CHAPTER II

LITERATURE REVIEW

General Information of the Mule

The mule (*Equus mulus*) is a cross-breed between domestic donkey (*Equus asinus*) (figure 1) and domestic horse (*Equus ferus caballus*) (figure 2). The Mules British Society explained the mule appearance as the horse body with the donkey extremities, such as long ears, short mane, low wither, and straight legs with small, hard, dense, upright, straight-sided hooves; as shown in figure 3. They are less susceptible to illness or lameness than horses and durable in critical temperature. They can cope very well to low quality or insufficient roughages. Because of these characters, they are strong, tough, and have long working lives (1). Untrained mules are stubborn but smart enough to be refined. A well-trained mule is calm, kind, patient and easy to be controlled.

In Thailand, mules are used for tacking and transporting military equipment to the places where are not accessible by other vehicles. The Third Livestock and Agricultural Division of the Veterinary and Remount Department, The Royal Thai Army, Chiang Mai, is the only unit responsible for mule reproduction in this country. The yearling male mules have to be castrated before training (2-4). The yearling male mules are untrained. Therefore, a standing castration is not suitable for this group of animal. General anesthesia is a type of chemical restrain. The technic provide whole body stablization and suitable for all type of surgical procedures, including male castration (2-4).

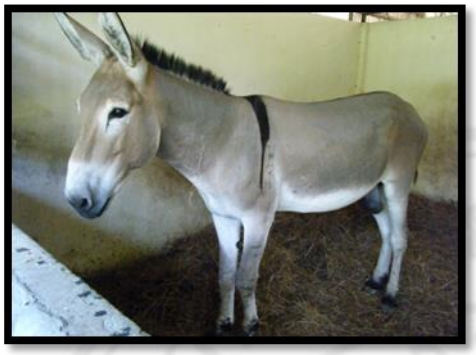

Figure 1. Domestic donkey (*Equus asinus*)

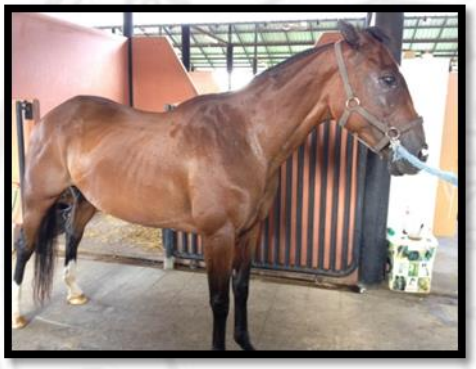

Figure 2. Domestic horse (*Equus ferus caballus*)

Figure 3. Mule (*Equus mulus*)

Anesthesia in Equine practice

Equine anesthesia, similarly to other species, consists of local and general anesthesia. General anesthesia involves the management of sedation (premedication) and the following anesthesia which can be divided into 2 phases; induction phase and maintenance phase. Induction phase is normally performed by using injectable drugs while maintenance phase can be accomplished by intravenous (total intravenous anesthesia: TIVA), inhalational, or combined inhalational and intravenous anesthesia (partial intravenous anesthesia: PIVA). In the field practice, TIVA is commonly applied because it is more economical and requires less complicated equipment than other two methods, especially in short procedures e.g. castration (7, 10, 22-23).

It has been proved that premedication with sedative drug prior to anesthesia, can help smooth induction and can reduce cost, time, amount of anesthetic drug, and risk of anesthetic complications (3, 5-11). In equine practice, there are number of preanesthetic agents or premedicants available: for example; phenothiazines, benzodiazepines, and alpha$_2$ adrenergic agonists. Acepromazine is a tranquillizer in phenothiazine group. It has long duration of action, but the onset is slow. It is suggested to combine acepromazine with other agents for better chemical restraint and premedication (7). Benzodiazepines, e.g. diazepam, are not directly for sedation purpose but rather for muscle relaxation; hence, they could not be used alone as a premedicant (6). Alpha$_2$ adrenergic agonists, including xylazine and detomidine, are more potent premedicants than the above two groups because of their rapid onset, analgesic effect, and muscle relaxant property (5-6, 10, 22-23).

