CHAPTER I

REVIEW OF THE LITERATURE

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the active ingredient largely responsible for the irritating and pungent effects of the fruits from the various species of *Capsicum*, including Mexican chile pepper and Hungarian red pepper (paprika). People in different parts of the world, particularly those in Asia, Central and Latin America, and Africa use capsicum fruit to make the food tasty and to stimulate appetite. Capsicum fruit contains approximately 0.1 - 1.0% capsaicin (Sirsat and Khanolkar, 1960). It has been estimated that people in the Northeastern part of Thailand may consume hot pepper as high as 5 gm or about 50 mg of capsaicin per 60 kg man per meal (Limlomwongse *et al.*, 1979).

Capsaicin is a rather stable compound which persists in dried pepper with apparently unchanged potency. Concentration of capsaicin as low as 10 ppm can be detected by tasting (Nelson and Dawson, 1923). Capsaicin is readily soluble in alcohols, acetone and other ketones, ether, petroleum ethers and chloroform. It is also soluble in alkali and soluble to some extent in concentrated hydrochloric acid as well as carbondisulfide. The compound is insoluble in cold water but freely soluble in hot water (Hodge *et al.*, 1941).

Over the past several decades, numerous investigators have demonstrated the pharmacological and physiological actions of capsaicin.

It has been shown to produce marked effects in wide range of physiological systems, for instance : thermoregulatory system, gastrointestinal tract, sensory nerve, cardiovascular and respiratory systems. Furthermore, capsaicin has also been found to be a cytotoxic substance which cause subcellular structural damages notably those of the mitochondria (Virus and Gebhart, 1979). Most recent evidence indicates that capsaicin is a substance P depleter (Nagy, 1982; Buck and Burks, 1983), and this action may be responsible for certain biological actions of capsaicin.

The present study concerns mainly with the interaction of capsaicin with selected cardiac drugs in the isolated rat atria, therefore, in the next section only the pertinent cardiovascular effects of capsaicin will be reviewed in some details.

The cardiovascular effects of capsaicin have received considerable attention from both pharmacologists and physiologists alike. Many investigators found that this compound produced variable effects on cardiovascular functions. Lille and Ramirez (1935) found that intravenous administration of capsaicin in anesthetized dogs exerted a potent hypotensive effect followed by a prompt return to normal. Two decades later, Porszasz *et al.* (1955) and Toh *et al.* (1955) studied the effects of acute intravenous administration of capsaicin in anesthetized cats and dogs. The Bezold-Jarisch reflex (hypotension, bradycardia and apnea) was observed after capsaicin injection. The apnea was followed by an increased respiratory rate. These reflexes were completely abolished by bilateral vagotomy, suggesting vagal reflexes mediated these responses. They attributed the hypotension, bradycardia and apnea evoked by intravenous capsaicin to the coronary chemoreflex and stimulation or sensitization of carotid sinus baroreceptors since cutting the vagi and

sectioning sinus nerve or cocaine applied directly to carotid sinuses completely obliterated the cardiovascular and respiratory effects. Further experiments in cats showed that atropine (1 mg/kg) or TEA (tetraethylammonium 5 mg/kg) blocked the hypotensive and cardioinhibitory actions of capsaicin but not the apnea. Capsaicin (25 µg) injected into the right auricle of cats produced apnea after a latent period of 1.5 sec ; but injection into the left ventricle produced hyperpnea instead. This suggested that the site of action of capsaicin in causing the apnea was probably in the pulmonary circulation and presumably on the stretch receptors. The apnea produced by capsaicin was greatly reduced or abolished by cooling the vagi to about 9-10℃, which is about the same temperature that blocks conduction in slowly adapting pulmonary stretch fibers. The authors then concluded that capsaicin acted largely by sensitizing the Hering-Breuer inflation reflex. In 1957, Porszasz demonstrated, however, in dogs that capsaicin stimulated baroreceptor rather than chemoreceptor. To prove this point the author blocked the chemoreceptor in the sinus with acetic acid, and nicotine was employed to test chemoreceptor blockade. When pH of the blood was lowered by adding acetic acid to the point that nicotine had completely no effect, capsaicin was injected into the carotid sinus. The hypotensive effect was still observed. Therefore, it is possible that capsaicin stimulated the baroreceptor to produce the triad of responses. Another notable finding was the absence of tachyphylaxis following repeated intravenous administration of capsaicin during the 2-3 hours period. Coleridge *et al.* (1964) recorded action potential from baroreceptor afferents in vagus nerve and found that injection of capsaicin into the carotid sinus activated or sensitized the baroreceptor fibers. This experiment

