PHARMACOLOGICAL ANALYSIS OF THE ACTIONS OF CALCIUM ENTRY BLOCKERS, FELODIPINE, NIFEDIPINE AND VERAPAMIL: INTERACTIONS WITH PRE AND POSTJUNCTIONAL ALPHA ADRENOCEPTORS

A Thesis

Presented to

The Faculty of the Department of Pharmacology

University of Houston

In Partial Fulfillment

of the requirements for the degree

Master of Science in Pharmacology

---

by

Eno-Obong J. Peter

August 1984

DEDICATION

To my mother,

A lady of patience,

A light in the darkness,

A friend throughout the years.

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ABSTRACT

The objective of the present study was to investigate the role of the autonomic nervous system in the hypotensive actions of the calcium entry blockers, felodipine, nifedipine and verapamil. Experiments were designed to determine the effects of these compounds on vascular sympathetic neuroeffector function under both *in vivo* and *in vitro* conditions.

Intravenous administration of cumulative doses of felodipine in intact urethane anesthetized rats produced a sustained and significant decrease in arterial pressure up to 60 minutes in duration at the highest dose (0.8 μmol/kg) tested. Higher doses of nifedipine (0.9 μmol/kg) and verapamil (0.9 μmol/kg) produced transient but significant decreases in mean blood pressure which lasted for 10 minutes. When cardiac compensation was prevented by either bilateral vagotomy plus atropine (1 mg/kg) or propranolol (1 mg/kg) alone or bilateral vagotomy together with atropine plus propranolol, nifedipine and verapamil were also able to reduce arterial pressure. However after ganglionic blockade (chlorisondamine plus atropine) only felodipine was effective in lowering arterial blood pressure.

In pithed rat preparations felodipine (0.5 μmol/kg), nifedipine (1.2 μmol/kg) and verapamil (0.9 μmol/kg) significantly attenuated pressor responses to spinal sympathetic stimulation and exogenous norepinephrine. While all three agents attenuated blood pressure responses to norepinephrine as well as spinal stimulation, felodipine appeared to be more effective in reducing pressor responses to intravenous norepinephrine than those of spinal stimulation.

In isolated perfused rat kidneys lower concentrations of felodipine (10^{-8} to 10^{-6} M), nifedipine and verapamil (10^{-8} to 10^{-7} M) failed to alter stimulus-induced release of [^{3}H]-norepinephrine at frequencies of 0.5 and 2.0 Hz, whereas higher concentrations of felodipine (10^{-5} M), nifedipine and verapamil (10^{-6} and 10^{-5} M) significantly enhanced [^{3}H]-norepinephrine release during periarterial nerve stimulation. On the other hand, the renal vasoconstrictor action of exogenous norepinephrine (16 and 32 ng) was significantly reduced by these agents in a concentration dependent manner (10^{-8} to 10^{-6} M) and abolished at the highest concentration (10^{-5} M). Exogenous norepinephrine caused a concentration dependent inhibition of stimulus-induced release of [^{3}H]-norepinephrine which was significantly antagonized by

phentolamine (2.1×10^{-7} M). While verapamil (10^{-5} M) reduced this presynaptic inhibitory effect of norepinephrine, similar concentrations of nifedipine (10^{-5} M) or lower concentrations of verapamil (10^{-6} M) were ineffective in blocking the actions of norepinephrine. Felodipine (10^{-5} M) significantly potentiated the inhibitory actions of the lower concentrations of norepinephrine (10^{-10} and 10^{-9} M).

These results suggest that autonomic effects could modify the hypotensive actions of these agents in intact rats. Felodipine is capable of decreasing blood pressure in the absence of an intact autonomic nervous system. These studies show that while all the three calcium entry blockers enhanced transmitter release from sympathetic nerve endings this effect was seen at very high concentrations which may not be of pharmacological relevance. Furthermore, it should be noted that concentrations of these agents required to produce any alterations in the release of neurotransmitter are far greater than those required to inhibit vasoconstrictor effects of norepinephrine. Therefore, this postjunctional effect of calcium entry blockers may contribute to their vasodilator action.

TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

FIGURES Page

I. Introduction and Statement of Problem

It is recognized that calcium plays a vital role in several biochemical and physiological processes (Ebashi et al., 1968; Kretsinger, 1976). The plateau phase of the cardiac action potential, and the action potential of vascular smooth muscle is dependent upon its movement into these muscles during the slow inward current (Bohr, 1964; Coraboeuf, 1978; Ringer, 1882; Reuter, 1979; Rüegg, 1971; Somlyo et al., 1970). This movement of calcium results in the activation of contractile elements and subsequent contraction of cardiac and vascular smooth muscle.

Recently, various investigators have carried out studies to describe the mode of action of a number of organic compounds collectively known as calcium antagonists. These studies were stimulated by the observation that excitation-contraction coupling in cardiac and vascular smooth muscle can be disrupted by calcium antagonists (Fleckenstein, 1964, 1967, 1977). Evidence in the literature, indicates that calcium entry blockers (calcium antagonists) interfere directly with transmembrane calcium ion fluxes (Fleckenstein, 1964, 1977;

Flaim et al., 1982; Godfraind et al., 1972). However, a growing body of evidence suggests that the actions of these agents are not homogeneous. Responses are variable depending on the tissue examined, due to differences in kinetic characteristics of slow channel blockade, effects on other transmembrane ion fluxes and interactions with autonomic nervous system reflexes (Henry, 1980; Lathrop et al., 1982). However, all of these agents share a common pharmacological feature in that they relax vascular smooth muscle and mediate negative inotropic cardiac effects (Antman et al., 1980; Fleckenstein, 1977; Grun et al., 1972; Godfraind, 1981; Nayler, 1980; Neuten et al., 1981). As a result of these properties, these drugs appear to have usefulness in several cardiovascular diseases, for example, supraventricular arrhythmias, angina pectoris (classic angina), prinzmental's angina (variant angina), and arterial hypertension.

The objective of the present study is to further investigate the mechanism of the hypotensive actions of felodipine, nifedipine and verapamil. Since these agents have the potential to affect the influence of the sympathetic nervous system on the vasculature in several ways including altering the release of norepinephrine from sympathetic nerves and altering the vasoconstrictor

capabilities of the transmitter norepinephrine, it is of interest to determine what influence the autonomic nervous system has on the cardiovascular responses of these agents. This investigation is particularly relevant for felodipine (structurally similar to nifedipine) since very little information is available in the literature concerning its hypotensive actions. Furthermore, since the three agents to be studied have been proposed to have different mechanisms of action, it will be interesting to compare the hypotensive actions of these agents as they relate to the influence of the autonomic nervous system.

The hemodynamic actions of these agents will be studied first in intact animals and then in animals where experimental manipulations of the autonomic nervous system have been performed. Several questions will be addressed. First, do these agents alter the influence of the sympathetic nervous system on the blood vessels in order to produce their hypotensive actions? Secondly, do these agents retain their hypotensive action in the absence of the influences of the autonomic nervous system? Finally, using an isolated perfused organ (e.g., rat kidney) an attempt will be made to determine whether or not these drugs directly affect sympathetic nerve function by measuring the release of norepinephrine during stimulation of

the sympathetic nerves in the presence of the compounds in question. In total, it is hoped that these investigations will provide additional insight into the hypotensive actions of these drugs.

II. Literature Survey

II. 1. Discovery of calcium entry blocker

Fleckenstein (1964) was the first to report that two newly synthesized coronary vasodilators with unexplained cardiodepressant side effects, prenylamine and verapamil, mimicked the cardiac effects of simple calcium ion withdrawal. He also found that these agents diminished calcium dependent high energy phosphate utilization, contractile force and the oxygen requirement of the beating heart without impairing the fast phase of the action potential.

In 1969, the term calcium antagonist was introduced by Fleckenstein since the effects of these agents were clearly distinguishable from beta receptor blockade. The actions of these agents could promptly be neutralized with elevated extracellular calcium concentrations, beta-adrenergic agonists, or cardiac glycosides; all of which are measures that increase the calcium supply to the contractile elements.

II. 2. The physiological role of calcium ions and the pharmacology of calcium entry blockers in the cardiovascular system and sympathetic nerve function.

The essential and basic function of the cardiac muscle cells is to generate adequate force of contraction to propel blood throughout the body. Excitation-contraction coupling describes in a phrase the complex processes linking the electrical depolarization of the surface membrane to the mechanical events in the myofibril. Calcium ions form the link between electrical activation and force generation. The cardiac muscle cells are made up of myofibrils which are the contractile apparatus. Depolarization of the cardiac muscle results in action potential. The action potential is characterized by an initial fast spike, followed by a plateau phase and a relatively slow phase of repolarization. The shape of the action potential varies among the different types of cardiac cells and can be divided into two characteristic groups, the fast-response and slow-response type (Cranefield, 1975; Coraboeuf, 1979). In slow-response type tissues, such as the sinoatrial node and atrioventricular node, the upstroke of the action potential is heavily dependent on the movement of sodium and calcium

ions into the cell through slow channels. As a result, calcium entry blockers have significant effects on the electrical properties of these cells (Antman et al., 1980; Fleckenstein, 1981; Singh et al., 1982). It is through interference with calcium entry during the upstroke of the action potential in slow-response cells that these agents provide beneficial effects in the treatment of supraventricular arrhythmias.

In contrast, fast-response tissues such as those of the his-purkinje network and atrial and ventricular muscle have an action potential characterized by very rapid depolarization followed by a plateau phase (Henry, 1980). The fast upstroke in these tissues is due to inward sodium movement through so-called fast sodium channels whereas the plateau phase (Zipes et al., 1975) is caused by inward calcium movement through slow channels. It is this inward calcium movement during the plateau phase which provides the link between electrical excitation and force generation in fast-response tissues, often referred to as excitation-contraction coupling (Landmark et al., 1978). Movement of calcium into these cells during the plateau phase serves to trigger the release of calcium from the large intracellular calcium storage sites in the sarcoplasmic reticulum of the myocardium. It is the release of

the intracellularly stored calcium and the availability of this calcium to the contractile elements of the myofibril that cause contraction (Fabito and Fabito, 1979). Thus, by regulating movement of calcium into the myofibrils, the slow channels modulate excitation-contraction coupling and it is at this level that the calcium channel blocking agents are proposed to influence myocardial force generation (Fleckenstein, 1977).

Evidence indicates that the pharmacological actions of the calcium entry blocking agents on myocardial contractility *in vivo* are complex (Ellordt et al., 1980; Nakaya et al., 1981). In intact animals and man, calcium entry blockers cause peripheral arteriolar dilation which results in a decrease in peripheral vascular resistance. The resulting fall in blood pressure can trigger compensatory mechanism through baroreceptor reflexes producing an increase in sympathetic activity. Therefore, the *in vivo* effect of these drugs on the heart are limited and changes seen in heart rate and contractile force are a composite of the direct effect on the heart plus compensatory responses due to alteration in blood pressure. However, *in vitro* where reflex compensation through the autonomic nervous system does not occur, all calcium entry blockers exert dose dependent negative inotropic,

chronotropic, and dromotropic effects that can be reversed by increasing the extracellular calcium concentrations (Fleckenstein, 1977; Stone et al., 1980; Schwartz et al., 1981). These effects are apparent at concentrations at which the fast sodium current is unaffected (Fleckenstein, 1977; Trithart, 1980), indicating the selectivity of this class of drugs for the myocardial slow channel.

In contrast to cardiac muscle, vascular smooth muscle depends heavily upon extracellular calcium for contractile purposes (Winquist et al, 1981). Like cardiac muscle, vascular smooth muscle tissue contains both actin and myosin which are the contracting elements. However, there are differences in other structural elements important in mechanical contractions. The sarcoplasmic reticulum which functions in sequestration of calcium is very sparse and poorly developed in vascular smooth muscle whereas it is extensively developed in cardiac smooth muscle. This difference is the primary reason why vascular smooth muscle depends primarily on extracellular calcium sources for contraction. However, there is substantial evidence indicating that the biochemical events associated with mechanical activity also are different in vascular and cardiac smooth muscle. It has been suggested that the key regulatory step in vascular smooth muscle contraction

is the phosphorylation of the P-light chain of myosin by a calcium-calmodulin activated kinase, initiating actin-myosin interactions (Adelstein and Eisenberg, 1980; Cooke and Stull, 1981; Small and Sobieszek, 1977).

Vascular smooth muscle cells do not only differ from cardiac muscle in biochemical events regulating contraction, but also the mechanism by which vascular smooth muscle can be mechanically activated. While cardiac muscle is primarily activated mechanically by electrical depolarization, in vascular smooth muscle excitation--contraction coupling is brought about by several mechanisms. Activation can result in (1) release of extracellular surface bound calcium, (2) opening of receptor operated calcium channels, (3) release of intracellular bound calcium and (4) depolarization with a consequent opening of potential-dependent calcium channels. The phrase pharmacomechanical coupling has been coined for receptor-operated calcium channel mediated excitation-contraction coupling to distinguish it from electromechanical coupling which is mediated by changes in membrane potential (Bolton, 1979; Casteel, 1980; Gabella, 1979). Evidence reveals that vascular smooth muscle is heterogeneous and one or more of the above listed mechanisms may be involved in producing mechanical activation of a given blood vessel (Bohr, 1973; Gillis, 1982; van Newten et al., 1981). For

example, it was the discovered by Bohr (1963) that in the rabbit aorta epinephrine induced contraction is composed of two phases: an initial fast phase, and a slower sustained phase. Investigations into this phenomenon (Bohr, 1963; Bevan et al., 1973, 1982; Hudgins et al., 1968) lead to the realization that the initial fast phase is not affected by removal of calcium and is probably caused by release of intracellular calcium by epinephrine. On the other hand, the slow phase appears to be the result of opening of alpha-adrenoceptor operated channels. This phase is sensitive upon extracellular calcium removal and accounts for the actions of calcium entry blockers. In contrast to this, Hinke et al. (1964) showed that potassium induced contraction of the same blood vessel results from muscle depolarization and is dependent on the opening of potential-operated channels for extracellular calcium to enter into the cell.

In addition to the discovery of the fast and slow phase of vascular responses to catecholamines, recent research has demonstrated that the mechanism of contraction of vascular smooth muscle may differ depending on the subtype of alpha-adrenoceptor involved in mediating contraction. The presence of both alpha 1- and alpha 2-adrenoceptors at postsynaptic locations, which

participate in the pressor responses of norephinephrine, in vascular smooth muscle of various mammalian species is well established (Starke, 1981; Timmermans et al., 1981). However, experiments have demonstrated that the distribution of these receptor subtypes throughout the cardiovascular system is not homogeneous and there appears to be great differences in relative contribution of these two receptors in catecholamine-induced vasoconstriction from vascular bed to vascular bed (McGrath, 1982). Interestingly, the evidence also indicates that the role of extracellular calcium in contractions mediated by these receptor subtypes is different (Gerold and Häusler, 1983; Langer and Shepperson, 1982).

Studies attempting to examine the mechanism of action of the calcium entry blockers in vascular smooth muscle have served to reinforce and expand our knowledge of the role of calcium in vascular smooth muscle contraction. The fact that these agents have been shown to reduce potassium induced contractions emphasizes the importance of extracellular calcium and potential-dependent channels in this response. In addition, calcium entry blockers diminish the slow phase of catecholamine-induced contraction of vascular smooth muscle without affecting the initial fast phase thus

indicating that these agents can affect receptor- as well as potential-dependent ion channels selectively. Perhaps most notable is the work which has been performed regarding the effects of the calcium entry blocking agents on pressor responses mediated by alpha 1- and alpha 2-adrenoceptors. Recent experiments in pithed rats (Cavero et al., 1981; van Meel et al., 1981a, 1981b, 1982) indicate that verapamil, nifedipine, D-600, and diltiazem reduce the pressor effects of B-HT 920, an alpha 2-adrenoceptor agonist, while responses to methoxamine, an alpha 1-adrenoceptor agonist, were much less affected. Similar findings were reported in ganglion blocked, vagotomized rabbits (van Meel et al., 1982) and in the autoperfused dog hindlimb preparation (Llenas et al., 1983a, 1983b). However, the preferential effect of calcium entry blockers on responses mediated by alpha 2-adrenoceptors is not universally accepted. Studies in the canine portal mesenteric vein (Vanhoutte et al., 1982) and in spontaneously hypertensive rats (Lefèvre-Borg et al., 1981) suggest that responses to alpha 1-adrenoceptors agonists are affected more than responses to alpha 2-adrenoceptor agonists. Similar findings were reported recently in the saphenous arteries and circumflex coronary arteries by Müller-Schweinitzer (1983).

