CHAPTER V

DISCUSSION

Ancistrotectorine is a new compound which has been suggested to inhibit intestinal smooth muscle and uterine contraction (Pasupat, 1985). It is interesting to investigate the effect of this compound in other kinds of smooth muscle for comparing its effects. From preliminary study, it was found that applying ancistrotectorine alone did not affected contraction of rat vas deferens. So the effect of ancistrotectorine was studied on contraction induced by some agonists.

The result obtained from both isotonic and isometric recording at the beginning of this investigation confirmed those of Paton (1975) who found that there was a close relationship between the two types of record. However in some experiments it was found that a load that employed in isotonic transducer for obtaining the same responses as recorded by isometric transducer should be changed when rat vas deferens contractions were induced by some kinds of agonist that made it difficult to find an arbitary element in the choice of load. Another difficulty was that itwas not easy to combine sensitivity to low doses of $CaCl_2$ with maximal doses of $CaCl_2$ in high K^+ deporalizing preparation in isotonic recording. A considerable isotonic load that was required in this preparation was too heavy for low doses of $CaCl_2$, because of low loading, the maximal response

was off the scale. The load must be suitably designed for isotonic recording, thus isometric recording was selected and suitable for many purpose in this experiment.

The phasic responses of rat vas deferens were induced by either $BaCl_2$, 5-HT or NA. In smooth muscle, increasing membrane permeability to Ca^{2+} as a result of membrane deporalization by KCl caused contraction (Bolton, 1979). The prostatic half was used in KCl induced contraction because of its higher amplitude and larger stimulation of ^{45}Ca uptake than epididymal half (Hay & Wadsworth, 1982a). In the rat vas deferens, about 25 % of the phasic response to KCl (160 mM) can be attributed to release of neuronal noradrenaline, but the remainder of the phasic response appears to be caused by a direct effect on the smooth muscle (Hay & Wadsworth, 1981).

Depending on the concentration of KCl and the type of smooth muscle, smooth muscles that readily generate action potentials (i.e. rat vas deferens, rat stomach, rat intestine), the initial peak of tension (phasic response) in response to high concentration (100 mM) of potassium seems to be associated with an initial burst of action potentials (Bolton, 1979). Another experiment which supports that KCl produce tension by stimulation of extracellular Ca^{2+} into the intracellular component was increasing of ^{45}Ca uptake by KCl in several vascular smooth muscles (Casteels, 1981). The organic calcium antagonists have been shown to inhibit KCl stimulated Ca^{2+} uptake into vascular, intestinal and vas deferens of rat (Thorens & Haeusler, 1979; Rosenberg, 1979; Hay & Wadsworth, 1984a).

An initial phasic contraction produced by 2 mM $BaCl_2$ was not mediated by the release of endogenous noradrenaline or activation either α-adrenoceptor, β-adrenoceptor, muscarinic receptor, or 5-HT receptor. Both the initial phasic and rhythmic components of the response to barium were presented in equal extent in both regions of the rat vas deferens but the prostatic half has the ability to produce more frequent rhythmic contractions (Hay & Wadsworth, 1983a).

The initial response to $BaCl_2$ may be mediated by uptake of a high affinity membrane bound Ca^{2+} source through Ca^{2+} channels sensitive to calcium channel inhibitors, as has been proposed for the initial contraction to barium in guinea-pig ileum (Clement, 1981).

In this experiment the maximum phasic response was induced by 5-HT 1.3×10^{-4}M. There are many factors which involve the mechanism of 5-HT contraction. Cytochemical and biochemical studies have shown that under appropriate conditions, exogenous 5-HT is accumulated into the neuronal adrenaline storage in the vas deferens (Jaim-Etchevery & Etchevery & Zieher, 1969). Nishino *et al* (1970) found that the 5-HT contraction in the rat vas deferen was blocked by phentolamine, reserpine and imipramine. It might therefore be expected that the contractile response with 5-HT would be mediated by release of endogenous noradrenaline. Ozawa & Katsuragi (1974) using the guinea-pig vas deferens in which they reported that the initial phasic component was mediated by post-junctional 5-HT receptors. Hay & Wadsworth's conclusion in the rat vas deferens were in agreement with Ozawa and found that the phasic component of 5-HT contraction is mediated via postsynaptic 5-HT receptors. Incubation of the rat vas deferens in

nominally Ca^{2+} free Krebs-Henseleit solution completely prevented both the phasic and rhythmic components of the 5-HT contraction (Hay & Wadsworth, 1982d).

