

CHAPTER II

LITERATURE REVIEW

Passiflora is the largest genus of about 500 species of flowering plant in the family Passifloraceae (the Passion flower family). They are mostly vines, with some being shrubs, and a few species being herbaceous. These species are found in the temperate and tropical regions. Several species are grown in the tropical area for their edible fruits; the most widely grown is *Passiflora edulis* Sims (Passion fruit or purple granadilla). Many others are planted outdoor in the warmer parts of the world or in the greenhouses for their exotic flowers. Various species of *Passiflora* have been used extensively in the traditional system of therapeutics in many countries. For example, *Passiflora incarnata*, a popular traditional medicine is used in treating spasmodic disorder, insomnia (Bergner, 2001), and anxiety (Sharma et al., 2001). *Passiflora edulis* has been used as a heart tonic, mild diuretic, digestive stimulant and a treatment for urinary infections (Zibadi and Watson, 2004).

Pharmacological effects of *Passiflora*

Regardless to a large variety of phytoconstituents found in *Passiflora* plants, there are only a few reports on the pharmacological investigations available. Most of the pharmacological works have been carried out on the CNS depressant effects of various species. The extracts of *Passiflora alata* reduced amphetamine-induced spontaneous motor activity, prolonged pentobarbital induced sleep time at 150 mg/kg i.p., increased

the onset time and survival period on pentylenetetrazole-induced-seizures in the animals at 75 and 150 mg/kg (Petry et al., 2001). Chrysin, a naturally occurring monoflavonoid in *Passiflora caerulea* was found to be a ligand for central as well as peripheral benzodiazepine receptors (Medina et al., 1990). Aqueous extract of *Passiflora edulis* exhibited non-specific CNS depressant effects in mice, rats and healthy human volunteers (Maluf et al., 1991). Maltol and ethyl maltol which were obtained by sublimation of 2N hydrochloric acid fraction of *Passiflora incarnata*, were able to potentiate hexobarbital-induced sleep, inhibit pentylenetetrazole-induced convulsions and also decrease amphetamine-induced hyperactivity in mice (Aoyagi et al., 1974). The aqueous extract of *P. incarnata* had sedative effects at doses 400 and 800 mg/kg. The hydro-alcoholic extract of *P. incarnata* did not show such sedative effect, by contrast, it enhanced locomotor activity (Speroni and Minghetti, 1988). Hence, the different extracts possessed various pharmacological activities, depending on the major phytoconstituents. The recent study suggested that flavonoid chrysin isolated from *P. incarnata* decreased the locomotor activity at 25 mg/kg and also exhibited anxiolytic activity at a dose of 1 mg/kg (Zanoli et al., 2000). *P. incarnata* methanol extract exhibited significant anxiolytic activity at a dose of 125 mg/kg in mice (Dhawan et al., 2001).

Passiflora foetida Linn.

Passiflora foetida Linn. (The Foetid Passion Flower or Stinking Passion Flower), also known as the Wild Maracuja or in Thai name as "Katokrok", is a climber; leaves 3-lobed, base cordate or sub-cordate. Flower white; coronal radiating threads white. Fruit covered by green, thread-like bracts (Figure 1A, 1B and 1C).



A) Flower

B) Fruits

C) Bracts

Figure 1 A) flower, B) fruits and bracts of *Passiflora foetida* Linn. or in Thai name, "Katokrok") (Wikipedia, 2007)

"Katokrok" is a creeping vine with an edible fruit and leaves with mildly rank aroma. It is native to northern South America, the West Indies and South East Asia, including Thailand. *P. foetida* is a popular folk-remedy plant in Central and South America. The leaf has been used as a medicine for vomiting, eczema, and chronic ulcer (Moongkarndi et al., 2004). In Nigeria, people use leaf infusion to treat hysteria and insomnia. In Brazil, *P. foetida* is used in the form of lotions for itching and skin diseases with inflammation (Dhawan et al., 2004).