However, the majority of the evidence in the literature is consistent with the greater role of extracellular calcium in the pressor responses induced by alpha 2-adrenoceptor activation as compared to that elicited by alpha 1-adrenoceptor activation, hence making alpha 2-adrenoceptor mediated vasoconstriction more susceptible to inhibition by calcium entry blockers.

Finally, a discussion of the vascular effects of calcium entry blockers would not be complete without mentioning the fact that some investigators believe that these agents not only affect the entry of calcium into vascular smooth muscle cells but also influence the actions within the cell as well (Church et al., 1980; Ljung et al., 1982). Some investigators (Böstrom et al., 1981) suggested that calcium entry blockers such as felodipine interact with calmodulin within the cell to prevent the calmodulin-calcium dependent phosphorylation of myosin. However, this interaction with calmodulin has been disputed (Daly et al., 1983). It has also been suggested that these agents may interfere with the Ca^{2+}-ATPase in the sarcolema (Mass-Oliva et al., 1980) and can stimulate Na^{+}, K^{+}-ATPase in rabbit aorta (Flaim et al., 1982). Nevertheless, the majority of the evidence supports the contention that these agents act primarily

by reducing calcium entry into smooth muscle cells.

In summary, calcium entry blockers, by affecting the transmembrane movement of calcium from extracellular to intracellular sites, are able to cause vascular smooth muscle relaxation. This appears to be true regardless of whether these vessels are activated by electrical excitation in which case blockade of potential-dependent channels is important, or activated by receptor stimulation, in which case blockade of receptor-operated channels are important. Since norepinephrine is released from sympathetic nerves and calcium entry blockers are known to reduce constriction caused by norepinephrine, it appears that these agents can reduce the influence of sympathetic nerves at the postsynaptic level through their actions on norepinephrine-induced constriction of vascular smooth muscle.

In addition to having a role in vasoconstriction in vascular smooth muscle, calcium ions play an important role in exocytosis which occurs at sympathetic nerve terminals during an action potential (Blaustein, 1979; Kostyuk, 1980). When the nerve terminal membrane is depolarized by the invasion of an action potential, extracellular calcium ions enter the cytoplasm through voltage sensitive calcium channels. This results in

subsequent fusion of vesicles containing the neurotransmitter with the plasma membrane and release of the transmitter. Evidence from separate studies by Bollin (1967) on the cat colon perfused in vitro and by Kirpeker et al. (1967, 1968) in the cat spleen perfused in situ indicated that removal of calcium from the perfusion fluid abolished the release of norepinephrine in response to nerve stimulation; furthermore, the norepinephrine output varied directly with the calcium concentration up to 75 μM. Calcium ions, in addition to playing an important role in release of neurotransmitter during electrical activation of the sympathetic nerves, are also important in presynaptic mechanisms that modulate noradrenergic transmission. Electrically evoked norepinephrine release can be modified by many endogenous and pharmacological agents that interact with presynaptic receptors located on postganglionic sympathetic nerve terminals and this action is known to be calcium dependent.

It is well established that activation of presynaptic alpha-adrenoceptors located on postganglionic sympathetic nerve terminals leads to inhibition of norepinephrine release during nerve stimulation. It is suggested that norepinephrine released during nerve stimulation activates these receptors and inhibits further

transmitter release. This forms a local negative feedback mechanism which modulates noradrenergic transmission (Kirpekar et al., 1978; Langer, 1980; Langer and Luchelli-Fortis, 1977). In support of the view that activation of presynaptic alpha-adrenoceptors reduces the availability of calcium for excitation-secretion coupling, Langer et al. (1975) demonstrated that the inhibitory effect of exogenous norepinephrine on transmitter release was potentiated when calcium concentration was reduced from 2.6 mM to 0.65 mM. Other evidence to support this postulated role of calcium has been obtained using rat vas deferens (Drew, 1978), rat superior cervical ganglia (Horn and McAfee, 1980), and mouse vas deferens (Marshall et al., 1977. Göthert (1977) also indicated that norepinephrine release induced by perfusion of the isolated rabbit heart with calcium free solution containing a low sodium and a high potassium ion concentrations was not affected by drugs that influence release via presynaptic mechanism. This further suggests that modulation of norepinephrine release through activation of presynaptic alpha-adrenergic receptors is mediated via changes in the availability of calcium ions for stimulus-release coupling.

Recently, Saeleans and Williams (1983) further

supported this idea by showing that in the cannine saphenous veins, clonidine induced inhibition of endogenous norepinephrine release was reduced by increasing the extracellular calcium concentrations, while enhancement of endogenous norepinephrine release in the presence of isoproterenol was reduced by decreasing extracellular calcium concentration. In summary, these findings support the concept that modulation of norepinephrine release induced by activation of presynaptic alpha adrenoceptor is mediated via changes in the availability of extracellular calcium ions for stimulus release coupling. It seems possible that calcium entry blockers may interfere with the release of norepinephrine by preventing influx of extracellular calcium during an action potential and/or by modifying the negative feedback influence of norepinephrine on neurotransmitter release.

Studies examining the actions of calcium entry blockers on sympathetic nerve terminals suggest a possible influence of these drugs on sympathetic neuroeffector function. Göthert et al. (1979), demonstrated that in the isolated rabbit heart, verapamil, D-600 and prenylamine decrease potassium ion or calcium ion-induced norepinephrine release by inhibiting influx of calcium ion into the nerve terminals. Higher concentrations of

verapamil and methoxyverapamil (D-600) were reported to inhibit the potassium stimulated calcium influx into synaptosomes from rat brain (Ichida et al., 1980; Nachshen and Blaustein, 1979). Zelis et al. (1982) also reported diminished release of [^{3}H]-norepinephrine after diltiazem in pulmonary arteries of the rabbit. However, this presynaptic effect of calcium entry blockers is disputed. In earlier studies, Haeusler (1972) found that verapamil did not alter the release of norepinephrine into the coronary perfusate in cat heart upon stimulation of the right stellate ganglion. Schoffelmeer et al. (1981) also reported that in rat neocortex slices D-600 did not inhibit electrically evoked release of [^{3}H]-norepinephrine, while potassium induced [^{3}H]-norepineprine release was reduced.

Recently, Galzin et al. (1983) demonstrated that in rabbit hypothalamic slices verapamil and not diltiazem increased electrically-evoked outflow of norepinephrine. In addition, Galzin et al., using alpha-adrenoceptor agonist and antagonist, reported that verapamil increased stimulus-induced release of norepinephrine by blocking the alpha 2-adrenoceptors that modulate transmitter release through a negative feedback mechanism, while diltiazem was devoid of this presynaptic alpha

2-adrenoceptor antagonist properties.

In summary, calcium entry blockers, by preventing fluxes of calcium ions through slow calcium channels of the heart, produce a decrease in cardiac output by inhibition of myocardial contratility. Along with this, their ability to inhibit excitation-contraction coupling in vascular smooth muscle brings about vasodilation in peripheral blood vessels. This results in a decrease in vascular resistance with a concomitant decrease in blood pressure. As calcium ions are needed for the exocytotic release of norepinephrine from sympathetic nerves, calcium entry blockers might interfere with the release of norepinephrine as well as decrease the postsynaptic effects of norepinephrine leading to a decrease in blood pressure. However, the autonomic nervous system, through the baroreceptor reflex, plays an important role in modulating alteration in blood pressure induced by various agents, including calcium entry blockers.

III. Material and Methods

III. 1. Pharmacological analysis of the cardiovascular actions of calcium entry blockers felodipine, nifedipine, and verapamil

Male Sprague-Dawley rats weighing between 180-390 g were anesthetized with urethane (150 mg/100 g i.p.). After endotracheal intubation, the animals were prepared for recording of blood pressure and heart rate. Blood pressure was measured with a Statham-P23AC pressure transducer from the left common carotid artery. Heart rate was obtained from the pressure wave by a cardiotachometer (Grass Model 7P4F). Blood pressure and heart rate were recorded on a Grass Model 7D polygraph. The right jugular vein was cannulated for administration of drugs. After surgery a 30 minute period was allowed for blood pressure and heart rate to stabilize.

A group of four rats was employed for studying each calcium entry blocker. Felodipine and nifedipine were prepared daily from a stock solution of 2 mg/ml in polyethylene glycol and verapamil in a solution of distilled water. Doses of drug used were: Felodipine

0.2 μmol/kg (80 μg/kg), 0.4 μmol/kg (160 μg/kg), and 0.8 μmol/kg (320 μg/kg) nifedipine (320 μg/kg), 0.2 μmol/kg (80 μg/kg), 0.5 μmol/kg (160 μg/kg), and 0.9 μmol/kg (320 μg/kg), verapamil 0.4 μmol/kg (200 μg/kg), and 0.9 μmol/kg (400 μg/kg). In each case the injection volume was 0.1 ml. In each group of rats the doses of felodipine, nifedipine, and verapamil were administered sequentially from lowest to highest dose. Blood pressure and heart rate were recorded at 10, 30, and 60 minutes after administration of each dose.

Experiments with each drug were performed in rats under each of the following experimental conditions:

1. Intact rats.
2. Bilateral vagotomy plus atropine (1 mg/kg) pretreatment
3. Propranolol (1 mg/kg) pretreatment.
4. Bilateral vagotomy plus atropine (1 mg/kg) and propranolol (1 mg/kg) pretreatment.
5. Chlorisordamine (1 mg/kg) and atropine (1 mg/kg) pretreatment.

III. 2. Evaluation of the effects of calcium entry blocker on sympathetic neuroeffector function

Pithed rat preparation was utilized for these

studies to evaluate the influence of felodipine, nifedipine, and verapamil on pressor response to total spinal nerve stimulation and to exogenously administered norepinephrine.

Male Sprague-Dawley rats weighing between 245-370 g were anesthetized with ether and the trachea was cannulated. The animals were pithed by insertion of a pithing rod through the right orbit and cranium into the spinal column, and immediately respired with a positive pressure ventilator (1 ml/100 g at a rate of 60 strokes/min). Both blood pressure and heart rate were recorded on a Grass Model 7D polygraph as described in Section III.1. All rats were bilaterally vagotomized to eliminate cardiac vagal influence during experimental procedures. The right jugular vein was cannulated for administration of drugs. After surgery, a 30 minute period was allowed for the blood pressure and heart rate to stabilize. Muscle twitching as a result of stimulating the motor fibers in the ventral roots was abolished by injecting d-turbocurarine (1 mg/kg i.v.), and propranolol (1 mg/kg i.v.) was administered to block beta-adrenoceptors.

By employing the method of Gillespie et al. (1967) a noncoated pithing rod was used as an electrode for activation of spinal sympathetic outflow and a stainless

steel needle placed under the skin served as the reference electrode. The spinal sympathetic fibers were stimulated with a Grass stimulator at frequencies of 0.25, 0.5, 1.0, and 2.0 Hz at supramaximal voltage (60 volts) and a pulse duration of 1 msec for a period of 15 sec at which maximum responses to blood pressure were recorded. Similarly, the responsiveness of vascular alpha-adrenoceptors to 0.1, 0.2, 0.4, and 0.8 μg/kg norepinephrine was assessed. These procedures were repeated after the administration of felodipine 0.5 μmol/kg (200 μg/kg), nifedipine 1.2 μmol/kg (400 μg/kg), and verapamil 0.9 μmol/kg (400 μg/kg) in separate groups of animals.

III. 3. Effect of calcium entry blockers on pre- and-postjunctional noradrenergic mechanisms in the rat kidney.

Experiments were performed to determine the effects of calcium entry blockers on: A. Norepinephrine release elicited during sympathetic nerve stimulation; B. Prejunctional alpha-adrenoceptor mediated inhibition of norepinephrine release; and C. Postjunctional alpha-adrenoceptor mediated renal vasoconstriction in the isolated perfused rat kidney.

III. 3A. Effect of calcium entry blockers on [^{3}H]-NE release elicited during periarterial nerve stimulation in the isolatd perfused rat kidney.

Male Sprague-Dawley rats weighing 225-300 g were used in this study. The right kidney was isolated under pentobarbital sodium (50 mg/kg i.p.) anesthesia as described previously (Ekas et al., 1981). Renal perfusion was initiated with a gravity flow system, then switched to a constant flow perfusion apparatus driven by a perfusion pump (Masterflex Model 7565-10, Cole-Parmer, Chicago Il.) for the duration of the experiment. The perfusion medium was Krebs-Ringer bicarbonate buffer (pH 7.4, 37°C) of the following composition (mM): NaCl 118.5, KCL 4.8, $CaCl_2 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 2.5, glucose 5 and EDTA 0.1. The buffer was continuously bubbled with 95% oxygen, 5% carbon dioxide. The perfusion flow rate was 6 ml/min and the perfusion fluid was not recirculated. Perfusion pressure was measured with a pressure transducer (Statham P23AC) and recorded on a polygraph (Grass Model 79D).

After a stabilization period of 20 to 30 minutes the neuronal norepinephrine storage sites were labelled with

l [7,8 ^{3}H]-norepinephrine ([^{3}H]-NE). Ten μCi of [^{3}H]-NE (specific radioactivity 11 Ci/mmol) was perfused through the kidney for 30 minutes followed by a 40 minute washout with [^{3}H]-NE free fluid. Bipolar platinum electrodes were placed around the renal artery for periarterial nerve stimulation. Stimulation parameters were 40 volts, 1 msec duration and a 20 sec stimulation period. To measure the amount of [^{3}H]-NE released during periarterial stimulation, 20 sec collection of the venous effluent were obtained before (pre), during and after (post) periarterial nerve stimulation. The during and post periarterial nerve stimulation collections were combined for the measurement of the total amount of [^{3}H]-NE released during stimulation. The actual amount of [^{3}H]-NE released during stimulation is expressed as the amount of [^{3}H]-NE in the post (during and after) collection corrected by subtracting the baseline efflux of [^{3}H]-NE collected in the pre stimulation period. Stimulus-induced release of [^{3}H]-NE is expressed as release ratio. A release ratio is calculated by dividing the amount of [^{3}H]-NE released during each stimulation period by the amount of [^{3}H]-NE released during the first control stimulation period. Two stimulations at a given frequency were performed 10 minutes apart in the absence of drugs

to obtain a control release ratio. A ratio of C_2/C_1 will represent any alterations in the release. Thus a ratio of 1.00 would mean that [^{3}H]-NE release is identical during two control stimulations. A release ratio of <1 is indicative of a decrease in [^{3}H]-NE release whereas an increase in release ratio (>1) will reflect an increase in [^{3}H]-NE release.

The first series of experiments were performed at a frequency of 0.5 Hz, and the effect of polyethylene glycol 400 (PEG-400 - vehicle in which felodipine and nifedipine were dissolved) on [^{3}H]-NE release elicited during periarterial nerve stimulation was determined. An initial stimulation at 4 Hz was performed to ensure that the electrodes were properly placed and the preparation was functioning. All subsequent stimulations were performed at 0.5 Hz, and each stimulation was separated by a period of 10 minutes. Two stimulations at 0.5 Hz were performed in Krebs-Ringer solution to obtain a control release of [^{3}H]-NE. After the second control stimulation, polyethylene glycol 400 (0.1 ml/100 ml) in Krebs-Ringer was perfused through for 40 minutes and nerve stimulation was performed every 10 minutes. The kidney was subsequently perfused with vehicle free solution and an additional measurement of [^{3}H]-NE release during

periarterial nerve stimulation was obtained.

In the next series of experiments, the effects of different concentrations of felodipine, nifedipine, and verapamil on stimulus-induced release of [^{3}H]-NE were determined at 0.5 and 2 Hz. The experimental protocol was similar to that described earlier. After the second control stimulation either felodipine, nifedipine, or verapamil (10^{-8} M) was perfused through the kidney for 10 minutes and periarterial nerve stimulation was performed in the presence of the calcium entry blockers at the end of 10 minute period. Increasing concentrations (10^{-7}, 10^{-6}, and 10^{-5} M) of calcium entry blockers were perfused through the kidney, for 10 minute period and their effect on stimulus-induced release of [^{3}H]-NE was determined. The kidney was subsequently perfused with drug free solution and an additional measurement of [^{3}H]-NE release obtained.

III. 3B. Effect of calcium entry blocker on prejunctional alpha-adrenoceptor mediated inhibition of norepinephrine release.