The rat vas deferen is well accepted as a predominantly adrenergic innervated tissue (Holman, 1975) and noradrenaline is known to cause vas deferens contraction by direct action on the smooth muscle cell interfering with specific action on the post-synaptic α-receptors. Noradrenaline as the physiological transmitter mainly causes liberation of calcium from binding site with smooth muscle cell (van Breemen and Lesser, 1971).

In case of rat vas deferens contraction induced by NA and KCl, phasic responses were followed by tonic contraction. A component of tonic phase when muscle is depolarized with KCl uses extracellulary located Ca^{2+} bound with high affinity but nevertheless is available to activate contraction (Haeusler, 1972), whilst NA induced tonic response is maintained by calcium influx (Bolton, 1979). It was concluded that both phasic and tonic components of rat vas deferens contraction were dependent on extracellular Ca^{2+} entering through potential-sensitive calcium channels. This study revealed that ancistrotectorine and verapamil reduced the phasic and tonic componets of induced contraction in rat vas deferens.

In agreement with Pasupat (1985), ancistrotectorine inhibited the contraction of rabbit small intestine and guinea-pig ileum induced by KCl, $BaCl_2$ and $CaCl_2$. The result from this study suggested that ancistrotectorine may possessed some mechanism of action which was

similar to the effect of verapamil on blockade of $(Ca)_o^{2+}$ through potential-sensitive calcium channel. Verapamil was more potent than ancistrotectorine. However some mechanism of action may be different, verapamil was more active against the tonic than against the phasic response. Ancistrotectorine as well as $LaCl_3$ which is known to antagonize Ca^{2+} in a variety of cellular and subcellular (Weiss, 1974) was more active against the phasic than against the tonic response. Although the majority of verapamil and $LaCl_3$ tentatively listed as calcium antagonists the mechanism of each antagonist appeared to differ. Another disagreement of the mechanism of verapamil and ancistrotectorine was found that the phasic response of NA induced contraction was less sensitive to inhibition by verapamil (by comparing with ID_{50} values). The reason may be noradrenaline does not increase calcium influx initially, contraction being caused by release. Verapamil blocked calcium channel at the surface of the membrane so the phasic contraction induced by NA was sligthly inhibited.

In addition, Bolton (1979) postulated two types of calcium channels : the potential-sensitive calcium channel is an ion channel population that opens as the potential across the membrane is reduced and the receptor-operated calcium channel is an ion channel controlled or operated by a receptor for a stimulant substance. The early part of the response to exogenous added noradrenaline may correspond to the receptor operated-channels (Hay & Wadsworth, 1983b).

The rhythmic contractions induced by $BaCl_2$ and 5-HT are likely to be mediated by the entry of a small amount of trigger Ca^{2+}, which subsequently release intracellular Ca^{2+} stores. The trigger Ca^{2+}

enters via a separate population of membrane channels that are relatively insensitive to calcium channel inhibitors (Hay & Wadsworth, 1980 and 1984b). Although both $BaCl_2$ and 5-HT induced rhythymic contraction were similar, they produced responses by different mechanisms. The rhythmic contraction produced by $BaCl_2$ was not mediated by activiation of any receptors whereas the rhythmic contraction induced by 5-HT was mediated by combined effects on 5-HT receptors and noradrenaline release. However the mechanism underlying the rhythmic response was not clearly inquired. Relatively high concentration of verapamil and ancistrotectorine were required to inhibit rhythmic contraction produced by $BaCl_2$ and 5-HT. Both amplitude and frequency of $BaCl_2$ induced contraction were reduced by verapamil $8,9 \times 10^{-5}M$ while ancistrotectorine had some selectivity for the amplitude by the same concentration as verapamil. The contractions induced by 5-HT were abolished by either verapamil or ancistrotectorine $8,9 \times 10^{-5}M$.