The phytoconstituents found in *P. foetida* comprise of alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds. These compounds include flavonoids pachypodol, 7,4'-dimethoxyapigenin, ermanin, 4',7-O-dimethyl-naringenin, 3,5-dihydroxy-4,7-dimethoxy flavone, C-glycosyl flavonoids chrysoeriol, apigenin, isovitexin, vitexin, 2''-xylosylvitexin, luteolin-7- β -D-glucoside, kaempferol, cyanohydrin glycosides tetraphyllin A, tetraphyllin B, tetraphyllin B sulphate, deidaclin, volkenin, linoleic acid and linolenic acid, and passifloricins (alpha-pyrone) (Dhawan et al., 2004). Flavonoids have been known for their effects on cardiovascular system (Formica and Regelson, 2000). The relative order of potency of the six active flavonoids for positive inotropic effect (PIE) was quercetin > morin = kaempferol = HEPTA > luteolin = apigenin (Itoigawa et al., 1999).

Recent studies revealed that extracts of *P. foetida* had antidepressant activity. The extracts *P. foetida* increased the active swimming time of mice in the open-space swimming test (Wijagkanalan, 2005). In addition, *P. foetida* extracts possessed marked binding affinity to D₁ and 5-HT_{1A} receptors isolated from rat brains (Wijagkanalan, 2005). It is possible that the extracts of *P. foetida* mediate the antidepressant activity through direct or indirect activation of these two monoaminergic receptors. In addition, the extracts of *P. foetida* may be potential candidates for developing as remedy in the treatment of depression. Studies have revealed that extracts of *P. foetida* (sub-fractions PF003-1 and PF003-2) elicited antidepressant effects (Wijagkanalan, 2005). However, the mechanism of action is still unclear at present.

Effects of antidepressant on the heart

There are several types of antidepressants for treatment of depressive disorders. These include the selective serotonin reuptake inhibitors (SSRIs), the tricyclic antidepressant (TCAs), and the monoamine oxidase inhibitors (MAOIs). The SSRIs and other medications that affect neurotransmitters such as dopamine or norepinephrine generally have fewer side effects than the TCAs.

Although the TCAs are effective against depression, they are associated with cardiovascular side effects including orthostatic hypotension, slow cardiac conduction, antiarrhythmic activity, and increased heart rate (Roose et al., 1999). TCAs have anticholinergic effects, caused excessive blockade of norepinephrine reuptake at the postganglionic synapse, direct alpha adrenergic blockade. Another mechanism of toxicity is cardiac sodium channel blockade, which results in slow the membrane depolarization (Thanacoody and Thomas, 2005, Yildiz et al., 2002). In comparison with TCAs that block the reuptake of serotonin (5-HT) and norepinephrine (NE), SSRIs are largely 5-HT reuptake blockers and have little effect on other neurotransmitters. In addition, TCAs may be associated with higher risk of myocardial infarction than SSRIs (Cohen et al., 2000). The cardiovascular side effects of SSRIs include modest slowing of heart rate, slightly effect on either resting or postural blood pressure, and little influence on electrocardiographic PR interval, QRS duration, or QTC interval (Glassman, 1998). However, there are case reported of QTC prolongation, first-degree block, and orthostatic hypotension in SSRI-treated patients (Rodriguez et al., 2001). SSRIs may

stimulate vasoconstriction with results in myocardial ischemia in patient with and without Coronary Heart Disease (CHD) (Fricchione et al., 1993). SSRIs are well tolerated in patients with cardiac diseases (Strik et al., 2000; Rasmussen et al., 1999; Roose et al., 1998).

Regulation of cardiac function

The primary function of the heart is to produce the driving force that propels blood through the vessels of the circulatory system. Along with the lungs, the heart works to distribute oxygenated blood and nutrients to tissue and organs of the body. The functional capabilities of the heart are directly related to its anatomic characteristics and autonomic system.

The autonomic nervous system

The autonomic nervous system is broadly divided into the body and exerts control over heart rate and velocity of impulse conduction. The efferent peripheral autonomic nervous system is consisted of sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). The heart is innervated by both sympathetic and parasympathetic nerves.

1. The sympathetic nervous system

Sympathetic innervation is widespread to all areas, including the ventricular myocardium. In general, sympathetic activation increases heart rate (chronotropic effect) and increases speed of conduction (dromotropic effect) as well as induced heart

muscle to contract more forcefully (inotropic effect). These effects are achieved by release of the neurotransmitter norepinephrine (NE) from sympathetic nerve endings. Binding of NE to receptors on heart muscle cell membranes results in opening of membrane ion channels, which allow more rapid sodium and calcium ion entry (Copstead LC and Banasik JL, 2005).

