RECENT PROGRESS IN THE NEUROTOXICOLOGY OF NATURAL DRUGS ASSOCIATED WITH DEPENDENCE OR ADDICTION, THEIR ENDOGENOUS AGONISTS AND RECEPTORS

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ABSTRACT — Nicotine in tobacco, tetrahydrocannabinol (Δ⁹-THC) in marijuana and morphine in opium are well known as drugs associated with dependence or addiction. Endogenous active substances that mimic the effects of the natural drugs and their respective receptors have been found in the mammalian central nervous system (CNS). Such active substances and receptors include acetylcholine (ACh) and the nicotinic ACh receptor (nAChR) for nicotine, anandamide and CB1 for Δ⁹-THC, and endorphins (1 and 2) and the μ (OPs) opioid receptor for morphine, respectively. Considerable progress has been made in studies on neurotoxicity, in terms of the habituation, dependence and withdrawal phenomena associated with these drugs and with respect to correlations with endogenous active substances and their receptors. In this article we shall review recent findings related to the neurotoxicity of tobacco, marijuana and opium, and their toxic ingredients, nicotine, Δ⁹-THC and morphine in relation to their respective endogenous agents and receptors in the CNS.

KEY WORDS: Anandamide, Dependence, Endomorphin, Morphine, Nicotine, Tetrahydrocannabinol

INTRODUCTION

One of the longest-used and most widespread drugs that is associated with dependence and is present in natural biological sources is nicotine, which is a psychoactive constituent of tobacco plants (Nicotiana tabacum and Nicotiana rustica). Three other popular drugs from plants are morphine from opium (Papaver somniferum), tetrahydrocannabinol (THC) from marijuana (Cannabis sativa) and cocaine from coca (Erythroxylum spp.). Nicotine and THC or morphine can be taken in by smoking dried leaves or the dried juice of unripe pods, and each has specific receptors which have their corresponding endogenous agonists both in the central nervous system (CNS) and in peripheral tissue. The endogenous agonists are acetylcholine (ACh) for nicotine (O’Brien, 1996; Taylor, 1996); anandamide for THC (Devane et al., 1992) and very recently sn-2 arachidonylglycerol for THC (Stella et al., 1997); and endorphins for morphine (Zadina et al., 1997) (Table 1). In spite of sharing common receptors, the endogenous and corresponding exogenous agonists exhibit no obvious structural similarities to each other (Fig. 1). ACh is a choline ester, anandamide is a derivative of arachidonic acid and endorphins are small peptides of four amino acids, while nicotine is an alkaloid, THC is a cannabinoid and morphine is an alkaloid.
Information on the nicotinic cholinergic system, cannabinoid transmission and \( \mu \)-opioid system has been increased and updated by cloning research, and by discoveries of endogenous active substances and the receptors in brain from mammals including humans (Albuquerque et al., 1997; Breivogel et al., 1997; Felder and Glass, 1998; Lord et al., 1997; Mansour et al., 1994; Olale et al., 1997; Zadina et al., 1997).

The interaction of nicotine, tetrahydrocannabinol and morphine with corresponding endogenous transmitting systems in the brain has received extensive interest in understanding the mechanisms underlying dependence, addiction or tolerance induced by chronic intake of tobacco, marijuana and opium narcotics in molecular levels in recent years.

Nicotine is not popularly viewed as narcotics are. Habitual users of tobacco are suggested to experience craving, tolerance, psychological dependence, probably milder physical dependence, relapse during abstinence and withdrawal symptoms (Benowitz, 1992; Dani and Heinemann, 1996). Therefore, an improved understanding of the mechanisms underlying the effects of nicotine on nervous function could provide a fundamental insight into drug-receptor interactions for studies of dependence, addiction or tolerance induced by chronic intake of marijuana and the opium narcotic.

Although our understanding of tolerance, dependence or addiction induced by nicotine, cannabinoid and morphine is still in its early stages, the discovery of endogenous cannabinoids and endogenous morphine in

Table 1. Relationships between abused drugs from natural sources and the corresponding endogenous systems in mammals.