Experiments were performed to establish the inhibitory effect of exogenously administered norepinephrine on the stimulus-induced release of [^{3}H]-NE at 0.5 Hz.

In the presence of cocaine (10^{-5} M) the effect of increasing concentrations of norepinephrine (10^{-10}, 10^{-9}, 10^{-8}, $2x10^{-8}$, 10^{-7}M) on stimulus-induced release of [^{3}H]-NE was determined.

In the next series of experiments felodipine (10^{-5} M) and either nifedipine or verapamil (10^{-6} and 10^{-5} M), concentrations that significantly increased [^{3}H]-NE release during periarterial nerve stimulation, were added to the perfusion medium containing cocaine (10^{-5} M). The effect of increasing concentrations of norepinephrine on stimulus-induced release of [^{3}H]-NE was determined in the presence of these calcium entry blockers. The kidney was subsequently perfused with norepinephrine free Krebs-Ringer and an additional measurement of [^{3}H]-NE release obtained.

III. 3C. Effects of calcium entry blockers on post-junctional alpha-adrenoceptor mediated renal vasoconstriction.

In the first series of experiments renal vasoconstrictor responses to exogenously administered norepinephrine were determined. The protocol was similar to the first series of nerve stimulation experiments except that norepinephrine was administered in place of

electrical stimulation. Norepinephrine (16 and 32 ng) was injected every 10 minutes in a volume of 0.1 ml into the renal artery. These responses were repeated over a period of 70 minutes to ensure the reproducibility of the vasoconstrictor responses.

In the next series of experiments increasing concentrations of either felodipine, nifedipine or verapamil (10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) were added to the perfusion medium and vasconstrictor responses to exogenous norepinephrine (16 and 32 ng) were obtained during perfusion with these compounds. After the highest concentration of each calcium entry blocker, the kidney was perfused with drug-free Krebs-Ringer and vasoconstrictor responses to norepinephrine were obtained.

III. 4. Statistical analysis and drugs

The results are expressed as mean ± standard error of the mean ($\bar{x}$ ± S.E.M.). Two way analysis of variance, randomized complete block design was performed and means were compared using Newman Keuls multiple range test. The results were considered statistically significant when $P < 0.05$.

In the isolated perfused kidney preparations, counts per minutes were obtained by aliquoting 0.5 ml of the

combined before and after stimulation samples into scintillation vials and adding 5 ml of scintillation coctail. The samples were counted in a Beckman LS750 Liquid scintillation counter. Each sample was counted for a period of 10 minutes. Counts obtained were converted to disintegrations per minute by dividing each counts by the sample channel ratio and then subtracting before from post stimulation (Table I).

Table 1: Release of [^{3}H]-NE is expressed as release ratio. Release ratio is defined as the amount of [^{3}H]-NE released above baseline during each stimulation period divided by the amount of [^{3}H]-NE released above baseline during the first control stimulation.

		C.P.M.	Time	S.C.R.	D.P.M.		R.R.
	1	161.3	10.00	.616	261	176	178/
Control	2	296.4	10.00	.677	437		/176 = 1.01
	1	146.2	10.00	.680	215	178	
	2	272.4	10.00	.692	393		
Drug	1	123.8	10.00	.622	199	122	122/
	2	223.0	10.00	.695	321		/176 = 0.69
Drug	1	161.3	10.00	.610	263	246	246/
	2	345.0	10.00	.677	509		/176 = 1.39

C.P.M. = Counts per minute

S.C.R. = Sample channel ratio

D.P.M. = Disintegration per minute

R.R. = Release ratio

The following drugs were used:

Felodipine (A. B. Haessle, Mölndal, Sweden), nifedipine (Pfizer Pharmaceutical: New York, N.Y.), verapamil (Knoll Pharmaceutical Co., Whippany, N.J.), l-propranolol (Ayerst Lab., Inc., New York, N.Y.), atropine sulphate (Mallinckrodt Chemical Works, St. Louis, Missouri), urethane, pentobarbital sodium, d-turbocurarine (Sigma Chemical Co., St. Louis, Missouri), chlorisondamine, phentolamine mesylate (Ciba Pharmaceutical Co., Summit, (N.J.), norepinephrine bitartrate, isoproterenol hydrochloride (Winthrop Lab., Div., New York, N.Y.), ether anhydrous, scintillation coctail (scinti verse I^{TR}) (Fisher Scientific, Fair Lawn, N.J.), cocaine hydrocholoride (Merck and Co.), l-(7,8 ^{3}H)-norepinephrine (New England Nuclear, Boston, Mass. or Amersham Inter. Pl., Amersham UK), polyethylene glycol 400 (Aldrich Chemical Co., Milwaukee, Wisconsin).

IV. Results

IV. 1. Pharmacological analysis of the cardiovascular actions of felodipine, nifedipine and verapamil.

In control rats intravenous administration of felodipine (0.2 μmol/kg) caused a decrease in mean blood pressure from 102 ± 12 to 81 ± 5 mmHg which was sustained for 10 minutes (Table 2). Blood pressure gradually returned back to control level at the end of 60 minutes. Administration of a higher dose of felodipine (0.4 μmol/kg) produced a decrease in mean blood pressure from 100 ± 11 to 79 ± 3 mmHg (Table 2) which was sustained for 30 minutes. Administration of felodipine (0.8 μmol/kg) decreased mean blood pressure from 90 ± 6 to 79 ± 1 mmHg and this was sustained for 60 minutes (Table 2). In essence, cumulative administration of felodipine resulted in a pronounced and sustained decrease in mean blood pressure. Felodipine also caused tachycardia which was more pronounced at the higher dose (Table 3). In contrast to what was seen with felodipine, the lower doses of nifedipine (0.2 and 0.5 μmol/kg) or verapamil (0.4 μmol/kg) failed to produce any significant decreases in

mean blood pressure (Tables 4 and 6). However, the higher doses of nifedipine (0.9 μmol/kg) and verapamil (0.9 μmol/kg) caused hypotension which was sustained for about 10 minutes (Tables 4 and 6). Like felodipine, cumulative doses of nifedipine and verapamil produced an increase in heart rate (Tables 5 and 7).

Bilateral vagotomy plus atropine (1 mg/kg) treatment significantly augmented the hypotensive action of felodipine as administration of (0.2 μmol/kg) produced a significant and pronounced decrease in mean blood pressure (105 ± 5 to 77 ± 1 mmHg) which did not return to control level for up to 60 minutes (Table 2). Subsequent administration of higher doses of felodipine (0.4 and 0.8 μmol/kg) produced further decreases in mean blood pressure (Table 2). In contrast to the tachycardia produced in control rats, felodipine produced a significant decrease in heart rate from 448 ± 9 to 341 ± 24 bpm (Table 3). This decrease in heart rate may explain the marked hypotension produced by felodipine. Unlike the observation made in intact rats, nifedipine (0.2 μmol/kg) significantly decreased mean blood pressure from 108 ± 3 to 95 ± 5 mmHg, which was sustained for 10 minutes (Table 4). Furthermore, higher doses of nifedipine (0.5 and 0.9 μmol/kg) caused greater decreases in mean blood

pressure which were sustained for 30 minutes and 60 minutes respectively (Table 4). Heart rate was also significantly decreased by nifedipine from 443 ± 19 to 285 ± 32 bpm (Table 5). Decreases in heart rate after this treatment may explain the hypotensive action of nifedipine which was not seen in control animals. Verapamil was not effective in decreasing mean blood pressure under this condition (Table 6).

In propranolol (1 mg/kg) treated rats, the hypotensive action of felodipine (0.2 μmol/kg) was of greater magnitude than that seen in intact rats in that mean blood pressure decreased from 105 ± 7 to 73 ± 3 mmHg (Table 3), and this was sustained up to 60 minutes. Higher doses of felodipine (0.4 and 0.8 μmol/kg) produced further decreases in mean blood pressure which were sustained for 60 minutes (Table 2). Heart rate was not significantly altered by felodipine (Table 3). Nifedipine (0.2 μmol/kg) produced sustained and greater decreases in mean blood pressure than those seen in intact rats although these effects were less than that of felodipine (Table 2). The higher doses of nifedipine (0.5 and 0.9 μmol/kg) caused further hypotension (Table 4). Heart rate was significantly decreased by nifedipine (0.2 - 0.9 μmol/kg) from 275 ± 19 to 246 ± 40 bpm (Table

5). Under these conditions, verapamil (0.4 and 0.9 μmol/kg caused an insignificant lowering of mean blood pressure from 149 ± 10 to 132 ± 10 mmHg (Table 6). Heart rate was not significantly altered by either dose of verapamil (Table 7).

In bilaterally vagotomized plus atropine and propranolol treated rats, felodipine (0.2 μmol/kg) produced a significant and sustained hypotension which did not return back to control level before the administration of the next higher dose (Table 2). Administration of felodipine (0.4 μmol/kg) resulted in a further decrease in mean blood pressure from 74 ± 4 to 65 ± 3 mmHg (Table 2). The highest dose of felodipine (0.8 μmol/kg) did not further reduce mean blood pressure (Table 2). Heart rate was not significantly changed by felodipine (Table 3). Administration of nifedipine (0.2 μmol/kg) nonsignificantly lowered mean blood pressure which returned back to near control level (Table 4). However, nifedipine (0.5 and 0.9 μmol/kg) did not significantly alter mean blood pressure (Table 4). Also nifedipine in doses used did not significantly change the heart rate (Table 5). Like nifedipine, the lower dose of verapamil (0.4 μmol/kg) did not significantly lower mean blood pressure (Table 6). However, the highest dose of verapamil

(0.9 μmol/kg) significantly decreased mean blood pressure. This effect was sustained for 60 minutes (Table 6). Heart rate was not significantly altered except during the first 10 minutes after administration of verapamil (0.4 and 0.9 μmol/kg) (Table 7).

After ganglionic blockade and atropine treatment, the mean blood pressure of these rats were significantly lower than in the previous groups (for felodipine 77 ± 2, nifedipine 73 ± 3 and verapamil 51 ± 4 mmHg). Felodipine (0.2 and 0.4 μmol/kg) caused decreases in mean blood pressure which were sustained for 30 minutes (Table 2). The highest dose of felodipine (0.9 μmol/kg) also produced a decrease in mean blood pressure for up to 60 minutes (Table 2). Heart rate was significantly decreased by these doses of felodipine (0.2 - 0.8 μmol/kg) from 313 ± 15 to 201 ± 11 bpm (Table 3). In contrast, nifedipine (0.2 - 0.9 μmol/kg) and verapamil (0.4 - 0.9 μmol/kg) did not significantly alter mean blood pressure in these rats (Tables 4 and 6). However, the heart rate was significantly decreased by the highest dose of nifedipine (0.9 μmol/kg) (Table 5). Verapamil (0.4 μmol/kg) also produced significant decreases in heart rate (Table 7).

Table 2: Effect of felodipine on mean blood pressure of urethane-anesthetized rats.[1]

	Time After Injection	Total Time Elapsed	MEAN BLOOD PRESSURE (mmHg) A	B	C	D	E
Before Felodipine	0	0	102±12	105±5	105±7	90±10	77±2
After Felodipine	10	10	81±5*	77±1*	72±3*	65±4*	58±2*
0.2 μmol/kg	30	30	89±8	84±3*	74±2*	69±3*	65±3*
	60	60	100±11	96±3*	83±7*	74±4*	74±4
	10	70	79±3*	75±4*	71±3*	65±4*	66±5*
0.4 μmol/kg	30	90	80±6*	78±4*	72±5*	68±4*	67±2*
	60	120	90±6	81±5*	80±5*	65±3*	70±4
	10	130	78±3*	72±4*	70±5*	65±8*	64±4*
0.8 μmol/kg	30	150	74±1*	73±6*	72±3*	63±6*	65±4*
	60	180	79±1*	75±6*	73±3*	65±8*	67±2*

[1]The doses of felopidine were administered sequentially and changes in mean blood pressure were recorded for up to 60 minutes after the administration of each dose A = control rats, B = bilateral vagotomy plus atropine (1 mg/kg) treated rats, C = propranolol (1 mg/kg) treated rats, D = bilateral vagotomy plus atropine and propranolol treated rats, E = chlorisondamine (1 mg/kg) plus atropine treated rats. Values given are $\bar{X}$ ± S.E.M., N = 4. *$p < 0.05$.

Table 3: Effect of felodipine on heart rate in urethane-anesthetized rats.[1]

	Time After Injection	Total Time Elapsed	HEART RATE (beats per minute) A	B	C	D	E
Before Felodipine	0	0	400±36	448±9	268±14	380±26	313±15
After Felodipine	10	10	420±49	430±14	252±13	350±32	263±14*
0.2 μmol/kg	30	30	455±49	433±13*	249±18	366±29	263±23*
	60	60	449±44	420±14*	263±21	380±30	268±19*
	10	70	431±51	386±13*	259±20	336±11*	226±19*
0.4 μmol/kg	30	90	451±59	391±10*	256±22	370±15	240±16*
	60	120	453±59	380±18*	271±21	390±21	255±10*
	10	130	465±62	328±19*	269±20	320±41	194±14*
0.8 μmol/kg	30	150	451±44	340±19*	280±24	375±10	189±15*
	60	180	481±50	341±24*	294±17	360±55	201±11*

[1]The doses of felodipine were administered sequentially and changes in heart rate were recorded for up to 60 minutes after the administration of each dose. A = control rats, B = bilateral vagotomy plus atropine (1 mg/kg) treated rats, C = propranolol (1 mg/kg) treated rats, D = bilateral vagotomy plus atropine and propranolol treated rats, E = Chlorisondamine (1 mg/kg) atropine treated rats. Values are $\bar{X}$ ± S.E.M. N = 4. *$p < 0.05$.

Table 4: Effect of nifedipine on mean blood pressure of urethane-anesthesized rats.[1]

	Time After Injection	Total Time Elapsed	MEAN BLOOD PRESSURE (mmHg) A	B	C	D	E
Before Nifedipine	0	0	94±4	108±3	104±3	89±6	73±3
After Nifedipine	10	10	88±5	95±5*	89±2*	80±4	70±4
0.2 μmol/kg	30	30	92±8	103±2	85±1*	77±6	69±4
	60	60	95±5	102±3	94±1*	80±6	77±4
	10	70	85±9	85±3*	81±3*	75±8	68±2
0.5 μmol/kg	30	90	87±8	94±3*	82±1*	79±6	75±4
	60	120	92±9	99±4	93±2*	85±7	78±4
	10	130	78±6*	85±2*	80±2*	77±6	69±4
0.9 μmol/kg	30	150	89±8	88±5*	86±2*	78±5	74±5
	60	180	92±9	96±4*	92±5*	77±4	85±7

[1]The doses of nifedipine were administered sequentially and changes in mean blood pressure were recorded for up to 60 minutes after the administration of each dose. A = control rats, B = bilateral vagotomy plus atropine (1 mg/kg) treated rats, C = propranolol (1 mg/kg) treated rats, D = bilateral vagotomy plus atropine and propranolol treated rats, E = chlorisondamine (1 mg/kg) plus atropine treated rats. Values given are $\bar{X}$ ± S.E.M. N = 4. *$p < 0.05$.

Table 5: Effect of nifedipine on heart rate in urethane-anesthesized rats.[1]

			HEART RATE (beats per minute)				
	Time After Injection	Total Time Elapsed	A	B	C	D	E
Before Nifedipine	0	0	350±4	443±19	275±19	384±38	294±26
After Nifedipine	10	10	365±12	393±19*	254±23*	373±44	286±21
0.2 μmol/kg	30	30	370±11	408±18*	255±32*	360±23	278±15
	60	60	383±11	400±14*	269±32	380±23	293±18
	10	70	388±22	320±32*	254±37*	373±28	281±32
0.5 μmol/kg	30	90	375±10	338±27*	254±34*	385±25	281±23
	60	120	380±20	336±31*	271±28	412±31	299±24
	10	130	388±28	251±23*	244±44*	383±31	253±28*
0.9 μmol/kg	30	150	398±24	264±27*	235±39*	386±28	271±29*
	60	180	383±17	285±32*	246±40*	388±27	304±37

[1]The doses of nifedipine were administered sequentially and changes in heart rate were recorded for up to 60 minutes after the administration of each dose. A = control rats, B = bilateral vagotomy plus atropine (1 mg/kg) treated rats, C = propranolol (1 mg/kg) treated rats, D = bilateral vagotomy plus atropine and propranolol treated rats, E = chlorisondamine (1 mg/kg) plus atropine treated rats. Values given are $\bar{X}$ ± S.E.M., N = 4. *$p < 0.05$.