supports the conclusion of Porszasz (1957) that baroreceptor was involved to produce the triad of responses. In addition, capsaicin injected directly into pulmonary artery was found to produce effects very similar to those elicited by intravenous injection, suggesting the involvement of receptors in the pulmonary vascular bed, as well as arterial baroreceptors. Thus, these results have provided strong evidence for the reflex cardiovascular changes produced by capsaicin. By sensitizing baroreceptors in the carotid sinuses and receptors in the pulmonary circulation, the chemical causes an increase in afferent vagal discharge resulting in reflex hypotension and bradycardia.

Toda *et al.* (1972) have compared the cardiovascular effects of capsaicin in anesthetized dogs and rabbits, and found that intravenous injection of capsaicin (10-300 µg/kg) in dogs caused a transient rise in mean systemic blood pressure followed by a sustained fall whereas in rabbits capsaicin caused only hypotension. Treatment of dogs and rabbits with atropine 2 mg/kg, blocked the hypotensive effect of capsaicin, suggesting the involvement of cholinergic mechanism, most likely the vagi. After treatment with atropine in anesthetized dogs, capsaicin caused only hypertension which was increased more than the non-treated dogs and was not followed by a sustained hypotension, whereas the heart rate was not altered or slightly rose. The hypertension observed in the dogs was not influenced by hexamethonium, tolazoline and phentolamine. In isolated dog and rabbit atria, capsaicin (0.02-2 µg/ml) did not change the atrial rate and contractile force significantly. However, capsaicin caused a sustained increase in the tension of spiral strips from coronary arteries, proximal and distal mesenteric arteries and

proximal and distal renal arteries of the dogs. In contrast, spiral strips from aorta, superior mesenteric and large pulmonary arteries did not respond to capsaicin. The contractile effect of capsaicin on dog mesenteric arteries was not significantly altered by phentolamine but was reduced by decreasing extracellular calcium ion to one-third of normal value. From these findings, the authors related the hypotensive response of dogs and rabbits to capsaicin with a cholinergic mechanism. The hypertensive action of capsaicin in the dogs was ascribed to the constriction of some peripheral vasculatures, and this action was not mediated by an adrenergic mechanism but most likely involved extracellular calcium ion.

In a much more recent experiment, Jancsó and Such (1983) applied capsaicin perineurally to the cervical vagus nerves and observed the cardiovascular and respiratory responses in urethane anesthetized cats. The results were separated into three phases. The first excitatory phase, lasting about 10-15 min, was characterized by marked decreases in the mean arterial blood pressure and heart rate. Electrophysiological studies showed an increase in the spike activity of the cat vagus nerve. This action was thought to result from direct excitatory action of capsaicin on the chemo-sensitive afferent fibers in the vagus nerve. The effect on heart rate, however, depended on initial cardiac frequency; at low initial heart rate there was tachycardia, while at higher initial heart rate there was bradycardia resembling Bainbridge reflex, the direction of which also depends on the initial heart rate. The second phase, subsequent to the first, was the transient block of impulse propagation in vagal afferent fibers. And the last phase, three to five days after

pretreatment of the cervical vagus nerves with capsaicin, phenyldiguanidine and veratrine given intravenously invariably evoked bradycardia, hypotension and apnea, while the reflex responses to intravenous injection of capsaicin and some of its pungent congeners were greatly reduced or even abolished. The authors concluded that vagal afferent fibers mediating cardiovascular and respiratory chemoreflexes are separated into chemo-specifically different populations, one of which can selectively and permanently be blocked by perineural capsaicin application and the others are capsaicin-insensitive and may be stimulated by phenyldiguanidine or veratrine. They also suggested that perineural application of capsaicin may be a useful method for elucidating the role of different populations of peptide-containing vagal afferent fibers in the regulation of cardiovascular and respiratory functions.