2. The parasympathetic nervous system

Parasympathetic innervation via vagus nerve, is localized primarily in sinoatrial (SA) and atrioventricular (AV) nodal areas. Parasympathetic activation results in a reduction of heart rate and speed of action potential conduction. Acetylcholine (ACh) is the neurotransmitter released by parasympathetic nerve endings. The binding of ACh to muscarinic receptors on plasma membrane of heart muscle cells increase membrane permeability to potassium ions (K^+), allowing K^+ leakage and hyperpolarization. Consequently, it is more difficult to reach threshold and initiate an action potential. The resting heart is normally under influence of parasympathetic system, which results in an SA discharge rate of about 75 beats per minute. If parasympathetic activity is blocked, the spontaneous discharge rate of SA nodal cells increases to about 100 beats per minute (Copstead and Banasik, 2005).

Cardiac excitation-contraction coupling

The term "excitation" refers to the transmission of a neural impulse (an action potential) through the peripheral nerves and eventually along the sarolemma and t-tubules. "Contraction" obviously refers to the muscle contraction and the biochemical

events responsible for the contraction. Then, excitation-contraction coupling (EC) refers to the process by which an action potential triggers a myocyte to contract.

When depolarization of myocyte occurs, calcium ions enter the cell during phase 2 of the action potential through L-type calcium channels located on the sarcolemma. This calcium triggers a subsequent release of calcium that is stored in the sarcoplasmic reticulum (SR) through calcium-release channels (ryanodine receptors). Calcium released by the SR increases the intracellular calcium concentration from about 10^{-7} to 10^{-5} M. The free calcium binds to troponin-C (TN-C) that is part of the regulatory complex attached to the thin filaments. When calcium binds to the TN-C, this induces a conformational change in the regulatory complex such that troponin-I (TN-I) exposes a site on the actin molecule that is able to bind to the myosin ATPase located on the myosin head. This binding results in ATP hydrolysis that supplies energy for a conformational change to occur in the actin-myosin complex. The result of these changes is a movement between the myosin heads and the actin, such that the actin and myosin filaments slide past each other thereby shortening the sarcomere length. Ratcheting cycles occur as long as the cytosolic calcium remains elevated. At the end of phase 2, calcium entry into the cell slows and calcium is sequestered by the SR by an ATP-dependent calcium pump (SERCA, sarco-endoplasmic reticulum calcium-ATPase), thus lowering the cytosolic calcium concentration and removing calcium from the TN-C. To a quantitatively smaller extent, cytosolic calcium is transported out of the cell by the sodium-calcium-exchange pump. The reduced

intracellular calcium induces a conformational change in the troponin complex leading, once again, to TN-I inhibition of the actin binding site. At the end of the cycle, a new ATP binds to the myosin head, displacing the ADP, and the initial sarcomere length is restored (Figure 2) (Copstead and Banasik, 2005; Kranias, 2005).

