INTRODUCTION

Mammalian physiologists have known for about a century that a vasoconstrictor material appears in serum when blood is allowed to clot. This material was unidentified until 1948 when investigators at the Cleveland Clinic isolated this vasoconstrictor substance as a crystalline complex and named it "serotonin" (Rapport *et al.*, 1948). Shortly thereafter, Rapport (1949) showed that the active moiety of this complex was 5-hydroxytryptamine (5-HT). This compound, when prepared synthetically by Hamlin and Fisher (1951) and others, proved to have all the properties of natural serotonin.

Source and chemistry

5-HT (serotonin) is 3-(β-aminoethyl)-5-hydroxyindole. It is widely distributed in the animal and plant kingdoms. It occurs in vertebrates, tunicates, mollusks, arthropods and coelenterates. Certain fruits such as pineapples, bananas, and plums also contain serotonin. In mammals, endogenous 5-HT is found in brain, blood platelets, enterochromaffin cell and myenteric plexus. In nervous tissues 5-HT is believed to function as a neurotransmitter. It is formed from the essential amino acid tryptophan by hydroxylation followed by decarboxylation. Normally the hydroxylase is not saturated; consequently, increased intake of tryptophan in the diet can increase brain serotonin content. After being released from serotonergic neurons, much of the released serotonin is

recaptured by an active reuptake mechanism and inactivated by monoamine oxidase to form 5-hydroxyindoleacetic acid (5-HIAA). This substance is the principal urinary metabolite of 5-HT, and the urinary output of 5-HIAA is used as an index of the rate of 5-HT metabolism in the body. In pineal gland, 5-HT is converted to melatonin. 5-HT stimulates or inhibits a variety of smooth muscles and nerves. These and other actions result in a wide spectrum of responses involving, in particular, the cardiovascular, central nervous system, and gastrointestinal system. Characteristically, responses to 5-HT are variable, they differ not only between species but also between animals of the same species. These variability depend on route and speed of injection, anesthetic state and spontaneous tone.

Action of 5-HT on central nervous system

Within central nervous system, the cell bodies of the 5-HT-containing neurons are located almost exclusively in the raphe nuclei of the brain stem, from which the axons project to other portions of the brain stem, to the spinal cord, and to the forebrain. It serves as a neurotransmitter. The hypothalamic neuroendocrine cells that release hormones which regulate adenohypophyseal secretion seem to be controlled, in part, by tryptaminergic neurons. 5-HT has been implicated as a possible physiological factor in stimulating the release of ACTH, GH and prolactin, and inhibiting the secretion of LH, FSH and TSH (Fuller and Clemens, 1981).

Action of 5-HT on gastrointestinal system

In human and animals, 5-HT is widely distributed in the gastrointestinal tract. The agent has been demonstrated in entero-chromaffin cells and in the myenteric plexus of the guinea-pig stomach and ileum. The effects of 5-HT on different parts of the gut are variable. The effects on the gut motor activity can be both excitatory and inhibitory (Brownlee and Johnson, 1963; Misiewicz et al., 1966; Bulbring and Gershon, 1967; Gershon, 1967; Rattan and Goyal, 1977), presumably due to stimulation of different receptor systems. 5-HT stimulates upper and lower small intestinal motility but inhibits activity of the stomach and colon (Misiewicz et al., 1966). Recently, Moen et al., (1983) have studied the effect of 5-HT on isolated guinea-pig fundus and antrum. They found that in the fundus 5-HT induced relaxation which gradually developed within 3 min. In the antrum 5-HT initiated phasic contraction, which culminated within 3 min and then returned to near or below pre-stimulatory values. 5-HT can act directly on the stomach of the rat to cause inhibition of gastric acid output (Canfield and Spencer, 1983). Intravenous administration of 5-HT causes dose-dependent contraction in the lower esophageal sphincter in the opossum (Rattan et al., 1977). Kamikawa and Shimo (1983) have studied the action of 5-HT on the esophagous muscularis mucosa of guinea-pig. 5-HT was found to a constriction by activating cholinergic nerves in the attached submucous plexus, and not by any direct action on the muscularis mucosa itself. Thus 5-HT-induced contractions were abolished by both tetrodotoxin and atropine (Kamikawa and Shimo, 1983).

Action of 5-HT on cardiovascular system

The contents of 5-HT in male and female rat hearts are approximately 0.70 ± 0.09 and 0.80 ± 0.10 µg/ml heart tissue respectively (Skillen *et al.*, 1962). This difference is not statistically significant. 5-HT is a potent vasoactive agent which produces a variety of actions depending on the tissues, doses and sympathetic activity. The effect of 5-HT on the vasculature is variable depending on the degree of sympathetic activity (Haddy *et al.*, 1959; Emerson *et al.*, 1973). In the pressence of an increased sympathetic tone, 5-HT exerts a vasodilator effect. On the contrary, in the pressence of decreased sympathetic tone, 5-HT produced vasoconstriction (Haddy *et al.*, 1959; Walsh, 1967; Edvinson and Hardebo, 1976).