Table 6: Effect of verapamil on mean blood pressure of urethane-anesthesized rats.[1]

			MEAN BLOOD PRESSURE (mmHg)				
	Time After Injection	Total Time Elapsed	A	B	C	D	E
Before Verapamil	0	0	124±5	104±8	149±10	141±7	51±4
After Verapamil	10	10	117±4	100±10	141±8	135±9	48±3
0.4 µmol/kg	30	30	118±4	105±14	144±8	143±10	49±3
	60	60	122±4	109±13	138±3	133±9	56±6
	10	70	108±6*	100±10	131±3	116±5*	52±6
0.9 µmol/kg	30	90	117±2	101±7	130±4	114±10*	52±5
	60	120	124±3	92±6	132±10	117±9*	58±4

[1]The doses of verapamil were administered sequentially and changes in mean blood pressure were recorded for up to 60 minutes after the administration of each dose. A = control rats, B = bilateral vagotomy plus atropine (1 mg/kg) treated rats, C = propranolol (1 mg/kg) treated rats, D = bilateral vagotomy plus atropine and propranolol treated rats, E = chlorisondamine (1 mg/kg) plus atropine treated rats. Values given are $\bar{X}$ ± S.E.M., N = 4. *$P < 0.05$.

Table 7: Effect of verapamil on heart rate in urethane-anesthesized rats.[1]

			HEART RATE (beats per minute)				
	Time After Injection	Total Time Elapsed	A	B	C	D	E
Before Verapamil	0	0	395±17	376±10	320±15	309±13	285±6
After Verapamil	10	10	393±15	360±7	311±13	291±9	245±5*
0.4 μmol/kg	30	30	413±9	365±12	345±13	326±11	258±5*
	60	60	428±6	351±18	361±13	348±9	275±3
	10	70	385±12	315±18*	324±3	285±9*	225±4*
0.9 μmol/kg	30	90	405±6	335±17*	355±11	314±4	250±5*
	60	120	403±8	330±18*	360±27	330±4	274±11

[1]The doses of verapamil were administered sequentially and changes in heart rate were recorded for up to 60 minutes after the administration of each dose. A = control rats, B = bilateral vagotomy plus atropine (1 mg/kg) treated rats, C = propranolol (1 mg/kg) treated rats, D = bilateral vagotomy plus atropine and propranolol treated rats, E = chlorisondamine (1 mg/kg) plus atropine treated rats. Values given are $\bar{X}$ ± S.E.M., N = 4. *$P < 0.05$.

IV. 2. Evaluation of the effects of calcium entry blockers on sympathetic neuroeffection function.

In this series of experiments, the resting mean blood pressure of rats after pithing ranged from 66 ± 2 to 71 ± 4 mmHg. Control responses to sympathetic nerve stimulation and to exogenous norepinephrine were obtained in the absence of the calcium entry blockers. Electrical stimulation of total spinal outflow and administration of exogenous norepinephrine produced frequency- and dose-dependent increases in mean blood pressure (Figures 1,2, 3). Administration of felodipine (0.5 μmol/kg) in these rats produced significant decreases in resting mean blood pressure ranging from 15 to 20 mmHg. The pressor responses to total spinal stimulation and exogenous norepinephrine were significantly reduced by felodipine (Figure 1). Felodipine appeared to be more effective in inhibiting pressor responses to injected norepinephrine than those of sympathetic nerve stimulation.

Like felodipine, administration of nifedipine (1.2 μmol/kg) in separate groups of rats produced significant hypotension ranging from 10 to 15 mmHg. Nifedipine significantly decreased the pressor responses to

sympathetic nerve and exogenous norepinephrine which unlike felodipine appeared to be of similar magnitude (Figure 2). In contrast to felodipine and nifedipine, administration of verapamil (0.9 μmol/kg) to pithed rats did not produce any significant alteration in resting mean blood pressure. However, verapamil significantly attenuated pressor responses to sympathetic nerve stimulation and to exogenous norepinephrine (Figure 3).

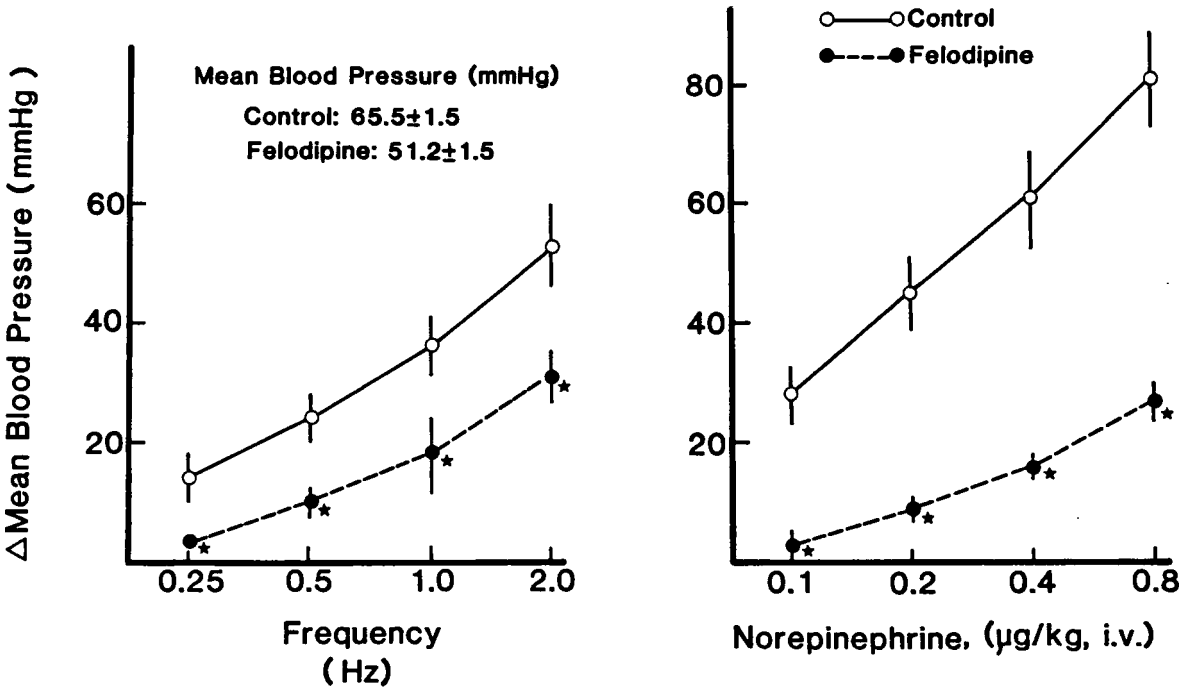

Figure 1: Effects of felodipine (0.5 μmol/kg) on pressor responses to spinal stimulation and to exogenous norepinephrine. Values given are $\bar{X}$ ± S.E.M., N = 6. *$p < 0.05$.

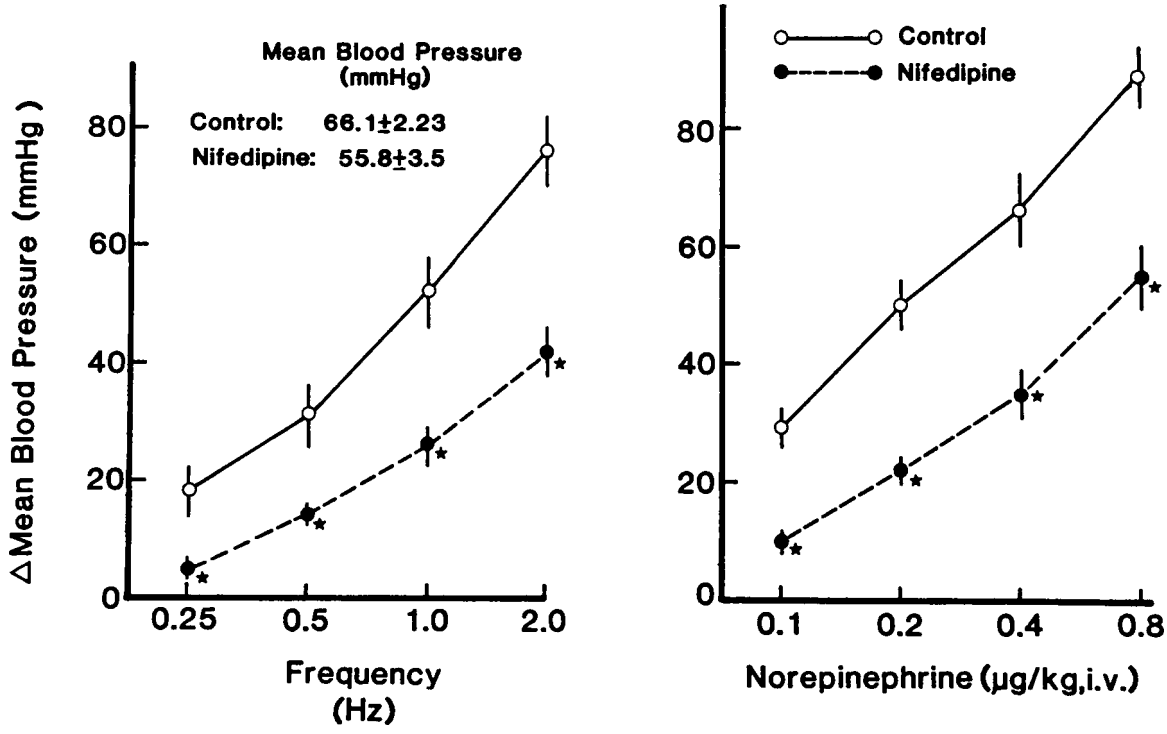

Figure 2: Effects of nifedipine (1.2 μmol/kg) on pressor responses to spinal stimulation and exogenous norepinephrine. Values given are $\bar{X}$ ± S.E.M., N = 6. *p < 0.05.

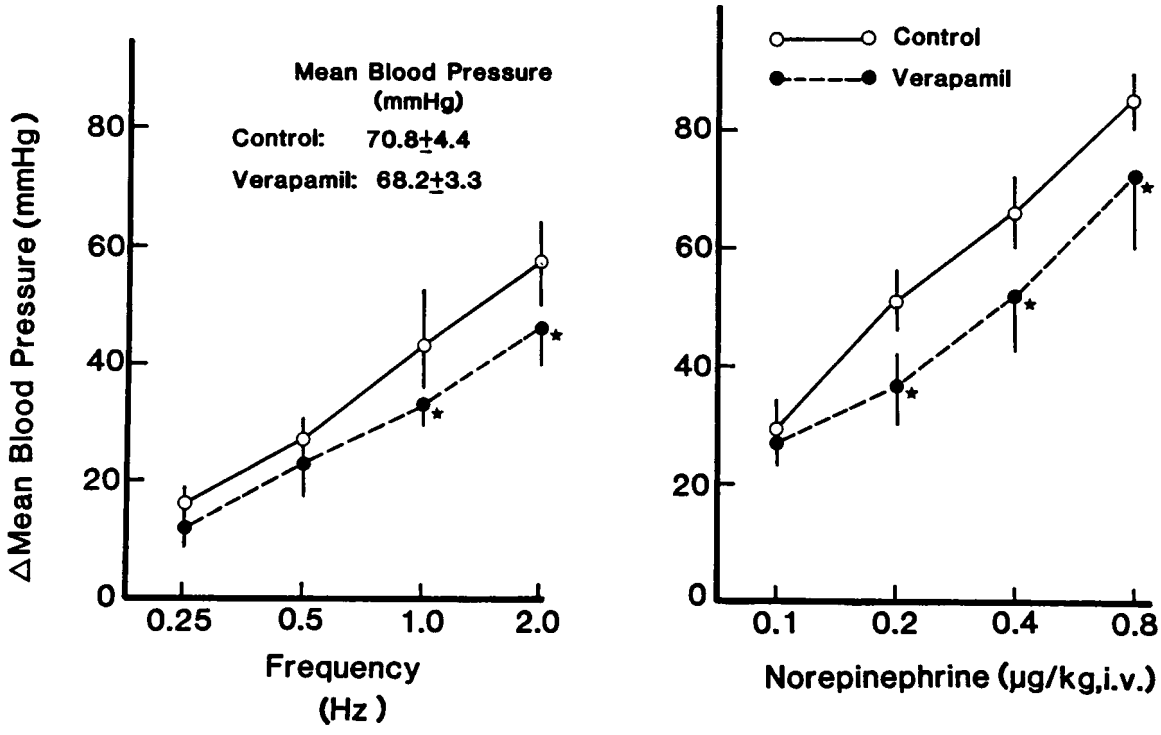

Figure 3: Effects of verapamil (0.9 μmol/kg) on pressor responses to spinal stimulation and to exogenous norepinephrine. Values given are $\bar{X}$ ± S.E.M., N = 6. *p < 0.05.

IV. 3A. Effects of calcium entry blockers on [^{3}H]-NE release elicited during periarterial nerve stimulation in siolated perfused rat kidney.

Periarterial nerve stimulation in the isolated perfused rat kidney at 0.5 Hz resulted in the release of [^{3}H]-NE which was 2204 ± 396 dpm. Previously it was shown from this laboratory that about 65% of the total [^{3}H]-NE released during periarterial nerve stimulation was intact [^{3}H]-NE (Ekas et al., 1983; Steenberg et al., 1983). Therefore, in this thesis, the release of total [^{3}H] measured during nerve stimulation will be referred to as [^{3}H]-NE release.

The effect of the highest concentration of the vehicle, polyethylene glycol 400 (0.1 ml) on [^{3}H]-NE release elicited during periarterial nerve stimulation is shown in figure 4. It is evident that perfusion of kidneys with polyethylene glycol did not cause any alterations in the stimulus-induced release of [^{3}H]-NE as reflected in the observation that the release ratios were identical in the absence and the presence of the vehicle.

The effect of increasing concentrations of felodipine (10^{-8} to 10^{-5} M) on [^{3}H]-NE release elicited during periarterial nerve stimulation at 0.5 and 2 Hz

is illustrated in figure 5. While the lower concentrations of felodipine (10^{-8} to 10^{-6} M) did not significantly alter stimulus-induced release of [^{3}H]-NE at either 0.5 or 2 Hz, the highest concentration of felodipine caused a modest but significant increase in [^{3}H]-NE release as reflected in the increase in the release ratio from 1.02 ± 0.01 to 1.29 ± 0.07 at 0.5 Hz and from 1.05 ± 0.01 to 1.34 ± 0.07 (Figure 5). This facilitatory effect of felodipine on stimulus-induced release of [^{3}H]-NE did not appear to be frequency dependent. Felodipine did not alter spontaneous (basal) efflux of radioactivity at any of these concentrations. The effect of felodipine was reversible since when the kidneys were perfused with felodipine free Krebs-Ringer solution, the release ratio returned almost back to control level (Figure 5).

The effect of nifedipine on [^{3}H]-NE release elicited during periarterial nerve stimulation at 0.5 and 2 Hz is illustrated in figure 6. Whereas the two lower concentrations (10^{-8}, 10^{-7}M) of this agent did not alter stimulus-induced release, the two higher concentrations (10^{-6} and 10^{-5}M) caused significant increases in [^{3}H]-NE release (Figure 6). This facilitatory action of nifedipine on [^{3}H]-NE release was more pronounced at

0.5 Hz as evidenced in the observation that release ratios increased from 1.05 ± 0.02 to 1.25 ± 0.09 (10^{-6} M) and 1.62 ± 0.13 (10^{-5} M) compared to from 1.01 ± 0.01 to 1.13 ± 0.06 (10^{-6} M) and 1.36 ± 0.04 (10^{-5} M) at 2 Hz (Figure 6). Nifedipine did not produce any changes in spontaneous (basal) efflux of radioactivity at any of these concentrations. The facilitatory effect of nifedipine was reversible since perfusion of the kidney with nifedipine free Krebs-Ringer resulted in a return of the release ratio close to control value (Figure 6).

Like felodipine and nifedipine, the lower concentrations of verapamil (10^{-8}, 10^{-7} M) did not significantly change stimulus-induced release of [^{3}H]-NE at either 0.5 or 2 Hz (Figure 7). The higher concentrations of verapamil (10^{-6}, 10^{-5} M) caused a pronounced increase in [^{3}H]-NE release as reflected in the increases in the release ratio from 1.05 ± 0.03 to 1.37 ± 0.08 (10^{-6} M) and 1.87 ± 0.20 (10^{-5} M) at 0.5 Hz and from 1.02 ± 0.02 to 1.34 ± 0.08 (10^{-6} M) and 2.14 ± 0.08 (10^{-5} M) at 2 Hz (Figure 7). Verapamil did not alter spontaneous (basal) efflux of radioactivity. The effect of verapamil was also reversible since in the absence of any drug, the release ratio returned to control level (Figure 7).

