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Cannabinoids: Forensic Toxicology and Therapeutics

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1. Introduction

Marijuana, *hashish* and other psychoactive products obtained from *Cannabis sativa* are the most produced and trafficked illicit drugs around the world (CND, 2006).
It is difficult to estimate the moment when man began to use some of the preparations from *cannabis sativa*. Thus, the reported consumption of the plant and its derivatives appears as an ancient practice, in many parts of the globe, from India to China, extending from the Middle East (Persia, Asia Minor and Egypt) to Africa, through the Christian culture, until the West (Ellenhorn & Barceloux, 1988; Ladrón de Guevara & Moya Pueyo, 1995; Rodríguez-Vicente et al., 1995). The effects that these compounds have on an individual brain have been addressed in several instances, such as religious practice, or simply in the search of pleasurable sensations. *Cannabis sativa* has been used in China nearly for five thousand years, being its cultivation related to fiber, oil and seeds production (Camp, 1936). However, Asians also knew its narcotic action in the seventh century BC, incorporating *cannabis* in their religious rituals and as a therapeutic agent, in neurological and psychiatric diseases (Mechoulam, 1991).
Cannabis consumption came to the Iberian Peninsula across North Africa, once conquered by the Arabs. But its presence was ephemeral, not achieving a significant presence on all the Christian kingdoms (Nahas, 1982). Ramazzini, an eighteenth century physician, studied its potential toxic effects, but it was O'Shaughnessy, an Irish surgeon who lived in India as a British colonial army doctor, the first scientific researcher to carry out pharmacological studies with the plant and the promoter of its application in therapy (Nahas, 1973; O'Shaughnessy, 1842). Thus, in 1842, after reviewing its therapeutic use in India and after some experimental research on animals, he introduced *Cannabis* in Europe (Robson, 2001). Indeed, this doctor was impressed with the outstanding application of this drug as a muscle relaxant, anticonvulsant, analgesic and anti-emetic. However, due to its uncontrollable power, there was a rapid decline in its therapeutic value. Thus, in 1840, the French physician Jacques-Joseph Moreau, considered as the father of psychopharmacology, described in his book "*Du Haschisch et de l'alienation mentale, Psychologiques études*" (1845), the toxic effects of

hallucinogens, calling attention to the danger of its use, since it could produce individual and social deterioration, and also cause addiction. Since 1971, the use of *cannabis* was controlled by the so-called "Drug Abuse Act", which forbade the use of both medical herbs and their active constituents, cannabinoids. Its use had already been removed from medical practice since 1932, the year it was eliminated from the British Pharmacopoeia. Ten years later, it was removed from the United States and 34 years later, from the Indian Pharmacopoeia. The controversy over its hallucinogenic actions on the brain has eclipsed its possible medical uses (Evans, 1997).
Cannabis consumption as a drug of abuse begins to spread in some European countries in the 60's and was popularized in the '70s and '80s. It is estimated that, presently, *cannabis* world production mainly occurs in America (46%), followed by Africa (26%) and by Asia (22%) (UNODC, 2007).

2. Botany: Cannabis sativa L

The genus *Cannabis* is composed of a single plant species, *Cannabis sativa* Linn., classified by Linnaeus in 1753, based on specimens from India but with different shapes. The morphological characteristics, fiber production, oils or resins, and even the size, are so varied that botanical classification becomes very difficult (Astolfi et al., 1979; Ellenhorn & Barceloux, 1988; Rodríguez-Vicente et al., 1995). Some botanists argue for the existence of three species: *Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*, while others admit the existence of only two. *Cannabis sativa*, hemp common name (*Cannabis* means hemp; *sativa* means sowing or cultivating), is a plant endemic to a geographic area between the Caspian and Black Seas, passing through Persia and India, growing up in the Far East since ancient times. Under normal conditions it can have one or two meters height reaching, in its highest development stage, up to four meters. *Cannabis sativa* has been cultivated, for centuries, due to the hemp present on the stems, seeds and oil and due to its biological active substance (Δ^9-Tetrahydrocannabinol) in the higher parts with flowers, varying its chemical composition according to the different parts of the plant. The plant growth favourable conditions are a moist soil with high nitrogen content, being the clayey soils the worse conditions to its growth (Wilsie & Reddy, 1946). This plant has been adapting to different climates, adaptation accompanied by morphological changes, especially related to the leaf (Eckler & Miller, 1912). In fact, plant cannabinoids chemistry is much more complicated than the pure Δ^9-THC and thus, multiple effects can be expected due to the additional cannabinoids presence as well as other chemicals (Turner et al., 1980).

3. Consumption patterns

The potency of *cannabis* products is determined by its Δ^9-THC content, usually given as a percentage of Δ^9-THC. ElSohly et al. (2000) estimated, in a study performed between 1980 and 1997 in confiscated *marijuana* samples, that Δ^9-THC percentage was between 1.5 and 4.2%, being, however, sometimes higher. The highest percentages were found in *marijuana* samples (29.9%), *hashish* (52.9%) and oil (47.0%). In 2005, the average or typical level of Δ^9-THC *Cannabis* resin at the retail level ranged between 1% and 17%, being this variation range difficult to explain given the common origin of most European resin.
Over the past 20 years, more modern farming methods and crops increasingly sophisticated have been developed, leading to increased potency on *Cannabis* products. In the so-called "flower power" days from the 60s and 70s, every *marijuana* cigarette contained about 10 mg

of Δ^9-THC. Currently, a cigarette can have about 150 mg Δ^9-THC (Δ^9-THC between 6 and 20%, corresponding to 60-200 mg/cigarette) or 300 mg, if mixed with *hashish* oil. Thus, nowadays, a *cannabis* consumer is frankly exposed to higher doses than in previous 60 or 70 decades (Gold, 1991; Mendelson, 1987; Schwartz, 1991). However, the Δ^9-THC content also varies extraordinarily, depending on the different C*annabis* sources and preparations. In fact, there are several *Cannabis* preparations, leading to different consumption forms and different names (even according to different countries), as well as different power degrees (Astolfi et al., 1979; Ellenhorn & Barceloux, 1988; Rodríguez-Vicente et al., 1995): the herb, consisting of several parts of the plant, presents a variable active ingredients quantity depending on the part of the plant used for its preparation. It is usually smoked alone, but it may be mixed with tobacco. It has different names according to the country (among others, in Portugal and in Mexico, *marijuana*; in Morocco and Spain, *grifa* or *marihuana*; in South Africa, *dagga*; in Great-Britain, *hemp*; in Brazil, *maconha*); the resin, also called *haxixe*, *hachis*, *hashis*, *hash*, *charas* or *chira*, is five to eight times more potent than the herb, being the product spontaneously secreted by plants in small drops, thus corresponding to the resin of the plant. It can also be extracted from the plant through organic solvents. It can be smoked in special pipes or in cigarettes, being the resin, after burning, mixed with tobacco; the hash oil, obtained by hot extraction of the plant or by hashish extraction with organic solvents and consequent evaporation, has, as a concentrated resin, a high power.

Class	Nº compound in the plant	Class	Nº compound in the plant
Cannabinoids	61	*Simple ketones*	13
Cannabigerol (CBG)	6	*Simple acids*	20
Cannabichromene (CBC)	4	*Fatty acids*	12
Cannabidiol (CBD)	7	*Simple esters and lactones*	13
$\Delta^{1(9)}$-THC	9	*Steroids*	11
$\Delta^{1(8)}$-THC	2	*Sugars and similar*	34
Cannabiciclol (CBL)	3	Monosaccharides	13
Cannabielsoin (CBE)	3	Disaccharides	2
Cannabinol (CBN)	6	Polysaccharides	5
Cannabinodiol (CBND)	2	Cyclitols	12
Cannabitriol (CBT)	6	Amino-sugars	2
Other Cannabinoids	13	Terpenes	103
Nitrogen compounds	20	Monoterpenes	58
Quaternary bases	5	Sesquiterpenes	38
Amides	1	Diterpenes	1
Amines	12	Triterpenes	2
Alkaloids spermidines	2	Mixture of terpenoid	4
Amino Acids	18	*Non-cannabinoid phenols*	16
Proteins, glycoproteins & enzymes	9	*Flavonoid glycosides*	19
Hydrocarbons	50	*Vitamins*	1
Simple alcohols	7	*Pigments*	2
Simple aldehydes	12	*Total*	421

Table 1. Compounds classes found in Cannabis sativa (Honório & Silva, 2006).

4. Chemical structure and properties

Cannabis sativa present a large number of different chemicals, as illustrated in Table 1, being cannabinoids the main class. These compounds vary in number and quantity, according to the climate, soil type, variety cultivated and the way the crop was performed.
The observed variations also depend on the part of the plant used for their extraction, the drugs preparation method for the consumption, as well as its storage conditions (Waller, 1971). *Cannabis* contains about 421 different chemical compounds, including 61 cannabinoids (Turner et al., 1980). During the consumption by smoking, more than 2000 compounds can be produced by pyrolysis. Eighteen different classes of chemicals, including nitrogen compounds, amino acids, hydrocarbons, sugars and fatty acids can contribute to the single known pharmacological and toxicological properties of cannabinoids (Huestis, 2002). The term "cannabinoid" was attributed to the compound's group with 21 carbon atoms present in *Cannabis sativa*, also added to their carboxylic acids, analogs and possible transformation products (Honório & Silva, 2006). They are usually formed by three rings, cyclohexene, benzene and tetrahidropiran. Some are the responsible for the power of the various psychoactive plant preparations (Mendelson, 1987).

