel et al., 1987; Bolton and Lim, 1989). However, in the present study
aortic strip preparations from chronically nifedipine, diltiazem, nimodipine, prazosin, yohimbine, atenolol or atropine-treated rats there was either partial or complete block of contractile response to HA suggesting that this may have been mediated by inhibition of inositol 1,4,5 triphosphate-induced release of calcium from intracellular stores [Fig. 84, 85, 86, 87 & 90].

KCl depolarizes the vascular smooth muscle membrane to allow Ca^{2+} influx into the cell through voltage-operated channels; NA (either exogenous or endogenous) admits Ca^{2+} by way of receptor-operated channels. Nifedipine has demonstrated activity consistent with its classification as inhibitor of voltage-operated channels at higher concentrations (Schuman, Gorlitz and Wagher, 1975; Towart, 1982; Godfraind, 1983). In the rabbit isolated mesenteric artery limited inhibition by nifedipine probably reflects a low dependence of this tissue upon extracellular calcium for contraction evoked by exogenous or endogenous NA (Clapham and Wilson, 1987). Nifedipine has been shown to inhibit both spontaneous and drug-induced (alphaPGF\_2\_alpha, oxytocin, vasopressin and potassium) contractile activity in isolated human pregnant myometrium at midterm and in the immediate post-partum period and uterine activity in menstruating, pregnant, post-partum and dysmenorrheic females (Andersson and Ulmsten, 1978; Andersson et al., 1979; Forman et al., 1979, 1982 a,b, Maigaard et al., 1983; Sandahl et al., 1979; Ulmsten et al., 1978, 1980). Nifedipine has also been shown
to inhibit the contractile responses to potassium and barium chloride in the human isolated bladder and ureter, but not to have any effect on bladder or urethral tone in vivo (Forman et al., 1978).

In the present study from chronically treated aortic strip preparations [Fig. 112, 113, 114, 115, 116, 117, 118, 119 & 124], there was inhibition of contractile responses to KCl in all preparations suggesting the possibility that there may be a down-regulation of the voltage operated channels.

**Diseased state**

Chronic treatment with DOCA - saline results in the development of hypertension (Selye et al., 1943). This model was successfully produced as indicated by the rise in blood pressure and increase in the maximal responses of aorta to NA [Fig. 55, 57, 58, 59 & 60]. Although there was no evidence of microscopic changes in the aorta, the ventricular tissue showed inflammatory lesions.

Alpha-adrenoceptor mediated contraction of vascular smooth muscle and beta-adrenoceptor mediated relaxation are an example of physiological antagonism. Increased alpha-adrenoceptor mediated vasoconstriction may be one mechanism contributing to the increased total peripheral resistance observed in hypertension (Doyle and Fraser, 1961; Mendlowitz, 1973). Alternatively, an increase in total peripheral
resistance may also result from decreased beta-adrenoceptor-mediated vasodilatation and unopposed alpha-adrenoceptor-mediated vasoconstriction (Cohen and Berkowitz, 1976; Bertel, Buhler, Kiowski and Lutold, 1980). Indeed, numerous investigators have reported a consistent reduction in vascular smooth muscle beta-adrenoceptor responsiveness in hypertension (Triner et al., 1975; Cohen and Berkowitz, 1976; Asano et al., 1982; Borkowski and Porter, 1984; Toal and Leenen, 1984; Fujimoto et al., 1987) and changes in the beta-adrenoceptor/adenyl cyclase complex (Feldman, 1987; Asano et al., 1988).

Alpha-adrenoceptors are involved in a variety of physiological processes, including regulation of blood pressure (Minneman, 1988). However, the role played by alpha-adrenoceptors in hypertensive disease remains unclear. Nevertheless, the blockade of alpha-adrenoceptors by appropriate antagonistic drugs is effective in lowering blood pressure, particularly with the selective alpha$_1$-antagonists such as prazosin (Cavero and Roach, 1980; Stanaszek et al., 1983; Titmarsch and Monk, 1987).

Prazosin's hypotensive effect is mainly due to decreases in total peripheral resistance, reflecting dilatation of arterioles as a result of vascular alpha$_1$-adrenoceptor blockade. Because the hypotensive activity of prazosin is abolished by either ganglionic blockade or by alpha-adrenoceptor blockade, prazosin clearly acts by
interference with peripheral sympathetic function and is devoid of direct vasodilator activity (Graham et al., 1977; Van Zwieten, 1990).