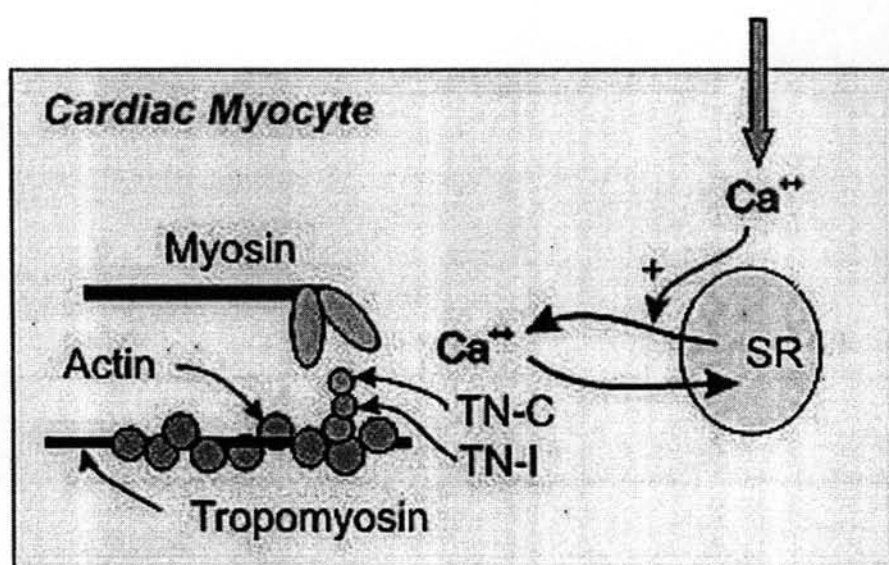
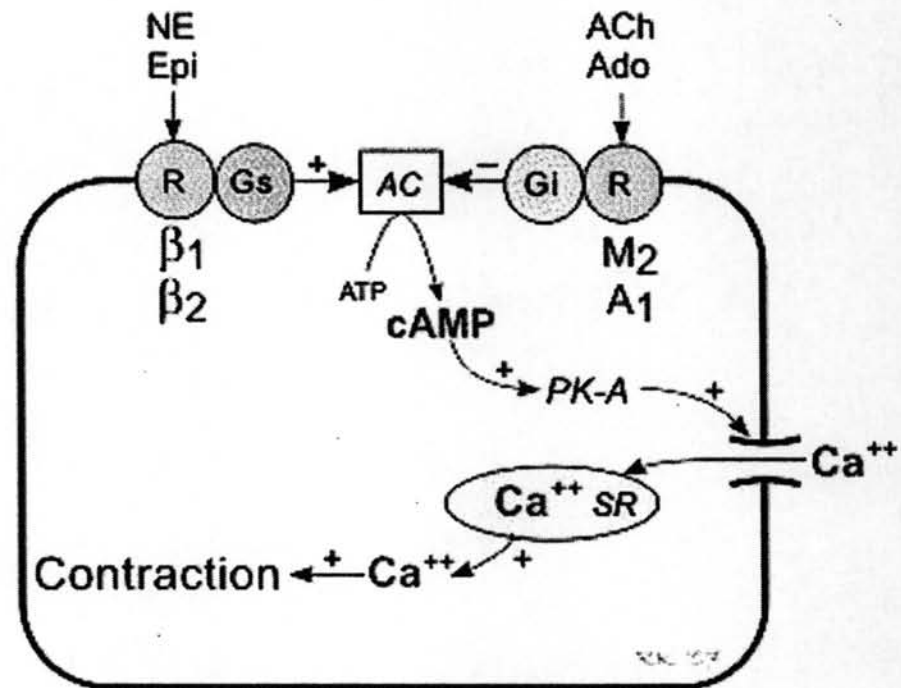


Figure 2 Cardiac myofilaments. Myosin. (thick filament) contains two heads having ATPase activity. Thin filament is made up of actin, tropomyosin, and troponin (TN). TN-C binds Ca^{2+} released by the sarcoplasmic reticulum (SR). TN-I inhibits actin-myosin binding until Ca^{2+} binds to TN-C (Kranias, 2005).

Mechanisms that enhance the concentration of cytosolic calcium increase the amount of ATP hydrolyzed and the force generated by the actin and myosin interactions, as well as the velocity of shortening. Physiologically, cytosolic calcium concentrations are influenced primarily by beta-adrenoceptor-coupled mechanisms (Figure 3).



R, receptor; Gs and Gi, stimulatory and inhibitory G-proteins; AC, adenylyl cyclase; PK-A, protein kinase A; SR, sarcoplasmic reticulum; α and β , alpha and beta-adrenoceptors; Epi, epinephrine; NE, norepinephrine; ACh, acetylcholine; M₂, muscarinic receptor; A₁, adenosine (Ado)

Figure 3 Mechanism of cardiac contraction mediated through beta-adrenergic receptor (Klabunde, 2007).