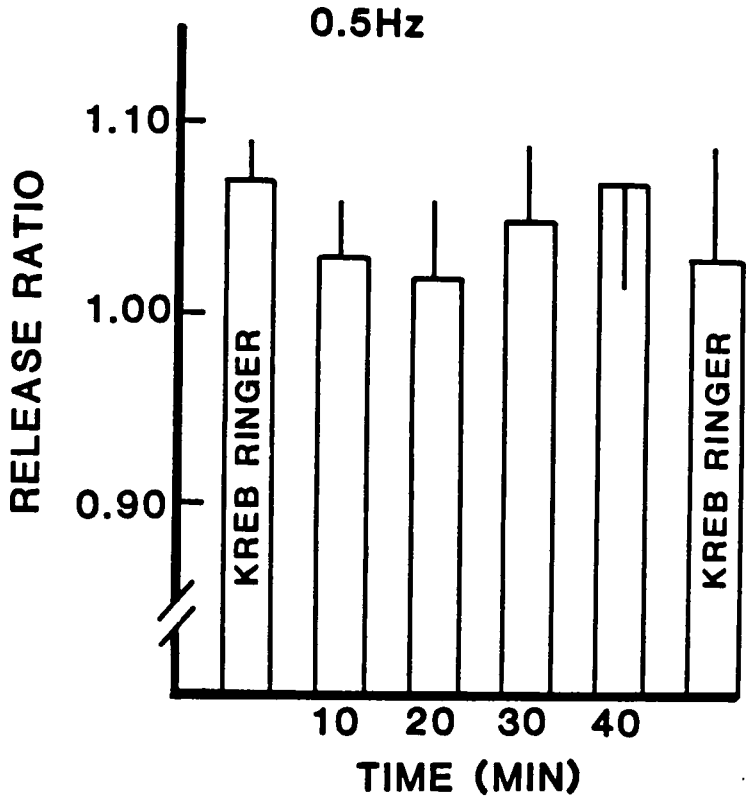

Figure 4: Effect of Polyethylene glycol 400 (P.E.G. 400) 0.1 ml on [^{3}H]-NE release elicited during periarterial nerve stimulation at 0.5 Hz. Release ratio is calculated by dividing the amount of [^{3}H]-NE release during each stimulation by the amount of [^{3}H]-NE released during the first control stimulation period. The first control release ratio = C_2 dpm/C_1 dpm (Kreb-Ringer). Release ratio in the presence of P.E.G. 400 would be P.E.G. dpm/C_1 dpm. Similarly, release ratios were calculated for all subsequent stimulation periods of 10 minutes apart. The final stimulation was performed in the absence of P.E.G. 400 Values given are $\bar{X}$ ± S.E.M., N = 4. *$p < 0.05$.

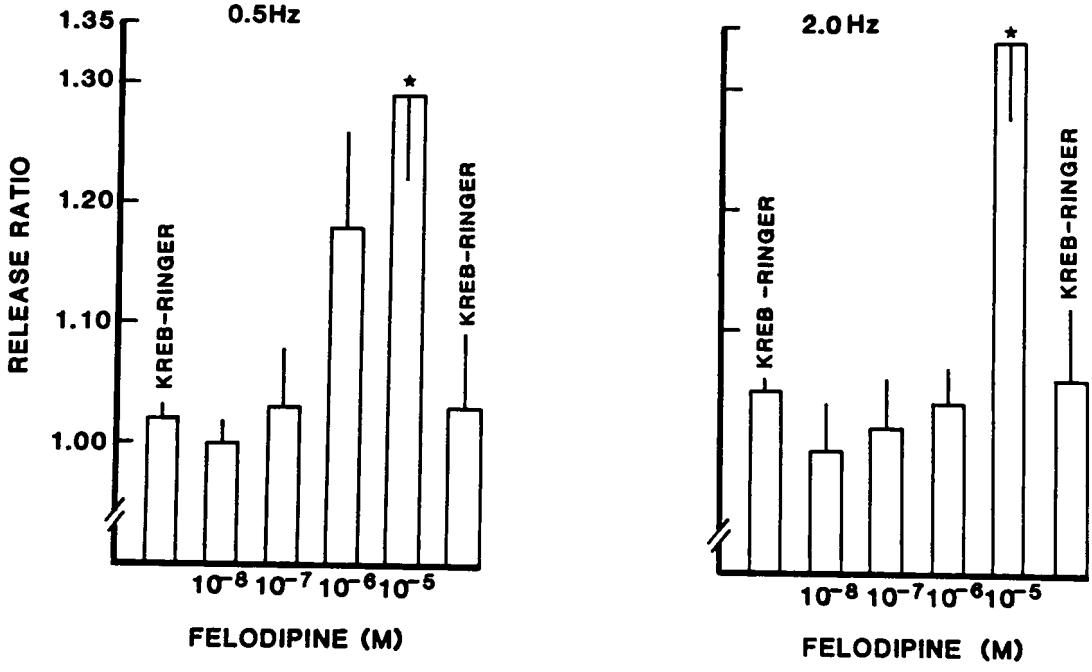

Figure 5: Effect of felodipine on [^{3}H]-NE release elicited during periarterial nerve stimulation at 0.5 Hz and 2.0 Hz. Release ratio is calculated by dividing the amount of [^{3}H]-NE released during each stimulation by the amount of [^{3}H]-NE released during the first control stimulation period. The first control release ratio = C_2 dpm/C_1 dpm. Release ratio in the presence of felodipine would be F dpm/C_1 dpm. Similarly, release ratios were calculated for all subsequent concentrations. Values given are $\bar{X}$ ± S.E.M., N = 6 (0.5 Hz), and 5 (2.0 Hz). *$p < 0.05$.

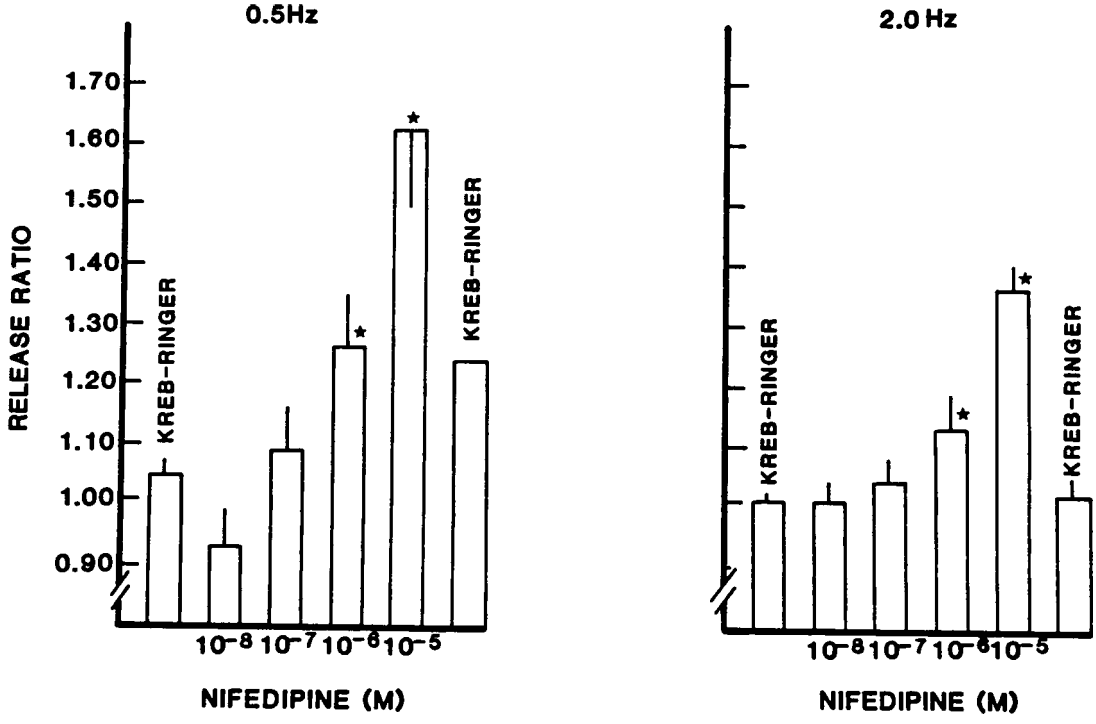

Figure 6: Effect of nifedipine on [^{3}H]-NE release elicited during periarterial nerve stimulation at 0.5 Hz and 2.0 Hz. Release ratio is calculated by dividing the amount of [^{3}H]-NE release during each stimulation by the amount of [^{3}H]-NE released during the first control simulation period. The first control release ratio = C_2 dpm/C_1 dpm. Release ratio in the presence of nifedipine would be N dpm/C_1 dpm. Similarly, release ratios were calculated for all subsequent concentrations. Values given are $\bar{X}$ ± S.E.M., N = 5 per group. *$p < 0.05$.

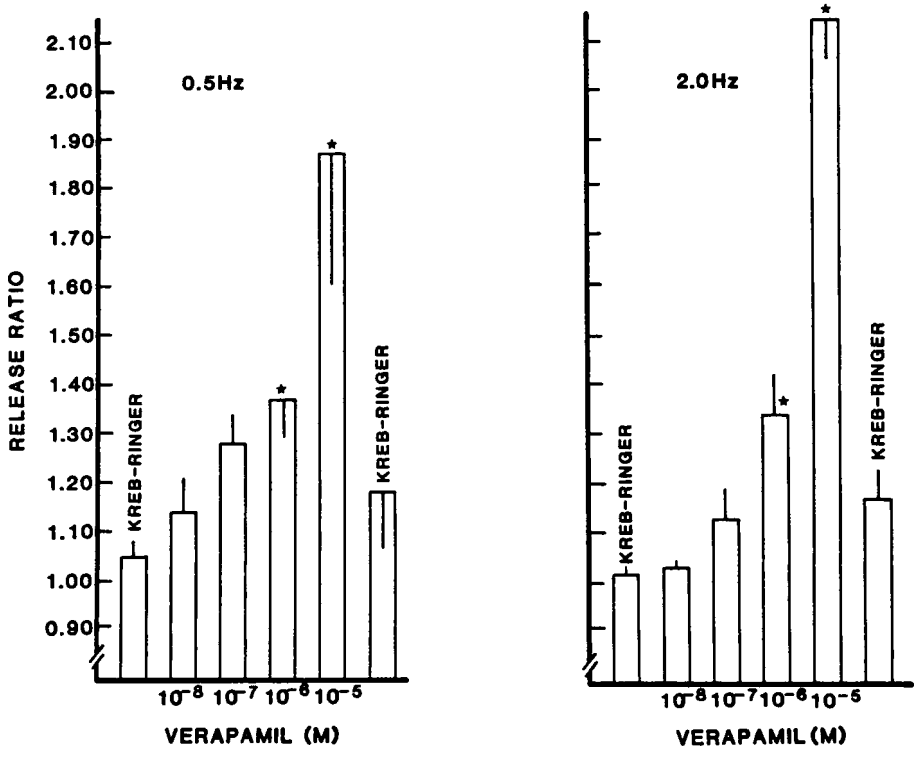

Figure 7: Effect of verapamil on [^{3}H]-NE release elicited during periarterial nerve stimulation at 0.5 Hz and 2.0 Hz. Release ratio is calculated by dividing the amount of [^{3}H]-NE released during each stimulation period by the amount of [^{3}H]-NE released during the first control stimulation period. The first control release ratio = C_2 dpm/C_1 dpm. Release ratio in the presence of verapamil would be V dpm/C_1 dpm. Similarly, release ratios were calculated for all subsequent concentrations. Values given are $\bar{X}$ ± S.E.M., N = 5 (0.5 Hz) and 6 (2.0 Hz). *$p < 0.05$.

IV. 3B. Effect of calcium entry blocker on the prejunctional alpha-adrenoceptor mediated inhibition of norepinephrine release.

These experiments were performed to determine whether calcium entry blockers influence prejunctional alpha-adrenoceptor mediated inhibition of norepinephrine release. When increasing concentrations of norepinephrine (10^{-10} to 10^{-7} M) were perfused through the kidney in the presence of cocaine (10^{-5} M), it produced significant decreases in the [^{3}H]-NE release elicited during nerve stimulation at 0.5 Hz (Figure 8). This inhibitory action of norepinephrine was reversible since perfusion with norepinephrine free Krebs-Ringer solution restored the release ratio back to control level (not shown in figure). In a separate group of experiments, when norepinephrine was perfused in the presence of cocaine plus phentolamine (2.1×10^{-7} M), the inhibitory action of norepinephrine on stimulus-induced release of [^{3}H]-NE was significantly antagonized (Figure 8). These results show that norepinephrine exerted its action via activation of prejunctional alpha-adenoceptors which are reported to be located on postganglionic sympathetic nerve terminals (Lokhandwala, 1979; Lokhandwala and Eikenburg, 1983).

In order to determine if the calcium entry blockers influence prejunctional alpha-adrenoceptor function in the kidney, the effect of these compounds on norepinephrine induced inhibition of the stimulus-induced release of [^{3}H]-NE was evaluated. In this series of experiments, control release ratio was obtained in the presence of cocaine (10^{-5} M) and either felodipine (10^{-5} M), nifedipine or verapamil (10^{-6} and 10^{-5} M) at 0.5 Hz. The concentrations of calcium entry blockers employed in this experiment were those that caused significant increase in stimulus-induced release of [^{3}H]-NE in previous studies. Cocaine and each of the calcium entry blocker were present in the perfusate throughout the duration of the experiment. After two control stimulations increasing concentrations of norepinephrine (10^{-10} - 10^{-7} M) were perfused through the kidney. As illustrated in figures 9, 10, and 11, felodipine (10^{-5}M), nifedipine (10^{-6} and 10^{-5} M) and verapamil (10^{-6} M) failed to antagonize the inhibitory effect of norepinephrine on stimulus-induced release of [^{3}H]-NE. Actually, felodipine significantly potentiated the inhibitory effect of the two lower concentrations of norepinephrine (10^{-10} and 10^{-9} M). However, verapamil (10^{-5} M) significantly antagonized the inhibitory effect of the higher concentrations of

norepinephrine (10^{-8} - 10^{-7} M) on [^{3}H]-NE release elicited during periarterial nerve stimulation (Figure 11).

In control experiments (not shown) it was established that felodipine (10^{-5} M), nifedipine and verapamil (10^{-6} and 10^{-5} M) in the presence of cocaine increased stimulus-induced [^{3}H]-NE release which was of the same order of magnitude as that observed in the absence of cocaine.

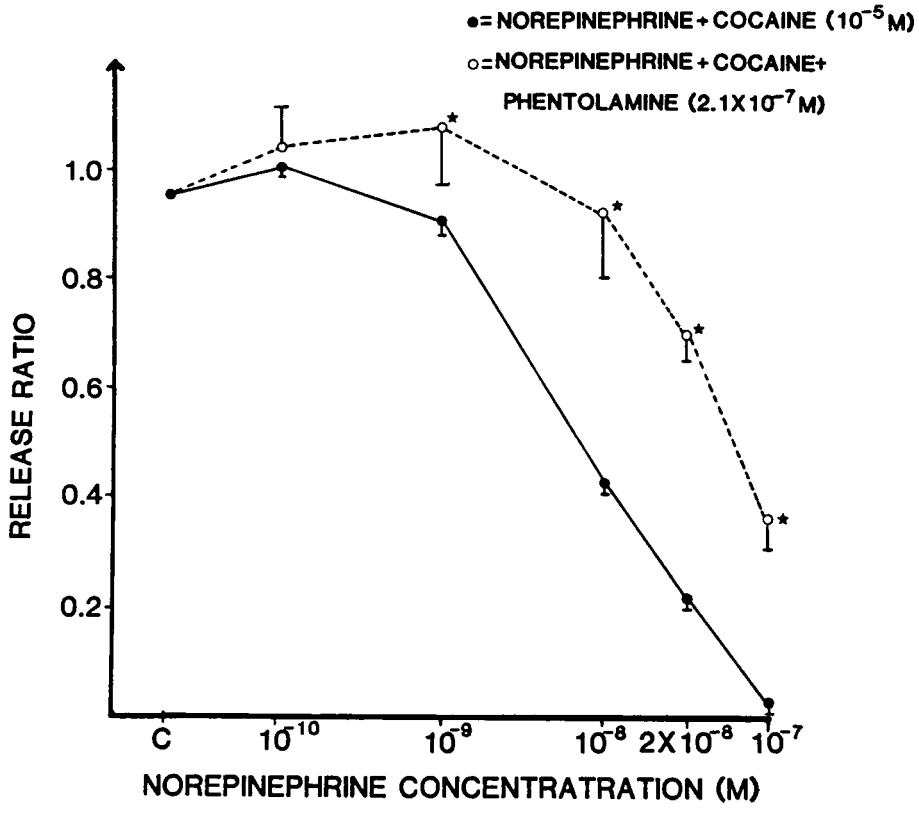

Figure 8: Effect of exogenous norepinephrine on [^{3}H]-NE release elicited during periarterial nerve stimulation (0.5 Hz) in the presence of cocaine (10^{-5} M) (●), and cocaine (10^{-5} M) plus phentolemine (2.1×10^{-7} M) (0). Release ratio is calculated by dividing the amount of [^{3}H]-NE release during each stimulation by the amount of [^{3}H]-NE released during the first control release ratio = C_2 dpm/C_1 dpm. Release ratio in the presence of norepinephrine would be NE dpm/C_1 dpm. Values given are $\bar{X}$ ± S.E.M., N = 5 per group. *$p < 0.05$.

