Chapter IV

Discussion

The present study was found that crude extract from C. citratus could increase tone of contraction of the isolated stomach and small intestine of rats, guinea-pigs and mice significantly, but did not on the amplitude and the rate of contraction of the isolated rat duodenum segment. Mechanisms of action of crude extract on tone of contraction were different in the isolated rat stomach fundus strip, duodenum and guinea-pig ileum segments. The crude extract increased tone of contraction of the isolated rat stomach fundus strip did not mediate via ACh-receptor because it could not block by atropine (75 µg/ml). The crude extract increased tone of contraction of the isolated rat duodenum and guinea-pig ileum segments which did not mediate via H_2- receptors because cimetidine (75 µg/ml) could not block this action. They could mediate via ACh-, H_1- and 5-HT receptors respectively, indicates that crude extract acts on non-specific receptors for induces contraction of these tissues. All receptor blocking agents used in this studies; atropine, chlorpheniramine, cimetidine and cyproheptadine are non-competitive antagonists for the crude extract induces contraction of the isolated rat stomach fundus strip, duodenum and guinea-pig ileum segments.

Kenakin (1984) described that the response of the tissue preparation to an agonist should be due solely to the direct action of

the agonist on one type of receptor. It should not be resultant of actions on more than one type of receptor, nor should it be due even partially to indirect action, which release an endogenous neurotransmitters such as noradrenaline, acetylcholine, histamine, 5-HT and substance P induce contraction. In addition, the concentration-response curve to an agonist that produces an effect by release of endogenous neurotransmitters (indirect agonist) will be shifted to the right by a competitive antagonist but also show a depressed maximal response. These results suggest that the crude extract has indirect action on the isolated tissues because crude extract could induce contraction mediated via more than one type of receptor and was abolished by antagonists by a non-competition which showed a depressed maximum response (Van Rossum, 1963).

Spasmogens are drugs able to induce a contraction in muscle tissues, especially in smooth muscle. Acetylcholine, histamine, 5-HT, barium chloride, nicotine and prostaglandine E_1, all of which have a spasmogenic action on the smooth muscle of the isolated stomach and small intestine of either rats or guinea-pigs (Henderson et al, 1968; Costa and Furness, 1979; Hara and Szurszewski, 1986). The crude extract from C. citratus also has spasmogenic action on the smooth muscle of the these isolated tissues. The crude extract caused significant increase in percentage maximum tone of contraction of the isolated rat stomach fundus strip and duodenum segment which were induced by acetylcholine, histamine and 5-HT. This finding suggests that crude extract is a spasmogen which has an indirect action on mucous membrane and stimulates directly or indirectly released

neurosubstances such as acetylcholine, histamine, 5-HT and other hormones which these substances bind to specific receptors and could increase tone of contraction. These spasmogens induced contraction of these isolated tissues at all concentration. This reason could bring to describe the mechanism that a cumulative-concentration of the crude extract causes increase contractile response more than a washed out-concentration of the crude extract on tone of contraction of the isolated stomach and small intestine of either rats or guinea-pigs.

Acetylcholine, histamine and 5-HT caused increase tone of contraction of the isolated stomach and small intestine of rats and guinea-pigs by both direct and indirect actions (Vaughan Williams, 1954; Vane, 1957; Mukai and Kubota, 1980; Balck et al, 1981; Yamada et al, 1982). Direct action is spasmogen which act on specific receptor on the cell membrane of smooth muscle whereas indirect action spasmogen act on the nerve pathway induced the contraction (Daniel et al, 1962; Day and Vane,1963;Brownlee and Johnson, 1965; Bolton, 1979).

The spasmogenic action of 5-HT is the result of an interaction with specific tryptamine receptors. These receptors are classified by Gaddum and Picarelli (1957) into two types : M- and D-tryptamine receptors. The M-tryptamine receptors are sited in the intramural parasympathetic ganglion cell (Brownlee and Johnson, 1963) and are blocked by morphine. The D-tryptamine receptors are localized in the membrane of smooth muscle cells and are blocked by phenoxybenzamine (Day and Vane, 1963). In the isolated rat stomach fundus preparation is only the D-tryptamine receptor (Vane, 1957; Offermeier and Ariens,

1966). This is in contrast to the guinea-pig stomach and ileum, in which both types of tryptamine receptors (Gaddum and Picarelli, 1957; Yamaguchi, 1972; Van Den Broucke and Lemli, 1980). The contraction of the isolated rat stomach fundus strip by 5-HT is believed to be the result of an interaction of 5-HT with two kinds of receptors : D-tryptamine receptors and excitatory α-adrenoceptors (Innes, 1962; Ogle and Wong, 1971; Frankhuijzen and Bonta. 1974; Black et al, 1981).