Adrenergic receptors

Adrenergic receptors (adrenoceptors) are a member of seven transmembrane G protein-coupled receptors. Adrenoceptors were classified into alpha (α) and beta (β) subtypes based on the responses to series of catecholamines (24). Beta adrenergic receptors (β adrenoceptors) consists of 3 subtypes; β_1, β_2 and β_3. Alpha adrenergic receptors (α adrenoceptors) were previously divided into 2 subdivisions; α_1 and α_2 base on the synapse location, postsynaptic and presynaptic respectively (25-28). But it has been proved later that the α_2 receptors could also be found postsynaptically and extrasynaptically (29). Therefore, nowadays the receptors are classified based on pharmacologic basis instead. The receptors that have antagonistically response to prazosin are classified to α_1 adrenoceptors while the ones that respond antagonistically to yohimbine, atipamezole and idazoxan are classified to α_2 adrenoceptors (30-32). After activation of α adrenoceptors by their agonists, the transmembrane signal transduction occurs and the following effects will depend upon the receptor subtypes.

Alpha$_2$ adrenergic receptors

The α_2 adrenoceptors are found in both central and peripheral nervous systems not only presynaptically, but also postsynaptically. The presynaptic α_2 adrenoceptors inhibit norepinephrine releasing after binding agonists. The postsynaptics are located on smooth muscle of blood vessels, platelets and causing platelet aggregation and vasoconstriction once activated.

The α_2 adrenoceptor consists of 3 homologous subtypes; α_{2A}, α_{2B}, α_{2C} based on pharmacological classification (6, 13-14, 33-34). In rodents, there is α_{2D} which appears functionally similar to the α_{2A} (6, 14). The α_{2A} receptors can cause many effects depended upon the organs they situate. They primarily mediate sedation by inhibit the release of norepinephrine presynaptically from the sympathetic nerve (8, 14). Similar action can be found with the α_{2C} receptors in other tissues. Inactivated α_{2C} receptors have higher affinity to norepinephrine than the α_{2A} receptors so the α_{2C} receptors can control neurotransmitter release even in low potential frequencies, while the α_{2A} receptors need stimulation with higher frequencies to response. The α_{2A} receptors can inhibit gastrointestinal motility (6, 8, 35). The α_{2B} response causes the initial hypertensive phase from vascular resistance. Contrarily, the α_{2A} response causes the long-acting hypotension. Both α_{2A} and α_{2B} receptors can mediate the antinociceptive effect (6, 8, 35). The α_{2C} receptors contribute to vascular regulation to mediate hypothermia. Both α_{2A} and α_{2C} receptors can also inhibit dopamine release in basal ganglia and inhibit 5-hydroxytryptamine (5HT) release in the hippocampus and the brain cortex. Apart from those, they can inhibit insulin release by decreasing cyclic adenosine monophosphate (cAMP) in pancreatic islet cells.

Transmembrane signaling via α_2 adrenoceptors

The transmembrane signaling mechanism of the α_2 adrenergic response involves 3 factors which are the receptor protein, the Guanine nucleotide binding protein (G protein) and the effectors (14, 34, 36). The receptor proteins consist of 415-480 amino acids in length and contain seven domains of hydrophobic amino acids, separated by segments of hydrophilic amino acids. The proteins interlace backward and forward with hydrophilic amino acids through the extracellular and intracellular membrane, forming the alpha helices (34). G proteins or Guanine nucleotide binding proteins are the amino acid sequences that are heterotrimeric with 3 subunits; alpha (α), beta (β) and gamma (γ) (34, 36). The G protein has molecular weight between 39-46 kD (37). The effectors, to the present knowledge, are categorized into 5 mechanism; adenylyl cyclase (AC), cGMP phosphodiesterase (cGMP PDE), phospolipase C (PLC), Ca^{2+} channels and K^+ channels (34).