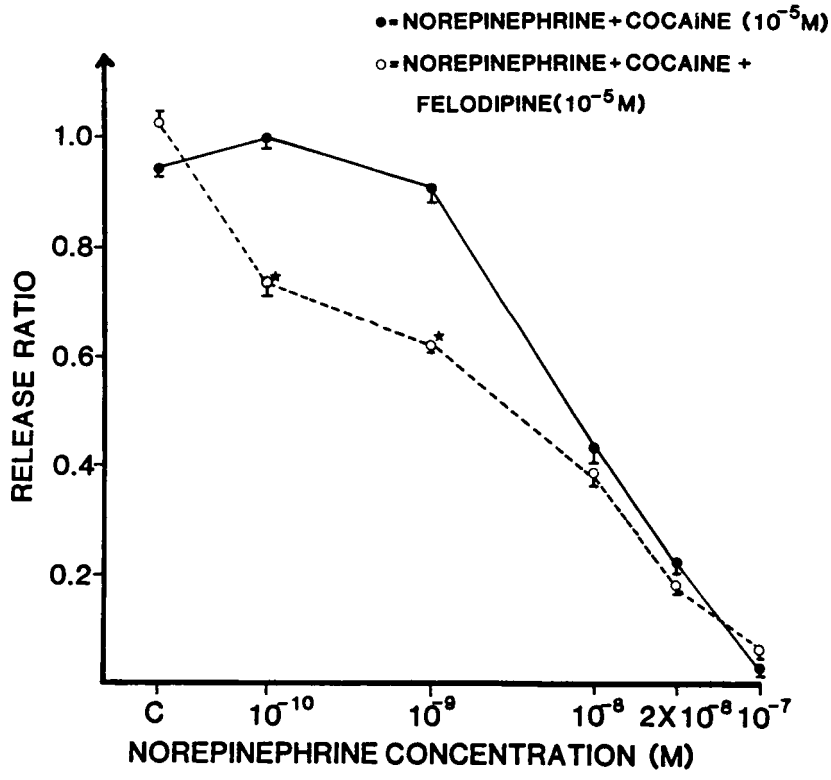

Figure 9: Effect of exogenous norepinephrine on [^{3}H]-NE release elicited during periarterial nerve stimulation (0.5 Hz) in the presence of cocaine (10^{-5} M) (●), and cocaine (10^{-5} M) plus felodipine (10^{-5} M (0). Release ratio is calculated by dividing the amount of [^{3}H]-NE release during each stimulation by the amount of [^{3}H]-NE released during the first control stimulation period. The first control release ratio = C_2 dpm/C_1 dpm. Release ratio in the presence of norepinephrine would be NE dpm/C_1 dpm. Values given are $\bar{X}$ ± S.E.M., N = 5 per group. *$p < 0.05$.

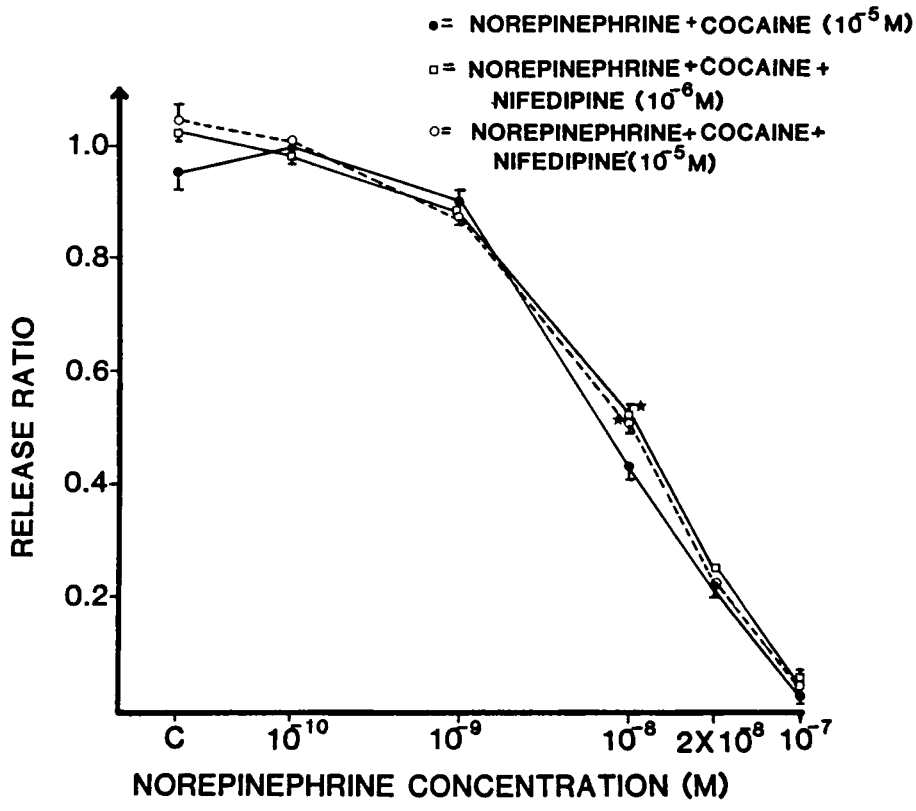

Figure 10: Effect of exogenous norepinephrine on [^{3}H]-NE release elicited during periarterial nerve stimulation (0.5 Hz) in the presence of cocaine (10^{-5} M) (●), cocaine (10^{-5} M) plus nifedipine (10^{-6} M) (□) and cocaine (10^{-5} M) plus nifedipine (10^{-5} M) (○). Release ratio is calculated by dividing the amount of [^{3}H]-NE released during each stimulation by the amount of [^{3}H]-NE released during the first control stimulation period. The first control release ratio = C_2 dpm/C_1 dpm. Release ratio in the presence of norepinephrine would be NE dpm/C_1 dpm. Values given are $\bar{X}$ ± S.E.M., N = 5 per group. *$p < 0.05$.

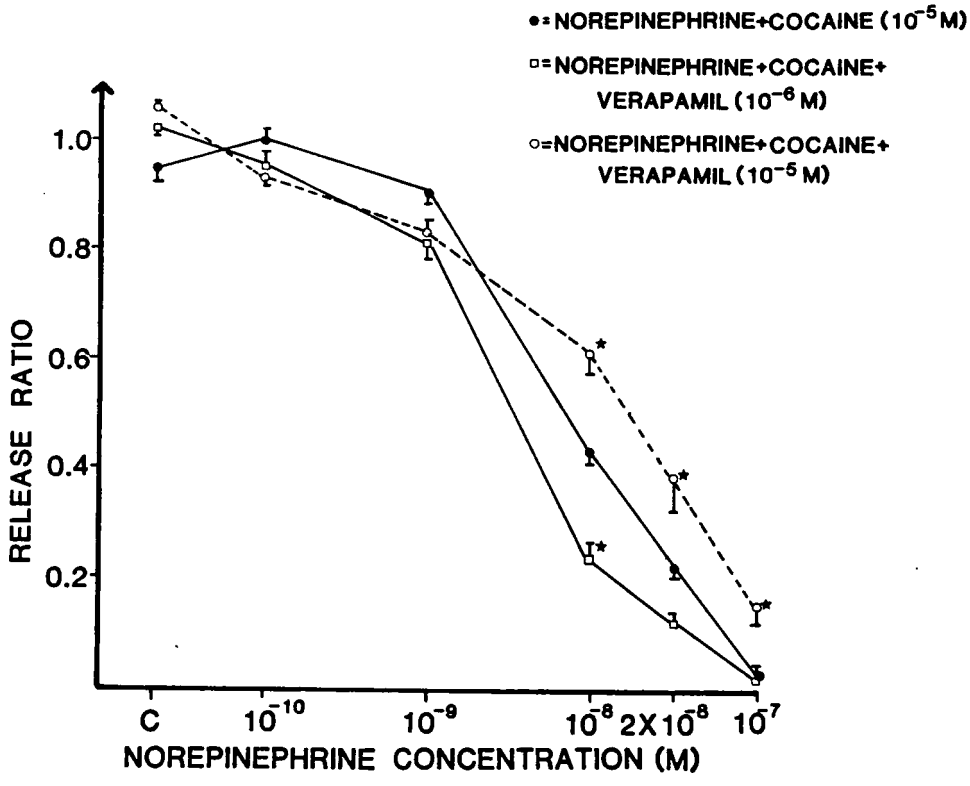

Figure 11: Effect of exogenous norepinephrine on [^{3}H]-NE release elicited during periarterial nerve stimulation (0.5 Hz) in the presence of cocaine (10^{-5} M) (●), cocaine (10^{-5} M) plus verapamil (10^{-6} M) (□), and cocaine (10^{-5} M) plus verapamil (10^{-5} M) (○). Release ratio is calculated by dividing the amount of [^{3}H]-NE released during each stimulation by the amount of [^{3}H]-NE released during the first control stimulation period. The first control release ratio = C_2 dpm/C_1 dpm. Release ratio in the presence of norepinephrine would be NE dpm/C_1 dpm. Values given are $\bar{X}$ ± S.E.M., N = 5 per group. *$p < 0.05$.

IV. 3C. Effect of calcium entry blockers on postjunctional alpha-adrenoceptor mediated renal vasoconstriction.

In order to determine whether these drugs influence postjunctional alpha-adrenoceptor functions, the effect of these drugs on the vasoconstrictor responses to norepinephrine was evaluated. In control experiments, it was established that norepinephrine produced renal vasoconstriction and this effect of norepinephrine was constant over a 70 min period (Figure 12).

As illustrated in Figures 13 and 14 after two control responses with norepinephrine (16 and 32 ng) perfusion of increasing concentrations of either felodipine or nifedipine (10^{-8} to 10^{-5} M) significantly antagonized the renal vasoconstrictor effect of norepinephrine in a dose dependent fashion. Perfusion with the lower concentration of verapamil (10^{-8} M) did not alter the vasoconstrictor effect of norepinephrine (16 and 32 ng) (Figure 15). However, perfusion of the higher concentrations of verapamil (10^{-7} to 10^{-5} M) significantly antagonized the renal vasoconstrictor action of norepinephrine in a dose dependent fashion (Figure 15). The renal vasoconstrictor response to norepinephrine (16 ng) was completely

abolished by verapamil (10^{-5} M) (Figure 15). This inhibitory effect of calcium entry blockers was partially reversible since perfusion of drug free Krebs-Ringer solution restored the renal vasoconstrictor effect of norepinephrine (Figures 13, 14, and 15).

In figures 16 and 17, the inhibitory action of calcium entry blockers on the vasoconstriction seen with 16 and 32 ng of norepinephrine is represented as percent of control responses. These figures allow us to make a comparison of the inhibitory actions of the three compounds on renal vasoconstriction elicited by norepinephrine. It is evident that the responses to both the concentrations of norepinephrine in the absence of any drugs were stable for up to the 60 minutes observation period. All of the three calcium entry blockers caused concentration-dependent inhibition of vasoconstrictor responses to norepinephrine and these actions appeared to be of similar magnitude for felodipine, nifedipine, and verapamil (Figures 16 and 17).

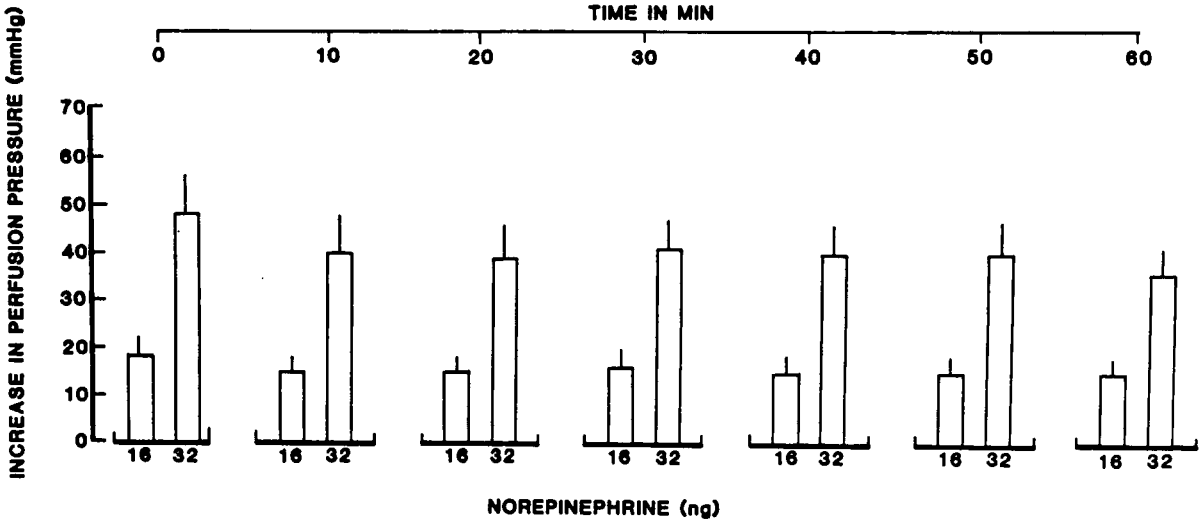

Figure 12: Vasoconstrictor responses represented as changes in perfusion pressure to exogenous norepinephrine (16 and 32 ng) in isolated perfused rat kidneys. Each set of two concentrations of norepinephrine was injected at 10 minutes intervals for a period of 60 minutes. Values given are $\bar{X}$ S.E.M., N = 6. *$p < 0.05$.

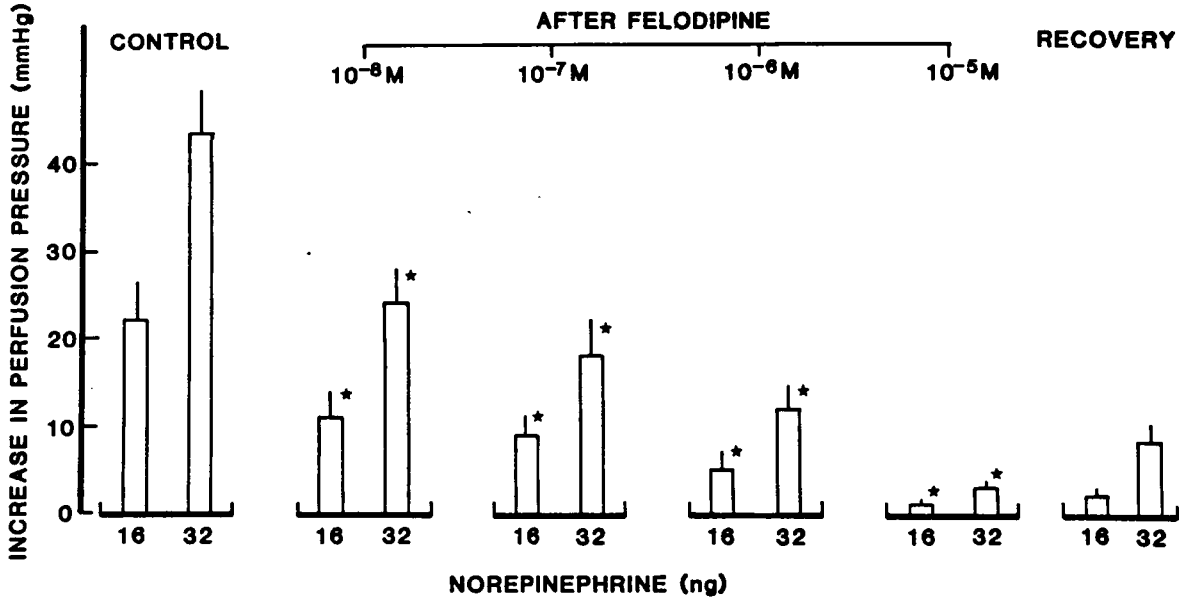

Figure 13: Influence of felodipine (10^{-8} - 10^{-5} M) on vasoconstrictor responses to exogenous norepinephrine (16 and 32 ng), in the isolated perfused rat kidney. Values given are $\bar{X}$ ± S.E.M., N = 6. *$p < 0.05$.