<table>
<thead>
<tr>
<th>Abused drug</th>
<th>Main active ingredient</th>
<th>Agonist</th>
<th>Receptors (R)</th>
<th>Distribution of receptors</th>
<th>Receptor type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco</td>
<td>nicotine</td>
<td>acetylcholine</td>
<td>( n\text{AChR} )</td>
<td>various brain regions (striatum, neocortex, hippocampus, thalamus, nucleus accumbens, amygdala)</td>
<td>ion channel</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( n\text{AChR} )</td>
<td>autonomic ganglion, adrenomedulla, chemoreceptors</td>
<td>ion channel</td>
</tr>
<tr>
<td>Marijuana</td>
<td>( \Delta ^{9}\text{-THC} )</td>
<td>anandamide, ( sn-2 )</td>
<td>CB1</td>
<td>CNS (basal ganglia, hippocampus, cortex, cerebellum, substantia nigra)</td>
<td>G-protein-coupled</td>
</tr>
<tr>
<td>Marijuana</td>
<td>( \Delta ^{9}\text{-THC} )</td>
<td>( sn-2 )</td>
<td>CB2</td>
<td>spleen (macrophages)</td>
<td>G-protein-coupled</td>
</tr>
<tr>
<td>Opium</td>
<td>morphine</td>
<td>endomorphin-1, endomorphin-2</td>
<td>OP3 R in the CNS</td>
<td>spinal cord, various brain regions (caudate putamen, neocortex, thalamus, nucleus accumbens, hippocampus, amygdala)</td>
<td>G protein-coupled</td>
</tr>
</tbody>
</table>

Abbreviations: \( \Delta ^{9}\text{-THC} \), delta-9-tetrahydrocannabinol; \( n\text{AChR} \), nicotinic acetylcholine receptor; OP3 R in the CNS, \( \mu \) opioid receptors in the central nervous system; \( sn-2 \), \( sn-2 \) arachidonylglycerol.
the brain, the development of competitive antagonists, and the characterization of brain nAChR subtypes provides a good opportunity to review tolerance, dependence or addiction induced by chronic intake of tobacco, marijuana and opium.

**TOBACCO**

**Toxins in tobacco**

The smoke from burning tobacco leaves contains many chemicals, which include nicotine, benzo(a)pyrene (a potent carcinogen), and other tumorigenic compounds (see Witschi et al., 1997). The acute toxicity of nicotine is very high and is sometimes lethal, especially in children.

**Tobacco and nicotine**

The relationship between tobacco and nicotine is well known, even to the general public. In brief, nicotine is one of the few naturally liquid alkaloids. It has a colorless, volatile base (pKa=8.5) that turns brown and acquires the odor of tobacco on exposure to air (see Taylor, 1996). Nicotine provides the reinforcement for smoking cigarettes. It induces an extremely durable dependence, as exemplified by the high failure rate among smokers who try to quit (Henningfield et al., 1983; Benowitz, 1992; O'Brien, 1996). Nicotine is readily absorbed through the lungs, as well as through the skin and mucous membranes (Henningfield et al., 1993). Since the pulmonary route produces discernible central effects in as little as 7 seconds, each puff on a cigarette, pipe or cigar produces some discrete reinforcement (O'Brien, 1996). The concentration of nicotine typically found in the serum of smokers is about 0.2 μM (see Olale et al., 1997).

Lennox Johnson, an American physician, found that intravenous injections of nicotine were pleasurable to smokers but not to nonsmokers, and that injections of nicotine might give even more pleasure than smoking itself (see Henningfield et al., 1983; O'Brien, 1996). Chewing tobacco is also an addictive method for delivery of nicotine that is very popular in the U.S. (Benowitz, 1992; O’Brien, 1996).

**Nicotinic acetylcholine receptors**

There are two major groups of ACh receptors (AChRs): nicotinic ACh receptors (nAChRs), which are coupled to ligand-gated ion channels; and muscarinic ACh receptors (mAChRs) which are coupled to a GTP-binding protein (see Ross, 1996) (Fig. 2). The former are characterized by the action of nicotine as an agonist. They are involved in fast synaptic transmission and their three-dimensional structure has been well described at the molecular level (Unwin, 1995). The latter are characterized by the action of muscarine as an agonist. They respond relatively slowly through coupling to a GTP-binding protein, and they are known to have seven transmembrane regions (Cooper et al., 1996) (Fig. 2). It is generally accepted that mAChRs are the predominant AChRs in the central nervous system, owing to the lower density of nAChRs (Cooper et al., 1996).

