CHAPTER IV

DISCUSSION

Pharmacological screening in the previous study, showed that contractions of intestinal smooth muscle and uterus in experimental mice were inhibited by ancistrotectorine (Pasupat, 1985). Moreover, this alkaloid also reduced contractions of rat vas deferens induced by KCl, NE, 5-HT and $CaCl_2$ (Ketkosol, 1985). The aims of the present study have formated on the background of these preliminary results so as to elucidate the mechanism of action of ancistrotectorine.

In the present study, various agonists; KCl, NE, 5-HT and histamine, are used to induce the contractions in rat aorta, since this isolated tissue did not possess spontaneous contractility. Moreover, $CaCl_2$ was also used as a stimulating agent in Ca^{2+}-free, high potassium depolarizing solution to induce the contraction (Hudgins. et al 1968)

Contractions induced in vascular smooth muscle by a high concentration of potassium are generally believed to be highly dependent on the concentration of extracellular calcium since these contractions are markedly decreased or abolished by the removal of calcium from the extracellular milieu (Deth, 1974). Another experiment supports the calcium involvement of

KCl produced contraction. By measuring the uptake and distribution of radiocalcium (^{45}Ca) in the vascular strips in the presence and absence of KCl, it was found that KCl increased the uptake of calcium (Greenberg et al, 1973). The experiment was repeated utilizing ruthenium red, an inhibitor of calcium transport. Ruthenium red reduced or abolished contractile responses of the tissues to KCl. The present observation agrees with these studies, KCl can produce the contraction. In addition, we observed a significance relaxant effect of ancistrotectorine, following pre-incubation of the rat aorta with ancistrotectorine for 15 min. This effect followed a concentration-related manner.

Ca^{+2}-antagonists (diltiazem and verapamil) were used in this experiment. Both of them inhibited the aortic contractions induced by KCl, although large differences in their inhibitory potency (calculated as EC_{50} value) exist, depending on the stimulating agents.

It has also been shown that NE could elicit contractions of the rat aorta. The ability of NE to induce the rat aortic contraction both in the absence and in the presence of calcium has not been clearly elucidated. Sutter (1976) reported that NE could contract rat aorta incubated in calcium-free physiological solution, whereas De Feliu et al, (1976)

reported that under these conditions the tissue lost its contractile response to NE. Recently, Cawin and Shalid (1984) demonstrated that NE-appeared to activate vascular smooth muscle of isolated rat aorta through stimulation of alpha-1 adrenoceptors which then led to Ca^{2+} influx and liberation of intracellular Ca^{2+}. This study is similar to the previous study of Hurvitz and Sunia (1971) in which they reported that NE combined with its receptors and caused an increase in membrane permeability to Ca^{2+} ion as well as displacements of Ca^{2+} from binding site within the cells. Our observation agree with De Feliu because NE elicited the contractile responses of rat aorta in Krebs-Henseleit solution. On the other hand in the absence of calcium, the contraction was abolished. We found that ancistrotectorine can inhibit the NE-induced contractions in the dose-dependent manner.

By using diltiazem or verapamil, we found that these agents can inhibit NE-induced contraction too.

In according to the effects of histamine and 5-HT, many have reported that an increased calcium influx through the receptors was associated with the contractile effects induced by these agents (Hudgins, 1968). Our study show that the extracellular is also important for these-agent-induced contractions because in Ca^{2+}-free solution the contractile responses were abolished. These results

illustrated the dependency on extracellular calcium for contraction. Ancistrotectorine also inhibited histamine and 5-HT-induced contraction in a dose-dependent manner. Many investigators demonstrated that vascular smooth muscle possesses various types of receptor such as alpha, beta-adrenoceptors, histaminergic H1, H2, serotonergic S1 and S2 receptors. (Kalkmam et al., 1984).

In the presence of methysergide, a 5-HT-blocker, the inhibition of the contractile responses were still obtained. In the presence of the combination of methysergide and ancistrotectorine, it seems that ancistrotectorine potentiates the inhibitory effect of methysergide.

The application of diltiazem or verapamil could inhibit the histamine and 5-HT-induced contractions $CaCl_2$ tranverses intracellularly when the membrane is depolarized (Massingham, 1983). We found that ancistrotectorine can inhibit $CaCl_2$-induced contractions. It is well known that a smooth muscle preparation immersed in a bathing medium containning a high potassium ion concentration will undergo a sustained membrane depolarization. One of the consequenees of the potassium-induced depolarization is an activation (opening or widening) of calcium channels in the muscle membrane (Ebashi S. and Endo. M. 1968).

This event leads to an influx of calcium ion and ultimately, to a smooth muscle contraction.

The application of diltiazem or verapamil also inhibit $CaCl_2$-induced contractions (Bristow et al., 1984).

Smooth muscle contraction is associated with intra and/or extracellular Ca^{2+} pool mobilization, with or without membrane depolarization. The excitation-contraction coupling mechanism depends on the agonist and on the smooth muscle preparation used (Cerrina et al., 1981). Our study show that ancistrotectorine inhibit the contractions induced by a variety of agents. This leads to the suggestion that ancistrotectorine may act by a non-specific antagomsm, this result is agreeded with Khwanta (1985).

The Ca^{2+}-channel antagonists, including the clinically available verapamil and diltiazem (Fleckenstien 1977, 1980, stone et al 1980) are of therapeutic benefit in a number of cardiovascular disorders. All drugs classed as Ca^{2+}-antagonists inhibit Ca^{2+}-induced contractions in K^{+}-depolarized smooth muscle preparation (Hof et al., 1982). From our experiment verapamil and diltiazem can inhibit Ca^{2+}-induced controctions, in k^{+}-depolarized rat aorta and also ancistrotectorine. The inhibilory effects of verapamil and diltiazem have been studied many years.

Janis and Triggle (1983) reported that verapamil and diltiazem are exclusively blockade of stimulated Ca^{2+} influx. Some workers indicated that verapamil block alphareceptors (Church J. and Zsotor. T. 1980). Major questions of mechanisms and sites of action remain. There exists multiple sites and mechanisms of action for the Ca^{2+} channel antagonists. In our study, it was found that the order of potency of the inhibitory effect on NE and $CaCl_2$-induced contraction was verapamil > diltiazem > ancistrotectorine. In the case of 5-HT, the order of potency was verapamil > diltiazem > methysergide > ancistrotectorine, the case of histamine, the order of potency was verapamil > diltiazem > ancistrotectorine and the last case, KCl, the order of potency was verapamil > diltiazem > ancistrotectorine. The present study show that the inhibitor effects of ancistrotectorine may by unknown mechanism with Ca^{2+} ion entry or involve a receptor mechamism. The mechamism of this compound has not been clearly elucidated by this study. In the future ancistrotectorine a may be usefull for the treatments of hypertension.