The action of transmembrane signaling is demonstrated in Figure 4. After activated by their agonists, the α_2 receptor changes its structure to be more attractive to the G protein leading to changing of an inactive guanine diphosphate (GDP)-bound G protein to an active guanine triphosphate (GTP)-bound G protein. The activated G protein then mediates the secondary messenger through the effectors, which; in this case, is the inhibition of the adenylyl cyclase activity resulting in decreasing of intracellular cAMP level. They may sometime regulate the ion channel activities as well as activate the enzyme in signal transduction (36).

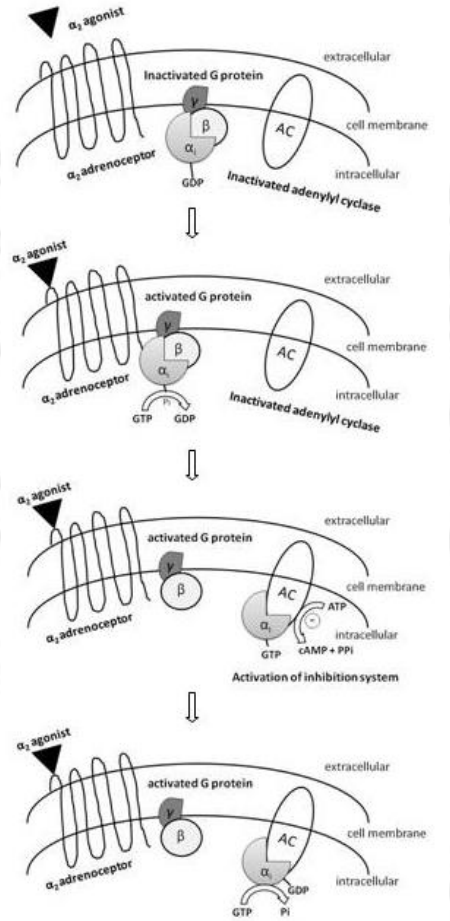

Figure 4. G protein-coupled receptor signaling transduction. The three subunits of a G protein called α, β and γ are bound by GDP in inactive form and bound to GTP when activated by coupling with activated α_2 adrenoceptors. Activated G-protein then dissociates from the receptor and causes the secondary messenger through to an effector. In case of the activation through the α_2 receptor, the effector can be the adenylyl cyclase inhibition. After that, GTP that bind to α subunit was hydrolyzes by GTPase to GDP. The G protein then returns to be inactive form again (34, 36-37).

Alpha$_2$ adrenergic agonists

Alpha$_2$ adrenergic agonists (α_2 agonists) are imidazole compounds that bind to adrenoceptors resulting in sedation, analgesia, and muscle relaxation. The α_2 agonists; for example, xylazine, detomidine, romifidine and clonidine are normally used for sedative and premedicative drugs in veterinary practice but xylazine and detomidine are commonly used in equine practice. The α_2 agonists can bind to both α_1 and α_2 adrenoceptors but much more selective to α_2 adrenoceptors. Xylazine and detomidine possess high affinity to α_2 adrenoceptors with the α_2:α_1 binding ratio of 160:1 and 260:1, respectively. However, in case of high dosages of these drugs, the excess drug may bind to α_1 adrenoceptors more (12-14).

Effects of α_2 agonists

- Central nervous system

Action of the α_2 agonists to the brain cause sedative and analgesic effects, while the action to the spinal cord involves analgesia and muscle relaxation.

- Sedative effect

Alpha$_2$ agonists activate postsynaptic α_2 adrenoceptors in the nucleus coeruleus of the brain. The decreasing of norepinephrine release from this area of the brain causes the signal transduction of hypnotic response by decrease the central nervous system (CNS) sympathetic outflow (16). So the animal shows signs of sedation such as head lowering, lower lip and eyelids drooping, ataxia and reduced environmental response. High dosage of α_2 agonists may adversely induce CNS excitation due to nonselective activation of α_1 adrenoceptors (14, 16).