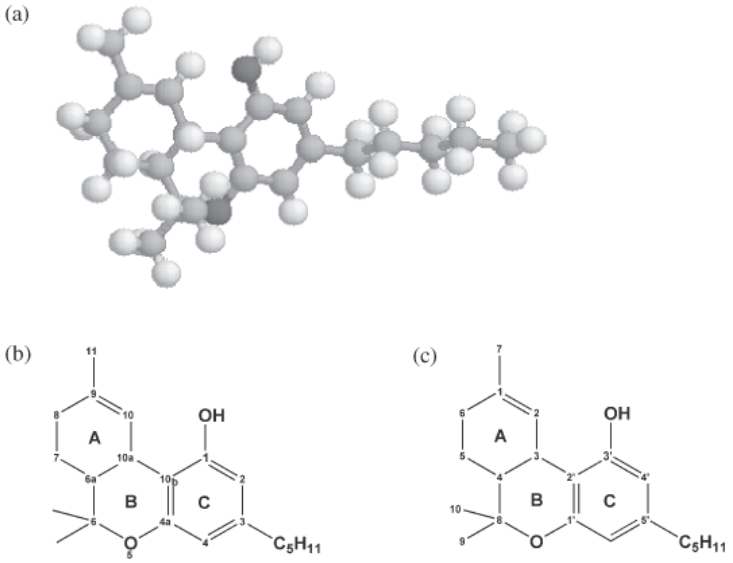

Fig. 1. Cannabinoid 3*D* chemical structure (a) and linear structures of two numbering systems used for cannabinoid compounds (b) and (c) (*Honório & Silva, 2006*).

The chemical structure of a cannabinoid type is shown in Figure 1, indicating the main numbering systems in the literature. The first report of proven isolation of the *cannabis* active ingredient in its pure form, Δ^9- Tetrahydrocannabinol or simply Δ^9-THC, dates from 1964, by Gaon and Mechoulam. Due to the great interest in the effects caused by the compounds extracted from *cannabis*, several studies have been conducted with the aim of identifying possible relationships between their chemical structure and their biological

activity. Cannabinol (CBN) was the first known cannabinoid, a phenolic compound resin obtained by Wood and collaborators in 1896. Canh, in 1932 got its cleansing in the crystalline acetate form, demonstrating that it is a phenolic derivative of dibenzopiran. Later, thanks to Cahn (1933) and Adams (1940), the structure of the CBN was established.
In 1940, Adams and Baker isolated another *cannabis* resin principle, which designated as Cannabidiol (CBD). Both CBN and CBD have lack active and enhancer effects. Later, in 1970, Mechoulam demonstrated that in the plant, there is a cannabinoids biosynthesis cycle that relates them, proving that the different components isolated by different authors were intermediate products. Although it is known that Δ^9-THC has six isomers (as an isomerization result), only the isomers Δ^9-THC e Δ^8-THC were isolated from the natural product. Of all the natural cannabinoids, Δ^9-THC is the most active compound, existing in the two forms mentioned above.

5. Cannabinoids properties

In fact, Δ^9-THC is the psychoactive cannabinoid with higher potency. Concerning the other cannabinoids present in the plant and about which there is some information:

i. Δ^8-THC presents a very similar pharmacological profile to that of Δ^9-THC, although lower, and thus, it has been studied for its possible use in drugs without psychoactive effects. This compound is only present is some plant varieties, being its concentration much lower when compared to Δ^9-THC (Mechoulam et al., 1992).
ii. CBN (Canabinnol) also has some psychoactive properties, among which are those related to the Δ^9-THC discriminative stimuli (Järbe & Mathis, 1992). This activity is, in animals, about one tenth of the described for Δ^9-THC. However, the results in humans have been quite contradictory, since some authors found that, by its intravenous administration, the CBN produces similar effects to those described for Δ^9-THC (Pérez-Reyes et al., 1973), contrary to those observed by Hollister (1974), who didn't detect any effect when administration was performed orally.

In fact, when comparing with Δ^9-THC, CBN has a higher affinity for the CB2 cannabinoid receptors than for the CB1 cannabinoid receptors (Munro et al., 1993). Being CB2 a peripheral receptor, CBN seems to participate in the immune system modulation, having been attributed, a long ago, to cannabinoids.
iii) CBD (Cannabidiol) is a cannabinoid practically devoid of psychoactive properties, since it is not capable to disconnect, from a CB1 receptor, neither an agonist nor an antagonist (Thomas et al., 1998). Since it is not a psychoactive substance, a detailed research has been developed in order to evaluate its possible clinical effects, and it has been described at least one case where its oral administration resulted in an effective long-term treatment of a psychosis framework (Zuardi et al., 1995).
We can, thus, say that cannabinoids properties depend on their chemical structure. Minimal variations in the THC molecule components can cause major changes in its activity.

6. Toxicokinetics

6.1 Absorption

Δ^9-THC absorption varies depending on the administration route. Inhalation is the most common administration route among *cannabis* consumers (smoke inhalation from water pipes or cigarettes), although there are also references to its use either orally (beverages or

food ingestion) or parenteral, providing a rapid and efficient method for drug distribution in the body. *Cannabis sativa* preparations (*hashish, marijuana*) are mainly consumed in cigarettes, and approximately 30% of the Δ^9-THC present in *marijuana* cigarettes or *hashish* are destroyed by pyrolysis during smoking (Huestis, 2002). The combustion heat leads to THC acids transformation to Δ^9-THC, as well as Δ^8-THC synthesis from CBD. Simultaneously, the existent Δ^9-THC is largely destroyed by smoking, originating CBN. This suggests that the maximum amount of Δ^9-THC absorbed during smoking does not exceed 70% of the Δ^9-THC content existent in the cigarettes. The intense pleasure effects can be produced due to the almost immediate CNS exposure to the drug. In fact, after smoking, there is a rapid absorption of Δ^9-THC through the respiratory tract into the bloodstream. However, about 18% of an inhaled Δ^9-THC dose is absorbed (Ohlsson et al., 1980), being an oral dose significantly less effective (Nahas, 1979). Moreover, Δ^9-THC bioavailability after inhalation is highly variable (Barnett et al., 1982, Lindgren et al. 1981; Ohlsson et al. 1980; Ohlsson et al., 1982, Pérez-Reyes et al., 1982), because it is affected, not only by the specific characteristics of the cigarette and its corresponding combustion, but also by the inhalation intensity, administration duration, among other factors. Experienced smokers inhale more efficiently than inexperienced people, being the Δ^9-THC bioavailability, in a *marijuana* cigarette with approximately 1-2% of the drug, between 16 and 40% for chronic users and between 13 and 14% for occasional users (Ohlsson et al., 1982). Cannabinoids oral ingestion leads to lower plasma Δ^9-THC levels than by inhalation, i.e., gastrointestinal absorption represents, approximately, one third of the achieved via inhalation. Orally, its bioavailability is reduced due to the gastric fluid acidity, the intestinal metabolism, as well as the first-pass enterohepatic system effect (Agurell et al., 1986). It has been observed that in acidic conditions (at a pH above 4.0), Δ^9-THC isomerizes, giving rise to Δ^8-THC or 9-hidroxihexahidrocanabinol. At a more acidic pH, the rupture of the pyran ring occurs, leading to the formation of several replaced cannabinoids. These changes could possibly explain the loss of Δ^9-THC activity after oral administration due to the acidic pH of the stomach (Garret et al., 1978). However, large intra-and inter-individual differences may also contribute to uncertainty in the effective dose distribution (Agurell et al. 1986; Ohlsson et al., 1982).

Δ^9-THC can be detected in plasma within seconds after inhaling the smoke of a *marijuana* cigarette ([a]Huestis et al., 1992), with plasma peak levels reached about 7 to 8 minutes after starting smoking, with euphoria and a maximum heart acceleration at about 20 minutes after (Perez-Reyes et al., 1982). However, after an oral administration, absorption is slow and irregular (Blaw et al. 1984; Ohlsson et al., 1980, Wall et al., 1983), reaching the highest Δ^9-THC plasma levels about 45 minutes after ingestion and remaining relatively constant for 4 to 6 hours (Wall et al., 1983). The clinical effects begin 30-60 minutes after oral consumption, reaching a peak 2-3 hours after ingestion (Isbell et al., 1967) and it can hardly be correlated with plasma levels. Bioavailability is reduced in about 20-40% after oral administration (Ohlsson et al., 1980, Wall et al., 1983) due to the drug degradation within the gastrointestinal tract (Perez-Reyes et al., 1973). We can, thus, say that a greater oral amount of Δ^9-THC is required to achieve the same physiological effects as by inhalation. Moreover, after oral administration, a gradual increase of its plasma concentration is produced and it can last for several hours, delaying the onset of their psychoactive effects (Cone and Huestis, 1993).

6.2 Distribution

Studies on Δ^9-THC bioavailability showed considerable differences between pulmonary and oral routes. Smoking seems to be the most effective method for drug administration. Δ^9-THC

entrance in the blood and its subsequent distribution to the tissues is very rapid, with very similar kinetics to the ones obtained after an intravenous administration.