Compared with the detailed information that is available about the structure, function and regulation of nAChRs in skeletal muscle and electric organs, much less is known about neuronal nAChRs, which are found in sympathetic ganglia and in the central nervous system (see Cooper et al., 1996; Lefkowitz et al., 1996).

**Fig. 2.** Schematic representation of nicotinic (upper) and muscarinic (bottom) acetylcholine receptors (nAChRs and mAChRs). Functional nAChRs are pentameric proteins (containing at least one type of α-subunit and one type of β-subunit).
The receptor in the muscle consists of four different protein subunits arranged as the pentamer α2βγδ or as the pentamer α2βεδ, upon replacement of the γ subunit by the ε subunit in a manner that depends on cell maturity and animal species (see Ross, 1996). By contrast, the available evidence suggests that the neuronal receptor is composed of agonist-binding α subunits and structural β subunits only (Whiting and Lindstrom, 1987; Lefkowitz et al., 1996; Olale et al., 1997; Albuquerque et al., 1997). It is generally believed that neuronal nACHRs are pentamers with at least two binding sites for ACh, α subunits, as is the case for the muscle nACHR, together with structural β subunits, or homoligomers of α7, α8 or α9 (Lefkowitz et al., 1996; Albuquerque et al., 1997). The subtypes of neuronal nACHR in the CNS are extremely diverse in terms of kinetics of activation and inactivation and in terms of sensitivity to nicotinic agonists and antagonists. Molecular biological studies of neuronal nACHRs have identified some variant subunits, namely α2 through α9, and β2 though β4. It is well established that nACHRs can exist in multiple forms with somewhat different pharmacological properties depending on the subunit composition. One subtype is thought to have an (α4)2(β2) stoichiometry and to represent most of the high-affinity nicotinic binding activity in the brain (Wada et al., 1989; Anand et al., 1991; Flores et al., 1992; Albuquerque et al., 1997). Nearly equal amounts of a subtype that is thought to have exclusively an (α7)2 stoichiometry are also found in the brain (Albuquerque et al., 1997). It is likely that many subtypes of nACHR in the brain are expressed in discrete populations of neurons with specific functional roles. Many of the nACHRs in the brain are thought to be located presynaptically, and they have been implicated in facilitation of the release of transmitters such as ACh, dopamine, glutamate and γ-aminobutyric acid (Albuquerque et al., 1997).

Nicotine and acetylcholine

Acetylcholine is an agonist for all AChRs, including all subtypes of nACHR and nACHR. Nicotine, by contrast, stimulates the nACHRs that are localized peripherally at autonomic ganglia that include the adrenal medulla and chemoceptors of carotid bodies and the aortic body, as well as nACHRs that are located centrally at cholinergic synapses in the brain and spine, in which Renshaw cell synapses are formed by collaterals of motor axons (Table 1).

Acetylcholine is hydrolyzed by acetylcholinesterase (AChE) at the synaptic cleft immediately (less than 1 msec) after it is released, while nicotine, which is not degraded by AChE, can persist after it has reached the cleft. Unless AChE is inhibited by an anticholinesterase, the time-course of depolarization induced by ACh is temporary or transient. By contrast, nicotine initially stimulates nACHRs by an ACh-like action and then blocks them because of a persistent depolarization, which results in desensitization of the receptors.

Dependence on nicotine

There is little doubt that most habitual smokers find it difficult to quit the habit because they have become addicted to the nicotine in the smoke that they inhale. A smoker who takes 10 puffs per cigarette and smokes one pack of 20 per day reinforces the habit 200 times daily (O'Brien, 1996). A variety of studies has suggested the critical importance of nicotine in maintaining tobacco smoking in humans. Moreover, nicotine has been self-administered by laboratory animals, as well as by humans. Nicotine affects many aspects of behavior, including learning and memory, via its interactions with neuronal high-affinity nACHRs (see Dani and Heinemann, 1996; Breese et al., 1997). A growing body of research suggests that nicotinic cholinergic systems are involved directly or indirectly in the pathophysiology of a number of neurological disorders. Nicotine is thought to exert its reinforcing actions indirectly by stimulating the function of dopaminergic systems of brain regions of the brain such as the mesolimbic, nucleus accumbens and nigrostriatal systems (Wonnacott et al., 1990).

