CHAPTER IV

DISCUSSION

There are only few published reports concerning the in vitro effect of capsaicin on isolated cardiac tissue preparations. Toda et al. (1972) have shown that capsaicin in concentrations ranging from 0.02 to 2 µg/ml has no influence on the rate and contractile force of isolated dog and rabbit atria. On the other hand, two research groups (Fukuda and Fujiwara, 1969; Molnar et al., 1969) have independently demonstrated the positive chronotropic and inotropic effect produced by low doses of capsaicin (0.5 µg/ml or less) on isolated guinea pig atria. The apparent discrepancy most probably due to different animal species used in these studies. The cardiac stimulation observed with atria from guinea pigs cannot be antagonized by propranolol or reserpine pretreatment, thus negates the participation of adrenergic mechanism. The results of the present study have shown yet another effect of capsaicin on isolated atria. Concentrations of capsaicin at 10 and 20 µg/ml have been found to depress both the rate and contractile force by isolated atria from the rats. The effect on the rate, which includes negative chronotropism, arrhythmogenesis, and heartbeat cessation, is ostensibly more pronounced than on the force. At 20 µg/ml capsaicin, all of 12 atria stop beating within 18 min after capsaicin addition whereas the contractile force of 5 electrically paced left atria is reduced by approximately 40 % following 30 min exposure to the same dose of capsaicin. In some experiments capsaicin produces the initial

stimulation of both the rate and contractile force; this is invariably followed by depression. The reason for inconsistent cardiac-stimulating effect of capsaicin observed in this study is unclear but may be related to the dose and animal species employed. The capsaicin concentrations used by Fukuda and Fujiwara (1969) and Molnar et al. (1969) to demonstrate the positive chronotropic and inotropic effect are much smaller than those employed in the present study, and atria were from guinea pigs instead of rats.

The negative chronotropic effect of capsaicin is independent of acetylcholine release since this action is not antagonized by atropine. Moreover, the fact that the washed atria pretreated with capsaicin appear more sensitive to the chemical indicates that the response is not tachyphylactic. There is considerable evidence suggesting the involvement of substance P and serotonin in the biological actions of capsaicin (Virus and Gebhart, 1979). If capsaicin slows heart rate through releasing substance P or serotonin or some unknown cardiac depressant, one would expect tachyphylaxis to ensue as the atrial contents of the released agents are depleted by repeated capsaicin additions. Therefore, the apparent lack of tachyphylaxis makes it unlikely that capsaicin reduces atrial rate indirectly by liberating endogenous cardiac depressant.

The capsaicin-induced reduction in the rate and contractile force are both reversed by catecholamines with β_1-agonistic activity. Of the three catecholamines tested, viz, isoproterenol, epinephrine, and norepinephrine, the most and least effective amines in antagonizing capsaicin effect on the rate are isoproterenol and norepinephrine respectively. This efficacy sequence is exactly the same as potency order of cardiac

stimulation. (Ahlquist, 1948). Thus isoproterenol, being the most powerful cardiac stimulant, is also the most active amine in reversing heart rate depression caused by capsaicin. Very low dose of isoproterenol added to the capsaicin-treated quiescent atria can restore heartbeat above the control rate, and the effect is sustained at least during the 60 min period of experimentation. This action of catecholamines is undoubtedly mediated through β_1 - adrenergic receptor since it is abolished by propranolol. It is well established that the β_1-stimulating catecholamines enhance cyclic AMP formation and transmembrane calcium ion influx by the myocardium (Mayer, 1974; Fleckenstein, 1977; Barany and Barany, 1981). The increased intracellular cyclic AMP level, through a cascade of enzyme activation, brings about lipolysis and glycogenolysis in the myocardial cells. The liberated free fatty acid and glucose are then oxidized by the mitochondria with the production of ATP. The increased mitochondrial ATP synthesis and elevated sarcoplasmic calcium ion concentration are unequivocally the basis for the positive inotropism produced by catecholamines. On the other hand, the mechanistic picture of the catecholamines-induced heart rate acceleration remains obscure at present. The difficulty arises mainly from the complex ionic movements during impulse generation in the automatic cells particularly the SA and AV nodes. Nonetheless, there is evidence indicating that implulse production and propagation in cardiac pacemaker require calcium ion (Fleckenstein, 1977; Fozzard, 1977). Calcium-antagonistic drugs such as verapamil, diltiazem, and nifedipine as well as certain divalent cations, e.g., Co^{2+}, Ni^{2+}, and Mn^{2+}, have been shown to suppress cardiac contractility and pacemaker activity (Fleckenstein, 1977). Since catecholamines are known to inerease heart rate by causing a more rapid

depolarization of pacemaker potential and to enhance calcium ion influx, these two effects may be causally related. It is a curious fact that the depressed contractile force of the capsaicin-treated atria is augmented by calcium ion whereas this ion cannot protect or attenuate capsaicin effect on the rate. This observation casts some doubt over the role of calcium ion in the pacemaker's electrical activity. At any rate, it is safe to state that the catecholamines relieve the capsaicin-mediated depression in heart rate and contractile force by interacting with β_1-adrenergic receptor and that cyclic AMP and calcium ion are intimately involved in this action of the catecholamines.