Only 3% of the Δ^9-THC detected in the blood is in its free form. About 97 to 99% is bound to plasma proteins, primarily (60%) to lipoproteins (α and β) (Hunt & Jones, 1980; Wall et al., 1983) and the remain to albumin at a 6: 4 ratio. For this reason, the free concentration in plasma is actually very low (Klansner et al., 1975), being in the erythrocytes in only about 10% (Garret & Hunt, 1974; Widman et al., 1974). With a large distribution volume (10L/Kg), high Δ^9-THC lipid solubility leads to increased concentration and prolonged retention of the drug in fatty tissues ([a]Johansson *e col*., 1989), like the nervous tissue. Indeed, concerning its effective distribution in the tissues, Δ^9-THC is pulled out from the plasma to the tissues in about 70% (Hunt and Jones, 1980), although the distribution (which only occurs for the free fraction) is limited by the low concentration of its free form in the blood. Therefore, this distribution will depend on each organ blood flow. Consequently, given the greater distribution through more vascularized organs, and due to its high lipid solubility, brain is the organ where higher Δ^9-THC concentrations are achieved: 3 to 6 times higher than in plasma and just in 30 minutes.

Initial studies in animals, after Δ^9-THC administration, marked with ^{14}C, showed that Δ^9-THC concentrations in the tissues (in many cases of Δ^9-THC and metabolites) were higher in the lung, liver, kidney, heart, stomach, spleen, fat gray, placenta, thyroid, pituitary and mammary gland, when compared with brain or blood (Kreuz & Axelrod, 1973; Leighty, 1973; Ryrfeldt et al., 1973, Siemens et al., 1979). Later studies in rabbits also suggested that the highest Δ^9-THC concentrations can be detected in fat and in the heart, but not in the brain (Leuschner et al., 1986). The relatively low Δ^9-THC levels found in the brain can be, mainly due to its strong irrigation and consequent rapid and constant Δ^9-THC transportation from the blood into and out of the brain. Afterwards, it distributes by adipose tissue, which is, together with the spleen, its major deposit, three days after administration (Rawich et al., 1979). Several weeks are needed for the drug to be completely eliminated, even after discontinuing the administration (Kreuz & Axelrod, 1973). A slow cannabinoids release from fatty tissues and a significant enterohepatic recirculation contributes to the long half-life in plasma, which has been estimated to be around 4 days, or even more in chronic *marijuana* users (Johansson et al., 1988). Moreover, cannabinoids may remain the double of the time, in the plasma of regular smokers (Mason et al., 1983). In fact, this gradual cannabinoids release from the tissues to the bloodstream extends its presence in the blood and subsequent entry into the brain, being this one possible explanation for the absence of a withdrawal syndrome when the administration is suspended (Agurell et al., 1986).

6.3 Metabolism

Only a minimal amount of Δ^9-THC is eliminated from the body in its original form (with less than 1% excreted by the kidneys in its unchanged form), and most appear as metabolites in faeces (68%) or in urine (12%). The drug is also present in other tissues and biological fluids such as saliva, hair and sweat. Δ^9-THC is almost completely metabolized in the liver, although metabolism can also occur in the lung and intestine (Agurell et al., 1986).

In man, Δ9-THC metabolism involves allylic oxidation, epoxidation, aliphatic oxidation, decarboxylation and conjugation reactions. The allylic oxidation at C-8 and C-11 and aliphatic oxidation at the side chain lead to the formation of hydroxylated metabolites. The mono- and di-hydroxy metabolites are then oxidized to form acids and hydroxy acids.

Thus, in studies performed in vivo and in vitro, it was been shown that Δ⁹-THC is primarily metabolized in its hydroxylated compound by the hepatic microsomal enzymes (cytochrome P450) by allylic hydroxylation at carbon 11. Δ⁹-THC is metabolized in 11-hydroxy-Δ⁹-tetrahydrocannabinol (Δ⁹-THC-OH) (Iribarne et al., 1996, Matsunaga et al., 1995), considered to be his true active metabolite (Lemberger et al., 1970). Similarly, Δ⁸-THC follows a very comparable degradation pathway (Agurell et al., 1981), being rapidly hydroxylated to 11-hydroxy-Δ⁸-tetrahydrocannabinol in the liver (Matsunaga et al., 1995).
Hydroxylation at position 11 is the most important Δ⁹-THC metabolism reaction in most species, including humans. The 11-hydroxy-Δ⁹-tetrahydrocannabinol (Δ⁹-THC-OH) has a similar pharmacological activity and potency than Δ⁹-THC. Δ⁹-THC-OH is quantifiable in plasma 10 minutes after a Δ⁹-THC intravenous administration. However, by oral administration, the relationship between this metabolite and the main drug is about 5 times higher than the one measured after an intravenous administration (Lemberger et al., 1971). Even so, plasma concentrations achieved after oral administration can range from 50 to 100% when compared to the detected Δ⁹-THC concentrations (Wall et al., 1983). After smoking *marijuana*, the detected Δ⁹-THC-OH concentrations are lower, about 10% of the Δ⁹-THC concentrations ([a]Huestis et al., 1992; Wall et al., 1983), reaching the maximum Δ⁹-THC - OH peak (Cmax) in approximately 13 minutes after smoking, with maximum concentrations of, around, 7 ng/ml, after a single *marijuana* cigarette.
After Δ⁹-THC administration, its psychological effects begin to occur about 10 to 20 minutes after, although the effects caused by Δ⁹-THC-OH are only evident about 3-5 minutes later (Lemberger et al., 1973). This difference may be due to their pharmacokinetic properties, particularly different distribution and transfer into the nervous system, since their effects are equivalent. This can also explain the fact that, after an oral administration, the pharmacodynamic effects are higher than those induced with the same Δ⁹-THC concentration, but reached after smoking (Ohlsson et al., 1980). However the biotransformation process continues, and the active metabolite Δ⁹-THC-OH may oxidize, giving rise to the corresponding carboxylic acid (Δ⁹-THC-COOH) or return to hydroxylate itself. In this second case, it converts to 8, 11-dihydroxy-Δ⁹-THC, i.e., 11-hydroxy derivative transformation occurs in the liver into dihydroxy-Δ⁹-THC. These compounds are then transformed into other hydroxylated metabolites, more polar, inactive, which are then excreted in urine and faeces. The Δ⁹-THC-OH oxidation leads to the production of an inactive metabolite, 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (Δ⁹-THC-COOH) (Lemberger et al., 1972), identified in plasma, urine and faeces (Wall & Perez-Reyes, 1981, Wall et al., 1983). Subsequently, conjugation with the glucuronic acid can occur, being Δ⁹-THC-COOH and its glucuronide conjugates the main end biotransformation products in many species, including humans (Halldin & Widman, 1983). Renal clearance of these polar metabolites is always slow due to its extensive plasma protein binding (Hunt & Jones, 1980). After smoking, Δ⁹-THC-COOH plasma concentrations gradually increase, becoming higher than Δ⁹-THC concentrations shortly after smoking, whereas Δ⁹-THC plasma concentrations decrease very rapidly ([a]Huestis et al., 1992). Hence, the Δ⁹-THC-COOH detection time is much higher than for Δ⁹-THC or for Δ⁹-THC-OH. The CBD (Cannabidiol) metabolism is quite complex, with the possible production of almost 83 metabolites (Harvey, 1991). The proportions of these compounds also vary between species (Harvey & Mechoulam, 1990).
The metabolism of CBN (cannabinol) is less complex than for other cannabinoids. In most species, the hydroxylation at C-11 predominates, although there is also an important side

chain hydroxylation. The excreted metabolites are mainly 11-hydroxy-CBN, the CBN acid-11-oic acid and its hydroxylated side chain analogues (Brown and Harvey, 1990).

6.4 Elimination

Over 65% of the drug is excreted in faeces (68%), with approximately 13% excreted in urine (Wall et al., 1983). A total of 80-90% is excreted in 5 days, mainly in the hydroxylated and carboxylated metabolites forms. Only minimal amounts are excreted in their free forms (Hunt & Jones, 1980, Wall et al., 1983). Therefore, we can say that both Δ^9-THC and Δ^8-THC are not eliminated in their free form but in the form of metabolites (THC-OH and/or THC-COOH) or by glucuronic acid conjugation, producing different glucuronides. This process takes place in the liver involving several enzymes. The glucuronides formed are highly hydrophilic and therefore easily eliminated in the urine. Δ^9-THC-COOH metabolite has been detected in either urine or faeces (Wall & Perez-Reyes, 1981), while Δ^9-THC-OH predominates in the faeces. In fact, biliary excretion, and the consequent elimination through the faeces is the major route of unconjugated metabolites elimination (Wall et al., 1983), although most of the metabolites are reabsorbed from the gut. This enterohepatic circulation, which leads to more than 15% of the metabolites (Nahas, 1979), is responsible for the delay in the final active metabolites disposition, contributing to a prolonged excretion and to its accumulation among different body tissues.