Molecular toxicology of dependence induced by nicotine

Since abused drugs have the ability to elevate mood and also to induce tolerance, with a pattern of declining response to repeated administration of the drug, a person who tries tobacco is likely to repeat the experience. Nicotine has been shown to induce the release of several neurotransmitters in the central nervous system via a direct receptor-mediated action on nerve terminals (Gray et al., 1996; McGehee and Role, 1995; Wonnacott et al., 1990).

Chronic treatment with agonists specific for most neurotransmitter receptor systems results in a decrease in numbers of respective receptors (down-regulation) (Creese and Sibley, 1981), as has been shown for β-adrenergic and muscarinic cholinergic receptors. However, the chronic administration of nicotine to mice and rats or smoking in humans (Fig. 3) often causes an increase in the density (up-regulation) of
Nicotine, tetrahydrocannabinol, or morphine, and their endogenous systems in CNS.

nACHRs in the brain, as monitored by binding of a selective agonist (Benwell et al., 1988; Wonnacott, 1990; Balfour, 1996; Breese et al., 1997). This up-regulation occurs before the development of tolerance to the effects of nicotine on several behaviors, such as locomotor activity, respiratory rate, heart rate, body temperature and the acoustic startle response (Dani and Heinemann, 1996). Continued smoking in humans results in nicotine tolerance. The dose-dependent increase in density of nicotinic receptors in human subjects that persists in smokers may, at least to some extent, influence the development of tolerance and addiction to nicotine (Dani and Heinemann, 1996). It has, however, also been reported that behavioral tolerance to nicotine is a consequence of a decrease in the density (down-regulation) of brain nACHRs. Studies on the levels of mRNAs for specific subunits of nACHRs should be conducted to characterize the mechanisms involved in the up-regulation (or down-regulation) of nACHRs in humans (Dani and Heinemann, 1996).

In a recent experiment, Xenopus laevis oocytes were injected with cRNAs for a7 nACHRs, a4B2 nACHRs and a3 nACHRs. Chronic exposure of such oocytes to nicotine at submicromolar concentrations, which are essentially equal to the concentration of nicotine typically found in the serum of smokers, irreversibly inactivated many a4B2 nACHRs and a7 nACHRs but inhibited a3B2B4a5 nACHRs to a much lesser extent (Olale et al., 1997). Since the behavioral effects of nicotine might reflect the sustained inhibition of a4B2 nACHRs and a7 nACHRs, in combination with the residual susceptibility of a3B2B4a5 nACHRs and also some other nACHR subtypes for acute activation, tolerance to nicotine exhibited by tobacco users might reflect the long-term, irreversible, functional inactivation of a4B2 and a7 nACHRs that is produced by chronic exposure to nicotine (Olale et al., 1997).

Despite many schizophrenics' extremely heavy nicotine use, nicotinic receptors were not previously thought to be involved in schizophrenia. The effects of nicotine in some diseases suggest that nACHRs may be involved in some way in their pathology. For example, neurobiological investigations in both humans and animal models indicated that decreased function of a7 nACHRs could underlie the physiological defect. The defect is linked to a dinucleotide polymorphism at chromosome 15q13-14, the site of the a7 nACHR (Freedman et al., 1997). The linkage data thus provide unique new evidence that the a7 nACHR gene may be responsible for the inheritance of a pathophysiological aspect of the illness.

An understanding of which nACHR subtypes are associated with the effects of chronic exposure to nicotine might provide better insights into mechanisms of nicotine dependence, tolerance and withdrawal and into the effects of medication with nicotine or nicotinic drugs.

