Chapter 4

Discussion

Results from primary screening indicated that the four indole alkaloids from *Uncaria salaccensis* had antispasmodic effects as shown by their inhibitory effects on spontaneous movement of isolated rabbit jejunum. Although, at the present time the mechanisms of the antispasmodic effects have not been deeply investigated, one such experiment shown in Fig. 6,7,23 and 24 suggested that I-1 and I-2 might have antiserotonergic properties. Thus, further test was performed on isolated guinea-pig ileum to study the effect of these on sustained contraction induced by various agonists, namely carbachol, 5-hydroxytryptamine(5-HT), histamine and $BaCl_2$. This study revealed that I-1 at all concentrations used ($8.5x10^{-6}M$, $11x10^{-6}M$ and $22x10^{-6}M$) reduced the maximum response of 5-HT in a non-competitive manner,as shown in Fig.6. Similarly, I-2 at higher concentrations, i.e. $11x10^{-6}M$ and $22x10^{-6}M$ produced the same effects (Fig.7). I-2 at concentrations $11x10^{-6}M$ and $22x10^{-6}M$ (Fig.11) and O-2 at contraction $11x10^{-6}M$ (Fig.13) reduced maximum contraction of carbachol, also in a non-competitive manner. This effect was only seen with I-1 at relatively high concentration, i.e. $22x10^{-6}M$.

Such results indicated that I-1 at low concentration produced antispasmodic action through blockade of serotonergic receptor, whereas at higher concentration the action became less specific as the blockade of muscarinic cholinoceptor was also evident. By contrast,I-2 showed affinity to both serotonergic receptor and cholinoceptor in both concentrations tested ($11x10^{-6}M$ and $22x10^{-6}M$) The results on isolated

rabbit jejunum preparation showed that I-1 possessed potent spasmolytic action in comparism to I-2,O-1 and O-2; ED_{50} of I-1, I-2 , O-1 and O-2 were $1.3x10^{-5}$, $3.7x10^{-5}$, $4.39x10^{-5}$ and $5.49x10^{-5}$ respectively.

Previous study on the effects of 5-HT on contraction of rabbit jejunum and guinea-pig ileum suggested that there are two types of tryptaminergic receptors in the guinea-pig ileum, namely the M receptors which can be blocked by morphine and the D receptors by dibenzyline. The M receptors are probably in the nervous tissue embedded in the muscular coats, and normally exert regulatory influence on muscular movement, whereas the D receptors are probably innate to the muscle fibers (Gaddum and Picarelli, 1957; Broucke and LemLi, 1980; Black *et al* , 1981 and Porquet *et al* , 1982). Most of the publications seem to conclude that receptors for 5-HT are present in the nerve-muscle complex of the gut (Brownlee and Johnson, 1963;Costa and Furness, 1979; Johnson et al, 1980; Broucke and Lemli, 1980;Gonella, 1981). 5-HT contracts the longitudinal muscle of the guinea-pig ileum mainly through M receptors in the nervous tissue by stimulating the intramural parasympathetic ganglion cells. Only little direct stimulation by 5-HT through the D receptors on the smooth muscle has been reported (Gyermek and Bindler,1962; Brownlee and Johnson,1963; Day and Vane,1963). This lack of direct serotonergic innervation of the muscle suggests that isolated guinea-pig ileum preparation may not be a good model for studying the serotonergic mechanism.

Aortic strip preparation obtained from rabbit has therefore been used as an alternative model for serotonergic mechanism in this study. 5-HT has been shown to be involved in vasoconstrictor action as examined in several effector regions. Black *et al* .,(1981) investigated 5-HT vascular mechanisms in several rabbit vessels, namely common carotid, external carotid, femoral arteries, ear arteries; as well as canine coronary

arteries, namely interventricular and circumflex. The results indicated that rabbit common carotid, femoral arteries as well as canine interventricular and circumflex coronary arteries contained both D-type 5-HT receptors and α adrenoceptors. However, external carotid arteries, like ear arteries, do not contain specific 5-HT receptors. To produce vasoconstriction, the action of 5-HT in the external carotid artery is mediated by a direct action at α adrenoceptors rather than through any specific 5-HT receptors (Apperly, Humphery and Levy, 1976).

In addition, Apperly, Humphrey and Levy (1976) concluded from their works in the isolated rabbit aorta preparations that both D-type 5-HT receptors (Gyermek, 1966; Black *et al*, 1981) and α adrenoceptors were shown to be present in rabbit aorta, that is the action of 5-HT on this vasculature is likely to be mediated through serotonergic receptors. The results of the present study demonstrated that two indole alkaloids, I-1 and I-2, at concentration 11×10^{-6}M, and 22×10^{-6}M mitigated the dose-response curves of 5-HT in the isolated aortic strip in a parallel manner. Such results therefore indicated that these alkaloids reduced the effect of 5-HT in a competitive pattern. With regards the potency, it was found from analysis of the pA_2 values (Table 4) that I-1 was more effective in blocking 5-HT effects as compared to I-2.

Another study on the same group of indole alkaloids from *Uncaria salaccensis* (Archongka, 1983) showed that these alkaloids have negative chronotropic, negative inotropic and negative dromotropic responses in isolated rat atria.

These effects were resistant to atropine. However the positive chronotropic responses of the tissue to 5-hydroxytryptamine was antagonized by these alkaloids. By contrast, responses to adrenaline and isoproterenol

remained unaffected . This study denoted that I-1, I-2, O-1 and O-2 have antiserotonergic properties.

In addition, the effects of these alkaloids were also tested on central serotonergic system in the rats using behavioral models (Chaisupamongkollarp, 1984). The result indicated that all of the four alkaloids significantly reduced the number of head shaking induced by injection of high doses of 5-HT precursor, 5-hydroxytryptophan(5-HTP) (200 mg /kg) which lead to over activation of central 5-HT system. Of all of the four alkaloids, I-1 had highest antiserotonergic potency.

In agreement with Archongka, (1983) and Chaisupamongkollarp, (1984), this study supported that indole alkaloids especially pentacyclic heteroyohimbines (I-1, I-2) have antiserotonergic properties, with I-1 being more specific and potent than I-2. In addition, O-2 has anticholinergic properties as suggested by the results from isolated guinea-pig ileum study (Fig. 13).