In urine a total of 20 Δ^9-THC metabolites were identified, two glucuronic acid and 18 unconjugated acids forms. Indeed, the Δ^9-THC-COOH glucuronide conjugate is the primary urinary metabolite formed (Williams & Moffat, 1980). All other unconjugated acids metabolites identified in urine, excepting the 11-nor-9-Δ^9-THC-COOH, undergo oxidation or are degraded, forming varied carboxylated or hydroxylated metabolites. The average life of the inactive metabolites is about seven days, staying in the body for up to thirty days (Sutheimer et al., 1985). Some authors even accept the metabolites presence in urine within 72 days after use (Ellis et al., 1985), despite having an estimated Δ^9-THC plasma elimination time of 4 days ([a]Johansson et al., 1989).

7. Action mechanism

For a long time ago, some hypotheses had been proposed to explain Δ^9-THC action mechanism, suggesting that Δ^9-THC may exert its actions through a nonspecific drug interaction with cell membranes and intracellular organelles (Hillard et al., 1985; Martin, 1986). However, it is notoriously difficult to delineate the precise action mechanisms of cannabinoids, given the evident Δ^9-THC activity in several places, including the receptors for opiates and benzodiazepines, as well as marked effects on prostaglandins synthesis and protein metabolism (Burstein et al. 1982; Welch & Eads, 1999). Cannabinoids inhibit macromolecular metabolism according to the dose, presenting a wide effects range on the enzyme systems, neurotransmitters and hormone secretion (Bloom, 1982, Chakravarty et al. 1975; Dalterio et al. 1977; Dalterio et al. 1987; Dill & Howlett, 1988; Pertwee, 1988). These numerous and diffuse effects supported the hypothesis of a nonspecific interaction. However, with cannabinoids pharmacology knowledge advance, it became obvious that some structural aspects would be required for the cannabinoids activity, including the receptor binding in the target cells (Mechoulam, 1991).

7.1 Cannabinoid receptors

Nowadays it is clearly known that cannabinoids act through its interaction with specific endogenous receptors, discovered by Devane et al. (1988) and later colonized. Indeed, a specific protein receptor was discovered, named CB1 (central receptors) (Matsuda et al., 1990, Munro et al., 1993), in the mouse nerve cells, being now known that it can also be found in the brain of the mouse, guinea pig, dog, monkey, pig and man, and peripheral nerves. The biology and behaviour associated with brain areas are consistent with the behavioural effects produced by cannabinoids (Table 2). The highest density of receptors is found in the basal ganglia cells, involved in coordinating body movements.

A) - Brain regions where cannabinoid receptors are abundant	
Brain regions	*Tasks associated with the region*
Basal ganglia	Motion control
Cerebellum	Coordination of body movements
Hippocampus	Learning, memory, stress
Cerebral Cortex	Cognitive functions
B) - Brain regions where cannabinoid receptors are moderately concentrated	
Brain regions	*Tasks associated with the region*
Hypothalamus	Maintenance of body functions (temperature, electrolyte balance, reproductive function)
Amygdale	Emotional response, fear
Backbone	Peripheral sensation, including pain
Brainstem	Sleep, temperature regulation, motor control

Table 2. Brain regions where cannabinoid receptors are abundant or moderately concentrated and functions associated with these areas *(Honório & Silva, 2006)*.

CB1 receptors mediate the majority of the cannabinoids responses in the central nervous system (CNS), being abundant in the cerebral cortex, hippocampus, amygdale, basal ganglia, cerebellum, and thalamus (Ashton, 2001; Robson, 2001). The high receptors density in the caudate nucleus and cerebellum are consistent with the marked cannabinoids effects in motor behaviour (Romero et al. 1995; Sanudo-Pena et al., 2000). The significant link within the cerebral cortex and hippocampus explains the marked cannabinoids effects on perception, cognitive aspects, memory, learning, endocrine function and body temperature regulation (Chait & Perry, 1994, Compton et al. 1993; Hampson & Deadwyler, 1999).

The CB1 receptors location in the hippocampus supports that cannabinoids play an import role in appetite and energy regulation. This supposition is strengthened by its presence in important peripheral organs, as the GI tract, liver, skeletal muscle and adipose tissue.

Despite being primarily viewed as a central receptor (presence in the nervous tissue) (Herkenham et al., 1990), this CB1 receptor was also found in the adipose tissue (Bensaid et al., 2003), myocardium (Bonz et al. 2003), vascular endothelium (Liu et al., 2000) and in sympathetic nerve terminals (Ishac et al., 1996).

In 1993, Munro et al. identified a second cannabinoid receptor, the CB2 receptor, present, preferentially, in the immune system cells, outside the CNS (peripheral receptor). Efforts have been made to improve the chemical manipulation of cannabinoids, to maximize the selectivity for these receptors CB2, avoiding the psychoactive effects (Robson, 2001). This distinctive peripheral cannabinoid receptor seems to play a major role in

immunomodulation (Lynn & Herkenham, 1994; Reggio et al., 1997), showing a significant anti-inflammatory and immunosuppressive activity. It has already been postulated the existence of a third receptor - CB_3 - (Fride et al., 2003), but the receptor itself has not been colonized yet. It is believed now that the two cannabinoid receptors - CB1 e CB2 - are responsible for many biochemical and pharmacological effects produced by the majority of cannabinoid compounds (Matsuda et al., 1990). The functional differences existent between the two receptors types are still not known, but structural differences raise that possibility.
In 1986, Howlett et al. showed that Δ^9-THC inhibited the intracellular enzyme adenylyl cyclase (AC) and that this inhibition occurred only in the presence of a G-protein complex, this is, in the presence of a cannabinoid receptor, which is a typical member of the largest known receptors family: G-coupled protein, containing seven transmembrane domains (Glass & Northup, 1999; Howlett et al., 1991). The body's cells respond in different ways when a ligand interacts with the cannabinoid receptor. The receptor intracellular surface interacts with G proteins that regulate effectors' proteins such as AC, or calcium and potassium channels, and via a protein kinase activated by mitogen (Bayewitch et al. 1996; Bidaut-Russell et al. 1990).

7.2 Endogenous cannabinoids

The cannabinoid receptors discovery prompted the search for an endogenous ligand to which the receptors naturally interact. For each biological receptor (in this case, a brain receptor) there is probably an endogenous agonist, i.e., a compound naturally produced by the body that interact with the receptor. The first endogenous cannabinoid discovered was arachidonoylethanolamine known as anandamide, derived from the Sanskrit word ananda, meaning "happiness." This substance was isolated from pig brain, by Devane et al. (1992), and these authors observed that, being chemically distinct from the cannabinoid plant, compared with Δ^9-THC, it has a moderate affinity for CB1 receptors, but with most of the Δ^9-THC actions, although having a short action period. In general, the affinity of anandamide for cannabinoid receptors is only ¼ to ½ the affinity displayed by Δ^9-THC, these differences being related to the cells or tissues used in those studies as well as with the other experimental conditions (Smith et al. , 1994). Anandamide mimics Δ^9-THC action since it binds to both receptor subtypes, CB1 and CB2, and has a similar pharmacological activity, despite having little power to exert some effects (Fride & Mechoulam, 1993; Howlett , 1995; Mechoulam & Hanus, 1996; Pertwee 1995). This ligand was found in several regions of the human brain (hippocampus, striatum and cerebellum) where CB1 receptors are abundant, suggesting the involvement of endogenous cannabinoids in the brain functions controlled by these areas. However, substantial concentrations of anandamide are also found in the thalamus, a brain area with few CB1 receptors (Di Marzo et al., 1994). An interesting fact is that anandamide was also found in small amounts in other parts of the body such as the spleen, where there are high concentrations of CB2 receptors, and heart (Ameri, 1999; Fowler, 2003; Howlett, 1998; Pop, 1999). It may, therefore, be concluded that the molecule anandamide has both central and peripheral impact (Di Marzo et al., 2000). Although Deutsch and Chin (1993) have proposed a biosynthetic pathway for anandamide, it appears that this gets rid of cell membranes followed by depolarization due to a calcium influx (Di Marzo, 1998, Evans et al., 1992). Also Sugiura et al. (1995) examined the effects of anandamide and another endogenous compound, 2-araquidonil glycerol (2-AG) in connection with a specific cannabinoid receptor, assuming that the latter substance can also

be an endogenous ligand with relevant role in the brain. Under normal conditions, the endocannabinoid system appears to be tonically active; instead, the endocannabinoids are produced as needed, act locally and are rapidly inactivated by cellular uptake and enzymatic hydrolysis (Giuffrida et al., 2001).
In addition to the identification of these ligands there were also synthesized some specific CB1 receptors antagonists (Rinaldi-Carmona, 1994) and CB2 (Portier et al., 1999). The SR141716 (now under the name of Rimonabant) was the first specific CB1 antagonist receptor, with high affinity, blocking the acute effects of Δ^9-THC and other CB1 agonists in vitro and in animals (Adams et al. 1998; Rinaldi-Carmona, 1994). In 2001, Huestis et al., conducting the first clinical studies on the pharmacokinetic and pharmacodynamic effects of Rimonabant, administered orally, in combination with *cannabis* smoking, demonstrated that *cannabis* administration alone produced the expected physiological responses with the consequent intoxication reflection and, when combined with Rimonabant, a dose-dependent blocking effect of *cannabis* was observed. Significant advances in cannabinoid research have opened new frontiers leading to an increasing interpretation of their effects and the role of endogenous cannabinoids in man.