MARIJUANA

Toxins in marijuana

Cannabis sativa, the source of marijuana, hashish or bhang, has been cultivated for centuries for two main purposes, the production of hemp fiber and the preparation of psychoactive materials. Marijuana is the most widely abused drug worldwide. Sixty-one cannabinoids have been identified in the smoke from cannabis leaves and one of them, Δ9-tetrahydrocannabinol (Δ9-THC), has been recognized as the predominant psychoactive constituent. There are binding sites for

![Fig. 3. Comparison of binding of nicotine to nicotinic acetylcholine receptors (nACHRs) in the hippocampus and thalamus of nonsmokers, smokers and former smokers. Numbers of nACHRs were estimated from the binding of [3H]nicotine to nACHRs prepared from the hippocampus or thalamus obtained at autopsy from nonsmokers (average age, 51.5±6.1 years, n=11), smokers (average age, 55.9±3.1 years, n=21) and former smokers (average age, 60.8±3.9 years, n=9) who had quit from 2 months to 30 years prior to death. Significantly different from nonsmokers (*p<0.01, **p<0.0004) and from former smokers (#p<0.018, ##p<0.0001). This Fig. was prepared from the data in Table 2 in the report by Breese et al. (1997).]
Δ⁸-THC in the central nervous system, and a receptor for cannabinoid agonists has been identified, in the brain, that is involved in the regulation of behavior, emotions, and coordination (Dewey, 1986). Δ⁸-THC produces a characteristic pattern of behavioral effects, such as catalepsy, antinociception, anticonvulsive activity, hypothermia, depression of motor activity and hyperexcitability, in a wide range of animal species (Olale et al., 1997; Dewey, 1986).

Marijuana and tetrahydrocannabinol

Although marijuana possesses properties of a hallucinogen, sedative and euphoria inducing, it is inadequately described by any single category. It is usually classified as an abused or/and addictive drug with other drugs, such as alcohol, cocaine, phenycyclidine and morphine (O’Brien, 1996; Willoughby et al., 1997). Since the cloning of the cDNA sequences of cannabinoid receptors and the isolation of an endogenous cannabinoid, anandamide, rapid progress has been made in marijuana research over the past six or seven years.

Cannabinoid receptors

The cannabinoid receptor has been cloned from rat and human sources and it is a member of the family of seven-transmembrane-domain GTP-binding protein-coupled (G protein-coupled) receptors (Howlett, 1995). Recently two subtypes of cannabinoid receptor, CB1 and CB2, were identified and cloned, providing important new tools for future investigations of the mechanisms of action of cannabinoids (Abood et al., 1997; Chakrabarti et al., 1995; Gerard et al., 1991).

The effects on the CNS of cannabinoids appear to be mediated exclusively by CB1, which is coupled to Gi to inhibit adenylyl cyclase activity and to a pertussis-sensitive G-protein for negative regulation of Ca²⁺ channels and positive regulation of K⁺ channels. Two splice variants of CB1 mRNA (CB1 and CB1A) have been identified in the mammalian brain. CB1 is abundant in regions of the brain such as the substantia nigra, hippocampus and cerebellum (Table 1). The effects of cannabinoids on memory, body temperature and motor function are consistent with the distribution of CB1 receptors in the hippocampus, hypothalamus and basal ganglia, but the signal transduction mechanisms that mediate these biological effects have not been well characterized. It has been reported that coupling to G-proteins by CB1 varies among brain regions and depends on the cellular environment (Breivogel et al., 1997).

Relatively little is known about the mechanisms that underlie the signal transduction that is mediated by CB2, which is referred to as a peripheral cannabinoid receptor. CB2 has been found in the promyelocytic leukemic line HL60, and mRNAs for this receptor were identified in rat splenic macrophages (Munro et al., 1993; Galiegue et al., 1995; Schatz et al., 1997), but not in rat brain (Holsapple et al., 1996).

Very recently, a review on molecular pharmacology of cannabinoid receptors and their endogenous cannabinoids has been published (Felder and Glass, 1998).