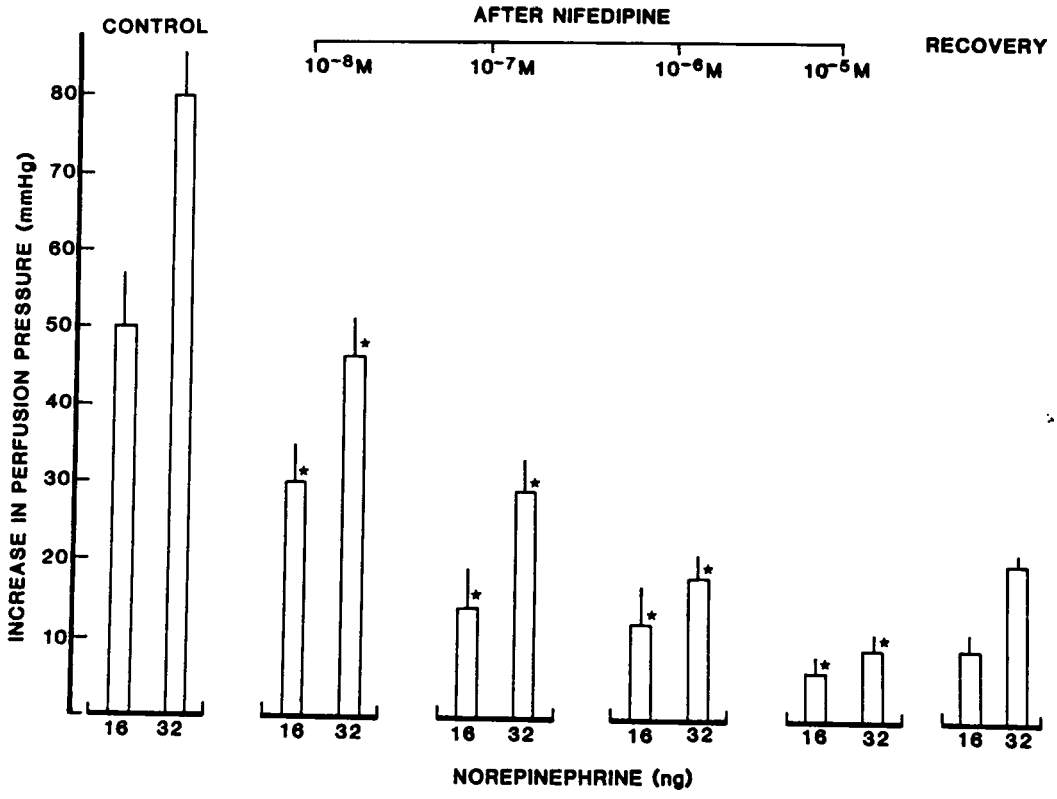

Figure 14: Influence of nifedipine (10^{-8} - 10^{-5} M) on vasoconstrictor responses to exogenous norepinephrine (16 and 32 ng), in the isolated perfused rat kidney. Values given are $\bar{X}$ ± S.E.M., N = 6. *$p < 0.05$.

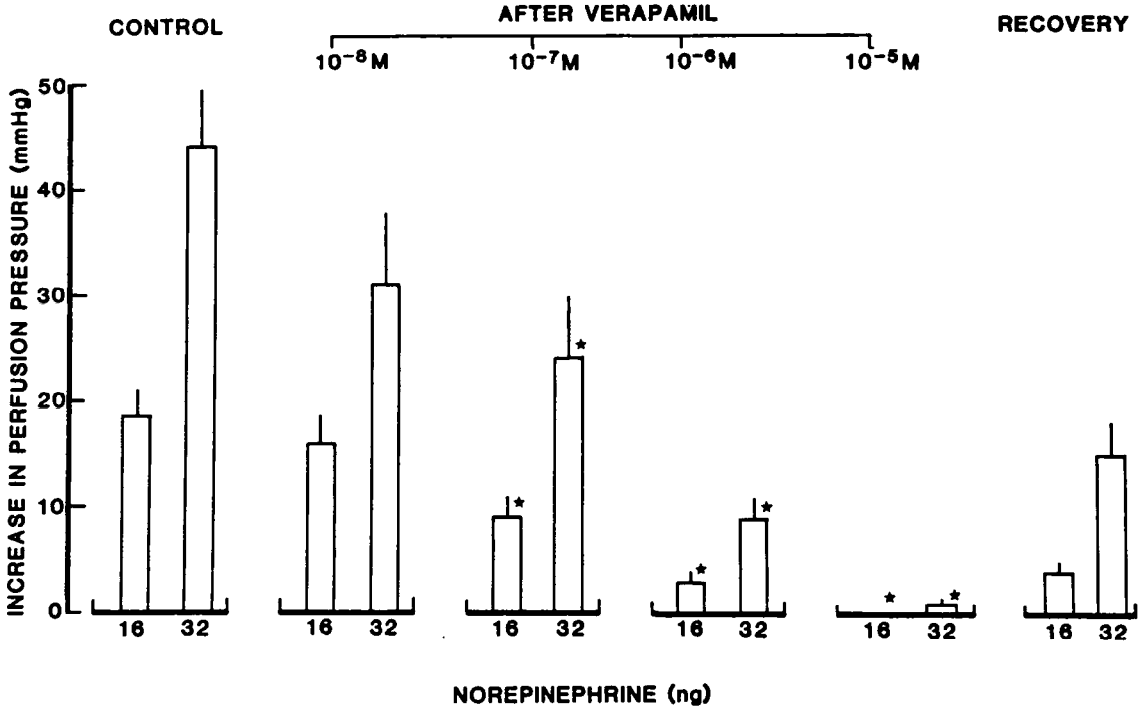

Figure 15: Influence of verapamil (10^{-8} - 10^{-5} M) on vasoconstrictor responses to exogenous norepinephrine (16 and 32 ng) in the isolated perfused rat kidney. Values given are $\bar{X}$ ± S.E.M., N = 6. *$p < 0.05$.

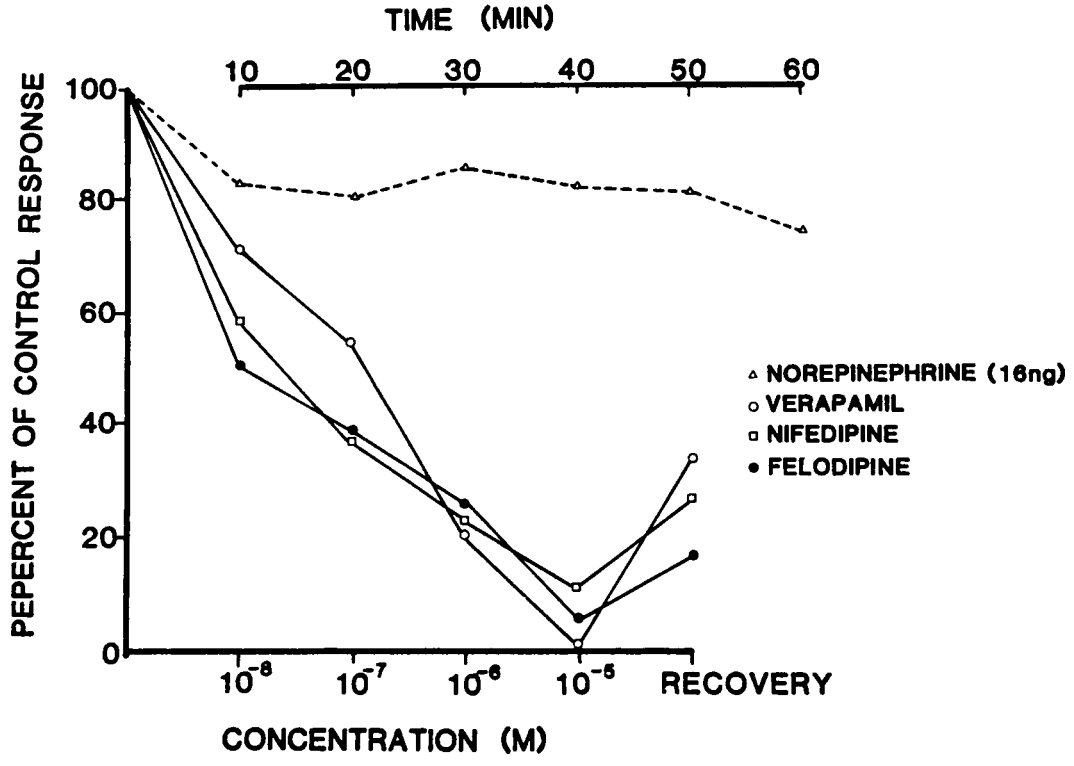

Figure 16: Percent of control response of the vasoconstrictor responses to norepinephrine (16 ng) alone (Δ) and in the presence of verapamil (10^{-8} to 10^{-5} M) (0), nifedipine (10^{-8} to 10^{-5} M) (□), and felodipine (10^{-8} to 10^{-5} M) (●). N = 6 per group.

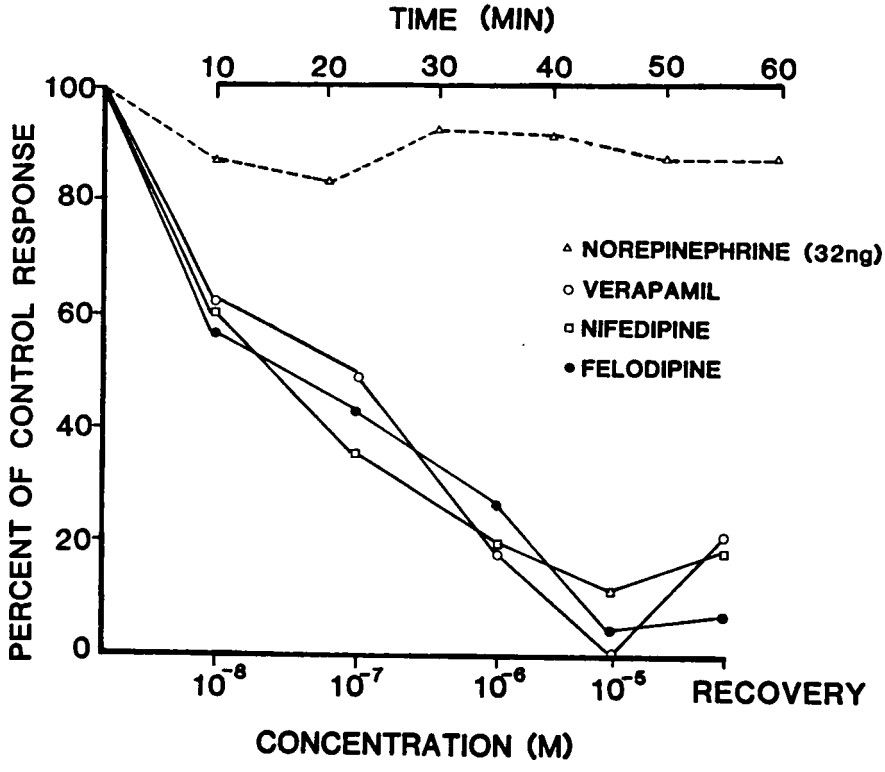

Figure 17: Percent of control response of the vasoconstrictor response to norepinephrine (32 ng) alone (Δ), and in the presence of verapamil (10^{-8} to 10^{-5} M)(0), nifedipine (10^{-8} to 10^{-5} M) (□), and felodipine (10^{-8} to 10^{-5} M) (●). N = 6 per group.

V. Discussion

V. I. Pharmacological analysis of the cardiovascular actions of felodipine, nifedipine and verapamil

This study examined the hypotensive actions of felodipine, nifedipine and verapamil in intact rats as well as in rats following either surgical and/or pharmacological alterations of the influences of the autonomic nervous system. These studies demonstrated that the autonomic nervous system, via the baroreceptor reflex mechanisms and other influences, plays an important role in the cardiovascular responses to these drugs.

Felodipine appears to be the most effective hypotensive agent of those tested, producing significant decrases in blood pressure over a dose range of 0.2 to 0.8 μmoles/kg. Furthermore, the hypotensive actions of this agent were of greater magnitude following removal of either the sympathetic or parasympathetic influences to the heart. This observation susggests that the autonomic influences on the heart serve to limit the hypotension produced by felodipine in intact animals.

Analysis of the data indicates that in intact animals, the fall in blood pressure is accompanied by reflex tachycardia while in vagotomized and atropine treated animals this is not observed. Finally, felodipine produced a significant decrease in blood pressure in animals following complete autonomic blockade to the cardiovascular system. This observation indicates that the hypotension produced by felodipine is caused, at least in part, by direct actions of the drug on the heart and/or vasculature, independent of autonomic effects.

Nifedipine, a dihydropyridine derivative structurally related to felodipine, had quantitatively different effects on blood pressure although qualitatively the actions of these drugs appeared similar. In intact rats, nifedipine produced only transient decreases in blood pressure, not lasting longer than 10 minutes. However, when either the parasympathetic or sympathetic influences on the heart were removed, nifedipine produced significant hypotension. This again suggests, as was observed with felodipine, that reflex cardiac compensation by the autonomic nervous system limits the hypotension produced by nifedipine in intact animals. Surprisingly, when all autonomic influences on the heart were removed, nifedipine did not significantly reduce blood pressure.

However, this observation must be viewed with caution since the percent change in blood pressure observed in these animals was similar to that seen after either parasympathetic or sympathetic blockade. Perhaps the greater variability of results in the animals with all autonomic influence on the heart removed is responsible for the insignificant fall in blood pressure in this group and a significant change would have been noted with a larger sample size. Finally, nifedipine did not produce a fall in blood pressure when all influences of the autonomic nervous system on the cardiovascular system were removed. This suggests that, at the doses employed, nifedipine requires an intact sympathetic tone to the vasculature for its hypotensive action and does not have the direct vascular relaxant action as seen with felodipine. Indeed, evidence available in the literature suggests that felodipine is probably a more potent vasodilator agent (Ljung, 1980; Sojöquist et al., 1983; Verdouw and Wolfenbuttel, 1983; Wathen et al., 1983), and this greater potency of felodipine may be due to the additional direct action on the vasculature which is not seen with either nifedipine or verapamil.

Verapamil, the third calcium channel blocking agent tested, appeared to be more like nifedipine than

felodipine. At the highest doses tested in intact rats the fall in blood pressure produced by verapamil did not last longer than 10 minutes. When parasympathetic or sympathetic influences on the heart were removed, verapamil did not produce a significant decrease in blood pressure, which is in contrast to the dihydropyridine derivatives nifedipine and felodipine. However, when all autonomic influences on the heart were removed verapamil at the highest dose tested, 0.9 μmoles/kg, produced a significant and sustained decrease in blood pressure. This again indicates the ability of the autonomic nervous system to reduce the hypotensive actions of these agents in intact animals. Lastly, verapamil, as was the case with nifedipine, failed to reduce blood pressure in rats following total autonomic blockade, indicating minimal direct actions on the cardiovascular system at the doses tested. This observation is consistent with other reports regarding the cardiovascular actions of verapamil which indicate that verapamil exerts greater effects on the heart than on peripheral vascular smooth muscle (Henry, 1980; Kawai et al., 1981; Taira, 1979).