8. Pharmacodynamic effects

Due to their action mechanism, cannabinoids exert multiple conducts, acting in almost all biological systems. Its activity is multiple and complex due to the variety of psychoactive products present in the plant whose toxic and pharmacological actions may overlap or be additive. The main factors influencing the toxicity of these substances are: dose, administration route, individual's personality, dependence degree, concomitant administration of other substances and chronological stage of the administration.
The behavioural and physiological cannabinoids effects have been increasingly reported over the recent decades (Adams & Martin, 1996; Dewey, 1986; Jones, 1987), including euphoria and relaxation feelings, times reaction changes, lack of concentration, learning and memory changing or mood states (such as panic reactions and paranoia). The spectrum of cannabinoids behavioural effects is unique, leading to a consequent classification of these drugs as stimulants, tranquilizers, or hallucinogenic (Benowitz et al. 1979; Hollister, 1986; Law et al., 1984). Other common physiological effects include increased appetite, dry mouth, vasodilation and decreased respiratory rate. Cannabinoids may affect the immune and endocrine systems, producing lung damage and influencing neonatal and child development of (Chandler et al., 1996, Day et al., 1991, Fried et al. 1999; Fried & Smith, 2001; Tashkin et al., 1991). The physiological effects of cannabinoids are most relevant for the main systems are:

a. Effects on Cardiovascular System

The Δ^9-THC effects of on the cardiovascular system depend on the dose, with a decrease in heart rate with low doses and increased at higher doses (which may exceed 160 beats/min). This substance may also lead to a decrease in contractile force and lead to progressive reduction in coronary blood flow (Tashkin et al., 1978). Acute administration of cannabinoids in humans produces vasodilation and tachycardia, resulting in an overall effect on systemic blood pressure (Huestis et al., 1992). However, the unrelenting use of Δ^9-THC results in hypotension mediated by CB1 receptors and bradycardia (Benowitz et al., 1975, Lake et al., 1997). Endocannabinoids induce vasodilation by acting, directly, on CB1

receptors located in the arterial smooth muscle of the brain (Gebremedhin et al., 1999). Moreover, the same occurs at other vessels, through an increased synthesis of nitric oxide (NO) endothelium-dependent (Deutsch et al., 1997). These and many other effects on CV can be an increased risk for individuals with pre-existing heart disease (especially in patients with heart failure and coronary), as already reported, for example, in acute cardiac accidents cases, often fatal for *Cannabis* consumers (Ashton, 2001).

b. Metabolic Effects

The endocannabinoid system appears to play a crucial role in regulating metabolism and body composition. The appetite stimulation (especially for sweets) and dry mouth due to decreased salivary secretion are usually adverse reactions produced in *cannabis* consumers. The consequent weight loss may suppose that there is a changing in glucose metabolism. However, several studies show that there is no agreement on the glucose levels change (Hollister et al. 1968; Lindemann, 1933, Weil et al., 1969). However, its effect on other metabolic processes is of great significance. Thus, control of food intake and body composition results from complex interactions between the adipocytes, the mesolimbic system, hypothalamus and gastrointestinal tract. The hunger feeling often existent in consumers is mediated by an intestinal hormone, ghrelin, which is produced in most circumstances of weight loss. Moreover, leptin, an endogenous hormone, can reduce food intake. The serum concentration of this hormone is directly proportional to the degree of adiposity, but obese people have lower sensitivity to the hormone. A protein produced in adipose tissue, adiponectin stimulates fatty acid oxidation and body weight decrease, being its concentration lower in obese individuals (Considine et al., 1996, Cummings et al. 2002; Fruebis et al. 2001).

Both cannabinoid receptors and their endocannabinoids ligands are present in tissues related to food intake. The concentration of endocannabinoids in the hypothalamus decreases after leptin administration (Di Marzo et al., 2001). Studies in animals (rodents) showed that the CB1 receptor agonists are potent inducers of hyperphagia (Jamshidi et al., 2001, Kirkham et al., 2002, Williams et al., 1999), while their antagonists prevent such effect (Di Marzo et al., 2001). In another study, it was found that mice do not express CB1 receptors resulting in spontaneous calories reduction (Cota et al., 2003). For all these interferences at the cannabinoid system, metabolic modulation of this pathway has been considered to be a greater possibility for therapeutic intervention in obesity (Feliciano et al. 2007; Francischetti et al., 2006).

c. Effects on Pulmonary System

Inhaling the smoke of *marijuana* cigarettes (or Δ^9-THC) produces acute bronchodilation in healthy subjects and asthmatics, bronchodilation that may last at least one hour (Tashkin et al. 1977; Vachon et al., 1973). It is important to note that *cannabis* smokers have a higher lung cancer risk than tobacco consumers because of the high aromatic hydrocarbons content in *marijuana* smoke, which has higher concentrations of irritant substances, such as sterols, terpenes, among others (Fehr and Kalant, 1972). Comparing a normal about five times tobacco cigarette with a *marijuana* cigarette, it is estimated that the latter produces more carboxyhemoglobin, with consequent maintenance increase in the respiratory tract (Benson & Bentley, 1995, Wu et al., 1988). Chronic use of *cannabis* cigarettes is associated with bronchitis and emphysema. It is estimated that 3-4 *cannabis* cigarettes per day equals more than 20 tobacco cigarettes a day, with subsequent evidence of acute and chronic bronchitis (Benson and Bentley, 1995).

d. Effects on Vision

The administration of *cannabis* cigarettes in normal individuals causes a slight constriction of the pupil, preserving light reflection, marked congestion of conjunctiva vessels, tearing and intraocular pressure reduction related to dose. In fact, vasodilatation and redness of the conjunctiva is a characteristic sign of *cannabis* use (Paton & Pertwee, 1973). Other changes include colour perception and light adaptation changes (Ohlsson et al., 1980).

9. Cannabinoids and driving influence

Experimental studies have been repeatedly demonstrating Δ^9-THC effects on an individual cognitive function and psychomotor skills, influencing learning and information acquisition, changing the individual's memory capacity, coordination and reaction times (Chait & Pierri, 1992; Kurzthaler et al. 1999; Leire et al., 1989). The biggest concern with cannabinoids acute effects is related to road traffic or labour accidents (Hall, 2001). Indeed, Δ^9-THC acute effects on cognitive function and psychomotor skills have been subject of extensive study, noting that, at doses between 40 and 300 mg/kg, cannabinoids can cause a dose-dependent reduction in tasks that require memory use, reaction times, in motor functions and coordination (Ameri, 1999; Curran et al. 2002; D 'Souza et al. 2004; Hall & Solowij 1998; Hampson & Deadwyler 1999; Lewek et al. 1998; Lichtman et al. 2002; Ramaekers et al., 2004).

The impaired state induced by cannabinoids has been studied by several authors in tests performed on drivers (Lamers & Ramaekers, 2001; Ramaekers et al., 2000), demonstrating that their driving risk effects increase with the dose, being more extensive and persistent in activities that require more careful attention. There is an enormous concern, both in the European Union and in the United States of America (USA) regarding the link between cannabinoids consumption and traffic accidents. However, from a legal standpoint, the evaluation and interpretation of the corresponding accuracy is still a big challenge, since the association between Δ^9-THC levels and the accidents risk is not perfectly clear. Currently, some authors claim that there is very little scientific evidence demonstrating that Δ^9-THC or Δ^9-THC-COOH detection in body fluids can be used as impairment evidence in any circumstance. They assume, for example, that Δ^9-THC or its metabolite can be detected in the body for days after smoking *cannabis* and thus, their presence may be indicative of a previous consumption and not of a recent use, not being certain that the presence of the drug in the body indicates an impairment state (Drummer et al., 2003). Papafotiou et al. (2005), through experimental studies in volunteers, acknowledged that the negative relationship between driving performance and Δ^9-THC levels found in blood is due to the fact that the Δ^9-THC peak concentrations are achieved in the CNS a few time after being achieved in blood. Similarly to what occurs with benzodiazepines, where the maximum influenced state is observed one hour after peak plasma concentrations are reached (Rush & Griffiths, 1996), the maximum influenced state after Δ^9-THC consumption occurs after achieving the maximum blood concentration. On the other hand, it has been shown that the influence and accident risk due to recent *cannabis* increases with dose, with an influenced state already present at low doses, being even worse at higher concentrations (Ramaekers et al., 2004). Note, however, that it is not perfectly acknowledged how to correlate the plasma Δ^9-THC levels variation with driver's behaviour, although this relationship has been simulated in experimental behavioural cannabinoid pharmacodynamics and pharmacokinetics studies. Ramaekers et al. (2006) developed, thus, a study in *cannabis* consumers, concluding that the impairment sate was progressively higher with increasing

Δ^9-THC concentrations. They admitted that already with Δ^9-THC concentrations between 2 and 5 ng/ml there was significant influence state; when Δ^9-THC was detected between 5 and 10 ng/ ml, about 75 to 90% of the individuals were under the influence and over 30 ng/ml, there was a were 100% influence. Cone and Huestis (1993) also conducted a similar research and concluded that the ability to drive may be influenced one hour after consumption, during the Δ^9-THC elimination phase, even when the concentrations decrease to 13 ng/ml. Berghaus et al. (1995) go even further, stating that with 6 ng/ml of Δ^9-THC the information processing is already affected, with attention and vision changes at 9 ng/ml and 12 ng/ml, respectively. They demonstrated that the Δ^9-THC driving influence is mainly evident in the first two hours after consumption, leading to performance changes, with higher prevalence on attention, psychomotor and cognitive capacities.