Beta-adrenergic stimulation, as occurs when sympathetic nerves are activated, increases cAMP which in turn activates protein kinase to increase in calcium entry into the cell through L-type calcium channels. Activation of the IP_3 signal transduction pathway also can stimulate the release of calcium by the SR through IP_3 receptors located on the SR. Furthermore, activation of the cAMP-dependent protein kinase phosphorylates a protein (phospholamban) on the SR that normally inhibits calcium uptake. This disinhibition of phospholamban leads to an increased rate of calcium uptake by the SR. Therefore, beta-adrenergic stimulation increases the force and shortening velocity of contraction (i.e., positive inotropy), and increases the rate of relaxation (i.e., positive lusitropy) (Klabunde, 2007).

Receptors and the regulation of cardiac function

The regulation of cardiac function involves receptors on cardiac tissues. The neurotransmitter that releases from nerve ending binds with receptor and regulates cardiac function.

The major receptors include muscarinic, adrenergic and serotonergic receptors. NE that releases from sympathetic nerve ending binds with α -adrenoceptor or β -adrenoceptor on the heart muscle cell (Figure 4). Expression of β -adrenoceptor on the cardiac muscle, especially β_1 receptor, is larger than another receptors. Activation of β_1 receptor results in elevation of rate and force of contraction. In contrast, ACh which releases from parasympathetic nerve ending binds with muscarinic receptor and

results in reduction of rate and force of contraction (Figure 5). Serotonin (5-Hydroxytryptamine, 5-HT) exerts many actions and has many species difference, making generalization difficult. The actions of serotonin are mediated through a remarkably large numbers of cell membrane receptors. The serotonin receptors that have been characterized thus far are described in Table 1. Seven families of 5-HT receptor subtypes (those given numeric subscripts 1 through 7) have been identified, six involving G protein-coupled receptors and one a ligand-gated ion channel (Kaumann and Levy, 2006; Borne, 1994). Among these receptor subtypes, several lack any recognized physiologic function. In cardiovascular system, serotonin directly causes the contraction of vascular smooth muscle. It binds with 5-HT receptor directly and causes small positive inotropic and chronotropic effects on the heart, mainly through 5-HT₂ receptors (Fig 6) (Katzung et al., 2004). In the guinea pig heart, it had been reported that the action of 5-HT is tyramine-like and was blocked by a β -adrenoceptor antagonist (Trendelburg U, 1960). Serotonin-induced tachycardia is an indirect effect mediated by the release of calcitonin-gene related peptide (CGRP) from sensory nerve terminals via activation of 5-HT₃ receptor (Nishio et al., 2002). In rat isolated atria, a high concentration of 5-HT can bind to β_1 -adrenoceptors and inhibit their activation by NE (Davy et al., 1997).

Hence, any xenobiotics that bind to these receptors can influence heart functions. For examples, flesinoxan, a very potent and selective 5-HT_{1A} ligand, decreased blood pressure and heart rate and its effects were antagonized effectively by

the putative 5-HT_{1A} antagonist, pindolol (Wouters et al., 1988). In conscious unrestrained rat, stimulation of CNS 5-HT_{1A} receptors with low dose of 8-OH-DPAT (5-HT_{1A} receptor agonist) produced cardiovascular activation, elevation of blood pressure and heart rate whereas at high doses, it produced depressor and bradycardia response (Dedeogin and Fisher, 1991). In addition, it has been demonstrated that 5-HT_{1A} was involved in cardiovascular stress responses (Van den Buuse and Wegener, 2005).

Taken together, it is possible that extracts of *P. foetida* have the effect on the heart. Thus, the present study aimed to investigate the effects of these extracts on the chronotropic and inotropic responses as well as its mechanisms of action. In addition, the action of *P. foetida* extracts on heart function may involve the adrenergic and serotonergic system.