Capsaicin has been employed by several investigators to demonstrate the possible role of several organs to reflexly regulate cardiovascular performances in laboratory animals. Capsaicin can mediate either reflex cardiovascular stimulation or depression depending on which organ has received the chemical. In 1955, Toh and coworkers administered capsaicin into the splanchnic circulation by injection into the superior mesenteric artery and into the leg by injection into the femoral artery of anesthetized cats. Capsaicin (10 µg) administered into the splanchnic circulation caused a rise in the systemic blood pressure which was not affected by cutting the vagi in the neck but could be abolished by extirpating the superior and inferior mesenteric plexuses. Capsaicin (10-20 µg) injected into the leg produced hyperpnea, occasional twitching of the leg muscles

and variable responses in blood pressure which were obliterated by sectioning the nerve supply to the muscle. These effects were thought to be due to stimulation of undetermined receptors in the intestines and in the skeletal musculature. Webb-Peploe and collaborators (1972) have corroborated this result. In anesthetized dogs with both vagi and carotid sinus nerves sectioned, capsaicin injected into one iliac artery caused several cardiovascular responses including : increases in aortic blood pressure, constriction of systemic resistance and splanchnic capacitance vessels, and dilatation of cutaneous vessels. These responses were unaffected by ipsilateral lumbar sympathectomy or skinning of the limb, but were abolished by limb deafferentation. This implied that the receptors were not situated in the skin. They also were not situated in the larger hindlimb veins since capsaicin injected into the lateral saphenous vein caused direct constriction of that vessel, and did not initiate the vascular reflexes noted with the injection of capsaicin into the arterial supply to that limb. This study therefore supports the earlier suggestion that the receptors are located in the muscle. Since the vascular reflexes caused by giving capsaicin into the limb were similar to those produced by electrical stimulation of afferent nerve fibers from hindlimb muscles, the authors suggested that the capsaicin-sensitive receptors in muscle may be those normally activated during exercise to reflexly cause redistribution of blood flow. Nearly one decade later, Crayton and coworkers (1981) have confirmed and extended the observations of Webb-Peploe *et al.* (1972). In anesthetized dogs, capsaicin (1-10 µg /kg) injected into a neurally intact donor-perfused hindlimb was found to produce a significant increase in mean aortic pressure, heart

rate, cardiac output and respiratory minute volume. Organ blood flows measurement during the injection of capsaicin showed a decrease in renal blood flow, but flows in liver, spleen, brain, heart and skeletal muscle remained near control values. These cardiovascular and respiratory changes are similar to those that occur during isometric exercise in dogs (Crayron *et al.*, 1979) and cats (Coote *et al.*, 1971; Mc Closkey *et al.*, 1972 and Mitchell *et al.*, 1977). Sectioning the afferent neural connection from the donor-perfused hindlimb abolished the cardiovascular and respiratory changes, indicating the responses are reflex in nature. Earlier studies demonstrated that cutaneous and venous receptors are not involved. Bones and joints are likewise probably not involved because intra-arterial capsaicin injection into isolated gracillis muscle caused reflex changes similar to those seen with capsaicin infusion into the entire leg. Therefore, the authors suggested that the capsaicin-sensitive receptors most probably located in or in proximity to the muscle. Subsequently, Kaufman and coworkers (1982) have attempted to determine which afferent fibers are stimulated by capsaicin injected into the arterial supply of the hindlimb by recording impulses from afferent fibers with endings in either the gastrocnemius or gracillis muscles in anesthetized dogs. Capsaicin (10-30 µg/kg)injected into the abdominal aorta, stimulated 24 of the 34 group IV (C fiber) afferents tested, but only 5 of the 19 group III afferents (Aδ fiber) tested. Impulse activity for the 24 group IV afferents increased from 0.7 ± 0.1 to a peak of 9.3 ± 1.4 impulses/second. Capsaicin had no significant effect on the firing rate of 30 group I and II muscle afferents. Bradykinin, an algesic substance, known to stimulate group III and IV muscle afferents (Guzman *et al.*, 1962) was also examined in this study and was found to stimulate a

greater proportion of group III than group IV afferents whereas capsaicin stimulated a significantly greater proportion of group IV than group III afferents. Thus capsaicin was found more selective than bradykinin on goup IV muscle afferents. These results strongly suggest that group IV muscle afferents are primarily responsible for the reflex increases in cardiovascular function evoked by injecting capsaicin into the arterial supply of the skinned hindlimb of dogs. Furthermore, the response of these afferent fibers to capsaicin is not tachyphylaxis. Thus, this substance may be a useful pharmacological tool in determining the reflex autonomic changes induced by stimulating group IV muscle afferents, and the connections of group IV muscle afferents to sites in the central nervous system involved in cardiovascular control.