10. Therapeutic perspectives of the endocannabinoid system modulation

Endocannabinoids (EC) are involved in several physiological functions, among which, special attention has been given to the regulation of appetite by central mechanisms and its influence on obesity (van Thuijl et al., 2008; Kirkham, 2009). Considering these innovative findings, the research for new pharmacological agents has drastically increased and the discovery of rimonabant, a synthetic antagonist of CB1 receptors, has confirmed the important role of endocannabinoid system on the modulation of food ingestion and energetic balance (Butler et al., 2009). These facts led to the first clinical studies using rimonabant as a new tool against obesity and its associated metabolic disorders. However, psychiatricside-effects, namely central, which include increased risk of depression and even suicide, US Food and Drug Administration declined permission for rimonabant, and in October 2008, rimonabant was also suspended across the EU. After rimonabant withdrawal, other CB1 antagonist drugs have also tested, including the taranabant, which was associated with weight loss in rats and in humans (Fong et al., 2007; Addy et al., 2008). However, due also to central side effects, including anxiety and depression, the clinical trials were stopped in October 2008 (EMEA. The European Medicines Agency recommends suspension of the marketing authorisation of Acomplia: http://www.emea.europa.eu 2008).

Although several other different influences of endocannabinoids have been discussed during the last years, including in inflammation, diabetes, cancer, affective and neurodegenerative diseases, and epilepsy (Izzo et al., 2009), the most recent findings are related to their cardiovascular actions (Durst & Lotan, 2011), which seem to be very ample bust also complex. *In vivo* experiments with rats have demonstrated the action of anandamide and 2-AG on the development of atherosclerotic plaque, as well as an effect on heart rate, blood pressure, vasoactivity and energy metabolism (action in dyslipidemia and obesity). Recent studies with an antagonist of CB1 receptors showed that the modulation of ECS can play an important role in reducing cardiovascular risk in obese and dyslipidemic patients. Similarly, studies in rats have demonstrated the action of CB2 receptors in adhesion, migration, proliferation and function of immune cells involved in the atherosclerotic plaque formation process. The ECS have been implicated in hypotensive stages associated with hemorrhagic chock, both endotoxic and cardiogenic, and even to advanced liver cirrhosis; on the other hand, recent evidence suggests that ECS plays an important role in cardiovascular regulation associated with hypertension, as well as a protective role in ischemia grafting. The development of atherosclerotic plaque and the metabolic stages associated to obesity are also matter of study of possible ECS pharmacomodulation.

Effects on myocardial ischemia/reperfusion and preconditioning

Initial studies used isolated preparations of heart to study the role of ECS in myocardial ischemia/reperfusion (I/R) and preconditioning. The involvement of ECS in preconditioning induced by the endotoxin (lipopolysaccharide: LPS) against the injury induced by I/R on myocardium has been implicated for the first time in 2001, based on the hypothesis that LPS could increase the production of endocannabinoids in inflammatory cells (Varga et al., 1998). A 90 minutes of low flow ischemia followed by 60 minutes of reperfusion with normal flow in isolated rat hearts pretreated with LPS was compared with a saline solution. The pretreatment with LPS reduced the infarct size and improved functional recovery after reperfusion when compared with control group, which could be attenuated by SR144528 (CB2 antagonist), but not by rimonabant (CB1 antagonist), suggesting the involvement of myocardium CB2 in the cardioprotection induced by LPS (Lagneux & Lamontagne, 2001). In a subsequent study, in which the preconditioning was triggered by heat stress, the SR144528 also abolished the effect of reducing the infarct size, unlike rimonabant (Joyeux et al., 2002).

These early studies suggested that the protection created by the preconditioning induced by heat stress or by LPS was mediated by the action of endocannabinoids in the CB2 receptors. In contrast, when preconditioning was induced by a brief period of ischemia (5 minutes), the blockade of CB1 and CB2 receptors did not raise the abolition of protection, and both receptors have been implicated in preserving endothelium-dependent vasodilatation induced by serotonin (Bouchard et al., 2003). The palmitoylethanolamide or the 2-AG, but not anandamide, when added to perfused isolated rat hearts offer protection against ischemia by reducing myocardial damage and infarct size and by improving the functional recovery of myocardium (Lepicier et al., 2003). The SR144528 completely blocked the cardioprotective effect of palmytoylethanolamide and 2-AG, whereas rimonabant only inhibited, partially, the effect of 2-AG (Lepicier et al., 2003). Similarly, ACEA and JWH015 (CB1 and CB2 agonists) also reduced the size of the infarct in this model (Lepicier et al., 2003). In contrast, it was found that anadamida's effect of reducing infarct area could also be antagonized by CB1 and CB2 antagonists; however, the same could not be mimicked by selective CB1 and CB2 agonists, suggesting an involvement of a different site of CB1 and CB2 receptors. Another recent study, which used a model of delayed preconditioning in rats, induced by transdermal treatment of nitroglycerin (as an NO donor) for 24 hours, suggested that the protective effect of nitroglycerin against myocardial infarction is mediated trough CB1 receptors. Nitroglycerin increased the concentration of 2-AG in myocardium, but did not increase anandamide (Wagner et al., 2006). These pioneer studies implicated a possible contribution of CB2 functional receptors in cardiomyocytes and the endothelial cells responsibility, at least in part, on the protective effects of preconditioning. Indeed, subsequent studies showed the presence of CB2 receptors in myocardium, in cardiomyoblast cells and in endothelial cells with different origins ((Mukhopadhyay et al., 2003; Blazquez et al., 2003). Concurrently with the beneficial effect of the activation of CB2 receptors in cardiomyocytes, a recent study showed that THC protected cardiomyoblast cells H9c2 submitted to hypoxia *in vitro*, presumably trough the activation of CB2 receptors and increased NO production (Shmist et al., 2006).

In an ischemia/reperfusion injury model in rats, both anandamide and HU-210 decreased the incidence of ventricular arrhythmias and reduced the size of the infarct, presumably trough the activation of CB2 receptors but not CB1 receptors (Krylatov et al., 2001). In an myocardic I/R injury model induced by ligation of coronary artery in rats, the reduction of

the second myocardic injury depending on leucocytes subsequent to the initial I/R injury was attributed to the activation of CB2 receptors, since the protection given by WIN 55.212-2 could be prevented by AM630, but not by AM251 (a CB1 antagonist) (Di Filippo et al., 2004). Two recent studies in myocardial infarct models, acute and chronic, in rats, showed that cannabinoids contribute to hypotension and cardiac depression associated to cardiogenic acute shock, which could be attenuated by antagonists of CB1 receptors (Wagner et al., 2003).

Overall, despite the role of CB1 receptors and of endocannabinoids in the protection given by the preconditioning against myocardic I/R, the issue remains controversial, recommending further investigation namely using mice with deletion of genes and more selective agonists of CB2 receptors. However, the findings that imply CB2 receptors' importance, presumably in both endothelial and inflammatory cells and perhaps in cardiomyocytes, are quite encouraging.

Cerebral ischemia/reperfusion (cerebrovascular accident)

The ECS may constitute an essential mechanism of neuroprotection, in both acute forms of neuronal injury, such as stroke or brain trauma, and in several chronic neurodegenative disorders, including multiple sclerosis, Parkinson's disease, Hungtington's disease, Alzheimer's disease and amyotrophic lateral sclerosis (Pacher et al., 2006a). Although the exact mechanisms of this neuroprotection are not yet completely understood, several processes dependent and independent of CB receptors seem to be involved: 1) modulation of the immune responses and release of inflammatory mediators by CB1, CB2 and not CB1/not CB receptors in neurons, astrocytes, microglia, macrophages, neutrophils and lymphocytes (Klein, 2005); 2) modulation of synaptic plasticity and excitatory glutamatergic transmissions via presynaptic CB1 receptors (Freund et al., 2003); 3) activation of cytoprotective signaling pathways (Pacher et al., 2006a); 4) modulation of calcium homeostasis and excitability trough interactions with calcium channels, potassium and sodium, gap junctions and intracellular calcium reserves, and with NMDA receptors (Freund et al., 2003); 5) central hypothermia mediated by CB1 receptors, presumably by the reduction of the metabolic rate of needed oxygen; 6) antioxidant properties of cannabinoids (Hampson et al., 2000); 7) modulation of endothelial activity and inflammatory response, leucocytes mobilization, adhesions to the endothelium, transmigration and activation presumably through CB2 receptors. The first evidence of a neuroprotective effect of cannabinoids has emerged in research studies on cerebrovascular accident, in which was used the non psychoactive cannabinoid dexanabinol/HU-211 that exerts its effect through CB1/CB2 independent mechanisms, in cerebral ischemia models *in vivo* in rats and gerbils (Pacher et al., 2006a). Further studies also investigated the neuroprotective effects of CB1 receptors stimulation with synthetic agonists. The synthetic cannabinoid WIN 55.212-2 attenuated the neurological damage in the hypothalamus resulting from cerebral global and transient ischemia in rats and reduced infarct size after permanent focal cerebral ischemia induced by cerebral middle artery occlusion, when it was administrated 40 minutes before or 30 minutes after occlusion, in a dependent way from CB1 receptors, since the protective effect was prevented by rimonabant (Nagayama et al., 1999). WIN 55.212-2, as well as anandamide and 2-AG, did also confer protection to cultured cortical neurons submitted to hypoxia and glucose deprivation *in vitro*, but these effects proved to be insensitive to antagonists of CB1 and CB2 receptors (Nagayama et al., 1999).