Tetrahydrocannabinol and anandamide

An endogenous ligand that binds to the cannabinoid receptor, anandamide, was identified by Devane et al. (1992) and, subsequently, two other cannabimimetic N-acetyl-ethanolamines were identified as docosatetraenoylthanolamide and homo-γ-linolenoylthanolamide in porcine brain by Hanus et al. (1993). It has been shown that rat brain neurons in primary culture produce and release anandamide and other N-acylthanolamines when they are stimulated with membrane-depolarizing agents or Ca²⁺ ionophores (Di Marzo et al., 1994). Cannabinoids inhibit the release of ACh, noradrenaline and glutamate from brain regions (Gessa et al., 1997; Giford and Ashby, 1996; Shen, et al., 1997; Schlicker et al., 1997). Felder and Glass (1998), however, have proposed that anandamide, as a lipophilic compound, would not be stored within the synaptic vesicle cytoplasm, but would diffuse freely across membranes.

Anandamide appears to have a 30-fold greater affinity for CB1 receptor in the brain than CB2 receptor found peripherally (Felder et al., 1995). Although anandamide and Δ⁸-THC produce similar pharmacological effects through the same cannabinoid receptor in the central nervous system (Crawley et al., 1993; Frize and Mechoulam, 1993; Smith et al., 1994), the duration of action induced by this endogenous cannabinoid is shorter than that by the latter (Willoughby et al., 1997). Most of the behavioral effects, such as hypothermia, hypomotility and catalepsy, but not antinociception, are almost completely dissipated by 30 min after administration of anandamide (Howlett, 1995). Since these two compounds have few structural similarities, they are expected to be metabolized through a different biochemical pathway, via rapid hydrolysis by a membrane-associated amidase for anandamide and via the P450 pathway for Δ⁸-THC (Willoughby et al., 1997).

Phenylmethylsulfonyl fluoride (PMSF) was found to be a potent inhibitor of this amidase (Compton and Martin, 1997).

Very recently, Stella et al. (1997) reported that sn-2
arachidonylglycerol is present in the brain in amounts 170 times greater than anandamide, and is a second endogenous cannabinoid ligand in the CNS. On the other hand, Adams et al. (1998) reported the possibility that anandamide may not be producing all of its effects by a direct interaction with the CB1 receptor, because the effects on spontaneous activity, body temperature, nociception and mobility in mice was not blocked by a pretreatment with SR 14176A, a selective CB1 antagonist.

**Dependence by marijuana**

The phenomenon of tolerance to most of the effects of Δ⁹-THC was demonstrated in a number of animal species and in human experimental procedures after repeated administration (Dewey, 1986). Tolerance developed rapidly after a few doses disappears soon, while tolerance to large doses persists for longer periods after cessation of drug use. It was reported that tolerance to cannabinoids was a true pharmacodynamic tolerance and not due to an alteration in absorption, disposition or metabolism of the parent compound (Dewey, 1986). The mechanisms underlying the development of tolerance to marijuana or Δ⁹-THC have yet remained to be characterized at the molecular level in the aspects of cannabinoid receptor, CB1 and anandamide system.

Psychological dependence, but not physical dependence, has been observed in human and experimental animals after repeated administration of marijuana. A withdrawal syndrome in human subjects, such as restlessness, irritability, mild agitation, insomnia, sleep EEG disturbance, nausea and cramping, has been described following close observation of marijuana users given regular oral doses of the agent (O’Brien, 1996). The mechanisms underlying the development of psychological dependence induced by marijuana or Δ⁹-THC have yet remained to be characterized at the molecular level in the aspects of cannabinoid receptor, CB1 and anandamide system.

**Toxicology of dependence induced by marijuana**

Since cannabinoids are associated with a feeling of mild euphoria and decreased cognitive abilities, a person who tries marijuana heavily is likely to repeat the smoking hourly or more to maintain a high throughout the day. Δ⁹-THC has been shown to affect the response of an effector system or the release of several neurotransmitters from nerve terminals, on which CB1 may locate, via a direct action in a receptor-mediated manner in the central nervous system (Dewey, 1986; Gessa et al., 1997; Gifford and Ashby, 1996; Shen et al., 1997; Schlicker et al., 1997).