- Analgesic effect

The α_2 agonists cause analgesia by activation of the α_2 adrenoceptors located at the dorsal horn neuron of spinal cord leading to the inhibition of the neurotransmitter release. These eventually increase threshold of nociceptive withdrawal reflex and threshold of temporal summation which use as measure of antinociceptive activity (16, 33, 38-39). For this reason, the α_2 agonists, either alone or combined with opioids, can block pain after epidural administration (14).

- Cardiovascular system

Inhibition of norepinephrine from peripheral presynaptic nerve endings to myocardium following the α_2 agonists administration causes atrio-ventricular (AV) blocks and bradycardia (14, 16, 40). Among α_2 agonists, xylazine has the shortest duration of bradycardia action and this effect is minimum comparing to the equisedative doses of detomidine and romifidine (41). The activation of postsynaptic α_2 adrenoceptors in vascular smooth muscle can cause both hyper- or hypotension, based on subtypes of the receptors α_{2A} or α_{2B} reaction. The α_{2B} response can cause hypertension in the initial phase of the α_2 agonists' effect (42-43). Contrarily, the α_{2A} response reduces the sympathetic outflow lead to decrease cardiac output and then causes hypotension (42-43). As the α_{2A} is the major subtype of the α_2 adrenoceptor, hypotension is more common side effect of α_2 agonists rather than hypertension which can be found only in the beginning of sedation or in higher dosage and prolong use of the drug (14, 16, 44).

- Other effects

Apart from nervous and cardiovascular systems, actions of α_2 agonists though activation of peripheral α_2 adrenoceptors in other organs were reported. Respiratory

system can be depressed by increasing airway resistance following by decreasing of the arterial oxygen while arterial carbon dioxide transiently increases (16). The α_2 agonists bind to postsynaptic α_2 adrenoceptors in islets of Langerhans in pancreatic cell to inhibit insulin releasing causes elevation of glucose concentration in the urine. The swallowing reflex is also suppressed after a α_2 agonist administration, so excessive salivation is usually found. Releasing of gastric acid and decreasing of gastrointestinal motility following α_2 agonists administration cause emesis in small animals (16) but this never happens to horses due to very strong cardiac sphincter (45-46). Alpha$_2$ agonists can also inhibit the release of antidiuretic hormone (ADH), so inhibit water reabsorption from the collecting ducts of kidneys (16). The skeleton muscle is inhibited by intraneural transmission of impulses in CNS causing muscle relaxation (16).

Xylazine and Detomidine in Equine practice

Xylazine and detomidine are α_2 agonists that are the most commonly used for sedative agents in many countries. The duration of action of xylazine is 20-40 min and detomidine is 90-120 min after IV injection (8). Detomidine provides longer duration of action in much lesser amount of the drug when compare to xylazine. They are rapidly metabolized by the liver and excreted in the urine. The side effects of α_2 agonists need to be aware of a veterinarian, including bradycardia, changes in blood pressure (hypertension in the beginning and then hypotension), severe respiratory depression, respiratory acidosis, ataxia, sweating, increased salivation, snoring, gut motility reduction, penile protrusion, and diuresis.

Xylazine

The chemical structure of xylazine is 2(2,6-dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazine-2-amine (47) or $C_{12}H_{16}N_2S$ as shown in figure 5. The common preparation in veterinary practice is for intravenous (IV) or intramuscular (IM) administration with the concentration of 20 mg/ml for small animals or ruminants and 100 mg/ml xylazine hydrochloride for horses.