In summary, these results indicate that all three calcium entry blockers have significant actions on the cardiovascular system which are somewhat modified in the

intact animal by cardiac compensation through the autonomic nervous system. However, the actions of these agents were not observed to be identical since only felodipine decreased blood pressure in the presence of total autonomic blockade. There are several possible explanations for this finding. First, one might suggest that only felodipine has significant direct effects on the cardiovascular system whereas the other agents lower blood pressure by interfering with the vasoconstrictor actions of norepinephrine. This is not likely since direct effects of these other agents on blood vessels and the heart have been reported by other investigators (Fleckenstein, 1977; Henry, 1980; Stone et al., 1980; Zoster and Church, 1983). Thus, it appears more likely that the inability of nifedipine and verapamil to reduce blood pressure in ganglionic blocked rats is the result of a difference in potency with regard to their direct actions on the heart and vasculature. The importance of the effects of these agents on the vasoconstrictor effects of norepinephrine should not be overlooked however. All three agents were able to reduce blood pressure when autonomic influences on the heart were removed but sympathetic tone to the vasculature remained functional. Under these conditions 0.8 μmoles/kg felodipine

reduced blood pressure 29% whereas 0.9 μmoles/kg nifedipine and verapamil lowered blood pressure 13% and 18%, respectively. After ganglionic blockade, felodipine reduced blood pressure by 14% and nifedipine and verapamil had no effect. Thus a considerable portion of the hypotensive effects observed with all three agents is dependent on neural influences to the vasculature. It should be pointed out that with regard to this action, the differences between these agents are minor. If one considers the decrease in blood pressure produced by felodipine without sympathetic tone to the vasculature as a contributing factor to the hypotension observed in animals with vascular sympathetic tone, then the response attributable to the effects of felodipine mediated via interference with the sympathetic nervous system is 15%, very similar to that of nifedipine and verapamil. Hence, in this respect these agents appear very similar. Experiments described in subsequent sections were specifically designed to examine the effects of these agents on sympathetic neurotransmission.

V. 2. Effect of calcium entry blockers on sympathetic neuroeffector function.

One of the objectives of the present study was to

determine the influence of the calcium entry blockers (felodipine, nifedipine, verapamil) on the responsiveness of pre- and postjunctional alpha-adrenoceptors to total spinal stimulation and intravenously administered norepinephrine. The pithed rat model was utilized for this study, since vasoconstriction of resistance and capacitance vessels can be conveniently elicited in this preparation without interference from central or reflex mechanisms. In these experiments, pressor responses to spinal nerve stimulation and intravenously administered norepinephrine were examined in the presence of propranolol, a beta adrenergic blocking agent. Propranolol was utilized to insure that cardiac beta-adrenoceptors did not participate in these responses.

The results of these experiments indicate that all three calcium entry blockers significantly reduced pressor responses to both sympathetic nerve stimulation and norepinephrine. However, the effects of these agents were not identical. In general the agents appeared to have similar effects on responses to nerve stimulation and norepinephrine, the exception being felodipine, which appeared to affect responses to norepinephrine to a greater extent. It has been reported recently that while postsynaptic alpha-adrenoceptors are involved in the

vasoconstrictor responses to neurally released norepinephrine, the effects of exogenous norepinephrine are due to activation of both alpha 1- and alpha 2-adrenoceptors (Langer, 1980, 1982). Also, several investigators have reported that calcium entry blockers are more effective in reducing responses to alpha 2-adrenoceptor activation than to alpha 1-adrenoceptor activation in several animal species (Bou et al., 1980; Cavero et al., 1983; Gerold and Hausler, 1983; Seed et al., 1983; Timmermans, 1983; van Meel et al., 1981a, 1982, 1983; van Zwieten et al., 1982). If one accepts the difference in the type of alpha-adrenoceptors activated by neurally released vs. exogenous norepinephrine, then one would have expected the results obtained with felodipine. However, our results indicate that the other two calcium entry blockers studied, nifedipine and verapamil, did not exhibit a preference for inhibiting responses elicited by nerve stimulation vs. exogenous norepinephrine. Thus it would appear that nifedipine and verapamil at the doses employed in this study affect responsiveness of both alpha 1- and alpha 2-adrenoceptors to a similar extent, since both agents had similar effects on responses to nerve stimulation and exogenous norepinephrine, whereas felodipine preferentially affects

responses to alpha 2-adrenoceptor activation. However, further experiments with selective agonists and antagonists would be necessary in order to verify this contention. It should be pointed out also that evidence in the literature indicates that some calcium entry blockers affect responses to both alpha 1- and alpha 2-adrenoceptor activation (de Mey and Vanhoutte, 1980, 1981; Müller-Schweinitzer, 1983; Timmermans et al., 1983; Vanhoutte and Rimele, 1982).

In addition to the differential effect of felodipine on responses to nerve stimulation and norepinephrine, the magnitude of the effects of the three agents on pressor responses to nerve stimulation and norepinephrine were different. Although the effects of felodipine and nifedipine on responses to nerve stimulation and norepinephrine appear to be of similar magnitude, this is misleading since the dose of nifedipine used to obtain this response is more than twice the dose of felodipine required to produce a similar effect. Thus, these data suggest that felodipine is more potent than nifedipine in reducing pressor responses to both nerve stimulation and exogenous norepinephrine. If one compares the effects of these agents to that of verapamil, an even greater difference in potency is suggested. While the

dose of verapamil used was almost twice the dose of felodipine, verapamil had minimal effects on responses to nerve stimulation and norepinephrine. However, one must be cautious in interpreting these data. While felodipine and nifedipine both significantly lowered resting blood pressure at the doses used, verapamil had no significant effect on resting blood pressure. Thus the basal vascular tone at which responses were elicited in the verapamil treated animals was significantly higher than that in the animals following treatment with the other two agents. This higher basal tone in the verapamil treated animals may be the reason why responses to nerve stimulation and norepinephrine were not reduced to a greater extent in these animals. Therefore, the differences between felodipine and nifedipine and verapamil in this study may indicate a significant difference in the potency of these drugs in their ability to reduce vascular responsiveness to norepinephrine or they may be secondary to the reduced basal tone in the felodipine and nifedipine treated animals. To resolve this question, one might maximally dilate the vasculature before the administration of these agents to insure that basal tone following the administration of the calcium entry blockers would be similar for each drug. This would permit a more accurate

estimation of the effects of these agents on the ability of norepinephrine to elicit smooth muscle shortening, independent of differences in basal vascular tone. However, the suggestion that verapamil is significantly less potent than felodipine and nifedipine in both its ability to relax vascular smooth muscle and to reduce pressor responses to norepinephrine is in accordance with findings in peripheral blood vessels by other investigators (Guazzi et al., 1980; Henry, 1980; Ljung, 1980; Stone et al., 1980; Zoster and Church, 1983). The in vitro experiments which are discussed in the succeeding sections also address the effects of these agents on vascular responsiveness to norepinephrine.

V. 3A. Effects of calcium entry blockers on [^{3}H]-NE release elicited during periarterial nerve stimulation.

The isolated perfused rat kidney is an established model that has been used for numerous pharmacological and physiological studies. In this in vitro preparation we demonstrated that the lower concentrations of felodipine (10^{-8} - 10^{-6} M), nifedipine (10^{-8}, 10^{-7} M) and verapamil (10^{-8}, 10^{-7} M) did not alter stimulus-induced release of norepinephrine. However, higher concentrations

of felodipine (10^{-5} M), nifedipine (10^{-6}, 10^{-5} M) and verapamil (10^{-6}, 10^{-5} M) significantly augmented stimulus-induced norepinephrine release at both 0.5 and 2.0 Hz. These findings are in accordance with the observation of Glazin and Langer (1983) who reported that verapamil increased stimulus induced norepinephrine release in a concentration dependent manner in rabbit hypothalamic slices while diltiazem in concentrations up to 100 μM had no significant effect. However, Göthert et al. (1979) reported that verapamil inhibited stimulus-induced release of norepinephrine from isolated rabbit hearts with an IC_{50} of 73 μM. Since we did not study the effects of verapamil at concentrations higher than 10 μM in the present study it is not known whether we would have observed inhibition of release in our studies if higher concentrations had been tested. In earlier studies, Häusler (1972) had reported that verapamil, in concentrations up to 2 μM, had no effect on the stimulus-induced release of norepinephrine from isolated cat hearts. In contrast, Starke and Schumann (1973) observed that nifedipine, at a concentration of 1 μM, decreased stimulus-induced norepinephrine release in the isolated rabbit heart. Unfortunately, considering the variety of animals and tissues employed in these studies, it is

difficult to relate these findings to the observations of the present study.

An important point to consider with regards to the results of our study is the role of extracellular calcium in exocytotic release of neurotransmitter from sympathetic nerve terminals. Since most evidence indicates that removal of calcium from the extracellular medium inhibits the release of neurotransmitter from sympathetic nerve terminals, one would have predicted that calcium entry blockers, which have been reported to inhibit calcium influx during excitation-contraction coupling, would inhibit norepinephrine release. Obviously, the facilitatory actions of these agents on transmitter release in the present studies cannot be explained simply by inhibition of calcium influx during excitation. Alternatively their action may be related to presynaptic mechanisms which are believed to regulate release of norepinephrine from sympathetic nerves (Langer, 1977; Lokhandwala, 1979; Westfall, 1980). Calcium entry blockers have been reported to interfere with alpha-adrenoceptor function and it has been suggested that some of these agents bind to these receptors, acting as antagonists (Fairhurst et al., 1980; van Meel et al., 1981a). Therefore, one could explain the present

findings by postulating that the calcium entry blockers were antagonizing the effects of norepinephrine at prejunctional alpha-adrenoceptors. Such an action would cause an increase in the release of neurotransmitter much in the same way as the alpha-adrenoceptor antagonists phentolamine and rauwolscine have been shown to do. This possibility was therefore tested.

V. 3B. Effect of calcium entry blockers on prejunctional alpha-adrenoceptor mediated inhibition of neurotransmitter release.

While all three calcium entry blockers increased the stimulus-induced release of norepinephrine in the present study, their effects on the alpha-adrenoceptor mediated negative feedback mechanism in the rat kidney were quite dissimilar. Verapamil (10^{-5} M) significantly reduced the inhibitory action of norepinephrine on neurotransmitter release, though it appeared to be much less potent than phentolamine in this regard. A similar action by verapamil on the alpha-adrenoceptor negative feedback mechanism has been reported by Glazin and Langer (1983) in rabbit hypothalamic slices. It should be noted that existing evidence suggests that stimulation of presynaptic alpha-adrenoceptors reduces the release of norepinephrine

during nerve stimulation by inhibiting calcium influx through voltage-sensitive calcium channels in the nerve endings (Drew, 1978; Horn and McAfee, 1980; Langer et al., 1975; Starke and Montel, 1974; Saelens and Williams, 1983). Therefore, the facilitation of neurotransmitter release by verapamil in the present study cannot be explained by inhibition of calcium influx during nerve depolarization but is more likely due to an antagonism of the action of norepinephrine at prejunctional alpha-adrenoceptors.

On the other hand, felodipine (10^{-5} M) significantly potentiated the inhibitory effect of the lower concentrations of norepinephrine on neurotransmitter release. Since activation of presynaptic alpha-adrenoceptors has been shown to inhibit the influx of calcium into adrenergic nerve endings, one might speculate that felodipine enhances this effect of prejunctional alpha-adrenoceptor activation. However, this hypothesis fails to explain why it was observed that felodipine has a facilitatory action on stimulus-induced neurotransmitter release at these same concentrations when studied alone. Nifedipine had no effect on the alpha adrenoceptor mediated negative feedback mechanism in the present study.

Based on the data obtained in this study, it is suggested that felodipine and nifedipine increase neurotransmitter release by a yet unknown mechanism. It is clear however that interference with the function of prejunctional alpha-adrenoceptors is not involved. Verapamil, on the other hand, may facilitate neurotransmitter release, at least in part, by antagonizing the influence of prejunctional alpha-adrenoceptors on norepinephrine release. However, in light of the effects of these agents on responses to nerve stimulation in the pithed rat, it appears that the effects of these agents on neurotransmitter release have little significance to the *in vivo* responses to these agents. This is well illustrated by the effects of these agents on vasoconstrictor responses to norepinephrine at concentrations which enhance neurotransmitter release, as discussed in the next section.

V. 3C. Effect of calcium entry blockers on postjunctional alpha-adrenoceptor mediated renal vasoconstriction.

All three calcium entry blockers diminished vasoconstrictor responses produced by exogenous norepinephrine in the rat kidney at concentrations which did not alter

stimulus-induced neurotransmitter release and completely abolished the effect of norepinephrine at higher concentrations. This is in agreement with the observations of many other investigators that vasoconstrictor responses induced by activation of alpha-adrenoceptors are diminished in the presence of these agents (de Mey and Vanhoute, 1980; Müller-Schweinitzer, 1983; van Meel et al., 1981). More importantly, verapamil appears to be less potent than felodipine and nifedipine in diminishing norepinephrine induced vasoconstriction in the isolated perfused rat kidney. Therefore, the data indicate that postjunctional alpha-adrenoceptors are much more sensitive to the effects of these agents than are prejunctional alpha adrenoceptors. This indicates that the effects of these agents on neurotransmitter release are of little pharmacological or therapeutic significance.

In light of the effects of these agents on the vasoconstrictor responses to norepinephrine, it is interesting to speculate on the type of alpha-adrenoceptor being affected. Unfortunately, a non-selective agonist was used in this study so that the receptor subtype mediating vasoconstrictor responses to norepinephrine is not clear. However, a recent study suggests that adrenergic vasoconstriction in the rat kidney occurs

primarily through activation of alpha 1-adrenoceptors and that while alpha 2-adrenoceptors are present in the rat kidney, they are associated with the renal tubules rather than the vascular smooth muscle (Schmitz et al., 1981). Thus, inhibitory action of these compounds on renal vasoconstriction elicited by norepinephrine was probably due to an interference with alpha 1-adrenoceptor mediated vasconstrictor mechanism.

Therefore, in agreement with what was observed in the pithed rat, the studies in the isolated perfused rat kidney indicate that alpha 1- as well as alpha 2-adrenoceptor mediated vasoconstriction can be affected by these agents.

VI. Summary and Conclusions

The following conclusions can be made from the present study.

1. Felodipine appears to be the most potent direct vasodilator of the agents studied *in vivo*. This is indicated by: the fact that at comparable doses in anesthetized, ganglionic blocked rats, only felodipine produced a significant fall in blood pressure.

2. The hypotensive actions of felodipine, nifedipine and verapamil are significantly influenced by the autonomic nervous system in anesthetized animals. The data suggest that partial impairment of the baroreceptor influences on the heart must be present in order for nifedipine or verapamil to lower blood pressure. Furthermore, vascular sympathetic tone appears to be essential for the blood pressure lowering effects of nifedipine and verapamil since neither of these agents reduced blood pressure when sympathetic tone to the vasculature was removed. This is in contrast to felodipine which lowered

blood pressure under these conditions.

3. An important effect of felodipine, nifedipine and verapamil on the pressor responses to norepinephrine and sympathetic nerve stimulation were verified in the pithed rat. At doses which produced comparable effects on blood pressure in the anesthetized rats all three agents appeared to reduce pressor responses to spinal stimulation and norepinephrine. Verapamil was the least effective in this regard.

4. When examined in the isolated perfused rat kidney, felodipine, nifedipine and verapamil reduced pressor responses to norepinephrine. The data suggest that verapamil may be slightly less potent in this regard.

5. Felodipine, nifedipine and verapamil increased the stimulus-induced release of norepinephrine from the isolated perfused rat kidney. Verapamil appeared to be slightly more potent. While the mechanism for this effect was not clearly elucidated, it would appear that this effect was not due to an action of these agents as an alpha-adrenoceptor antagonist. However, verapamil did appear to interfere with the inhibitory action of norepinephrine on stimulus-induced neurotransmitter release. This action did

not appear to be of a competitive nature however and the mechanism for this effect remains unclear.

6. The effects of these agents on neurotransmitter release, while interesting, did not appear to be of pharmacologic or therapeutic significance with regard to the hypotensive actions of these agents. This suggestion is based on the observation that vascular responses to norepinephrine were eliminated or reduced drastically at concentrations which affected the release of neurotransmitter.

7. The effects of these agents on pressor responses to sympathetic nerve stimulation and norepinephrine in the pithed rat and their effects on pressor responses to norepinephrine in the rat kidney agree with the observations in anesthetized rats that verapamil is the least potent of the three agents tested. While verapamil did appear to be slightly more effective in increasing stimulus-induced norepinephrine release, its postjunctional actions are of greater importance. Therefore, it would appear that the greater hypotensive action observed with felodipine in the intact anesthetized rat can probably be attributed to both its greater effects on vascular

responsiveness to norepinephrine as well as it greater ability to directly relax vascular smooth muscle. The results suggest that these agents differ primarily in terms of potency rather than fundamental differences in their mechanisms of action.

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