Tolerance is expected to develop after chronic exposure to agonists. Since chronic treatment with agonists for most neurotransmitter receptor systems results in a decrease in receptor number (down-regulation), one would predict that chronic exposure to Δ⁹-THC would result in a decreased number of cannabinoid receptors in brain. The chronic administration of Δ⁹-THC to rats or chronic smoking in monkeys, however, does not cause irreversible alteration in properties of CB1 receptors (Westlake et al., 1991). Impairment of memory, mathematical skills and verbal expression has also been observed in humans (Fig. 4) after chronic consumption of marijuana (Block et al., 1992; Block and Ghoneim, 1993; Solowij et al., 1995). It would be expected that chronic exposure to Δ⁹-THC would result in some alteration in properties of CB1 receptors.

**Fig. 4.** Impairments of selective attention due to frequency and duration of cannabis use. Performance measures of reaction time, hit rate and false alarm rate were determined in non-users (controls, n=16) and users of long (n=16) and short (n=16) duration, and of heavy (n=16) and light (n=16) frequency of cannabis use. The overall means total users (n=64). The data were expressed as relative values to the respective control value as 1.0. Values more than 1.0 for reaction time and false alarm, and values less than 1.0 for hit rate represent impairments of performance, respectively. Significantly different from control (*p<0.05, **p<0.02, #p<0.002, ψ<0.0005). This Fig. was prepared from the data in Table 1 in the report by Solowij et al. (1995).
It has recently been demonstrated that $^{[125]}$I AM281 [N-(morpholin-4-yl)-5-([4]-[125]iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-[1H-pyrazole-3-carboxamide], a selective CB1 antagonist, has appropriate properties for imaging cannabinoid CB1 in vivo (Gatley et al., 1998). Positron emission tomography (PET) or single photon emission tomography (SPECT) imaging techniques have been used to study the pharmacokinetics and pharmacodynamics of abused drugs such as cocaine in the human brain (Gatley et al., 1998). Thus, it will enable for the first time the use of SPECT with $^{[125]}$I AM281 to map cannabinoid receptors in the living human brain in the near future. Similar studies of cannabis binding sites would be expected to further our understanding of both the neurotoxicity of marijuana and of the medicinal effects of cannabinoid receptor ligands.

OPIUM

Toxins involved in opium

Opium contains more than 20 alkaloids (Reisine and Pasternak, 1996). The opiates are a class of naturally occurring alkaloids, of which the best known is morphine and the better may be codeine, derived from opium which is obtained by scoring the unripe seed capsule of the poppy Papaver somniferum and drying the exudate, and also include a wide variety of semisynthetic congeners derived from the natural alkaloids and thebaine, another component of opium. Opioid drugs are more inclusive. They include naturally occurring drugs and chemically synthesized agents which mimic or antagonize the pharmacological effects of morphine. Among the opioids, the most commonly abused drug is heroin (diacetylmorphine), which is prepared by acetylation of the phenolic and allylic hydroxyl groups of morphine, and the first pure antagonist identified is naloxone (Reisine and Pasternak, 1996).

Opium and morphine

Preparations of opium have a long history as therapeutic agents to relieve pain, to suppress coughing and to treat severe diarrhea. Papaver somniferum, the source of opium, has been cultivated for more than 5000 years mainly for two purposes, therapeutic use and psychological use or abuse drugs (Dhawan et al., 1996; Reisine and Pasternak, 1996). Since opioids induce a pleasurable state of euphoria in humans, their abuse and chronic use lead to psychological and physical dependence. The active principle in opium is morphine (Fig. 1). The actions of opiates and opioids are often able to be explained by actions on multiple opioid receptors. There are at least three types of opioid receptor, $\mu$, $\delta$ and $\kappa$. The $\mu$ receptor was named after morphine, which induces analgesia, miosis, bradycardia, hypothermia and indifference to environmental stimuli (Reisine and Pasternak, 1996).