Results from several studies have demonstrated that NA levels in a variety of vascular and non-vascular tissues are elevated in spontaneously hypertensive rats compared to their normotensive genetic control, the Wistar-Kyoto rats (Donohue et al., 1988). The tissues examined include mesenteric artery (Head et al., 1985), caudal artery (Cassis et al., 1985), kidney, aorta and vas deferens (Head et al., 1985). There is evidence to suggest that the elevated NA concentration in these tissues is due to a greater sympathetic innervation in the hypertensive strain.

All calcium channel blockers except nimodipine blocked responses to the different agonists. With nimodipine treatment there was leftward shift of the concentration-response curve and increase of maximal response to phenylephrine [Fig. 75]. As suggested earlier this could be related to the occurrence of different isoforms of L-type calcium channels (Godfraind et al., 1992), suggesting that the several agonists employed utilise different sources of calcium as discussed above.

Of the different antagonists employed, yohimbine and atenolol shifted the concentration response curves of phenylephrine [Fig. 76 & 77] and 5-HT [Fig. 96 & 97] to the left and increased the maximal responses. The potentiating
action of yohimbine is in line with its known presynaptic inhibitory action on \( \alpha_2 \) receptors accounting for the potentiation. No explanation can be offered for the potentiating action of atenolol. As expected, with all the other antagonists the responses to several agonists were either unaffected or depressed completely or partially with decreased maximal responses.

Compared to the well reported structural cardiac changes engendered by hyperthyroid state (Everett et al., 1986), the vasculature does not seem to get affected. In fact concentration–response curves of all agonists were shifted to the right with depressed maximal responses. This could be secondary to the primary effect exerted on the heart.

With a few exceptions all antagonists shifted the concentration–response curves of the several agonists to the right and depressed the maximal responses. Yohimbine and atenolol shifted the concentration–response curve of histamine to the left [Fig. 85 & 90] and prazosin [Fig. 122] shifted the concentration–response curve of KCl to the left and increased the maximal responses. The results with NA and phenylephrine can be explained by the decreased alpha receptor density in hyperthyroid state reported by Gunasekera and Kuriyama (1990).

The block of responses to other agonists by the antagonists employed may be due to generalised reduced
sensitivity of the rat aorta. No explanation can be offered for the leftward shifts produced by yohimbine and atenolol.

Hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction are seen more commonly in India today than ever before. The increase in life expectancy engendered as a result of public health programmes undertaken by the Government and the availability of potent chemotherapeutic agents are obviously responsible for the increasing number of cardiovascular disorders which are related to the senescent process. Hyperthyroidism also contributes to some of the above-mentioned cardiovascular problems. Our attempt to study interactions of calcium channel blockers and some antagonists with agonists in the heart and arterial tissue of normal, hypertensive and hyperthyroid rat was meant to get an understanding of the mechanisms involved in relation to calcium ion movements and receptor density in health and disease. In the normal ventricle some calcium channel blockers appeared to decrease (verapamil and nifedipine) beta-receptor density and some to increase (diltiazem and nimodipine) it, although all the calcium channel blockers reduced Ca$^{++}$ influx. Thus calcium ion movements have no bearing on the receptor density and vice versa. In the hypertensive state where the density of beta-receptors was increased by the hypertensive process both nifedipine and nimodipine decreased their density. Both agents are useful in postmyocardial infarction subjects. In the hypertensive artery, the sensitivity of alpha-receptor was not affected
but the calcium influx appeared to have been enhanced. The calcium channel blockers in general reduced the receptor density and calcium influx. Thus the action both on the heart and the vasculature would explain the effects in hypertension, coronary artery disease and cardiac arrhythmias.

In the hyperthyroid state there was a decrease in beta-receptor density (rightward shift of concentration response curve), but no effect on the maximal response. Chronic nifedipine and nimodipine treatment further reduced beta-receptor density and also reduced calcium influx. A decreased beta-receptor density has been observed by Hawthorn et al. (1988) on chronic thyroid hormone treatment. In the aorta responses to alpha-receptor activation and calcium influx were reduced by calcium channel blockers. Thus the beneficial effect of the calcium channel blockers in the cardiovascular complications of hyperthyroidism may be through their cardiac and vascular actions.

The present study has tried to answer a number of questions in relation to some commonly used CCBs, agonists and antagonists. While some questions have been answered successfully, others are still unsolved. Some leads have been obtained which because of constraints of infrastructural facilities in the laboratory cannot be pursued further. Facilities for radioligand binding assays, electrophysiological recording and biochemical estimations such as calcium storage compartments including those of cyclic AMP and inositol 1,4,5 triphosphate if made available could go a long way in unraveling the unresolved problems.