In models of cerebral middle artery occlusion in rats, the agonist BAY38-7271 reduced the size of the infarct, even when administered intravenously 4 hours after the occlusion (Mauler et al., 2002). The pre-treatment with rimonabant partially attenuated the effect of HU-210, indicating the involvement of CB1 receptor. However, the protective effect of HU-210 could be completely abolished by warming the animals' body until the controls temperature, showing that hypothermia mediated by CB1 receptors was responsible for the beneficial effects observed (Leker et al., 2006). Similarly, hypothermia mediated by CB1 receptors was responsible for the neuroprotective effects of THC in a model of cerebral ischemic injury in rats (Hayakawa et al., 2004), and in a model of global cerebral ischemia injury in rats (Louw et al., 2000). Concurrently with the neuroprotection mediated by CB1 receptors, mice without CB1 receptors showed an increased neurotoxicity to NMDA and high mortality levels in permanent focal cerebral ischemia, and an increased infarct area, with neurological deficits more severe after transient focal cerebral ischemia and decreased blood flow in brain in ischemic penumbra during reperfusion, when compared with controls under the same aggressions (Parmentier-Batteur et al., 2002). In contrast, several recent studies do not support the neuroprotective role of endocannabinoids in the activation of CB1 receptors. In fact, rimonabant and LY320135 (CB1 receptors antagonist) reduced the size of the infarct and improved the neurological function in a cerebral ischemia model in rats, induced by brain middle artery occlusion (Muthian et al., 2004), while low doses of WIN 55,212-2 showed no protective effects (Muthian et al., 2004). Recent studies have evaluated the effect of selective CB2 agonists (O-3853, O-1966) in a model of cerebrovascular accidents. CB2 agonists significantly decreased cerebral infarct and improved motor function after cerebral middle artery occlusion for one hour, followed by 23 hours of reperfusion in rats, by attenuation of the increased mobilization of leucocytes and their adherence to vascular endothelial cells induced by transient ischemia (Zhang et al., 2007). The role of CB2 receptors in I/R injury was also supported by the increased accumulation of CB2-positive macrophages derived from resident microglia and/or from invading monocytes resulting from I/R cerebral injury (Ashton et al., 2007).

In general, it seems clear that both agonists and antagonists of CB1 receptors may play a neuroprotective effect on cerebral I/R injury. The reason for the contradictory effects of the pharmacological blockade versus genetic "knockout" of CB1 receptors is still unclear, but could be related with effects that are independent from CB1 antagonists, and that's the reason why this subject requires further clarification. In the case of CB2 agonists, the most likely protection mechanism is the reduction of increased leukocyte infiltration, mobilization and adhesion to vascular endothelial cells and consequent activation, in a process induced by I/R transient injury.

Circulatory shock (organ/body ischemia and/or ischemia/reperfusion)

In addition to its well-known immunologic and neurobehavioral actions, cannabinoids and their synthetic endogenous analogs exert complex cardiopressant and vasodilator effects, which were implicated in the mechanisms underlying hypotension associated to hemorrhagic shock, cardiogenic and septic, advanced liver cirrhosis, cirrhotic cardiomyopathy, heart failure induced by doxorubicin and shock associated to necrotizing pancreatitis (Lamontagne et al., 2006; Ashton & Smith, 2007; Ribuot et al., 2005; Moezi et al., 2008; Sarzani, 2008). These depressant effects of the cardiovascular system could be prevented or reversed by the pretreatment with CB1 receptor antagonists, and they have been analyzed in many recent studies. CB receptors antagonists (eg. rimonabant, AM281,

AM251 and SR144528) prolonged the survival in septic shock or in necrotizing pancreatitis (Varga et al., 1998), increasing mortality in hemorrhagic (Wagner et al., 1997) and cardiogenic (Wagner et al., 2001) shock, despite the increase in blood pressure. One possible explanation for this intriguing controversy is the hypothesis that vasodilatation mediated by endocannabinoids can provide a survival value by increasing tissues oxygenation, neutralizing the excessive sympathetic vasoconstriction triggered by hemorrhage or by myocardium infarct, which could be avoided by blocking CB1 receptors. In contrast, the blockade of CB1 receptors could increase survival in endotoxic shock by preventing the primary hypotensive response to LPS (Pacher et al., 2006a). Even more complicated is the fact that, in hemorrhagic shock, both cardiogenic and septic, UH-210, WIN 55,212-2 and THC (CB agonists) are able to improve endothelial function and/or survival (Varga et al., 1998). Since cardiovascular failure and dysfunction in many of the cited studies are triggered by I/R injury and/or ischemia, and consequently oxidative/nitrosative stress and inflammatory response associated to the activation of several cell death pathways downstream (Pacher et al., 2007), another explanation for the different beneficial effects of agonists and antagonists in circulatory shock could reside in their various anti-inflammatory and/or antioxidant properties (Klein, 2005). These could be attributed to their inverse agonist properties or to mechanisms independent from CB1 and CB2 receptors (Pertwee, 2006).

In global terms, it seems clear that both cannabinoids and antagonists of CB receptors may exert several beneficial effects in shock models in rats; however, the specificity of these effects and their importance for the circulatory shock in humans requires further investigation.

Role of endocannabinoid system in hypertension

The potential use of cannabinoid ligands as antihypertensive agents was even considered since 1970 (Archer, 1974; Birmingham, 1973), and were further reviewed (Sarzani, 2008; Pacher et al., 2005). Cannabinoids decrease blood pressure in hypertensive rodents primarily because of decrease cardiac contractility, suggesting that could have a therapeutic role on hypertension and cardiac hypertrophy. Rimonabant, the CB(1) receptor blocker induced a significant increase in cardiac contractility and blood pressure in hypertensive rats but, on the contrary, contributed to decrease blood pressure in weight-loss clinical trials especially in obese patients with hypertension, which suggests that the overactivation of the ECS in intra-abdominal obesity could be a deleterious effect, in particular from a cardiometabolic opinion (Sarzani, 2008). In addition to the studies in animal models that were already mentioned, it was found that inhalation of THC causes a greater and more lasting fall in blood pressure in hypotensive subjects when compared with normotensive subjects (Crawford et al., 1979). Although the mechanism underlying this increased sensitivity is not cleared yet, it suggests a role of endocannabinoid system in the regulation of cardiovascular functions in hypertension. In a recent study, using three different experimental models of hypertension to explore this possibility, the authors found a significant endocannabinergic tone in hypertension that limits the blood pressure rise and cardiac contractility through the activation of cardiac and vascular CB1 receptors (Bátkai et al., 2004b). It was also found, that over-regulation of these same receptors contributes to potentiate of this tone, maybe trough the inhibition of the activation of endogenous anandamide, stabilizing blood pressure and the contractility of the heart in hypertension.

These findings contribute to the interesting possibility of using inhibitors of fatty acid amide hydrolase in the treatment of hypertension. More clinical studies will be needed to clarify this interesting possibility in a near future.

Role of endocannabinoid system in atherosclerosis

Cannabinoids, endogenous and synthetic, have complex cardiovascular actions through the activation of CB1 receptors (vascular and myocardial) (Steinberg et al., 2007). The decline of cardiac function associated with age and the changes in inflammation genes expression, nitrative stress and apoptosis in rats FAAH$^{-/-}$ compared with wild type rats was analyzed (Batkai et al., 2007; Mach & Steffens, 2008). The authors found that increased levels of anandamide in FAAH$^{-/-}$ rats have a protective effect, which is consistent with the protective role of cannabinoids in inflammatory disorders, such as atherosclerosis. Besides that, anandamide demonstrated its capacity to attenuate, in a dose-dependent manner, the expression of ICAM-1, induced by TNF-α, and of VCAM-1 in endothelial cells of human coronary arteries, and also THP-1 monocytes adhesion in a process dependent on CB1 and CB2 receptors (Batkai et al., 2007). Contrary to the potential beneficial effect in cardiovascular disease, the endocannabinoids may exhibit some prothrombotic effects. In fact, both anandamide and 2-AG were described as activators of human and rodent platelets. The platelets are cellular anucleated fragments that circulate on blood stream. Besides their recognized role in homeostasis and in thrombus formation, platelets may also have proinflammatory properties and be growth regulators, contributing to the progression of atherosclerosis (Leite et al., 2009). Endothelial cells, macrophages and platelets may, by itself, increase their synthesis of endocannabinoids during the formation of atherosclerotic plaque, leading to the activation of platelets. Alternatively, these cells are able to metabolize 2-AG and anandamide, which can offset the increased levels of cannabinoids. CB1 blockade with rimonabant, besides reduce weight and abdominal adiposity, improves cardiometabolic profile, due to multiple influences, including increased levels of high density lipoprotein (HDL)-cholesterol and reduced triglycerides (Despres et al., 2005; Van Gaal et al., 2005). A possible role for CB2 receptors on the progression of atherosclerosis was suggested in an experimental model. The authors found that oral low-doses THC treatment could inhibit the development of atherosclerostic plaque, which was reversed using SR144528, an antagonist of CB2 receptors (Klein et al., 2003). The progression of atherosclerosis was associated with a reduced infiltration of macrophages in the atherosclerotic lesions. The mobilization, adhesion and trans-endothelial migration of leukocytes are triggered by the local production of chemokines, its receptors and adhesion molecules (Braunersreuther & Mach, 2006). Cannabinoids, endogenous or synthetic, have shown to modulate the migration of several cell types, including immune cells trough activation of CB2 receptors (Miller & Stella, 2008). In overall, despite some interesting findings, a specific role of endocannabinoid signaling during atherosclerosis remains to be better elucidated.