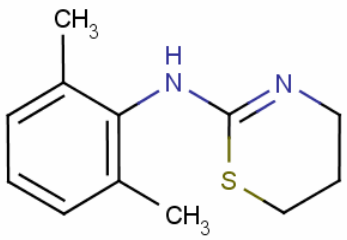

Figure 5. The chemical structure of xylazine (48)

Pharmacokinetics: Xylazine is metabolized by liver and excreted by kidney. Garcia-Villar and his colleague (1981) reported that systemic half life and transient distribution phase with half life were 50 min and 5.9 min, respectively after IV injection of a single dose in horses (49). The bioavailability, measured by the ratios of the area under the plasma concentration versus the time curve, of IV and IM injection were 40% and 48%, respectively (49). At the dose of 0.6 mg/kg via IV route, the half-life of the drug in the mules seems to be much shorter. Latzel (2012) reported that the elimination half-life of xylazine at this dose in mules was only 32 min (50). However, the authors concerned that this dose was probably too low for the mules identified by unsatisfied sedative level and rapid recovery (50). Latzel found that velocity constant during elimination of mules (0.023/min) was longer than horses (0.015/min) and velocity constant for distribution from peripheral to central compartment of mules (0.097/min) was faster than of horses (0.043/min) (50).

Pharmacodynamics: In horses, the maximum sedative and analgesic effects of xylazine reach within 5 min and remain for 30 min before gradually decreasing in the next 30 min after administration at the dose 1.1 mg/kg IV. Xylazine must be used with care in horses undergoing inhalational anesthesia because it required to procedure surgical plane decreases the minimum alveolar concentration (MAC) of halothane and isoflurane, so it is recommended to reduce any anesthetic drug following xylazine administration. The suggestion is also applied to donkey (18). A study comparing between horses and mules demonstrated that the reaction and behavior of the horses responded to xylazine was rapid and was more satisfied than mules (50). At 2 min after receiving xylazine, the HR of the mules decreases to the

lowest value similarly to horses but recovers quickly within 10-15 min to the baseline values (50).

Clinical use: In horses, xylazine can be used alone or in combination with butorphanol or other opioids for sedation, and also used as a premedication. It can also be used as one of the agents in combination of triple drip (xylazine, ketamine and guaifenesin) for IV anesthesia. Xylazine dose range is from 0.3-1.1 mg/kg (51). Adversely, excitement, seizure and collapse can occur after receiving IV overdose or after an accidental intracarotid injection by central α_1 receptors activation (52).

Detomidine

Detomidine is an imidazole compound called 4-([2,3-dimethylphenyl] methyl)-1H-imidazole or $C_{12}H_{14}N_2$ (53) (figure 6). The IV and IM injectable preparation for horses is the solution with concentration at 10 mg/ml detomidine hydrochloride. The sublingual administration for horses is the gel containing 7.6 mg/ml detomidine hydrochloride (54).

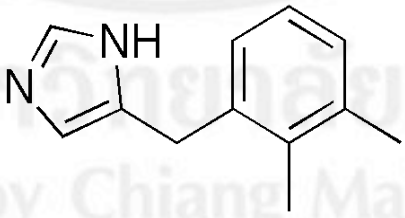

Figure 6. The chemical structure of detomidine (55)

Pharmacokinetics: Detomidine is metabolized by liver and excreted by kidney. The higher concentration of detomidine leads to the longer elimination half

life, larger amount of the volume distribution but lower velocity of clearance. In horses receiving detomidine at 50 µg/kg IV, the elimination half-life, volume of distribution and clearance were reported to be 9.7 h, 6.8 L/kg and 8.1 ml/min/kg, respectively (8) but at 30 µg/kg, these parameters were 30 min, 470 ml/kg and 12 ml/min/kg, respectively (56).