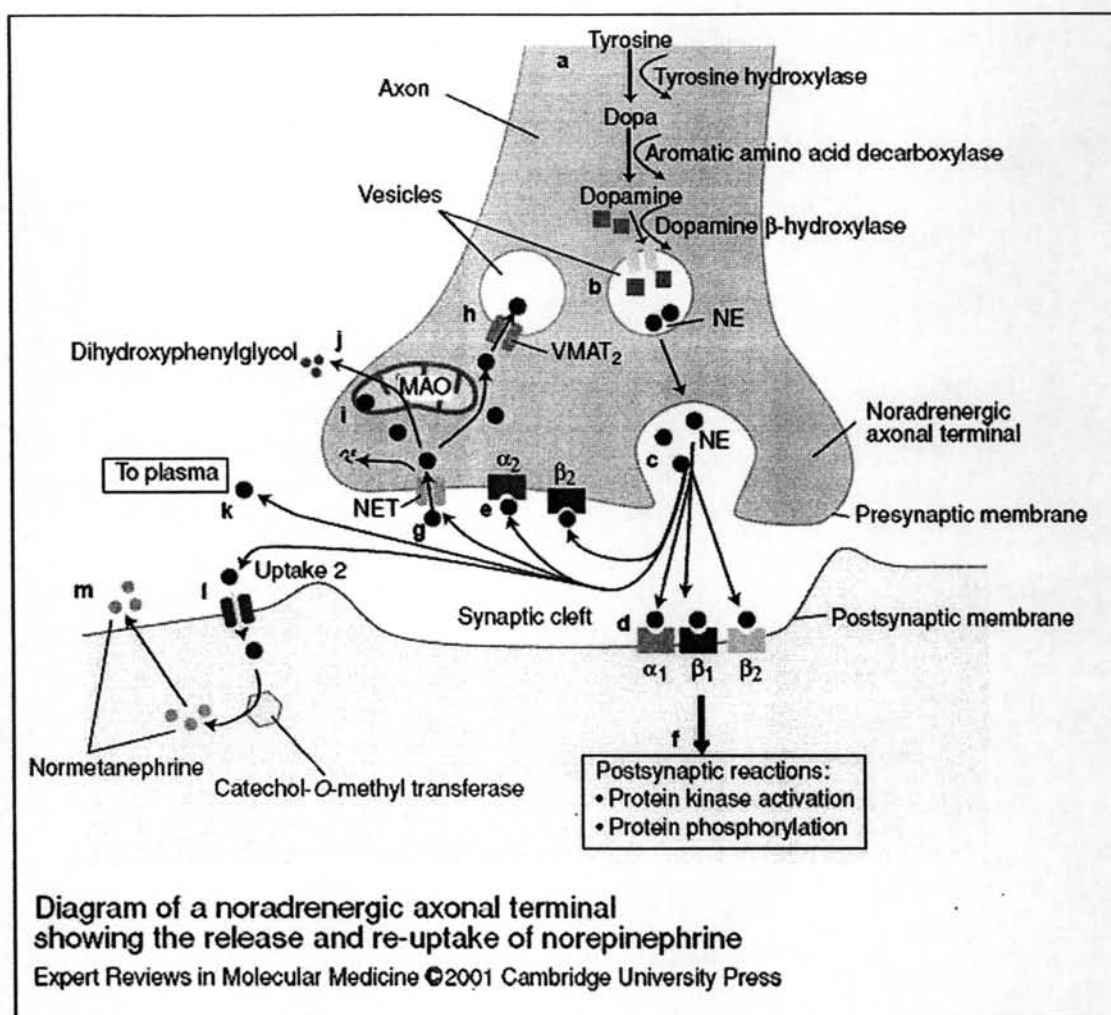


Figure 4 Diagram of a noradrenergic axonal terminal showing the release and re-uptake of norepinephrine (Tellioghr and Roberson, 2001).

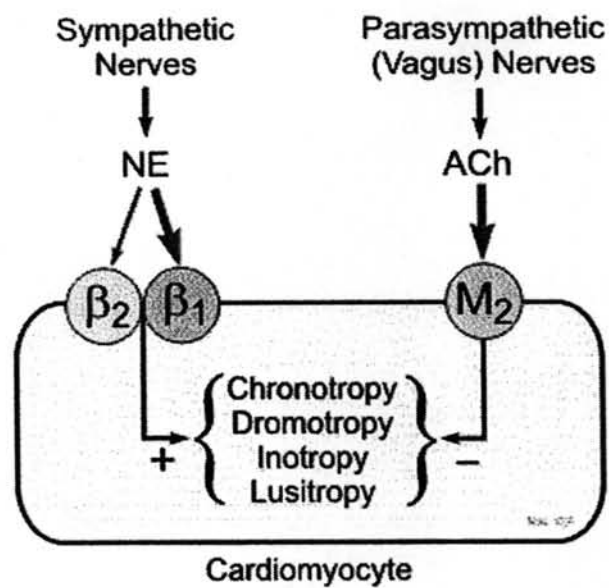


Figure 5 The activation of NE and ACh on cardiomyocyte (Klabunde, 2004).