The D-tryptamine receptors and the α-adrenoceptors are very similar, from literature it is known that 5-HT an affinity towards α-adrenoceptors, as rat vas deferens (Offermeier and Ariens, 1966), isolated strips of cat spleen (Innes, 1962), and isolated rabbit ear artery, common carotid and femoral arterial strips (Black et al, 1981). All the contractile response of these tissues antagonized by phentolamine and pizotifen, α-adrenoceptor blocking agents. Induces contraction of the isolated guinea-pig ileum by 5-HT mainly mediate through the M-tryptamine receptors in the nervous tissue. It stimulates the intramural parasympathetic ganglion cells to produce the releasing of acetylcholine from nerve terminals. Only little direct stimulation of 5-HT through the D-tryptamine receptors on the membrane of smooth muscle are recorded (Brownlee and Johnson, 1963; Day and Vane, 1963).

Immunohistochemical studies have revealed that 5-HT like substance is indeed present in nerve cell bodies in the myenteric plexus and in varicose nerve fibres in the ganlion of the myenteric and submucous plexus. Additionally, there is increasing evidence that

5-HT is an enteric neurotransmitter, and some intrinsic intestinal neurones which are also capable of taking up and storing indoleamine and 5-HT taken up into intestinal nerves can be released by electrical stimulation (Costa and Furness, 1979; Johnson et al, 1980; Holzer and Skofitsch, 1984).

Histamine receptors on the isolated stomach and small intestine of rats and guinea-pigs are classified into two types : H_1- and H_2- receptors. On rat stomach both H_1- and H_2- receptors are localized in the membrane of smooth muscle cells which are blocked by chlorpheniramine and cimetidine respectively. In the isolated small intestine of rats and guinea-pigs the H_1- receptors are localized in the membrane of smooth muscle cells which will be blocked by chlorpheniramine. The H_2- receptors are sited in the myenteric plexus, soma-dentritic region of cholinergic nerves and dentrito-dentritic synapes close to presynaptic terminal of cholinergic neurones which are blocked by cimetidine and atropine (Harry, 1963; Ash and Schild, 1966; Henderson et al, 1968; Rubinstein and Cohen, 1985).

It has been reported that acetylcholine and histamine acts directly on the smooth muscle cells of the guinea-pig ileum, interfering with specific receptors for these spasmogens (Day and Vane, 1963; Paton and Aboo Zar, 1968; Van Den Broucke and Lemli, 1980). Paton and Aboo Zar (1968) described that the origin of acetylcholine in guinea-pig ileum comes from nerve fibres and have some of the output of acetylcholine from the intestine comes from the smooth muscle itself, acts as a "local hormone" involved in the

maintenance of tone, motility and rhythmicity. Stimulation of H_2-receptors by dimaprit, a highly selective H_2- agonist, caused the release of contractile substances in the isolated guinea-pig ileum segment, the substances were tentatively identified as acetylcholine, substance P, 5-HT and others (Barker and Ebersole, 1982).

The contraction of smooth muscle can be brought about by agents that initiate cellular reactions which result in an increase in the free calcium ions concentration in the cytoplasm (Durbin and Jenkinson, 1961; Edman and Schild, 1961; Bozler, 1962; Bohr, 1964; Somlyo, 1985). Histamine, 5-HT and KCl are almost entirely dependent on extracellular calcium for inducing smooth muscle contraction, while acetylcholine may utilize calcium from an intracellular source to cause a contraction (Edman and Schild, 1961; Weinstock and Weiss, 1979; Reuter, 1983; Rubinstein and Cohen, 1985). Weinstock and Weiss (1979) found that 5-HT is much more dependent on the presence of calcium ions in the extracellular fluid to elicit a contraction of rat stomach fundus strip than is acetylcholine. This indicates that 5-HT acts by increasing the permeability of the muscle membrane to external calcium ions, as originally suggested by Wooley and Gommi (1963).

Coaxial electrical stimulation on the isolated guinea-pig ileum segment will stimulate postganglionic cholinergic nerve fibres. Measurable increase in the output of acetylcholine has its origin in the nervous structure of the wall of the ileum (Paton, 1955; Brownlee and Johnson, 1965). The contractile response of the isolated tissue to electrical stimulation by coaxial electrodes is abolished by small

doses of atropine (10^{-8}M), which is greatly augmented and prolonged by eserine or the anticholinesterase. It is insensitive to mepyramine, to desensitization to histamine or 5-HT, or to concentration of hexamethonium. Additionally, morphine in low concentration has a depressant action on the postganglionic cholinergic nerve fibres of the guinea-pig intestine, reducing both the resting output of acetylcholine (20-40%) and the output from the nerve endings when they are stimulated (Paton, 1955 and 1957).

The crude extract from C. citratus caused significant increase the amplitude of contraction of the isolated guinea-pig ileum segment which was induced by coaxial electrical stimulation and this effect was blocked by atropine (75 µg/ml). The result suggests that crude extract stimulate directly or indirectly on mucous membrane involving the release of neurosubstances caused increase the amplitude of contraction of the isolated guinea-pig ileum segment.