The decrease in heart rate and contractile force induced by capsaicin as well as the reversal of this effect by catecholamines resemble that of verapamil and other calcium antogonists (Fleckenstein, 1977). Verapamil and other drugs in this class have been called calcium antagonists because they are believed to produce cardiac effect by interfering with transmembrane calcium supply. Electrophysiologic study has shown a drastic reduction in transmembrane calcium conductivity produced by verapamil. Although similar electrophysiologic experiment has not been performed with capsaicin in the present investigation, It is dubious whether the cardiac actions of capsaicin and verapamil have common mechanism. Verapamil is substantially more effective in reducing cardiac contractility than slowing the rate, while the opposite is true for capsaicin. Nevertheless, it is interesting to note that calcium ion is not very active in alleviating verapamil effect on the rate but is effective with regard to force- a situation analogous to capsaicin.

The negative chronotropic effect of capsaicin (10 and 20 µg/ml) is invariably terminated with the atria in the quiescent state. When

these dormant atria are stimulated electrically, regular beating resumes. This observation strongly indicates that capsaicin stops heartbeat by affecting the SA node rather thanthe atrial musculature. Should capsaicin induce failure in the excitation-contraction coupling mechanism, then no response to electrical stimulation is to be expected. Thus, the heartbeat cessation caused by capsaicin must be due to the lack of electrical impulses from SA node which normally activate atrial contraction. How capsaicin interferes with electrical activity of the SA node and consequently slows atrial rate is not known at present. The most likely possibility is that capsaicin may reduce heart rate by decreasing the rate of impulse generation and/or the conduction velocity of impulse in the SA node. Heartbeat will stop when impulse production and/or propagation is completely blocked. Direct electrophysiologic measurements are needed to substantiate this point. In this respect, capsaicin may prove a valuable new pharmacological tool to delineate the intricate electrophysiologic processes in the nodal tissues. Because the capsaicin-induced, non-beating atria can be electrically paced to beat as fast as 300/min with no arrhythmias observed, impulse conduction by atrial muscle is apparently unimpaired.

The effect of methyl capsaicin, the non-phenolic capsaicin congener, on the rate of isolated rat atria is similar to that produced by capsaicin. Methyl capsaicin causes a gradual diminution in the rate, and the atria eventually cease to beat. Heartbeat can be restored by repeated washings or by addition of isoproterenol. This finding suggests that both compounds induce cardiac depression by the same or similar mechanism. However, methyl capsaicin appears less potent than the parent compound since in the presence of the former the atria

continue beating for a longer period than with capsaicin present. Therefore, methylating the phenolic group in capsaicin molecule reduces its activity on the rate. Thus, although the presence of phenolic structure is not an absolute requirement for capsaicin to exert its action on the rate, this functional group ostensibly renders the chemical more active. The reason behind this is not clear but may be related to change in lipid solubility as methyl capsaicin is obviously less polar than capsaicin.

The mechanism of capsaicin action on the rate and contractile force by isolated rat atria is at present a matter of conjecture. Capsaicin does not appear to act by releasing acetylcholine or other endogenous cardiac depressants since this action is not obliterated by atropine and the response is not tachyphylactic. It has been shown by Chudapongse and Janthasoot (1976, 1981) that capsaicin inhibits mitochondrial electron transport. Consequently, the ability of the mitochondria to synthesize ATP is impeded. In cardiac muscle, the major source of energy for contraction is the ATP derived from mitochondrial oxidative phosphorylation (Bing, 1965; Opie, 1969). Since capsaicin impairs mitochondrial ATP synthesis, the reduction in myocardial ATP level is conceivable. One may further speculate that the diminished energy supply to the contractile machinery then leads to a less forceful contraction. However, the mitochondria inhibition seems unlikely to be the mechanism responsible for capsaicin effect on the rate. The action on the cardiac cell membrane appears more plausible since heartbeat can be restored simply by repeated washings. It should be pointed out in this connection that capsaicin molecule contains both the hydrophilic phenolic group and the lipophilic alkyl chain similar

to the phopholipid component of cell membrane. Thus, capsaicin may be envisaged to embed itself in the SA nodal cell membrane and thereby disturbs the membrane functions resulting in altered electrical activity of the SA node. Clearly much more experiments are needed to determine the exact mechanism of the cardiac depression induced by capsaicin. Finally, the deleterious action of capsaicin on isolated rat atria described here should alert heavy chili consumers to the possible adverse effect capsaicin might have on cardiovascular functions.