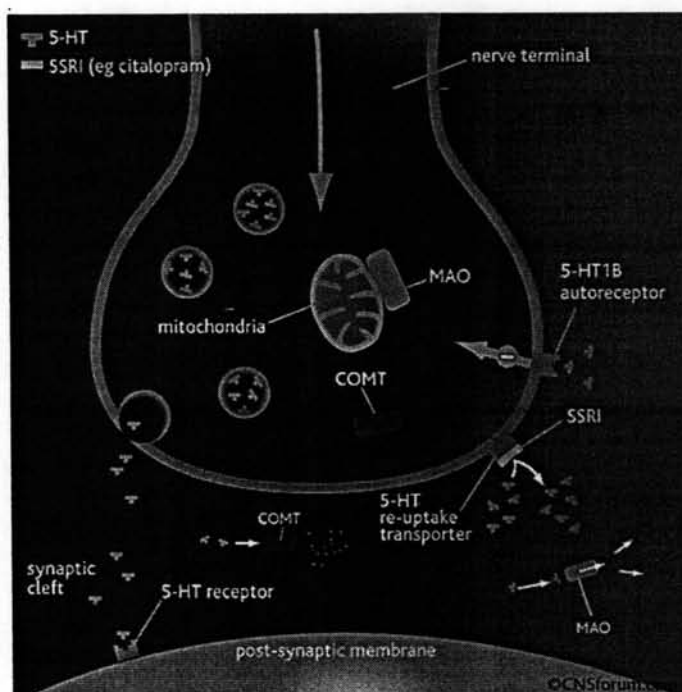


Figure 6 Diagram shows the release and reuptake of serotonin (Rang et al., 2001)

Receptor	Actions	Agonists	Antagonist
5-HT _{1A}	CNS: neuronal inhibition, behavioural effects (sleep, feeding, thermoregulation, anxiety)	bupirone, psilocin	spiperone, methiothepin, ergotamine, yohimbine
5-HT _{1B}	CNS: presynaptic inhibition, behavioural effects; vascular: pulmonary vasoconstriction	ergotamine, sumatriptan	methiothepin, yohimbine, metergoline
5-HT _{1D}	CNS: locomotion; vascular: cerebral vasoconstriction	sumatriptan	methiothepin, yohimbine, metergoline, ergotamine
5-HT _{2A}	CNS: neuronal excitation, behavioural effects, learning; smooth muscle: contraction, vasoconstriction / dilatation; platelets: aggregation	α -methyl-5-HT, LSD (CNS), psilocin, DOI	Nefazodone, trazodone, mirtazapine, ketanserin, cyproheptadine, pizotifen, LSD, atypical antipsychotics (PNS)
5-HT _{2B}	stomach: contraction	α -methyl-5-HT, LSD (CNS)	yohimbine, LSD (PNS)
5-HT _{2C}	CNS, choroid plexus: cerebrospinal fluid (CSF) secretion	α -methyl-5-HT, agomelatine, LSD (CNS), psilocin, DOI	mesulergine, agomelatine, LSD (PNS), fluoxetine
5-HT ₃	CNS, PNS: neuronal excitation, anxiety, emesis	2-methyl-5-HT	metoclopramide (high doses), renzapride, ondansetron, alosetron, mirtazapine, memantine
5-HT ₄	GI, CNS: neuronal excitation, gastrointestinal motility	5-methoxytryptamine, metoclopramide, renzapride, tegaserod	GR113808
5-HT _{5A}	CNS (cortex, hippocampus, cerebellum): unknown	5-carboxytryptamine; LSD (partial agonist) ⁽¹⁾	unknown
5-HT ₆	CNS: unknown	LSD	SB271046
5-HT ₇	CNS, GI, blood vessels: unknown	5-carboxytryptamine, LSD	methiothepin, risperidone

Table 1 Summary of characterized 5-HT receptors, with selected agonist/antagonist agents.