Longhurst *et al.* (1980) used capsaicin to demonstrate the potential function of the stomach as a reflexogenic organ which regulated the cardiovascular activity. They developed an autoperfused canine stomach preparation from a dog anesthetized with α-chloralose so that capsaicin could be injected into the left gastroepiploic artery supplying the greater curvature of the stomach. Control injections were given into inferior vena cava to determine capsaicin effects on areas downstream from the stomach. Capsaicin (25-500 µg) administered into left gastroepiploic artery caused significant increases in systemic blood pressure, heart rate, myocardial contractility and systemic vascular resistance but no changes in left ventricular end-diastolic pressure and aortic flow. On the other hand, control capsaicin injections caused significant decreases in systemic blood pressure, heart rate, myocardial contractility and aortic flow,

but no changes in systemic vascular resistance or left ventricular end-diastolic pressure. The cardiovascular responses obtained by intragastroepiploic arterial injection of capsaicin were attenuated to a large extent by diaphragmatic celiac nerve severance (sympathetic) and to a smaller extent by diaphragmatic vagus nerve section (parasympathetic). The opposite nature of the cardiovascular responses to gastric and vena caval injections of capsaicin, and the results of nerve section suggested that the effects from gastric injection were specific and limited to the stomach or adjacent regions. These authors concluded that capsaicin stimulated gastric or perigastric receptors to induce a significant activation of the cardiovascular system.

Ashton and collaborators (1982) have studied the reflex cardiovascular changes evoked by capsaicin injection into canine liver. They developed an animal model in which the venous return from the inferior vena cava was discarded while total venous return to right heart was maintained constant. This model could prevent systemic circulation of capsaicin when the drug was injected into portal circulation of the liver. Capsaicin (500 µg)was found to rapidly decrease left ventricular systolic pressure, mean arterial pressure, heart rate, renal vascular resistance and myocardial contractility. Left ventricular end-diastolic pressure did not change. Vagus nerve interuption at the level of the diaphragm did not alter the hemodynamic changes occurring during capsaicin injections, but interuption of anterior hepatic nerve eliminated the responses. The authors concluded that the liver tissue contained capsaicin-sensitive afferent endings, most likely type C fibers, activation of which resulted in reflex cardiovascular depression and this afferent neural pathway is

predominantly in the anterior hepatic nerve plexus.

Most recently, cardiovascular reflexes arising from the gallbladder stimulated by capsaicin was reported by Ordway *et al.* (1983). Application of capsaicin to the serosal surface of the gallbladder in anesthetized cats evoked cardiovascular responses including increases in mean arterial pressure, heart rate, myocardial contractility and systemic vascular resistance while there was no change when this substance was applied to the surface of the liver. Similar experiment with bradykinin, an endogenous algesic polypeptide, caused an activation of the cardiovascular system similar to that seen with capsaicin. Bilateral vagotomy at the level of diaphragm did not alter the cardiovascular responses to capsaicin and bradykinin but these responses were eliminated by bilateral splanchnic nerve section or selective denervation of the gallbladder. The investigators concluded that capsaicin or bradykinin stimulated receptors which were localized in the gallbladder of cats. Stimulation of these receptors reflexly activates the cardiovascular system through a spinal afferent pathway.

Most of the aforementioned investigations have been carried out in whole animals; and reports concerning the effects of capsaicin on isolated cardiac tissue preparations are scanty. In 1935, Lille and Ramirez reported that capsaicin had a slight and insignificant inotropic effect on the frog heart. Subsequently, Molnar and collaborators (1969) demonstrated the stimulatory effect of capsaicin on isolated guinea-pig atria. Capsaicin (0.05 - 0.1 µg/ml) was found to produce both positive inotropic and chronotropic effects. However

a long-lasting and highly specific tachyphylaxis to capsaicin rapidly developed. Catecholamines, tyramine and nicotine were found to produce their normal effects on capsaicin-desensitized atria. Neither propranolol nor cocaine could antagonize the positive inotropic and chronotropic effects of capsaicin on isolated guinea-pig atria, indicating that releasing of endogenous catecholamines was not involved. Similar results were also obtained by Fukuda and Fujiwara (1969) and Ueno (1971). However, Toda *et al.* (1972) failed to demonstrate capsaicin effect on isolated atria from dogs and rabbits.