New therapeutic opportunities of ECS in cardiovascular disorders

Obesity remains a continuous healthy problem and research issue, which is explained by the serious consequences associated with it, as well as by the increasing incidence of type 2 diabetes and associated obesity, including in younger individuals. In this way, the ECS, due to its well known properties of weight control and energy balance, appeared as a promising

target for the treatment of obesity, namely by blocking its receptors. The blockade of these receptors was effectively done by rimonabant, which was viewed as a promising drug for the treatment of obesity. Besides its action on obesity, rimonabant has also proved to be efficient in controlling vascular diseases in several clinical trials and, therefore, this drug was presented as an effective therapeutic approach for treating obesity and cardiovascular disease. However, despite the proven effectiveness in weight loss, rimonabant clinical use was associated with several side effects, which mainly includes the following three groups: the first one includes psychiatric disorders such as depression and anxiety; the second one is associated with gastrointestinal disturbances such as nausea; and finally the third group with regard to neurological changes that are reflected in headaches and vertigo. Despite these adverse effects, which originated its removal from the marker, since the blockade of CB1 receptors continues to prove an asset in the management of obesity and its associated risks (such as reduction of lipogenesis, decreased waist circumference, insulin resistance and dyslipidemia), research in the modulation of the ECS has continued.

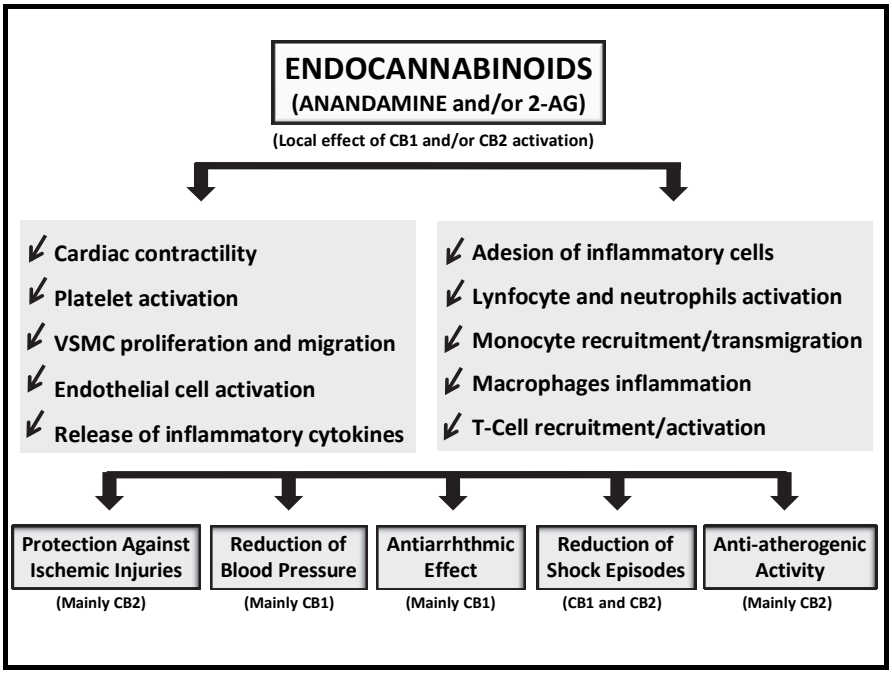

Fig. 3. Endocannabinoids pleiotropic effects on cardiovascular physiology, demonstration protection activity on several tissues, together with positive impact on various other cells that contribute to cardiovascular/atherosclerotic pathologies, such as the monocytes, macrophages, lymfocyttes, leucocytes, neutrophils and other inflammatory cells.

Although CB1 receptors seem to be the main target for new therapeutic interventions, CB2 receptors are also involved in several mechanisms and, since they are present in immune cells and are, apparently, involved in modulating immune responses, they are extremely important and may be seen as a therapeutic target as well. Several line of evidences have

been clearly suggesting that cannabinoids and their endogenous and synthetic analogs can promote important cardiac effects, which includes hypotension and cardiodepression. The actions seem to be mediated by complex mechanisms, including both direct and indirect effects both on the vasculature and on the myocardium. Furthermore, the ECS, including endocannabinoids and cannabinoid receptors, have been implicated in the myocardial and cerebral ischemia/reperfusion, in hypotensive state associated with hemorrhagic, endotoxic and cardiogenic shock, and in advanced liver cirrhosis. There is also promising evidences hypothesizing a key role for the endocannabinergic system in the cardiovascular regulation in hypertension, as well as a beneficial action on atherosclerotic plaque. Resuming, cannabinoids are able to modulate a countless number of physiologic functions, demonstration a pleiotropic protective action on the cardiovascular physiology (Figure 3) and therefore, endocannabinoid system is a potential target for the treatment of several diseases and the research about this subject still have a long way to go. The evidence so far gathered shows that the modulation of ECS (as agonism or antagonism of its receptors) is an enormous potential field for research and intervention in multiple areas of human pathophysiology. The development of selective drugs for the CB1 and CB2 receptors may open a door to new therapeutic regimens, in particular in several cardiovascular disorders.

11. Conclusion

The recreational use of the plant *Cannabis sativa* and the attempt to exploit their potential therapeutic use have been described over the centuries. The popularity of *marijuana*, one of the most common forms of consumption as a recreational substance and as a drug, reflects its ability to later sensory perceptions and to reduce anxiety. Experimental studies have been repeatedly demonstrated Δ^9-THC effects on an individual cognitive function and psychomotor skills, influencing learning and information acquisition, changing the individual's memory capacity, coordination and reaction times. The biggest concern with cannabinoids acute effects is related to road traffic or labour accidents. Indeed, Δ^9-THC acute effects on cognitive function and psychomotor skills have been subject of extensive study, noting that, at doses between 40 and 300 mg/kg, cannabinoids can cause a dose-dependent reduction in tasks that require memory use, reaction times, in motor functions and coordination. It has been shown that the influence and accident risk due to recent *cannabis* increases with dose, with an influenced state already present at low doses, being even worse at higher concentrations. Δ^9-THC plasma concentrations can be very variable, with values between 1 and 35 ng/ml in suspected impaired drivers and between 1 and 100 ng/ml in fatal road traffic drivers.

Non-psychoactive actions of *marijuana*, like pain relief, were also described in ancient texts. However, the biochemical and pharmacological study of this substance has a fairly recent start. Endocannabinoids are involved in several physiological functions, among which, special attention has been given to the regulation of appetite by central mechanisms and its influence on obesity. Considering these innovative findings, the research for new pharmacological agents has drastically increased and the discovery of rimonabant, a synthetic antagonist of CB1 receptors, has confirmed the important role of endocannabinoid system on the modulation of food ingestion and energetic balance. Although several other different influences of EC have been discussed during the last years, including in inflammation, diabetes, cancer, affective and neurodegenerative diseases, and epilepsy, the

most recent findings are related to their cardiovascular actions, which seem to be very ample bust also complex. The ECS, which includes the endocannabinoids and its receptors, have been implicated in hypotensive stages associated with hemorrhagic chock, both endotoxic and cardiogenic, and even to advanced liver cirrhosis; on the other hand, recent evidence suggests that ECS plays an important role in cardiovascular regulation associated with hypertension, as well as a protective role in ischemia grafting. The development of atherosclerotic plaque and the metabolic stages associated to obesity are also matter of study of possible ECS pharmacomodulation.

The continued approach of biophysics and molecular characterization of ligands for the cannabinoid receptor will contribute decisively to the success of cross-level research of ECS. Those advances will be pivotal for the development and definition of the profile of new chemical entities as therapeutic endocannabinoid modulators. They may also facilitate the identification of new dynamics of the ECS to be used as predictive and/or diagnostic orientation biomarkers for the patients, as well as therapeutic based on ECS pharmacomodulation. The therapeutic approach of cardiovascular system starting from the modulation of ECS appears to be a promising and multidisciplinary issue of study that is still in its early stages but that could be a field for better therapeutic intervention in several disorders, including of cardiovascular and cardiometabolic nature.

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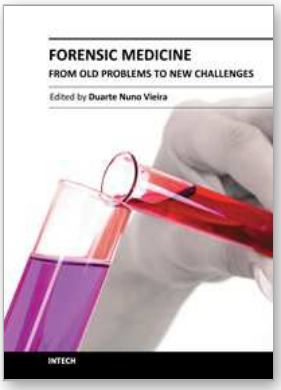

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