Previous study found that 5-HT had the same effect of the contraction when applied on mucosal and serosal side of the isolated rat stomach induces. These results indicated that spasmogen had direct action on the membrane of smooth muscle cells (Hara and Zweifach, 1963; Offermeier and Ariens, 1966). In addition, acetylcholine and histamine had direct actions on the membrane of smooth muscle cells which induced contraction as same as the action of 5-HT (Day and Vane, 1963).

In the present study the crude extract from C. citratus had effect on tone of contraction of the isolated mouse stomach, and guinea-pig ileum segment which could be significantly increased tone of contraction only by applying the extract on the mucosal side. This effect differed from applying the extract at serosal side. It could suggest that crude extract has indirect action to induce contraction of the isolated these tissues by act directly on mucous membrane and stimulates directly or indirectly on the same release of some substances such as acetylcholine, histamine and 5-HT.

Stimulation of autonomic nerves supplying to smooth muscle tissues often evokes either an excitatory junction potential or an inhibitory junction potential which involves a shift in the membrane potential. The entry of calcium into the cell through voltage-sensitive calcium channels is likely to be increased or decreased respectively. In smooth muscle which freely discharges action potentials either spontaneously (intestinal smooth muscle), the change in membrane potential will alter the rate of action potential discharge and so the tension developed by the smooth muscle. In this way there is modulation of the rate of entry of calcium, or its release within the cell, by a voltage-dependent mechanism entrained by the junction potential, as the action potential represents the operation of voltage-dependent calcium channels (Sperelakis, 1962; Bolton and Large, 1986).

The crude extract from C. citratus has indirect action by act on mucous membrane and stimulates released neurosubstances;

acetylcholine, noradrenaline, histamine, 5-HT and substance P induce contraction of the isolated stomach and small intestine of rats and guinea-pigs which may be mediated by voltage-dependent mechanism. Acetylcholine, histamine and 5-HT induces contraction of these isolated tissues mediated by a voltage-independent mechanism, was described by Bolton and Large (1986). They described that activation of receptors on smooth muscle cells caused by bathing smooth muscles in solutions containing excitatory transmitters (acetylcholin, noradrenaline, histamine, 5-HT and others) can bring into operation processes which are not voltage-dependent and which do not depen on modulation of the rate of action potential discharge. In this condition the contraction is not dependent solely on the opening of voltage-dependent calcium channels but can be elicited directly by calcium entry through receptor-operated calcium channels.

Previously study by Sperelakis (1962) described that Ca^{2+} and Sr^{2+} concentrations in the intracellular appears to be necessary for contraction of all type of muscles and the field contraction of depolarized smooth muscle. Calcium may have three sites of action : 1) in the cell membrane, at which it lowers membrane excitability, 2) in the excitation-contraction coupling process, and 3) in the contractile machinery. In addition, Sr^{2+} replaced Ca^{2+} in the field contraction but not in the normal contraction of polarized muscles, Sr^{2+} cannot penetrate through the polarized cell membrane but can penetrate through the depolarized membrane. One possible explanation for this is that Sr^{2+} is a good substitute for Ca^{2+} in the contractile mechinery, but not is the cell membrane response. That is,

substitution of Sr^{2+} for Ca^{2+} in the membrane structure may result in decrease in tension. And the postganglionic nerve fibres supplied smooth muscle cells can no longer be excited by electrical stimulation (Day and Vane, 1963). This experiment confirm the present result on tracing of the decrease in the amplitude of contraction of the isolated guinea-pig ileum segment during voltage stimulation by coaxial electrodes (Fig 27 and Fig 28).

Morphologically and electrophysiologically of intestinal smooth muscle in several mammals can be divided into at least three layers : longitudinal, inner circular and outer circular muscle layers. In addition, cells on the serosal-facing surface of the outer circular muscle have properties which suggest that they may function as pace-makers of small intestine motility (Hara and Szurszewski, 1986; Hara et al, 1986). Voltage and tension relationship have been determined for fundal, corporal and antral muscle of the dog stomach (Morgan et al, 1981) ; in antral and corporal muscle, the voltage threshold for contraction is approximately-42 mV whereas in fundal muscle it is appoximately-50 mV. The different voltage thresholds together with differences in spontaneous electrical activity, account in part for the marked differences in the physiological function of these muscle in vivo. The voltage-tension relationship for intestinal smooth muscle is not known (Hara and Szurszewski, 1986).

In summary, the crude extract from C. citratus caused significant increase in : 1) tone of contraction of the isolated stomach and small intestine of rats, guinea-pigs and mice. It mediated

via different receptors which induce contraction of the isolated rat stomach fundus strip. This effect did not mediate via ACh-receptor whereas induce contraction of the isolated rat duodenum and guinea-pig ileum segments did not mediate via H_2-receptor 2). The percentage of maximum tone of contraction of the isolated rat stomach fundus strip and duodenum segment which were induced by acetylcholine, histamine and 5-HT. 3) The amplitude of contraction of the isolated guinea-pig ileum segment which was induced by coaxial electrical stimulation and 4) The tone of contraction only the applying the extract on mucosal side but no affect on serosal side. All results in this investigation suggest that the crude extract has indirect action to induce contraction of the these isolated tissues.