Most recently, the effect of capsaicin on isolated rat atria has been investigated in two different laboratories. Tantipongse (1983) studied the effect of capsaicin on the rate and contractile force of isolated rat atria. Relatively high doses of capsaicin (10 and 20 µg/ml) were found to depress both the rate and contractile force. Irregular beats were also observed in many experiments and complete cessation of heartbeat was the usual end result. The capsaicin-induced quiescent atria resumed normal beat after repeated washing the atria with capsaicin-free medium, or addition of catecholamines, or electrical stimulation. Atropine or calcium chloride could not mitigate capsaicin effect on atrial rate. The depressive effect of capsaicin on isometric tension by electrically paced left atria was reversed by catecholamines as well as calcium chloride. The author suggested that capsaicin acted on the cell membrane to interfere with impulse generation and/or propagation in the SA node. In contrast to these findings, Pongprayoon (1983) reported that capsaicin (0.01 - 100 µM) produced positive chronotropic

effect reaching a peak at 2-3 min after exposure. Capsaicin at the concentration of 0.1 μM produced the largest effect on rate. Positive inotropic effect was also observed but it was slight and insignificant. Tachyphylaxis occurred in tissue exposed to the drug longer than 2 min. Propranolol, phentolamine, phenoxybenzamine, curare, atropine, diphenhydramine and metiamide did not produce any modification of capsaicin action. Thus adrenergic, cholinergic and histaminergic mechanisms appeared unlikely to involve. Only cyproheptadine was found to partially antagonized capsaicin effect, suggesting that serotonin may play a role in capsaicin aciton.

The mechanism of capsaicin action on the cardiovascular system is unclear at present. Recent findings have implicated substance P in the biological actions of capsaicin (Nagy, 1982). Substance P has been suggested a neurotransmitter in primary sensory neurons of the baroreceptor reflex since nerve endings with substance P-like immunoreactivity are found in the carotid sinus, in the wall of the aortic arch, in the petrosal and nodose ganglia which contain the cell bodies of baroreceptor and chemoreceptor nerves, and in the nucleus tractus solitarius of the medulla oblongata, a region where many baroreceptor and chemoreceptor primary afferent nerves terminate (Cuello and Kanazawa, 1978; Ljungdahl *et al.*, 1978; Lundberg *et al.*, 1978; Gillis *et al.*, 1980; Helke *et al.*, 1980). Substance P-like material has also been detected in guinea-pig and human heart nerve fibers (Reinecke *et al.*, 1980). Microinjection of substance P (or of capsaicin to displace substance P from sensory nerve endings) into the intermediate parts of the nucleus tractus solitarius or systemic administration

of the substance results in bradycardia and hypotension in rats and cats, such effects are similar to those obtained when baroreceptor afferents are stimulated (Haeusler *et al.*, 1980 a, b; Talman and Reis, 1981). These findings have led to the suggestion that substance P is a neurotransmitter at the central terminals of baroreceptor and chemoreceptor afferent nerve endings. However, the distribution of substance P nerve throughout the cardiovascular system is widespread and not limited to areas of baroreceptor afferent endings (Furness *et al.*, 1982b). It has been demonstrated in both peripheral and central nervous system that acute treatment of capsaicin produces an initial release of substance P while prolonged treatment causes depletion of substance P (Jessell *et al.*, 1978; Yaksh *et al.*, 1979; Gamse *et al.*, 1980). A series of subcutaneous injections of capsaicin in adult guinea-pigs depletes substance P nerves of the heart and blood vessels (Papka *et al.*, 1981; Furness *et al.*, 1982b). Furthermore, systemic administration of capsaicin to new born rats causes degeneration of the unmyelinated primary afferent fibers including those containing substance P (Jancsó *et al.*, 1977; Nagy *et al.*, 1980; Scadding, 1980; Gamse *et al.*, 1981). Lorez *et al.* (1981) stated that treatment of neonatal rats with capsaicin did not change baroreceptor reflex function in adult animals although there was a reduction in the number of primary afferent substance P - containing fibers in the rootlets of cranial nerves IX and X and a marked decrease of those terminating in the nucleus tractus solitarius. In contrast, Bond and coworkers (1982) reported a significant suppression of baroreceptor reflex response to bilateral carotid occlusion in anesthetized adult rats which had been treated neonatally with

capsaicin. In the same year, Furness *et al.* (1982a) demonstrated that a series of subcutaneous injections of capsaicin in adult guinea-pigs caused a substantial reduction in substance P levels in vascular and cardiac nerves. No changes in resting heart rate, blood pressure or baroreceptor sensitivity were seen in the concious animals. They concluded that the widespread substance P nerves associated with the heart and blood vessels were not necessary for baroreceptor reflex function. Thus, the results of these studies appear contradictory. The role of substance P fibers which end in the carotid arteries and sinuses and run in the vagus nerves remains to be established. Undoubtedly, more investigations are needed to prove whether capsaicin produces reflex cardiovascular changes through substance P release and/or depletion.