Pharmacodynamics: Detomidine at 20 µg/kg is equivalent, in terms of sedative and analgesic effects, when compared to 1.1 mg/kg xylazine but has longer duration of action (57). However, it was reported that, in conscious horses, detomidine at 10 to 20 µg/kg IV can adversely cause decreasing of heart rate and cardiac output (58). A study showed that 30% of horses were diagnosed to AV block within 5 min after receiving detomidine IV or IM at a dose of 40 µg/kg (58). Reduction of heart rate and cardiac output could prolong for 45 min to 1 hr (58). Mean arterial blood pressure dropped for 90 min to 2 hr. Detomidine can also cause respiratory and gastrointestinal suppression both in horses and ponies (58). On the other hand, in case of colic, it can be very effective visceral analgesic.

Clinical use: Detomidine produces sedation, analgesia and muscle relaxation in horses. It can be used alone and combined with butorphanol or other opioids for standing sedation and analgesia in abdominal-pain horses. The range of detomidine dosage in horses is 5 to 20 µg/kg for IV administration but it can be given intramuscularly with 10 to 40 µg/kg (8). Excited or nervous horses may require higher doses of detomidine but must be kept an eye for ataxia and cardiovascular side effects.

Barbiturates

Barbiturates are derivatives of barbituric acid combining malonic acid. They can be given as a bolus injection following premedication with acepromazine, α_2 agonists or without premedication. They can adversely produce cardiovascular depression and transient apnea. The barbiturates are classified in to 4 types based on duration of action; long, intermediate, short, and ultrashort acting. Example of barbiturate drugs available for veterinary used are phenobarbital sodium, pentobarbital sodium, and thiopental sodium. Thiopental sodium, a common equine anesthetic drug, is classified into the ultrashort acting barbiturate.

Thiopental sodium (Thiopentone) has very short action in anesthesia approximately 5-15 min. A bolus injection at dosage of 10-15 mg/kg IV induction can be given to horses without premedication (7). With premedication using xylazine 1 mg/kg or detomidine 20 µg/kg, thiopental induction can reduce to 5-6 mg/kg (7). Before falling down, the animal should be restrained quietly but firmly. They usually sink down slowly into sternal position and then lateral recumbency within 1-2 min after bolus injection. The anesthesia can be prolonged with incremental doses of thiopentone (one-quarter to one-half of the induction dose) but the total recommended doses for one time general anesthesia in horses should not exceed 11 mg/kg (7). Thiopentone can produce cardiovascular depression and transient apnea. A solution of thiopentone can be irritation so a proper intravenous catheterization is recommended for intravascular injection of the solution (7). The solutions should be diluted to 5-10 %w/v before administration to the large horses. Recovery depends on the metabolism to eliminate thiopentone within the circulation and tissues. Remaining of thiopentone within the tissues takes time to clear out. The prolonged recovery can

be affected from incremental dosage. Thiopentone is highly protein bound; therefore, dose reduction is advised in hypoproteinemia animal (7). Because thiopentone has no analgesic effect, premedication with agents such as α_2 agonists is recommended.

Anesthesia in donkeys/mules

Donkeys and mules have different behaviors and may respond differently to stimulants comparing to domestic horses. The effect of α_2 agonists is varied among equine species. It is suggested that mules require 50% more sedative drug than donkeys and horses to make a sufficient sedation comparable to horses (18-21, 50). Mule and donkey's plane of anesthesia are more difficult to observe. Eyes signs, such as nystagmus, palpebral, and corneal reflexes are not always reliable for the depth of anesthesia. Frequently, the eyes are stable until the mules or donkeys move without nystagmus (18). The normal respiratory rate range of mules and donkeys is higher but the movement of thoracic wall is less extent than horses. The most effective and sensitive indicator of the depth of anesthesia is blood pressure which can be measured by either non-invasive or invasive techniques similarly to horses. Blood pressure will increase when plane of anesthesia becomes lighter (18). The recovery is usually smoother in donkeys when compare to horses but still need assisting of holding the head and tail during standing up. For pain management in donkeys and mules after operation, phenylbutazone or flunixin meglumine can be given (18).