Although high concentration of antagonists were used, the rhythmic contractions induced by $BaCl_2$ were not abolished by verapamil and ancistrotectorine. The reason may be that the concentration of $BaCl_2$ was too high for the antagonists to inhibit its effect.

An wondering result from the study was the frequency of rhythmic response induced by 5-HT and $BaCl_2$ were potentiated by ancistrotectorine ($1.2 \times 10^{-5} - 3,5 \times 10^{-5}M$). Increasing the frequency of NA induced rhythmic contraction was also affected by ancistrotectorine ($1.2 \times 10^{-5} - 2.3 \times 10^{-5}M$). Ancistrotectorine's stimulant action may result from blockade of potassium channels, as the following evidence suggests. Tetraethylamonium which is known to block

potassium channels produces rhythmic contraction in the rat vas deferens (Hay & Wadsworth, 1982c).

One interesting observations in the experiments involving the inhibitory effect of ancistrotectorine against barium and 5-HT induced rhythmic contractions. Elevation of the $(Ca^{2+})_o$ reversed the inhibitory effect of verapamil on $BaCl_2$ and 5-HT induced rhythmic contractions but did not reverse the inhibitory effect of ancistrotectorine on responses produced by 5-HT. The inhibitory action of ancistrotectorine was slightly reversed by increasing $(Ca^{2+})_o$. It is possible that the lack of reversal of the inhibition of ancistrotectorine on $BaCl_2$ and 5-HT induced rhythmic contraction may be because verapamil and ancistrotectorine were acting at different site or different mechanism.

Use of K^+ deporalized preparation has been of value in comparing the effect of ancistrotectorine with verapamil, the mechanisms involved in the coupling process have been examined with particular emphasis on the role of Ca^{2+}. All drugs classed as Ca^{2+} antagonist inhibit Ca^{2+} induced contraction in K^+ deporalized smooth muscle preparation (Hof *et al.*, 1982). From this experiment, lower dose of ancistrotectorine (2.1×10^{-5}M) was shown to have a dual mechanism (competitive and non-competitive) : higher concentration point out to a typical non-competitive blockade (Fig. 22). These results confirm earlier work that ancistrotectorine blocked 5-HT, $BaCl_2$, Ach and histamine induce contraction of guinea-pig ileum in a non-competitive manner (Pasupat, 1985). In agreement with Phusiraphun (personal communication) ancistrotectorine also antagonized NA, 5-HT, histamine, KCl and $CaCl_2$ induced contraction of rat aortic strip non-competitively.

Inhibition of smooth muscle contractility may be the results of either (1) inhibition of neurotransmitter release from nerve terminals; (2) blockade of specific membrane receptor sites; (3) stabilization of muscle membranes; (4) interference with the availability of Ca^{2+} at a step or steps in the contraction sequence subsequent to membrane activation; (5) interference with the normal function of the regulatory proteins involved in contraction and relaxation e.g. troponin, tropomyosin; or (6) inhibition of actomyosin ATPase and/or subsequent chemomechanical transduction (Malagodi & Chion, 1974). Ancistrotectorine exhibited no selectively in the blockade of drug receptors as demonstrated by concentrations were not marked different from those that blocked response to specific agonists and those to nonspecific stimulants and by the insurmountable antagonism of the agonists that are belived to activate specific receptors (Ach, norepinephrine, 5-HT).

In conclusion, the author wishes to stress that the purpose of this work was not to elucidate the mechanism of action of ancistrotectorine, but rather to screen and compare its effect with a Ca^{2+} antagonist, verapamil. The inhibitory effect of ancistrotectorine on rhythmic responses at high concentration was not obviously explained. The result of present study indicates that in the smooth muscle of rat vas deferens ancistrotectorine posseses potent spasmolytic properties which may be interfering with Ca^{2+} ion movement through potential-sensitive channels. All contractions induced by various spasmogens were reduced or blocked. This points to an unspecific antagonism.