5-HT has been shown to stimulate cardiac function in several species. Effects of 5-HT on the heart of intact animals are variable depending on animal species, and on route and rapidity of 5-HT administration (Erspamer, 1966). In man rapid intravenous injection, intravenous infusion, and infusion into the pulmonary artery generally produce an increase in heart rate (Le Messurier *et al.*, 1959; Erspamer, 1966) and exert a direct positive inotropic effect on the myocardium (Buccino *et al.*, 1967; Benfey *et al.*, 1974). In the dog, rapid intravenous injection of 5-HT usually produces bradycardia or initial bradycardia followed by marked tachycardia or sinus tachycardia. Fifteen to thirty minutes after intraperitoneal injection of 4 µg/kg 5-HT to the rat, basal cardiac output is unchanged, whereas blood flow in the myocardium is increased.

Conversely, intravenous infusion of 2.3 µg/kg/min 5-HT produces an increase in cardiac output. In isolated perfused rabbit heart, the positive inotropic and chronotropic effects induced by 5-HT are considered to be mediated by catecholamines since they disappear after reserpine pretreatment (Jacob and Poite-Bevierre, 1960). Injection of 30-300 µg 5-HT into the perfusion fluid of an isolated dog heart produces a maked increase in the heart rate and clear increase in coronary flow whereas the increase in contractile force is small (Schneider and Yonkman, 1954). In the cat, 2-200 µg 5-HT infused into the isolated cat heart caused an increase in heart rate, coronary flow, and contractile force (Schneider and Yonkman, 1954).

The effect of 5-HT on the isolated atria of rabbit, cat, and guinea-pig has been studied by Trendelenburg (1960). In rabbit, 5-HT at 0.1-10 µg/ml was found to produce a pure stimulatory effect (increase in amplitude of contraction and beating rate). Rabbit atria pretreated with reserpine showed very little or no response to 5-HT (Trendelenburg, 1960). Guinea-pig atria responded to 5-HT (1-10 µg/ml) with a pronounced and regular increase in amplitude of contractions and in rate of beating. The effect of this amine is mainly direct (LSD-sensitive), but indirect effects (morphine-and cocaine-sensitive) may also contribute to the stimulant response to 5-HT. The responses of atria from animals pretreated with reserpine are not significantly different from that of atria isolated from untreated animals (Trendelenburg, 1960). The response of cat atria to 5-HT (1 µg/ml) consists chiefly of an increase in the rate. Changes in amplitude of contractions are observed, but are small

and variable. The effect of 5-HT is blocked by LSD, but is not affected by pretreatment with reserpine (Trendelenburg, 1960). Two alternative mechanisms by which this stimulation is achieved have been proposed. The first is the indirect action in which 5-HT acts on presynaptic receptors of sympathetic neurons innervating the heart and thereby causes the release of catecholamines from these neurons. This mechanism has been demonstrated in the rabbit (Trendelenburg, 1960; Fozard and Mwaluko, 1975) and the dog (Chiba, 1977). In the cat and guinea-pig however, a different mechanism has been suggested (Trendelenburg, 1960). In these species the mechanism appears to be by direct stimulation of specific receptors on the cardiac cells. Sakai and Akima (1979) showed that in isolated rat hearts perfused at a fixed flow rate, single injection of 5-HT in microgram range (0.1-3.0 μg) into the coronary circulation produced a dose-dependent increase in left ventricular dP/dt max and perfusion pressure, but no clear change in heart rate. The increases in the left ventricular dP/dt max and perfusion pressure were not significantly affected by treatment with propranolol, while they were abolished by methysergide. This finding suggests that in the rat, the cardiac effects of 5-HT do not involve catecholamines action at the cardiac β-adrenergic receptors and, in this respect, most closely resembles the cat and guinea-pig rather than rabbit and dog. Recent data from cardiac cell cultures studies (Higgins *et al.*, 1981a) are in agreement with the above observations. Higgins *et al.*(1981b) showed that low concentrations of 5-HT (up to 10^{-5} M) have direct action on cardiac muscle, but at high concentrations (above 10^{-4} M) such an effect

can not be separated from an action by which catecholamines are released from intra-cardiac stores.

Gaddum and Picarelli (1957) first defined two peripheral sites of action of 5-HT using guinea-pig ileum. By means of specific antagonists, they differentiated two subtypes of 5-HT receptors. The first which they designated the D (musculotropic) type serotonin receptor is competitively blocked by cyproheptadine and methysergide, and is located on the smooth muscle cells. The second is on the cholinergic neurons of Auerbach's plexus as well as the presynaptic sympathetic neurons which believed to modulate norepinephrine release. This type of 5-HT receptor has been named the M (neural) receptor and is not blocked by cyproheptadine (Feniuk *et al.*, 1978).

The aim of this study are to investigate the stimulatory effects of 5-HT on the isolated rat left atrial force of contraction and right atrial rate, and to ascertain to what extent these stimulations are dependent on direct stimulation and on catecholamine release. Two specific 5-HT antagonists, cyproheptadine and methy-sergide as well as propranolol, a beta receptor antagonist are used in these investigations. The results obtained from this study may further improve our understanding on the role of 5-HT in controlling various physiological functions of the rat auricle.