Chapter IV

Discussion and conclusion

From the primary screening, the four indole alkaloids from *Uncaria salaccensis* have hypotensive effects. Although the mechanisms of depressor effect have not been investigated in deep in the present study, one such experiment, shown in Fig. 3-6, implicated that I-1 may cause peripheral vasodilatation. Such tentative conclusion was suggested by the observed decrease in diastolic pressure which occured in preference to a decrease in systolic pressure. An attenuation of peripheral resistance, because of arteriolar dilatation, will temporarily increase the runoff from the arterial reservoir. If cardiac output remains constant, this imbalance between input into the circulation and output through the capillary bed will result in a decrease in both arterial volume (diastolic volume) and mean arterial pressure (Little, 1981). In addition to the depressor effect, the alkaloids also produced significant decrease in heart rate. Interestingly, the decline in cardiac rate probably occured against baroreceptor reflex, since baroreceptor reflex mechanism would tend to elevate the heart rate during the periods when blood pressure was reduced (Little, 1981). Similarly, in the isolated heart preparation, I-1 also induces negative chronotropic response. At the dose of 24 mg/kg B.W., I-1 was capable of inhibiting or abolishing the contraction, which was characterized by arrhythmia and heartbeat cessation.

I-2 possesses a biphasic response on blood pressure comprising an initial transient pressor effect follow by a more pronounced longer-lasting depressor phase. In the initial phase, both systolic and diastolic pressure increase were probably due to peripheral vasoconstriction (Little, 1981). After 10-20 s, both blood pressure and heart rate reduced. The decline of diastolic pressure was more pronounced than that of the systolic and the pulse pressure became wider than control values. Possibility exists that these observation could be due to peripheral vasodilatation and direct depression of cardiac contraction. In isolated preparation, I-2 induced both of negative chronotropic and inotropic response, although the effect on the rate was ostensibly more pronounced than on the force. Compensation of the force when the rate decrease is strikingly apparent. At 24 mg/kg B.W. *in vivo* I-2, the respiratory rate is stimulated whereas the blood pressure increases.

At a low dose, O-1 (3 mg/kg B.W.) produced a transient increase in blood pressure in the rats. In contrast there were dose - dependent blood pressure reduction without compensatory tachycardia. The heart rate was depressed and pulse pressure wider. Suggestively, mechanism of actions of O-1 on blood pressure was similar to those of I-1 and I-2, but the apparent potency of O-1 is some what lesser than those of I-1 and I-2

The action of O-2 on blood pressure was resemble to that of O-1. The lethal dose is 96 mg/kg B.W., as potent as O-1.

In the isolated heart preparations, three indole alkaloids (I-1, I-2, O-1) have been found to depress both the rate and contractile force. By contrast, O-2 produced no discernable effect on the

contractile force of the electrically driven left atrium, although this alkaloid suppressed the rate. The effects of I-1, I-2 and O-1 on the rate, which include negative chronotropism, arrhythmogenesis, and heartbeat cessation, were ostensibly more pronounced than on the force. From the concentration - response curves, pentacyclic heteroyohimbines (I-1, I-2) was apparently more potent than the oxindole counterparts (O-1, O-2). The findings that all indole alkaloids tested reduced the maximum frequency of stimulus to which the heart tissue could respond indicated that the effect on the rate of the alkaloids such as arrhythmogenesis and heartbeat cessation was probably due to negative dromotropic effect or defection of conducting impulse of cardiac muscle (Brittain, Drew and Levy, 1982).

In order to determine whether the negative chronotropic effect of alkaloids involved cholinomimetic actions, experiments were performed in the presence of atropine. As shown in Fig. 11, 12, 13 and 16 preincubation of the atrial tissue in the presence of a muscarinic cholinergic antagonist atropine at concentration as high as 2×10^{-6} M did not mitigate alkaloid effects on atrial rate. Since the same concentration of atropine effectively nullified the action of acetylcholine, as shown in Fig. 15, the negative chronotropic effect of indole alkaloids are independent the involvement of cholinomimetic mechanism.

It is well known that catecholamines possessing ß-stimulating activity, for instance adrenaline and specific $ß_1$-stimulating isoproterenol, have direct positive chronotrcpic and inotropic effects on the heart. Since the alkaloids in the present study are found to depress rate, it was therefore interesting to investigate whether

these alkaloids could modify the positive chronotropic effect of catecholamines on isolated atria. From the experiments involving preincubation of the heart tissue with the alkaloids, it was observed that indole alkaloids did not alleviate or block the effect of either adrenaline or isoproterenol. These observations therefore suggest that these indole alkaloids do not possess ß-Adrenergic blocking property.

In addition, the effect of alkaloids on responses to 5-hydroxytryptamine (5-HT) in the isolated rat atria were also investigated. 5-HT has been shown to stimulate cardiac function in several species. Two alternative mechanisms by which this stimulation is achieved have been suggested. The first is an indirect action of 5-HT on presynaptic receptors which lie on the sympathetic neurones innervating the heart. Such stimulation results in the local release of catecholamines. This mechanism has been demonstrated in the rabbit (Trendelenberg, 1960; Fozard and Mwaluko, 1976; Fozard and Mobarok Ali, 1978) and the dog (Chiba, 1977). In the cat and guinea - pig, however, a different mechanism has been suggested (Trendelenberg, 1960). In these species the mechanism appears to be by direct stimulation of specific receptors on the cardiac cells. In the rat, Sakai and Akima (1976) concluded from their work in the blood purfused rat heart that 5-HT in the micromolar range produced its effects by a direct mechanism and not involving catecholamines, and in the respect most closely resembled the cat and guinea - pig rather than rabbit and dog. However, some studies raise a contradiction to the previous suggestion. Recently, Higgins, Allsopp and Bailey (1981) showed that low concentrations of 5-HT (upto 10^{-5} M) have a direct action on cardiac muscle, but at high concentrations (above 10^{-5} M) such an effect

cannot be separated from an action by which catecholamines are released from intracardiac stores. In spite of the existing controversy, the results of the present study demonstrated that four indole alkaloids mitigated the positive chronotropic effect of 5-HT. As regards the potency, it was found from the pD_2 values (Table 4) that I-1 was the most effective in blocking 5-HT effects, whereas I-2, O-1 and O-2 being of lesser potency respectively.

The mechanism of indole alkaloids action on the blood pressure is at present a matter of conjecture. Suggestively, the depressor effect is probably due to peripheral vasodilatation and direct depression of cardiac contraction. In the isolated rat atria preparation, the mechanisms on the negative chronotropic and inotropic responses of the indole alkaloids, which include arrhythmogenesis, and heartbeat cessation, are probably due to negative dromotropic effect or defection of conducting impulse of cardiac muscle and antiserotonergic properties.