Opioid receptors

The endogenous opioid system consists of three distinct families of endogenous opioid peptides and three major classes of opioid receptors. Opioid receptors are present not only in the central nervous system but also at the peripheral tissues such as intestine and vas deference (Reisine and Pasternak, 1996). Identification of these receptors, $\delta$, $\kappa$ and $\mu$, by molecular cloning has confirmed pharmacological studies that previously postulated the existence of these three receptors. IUPHAR (The International Union of Pharmacology) recommends the nomenclature of opioid receptors as OP$\delta$ for $\delta$, OP$\kappa$ for $\kappa$ and OP$\mu$ for $\mu$ (Dhawan et al., 1996, 1998).

The cloned opioid receptors are highly homologous in their amino acid sequences and belong to the family of seven-transmembrane, G protein coupled receptors. Opioids inhibit adenyl cyclase via the activation of a pertussis toxin-sensitive G protein, Go/Gi (Emmerson et al., 1994). The amino acid sequences of OP$\delta$ and OP$\kappa$ receptors, OP$\delta$ and OP$\kappa$ receptors, and OP$\delta$ and OP$\kappa$ receptors are 66, 68 and 58% identical respectively, with the primary variations occurring in the extracellular N-terminus and intracellular C-terminus domains and external loops (Dhawan et al., 1996; Childers, 1991).

The OP$\delta$ ( $\delta$ ) receptor was initially named after the vas deference ( $\delta$ , for deference), in which enkephalins are more potent than morphine in inhibiting evoked release of neurotransmitters and their effects are relatively insensitive to naloxone. Most of the agonists for OP$\mu$ receptor are enkephalins in mammals (Lord et al., 1977; Dhawan et al., 1996, 1998). The OP$\delta$ receptors have a more restricted distribution than other opioid receptors in the central nervous system, in which olfactory bulb, neocortex, caudate putamen and nucleus accumbens have high densities in these receptors (Waksman et al., 1986; Tempel and Zukin, 1987; Dupin et al., 1991; Renda et al., 1993; Mansour et al., 1994). The role of OP$\kappa$ receptors involves analgesia, motor integration, gastro-intestinal motility, olfaction, cognitive function, mood-driven behavior, etc. (see Dhawan et al., 1996).

The OP$\kappa$ ( $\kappa$ ) receptor was initially named after
ketocyclazocine, which has an agonist effect markedly different from that of morphine in vivo. To date, the dynorphins are the most probable endogenous ligands of OP2 receptors (Dhawan et al., 1996, 1998). The highest density of OP2 receptor is found in the inner layers of the cerebral cortex in guinea pigs and nucleus accumbens, claustrum, dorsal endopiriform nucleus and interpeduncular nucleus in rats (Nock et al., 1989; Hunter et al., 1990; ). The role of OP2 receptors involves the regulation of nociception, diuresis, feeding and neuroendocrine secretions (see Dhawan et al., 1996).

Morphine and other morphine-like opioid agonists produce pharmacological effects, such as analgesia, respiratory depression, miosis, reduced gastrointestinal motility, and feelings of well-being (euphoria), primarily through interaction with OPs (μ) receptors (see O'Brien, 1996; Dhawan et al., 1996). Most of the clinically used opioids are relatively selective for OP3 receptors, reflecting their similarity to morphine. Morphine shows about a 50-fold higher affinity for OP3 receptors than for OP1 receptors (Emmerson et al., 1994; Dhawan et al., 1996). To date, the most potent and selective agonist at OP3 receptors is etonitazene, a benzimidazole opioid, and endorphin-1 and -2, endogenous peptides (Table 2) (Zadina et al., 1997; Dhawan et al., 1998). β-Endorphin binds with equal affinity to both OP1 and OP3 receptors (Zadina et al., 1997). Although no endogenous mammalian peptides with both high affinity and selectivity for the OP3 receptor were known, endorphin-1 and -2 have been reported to have a high affinity for OP3 receptors (Zadina et al., 1997) (Table 2). OP3 receptors distribute in the superficial layer of the dorsal horn of the spinal cord, where the nociceptive primary afferent fibers are included, and in various regions of the brain (Chaillet et al., 1984; Porreca et al., 1984, 1987; Fang et al., 1986; Paul et al., 1989; Besse et al., 1990). The order of density of OP3 receptors is caudate putamen > neocortex > thalamus > nucleus accumbens > hippocampus > amygdala (see Dhawan et al., 1996). The major role of OP3 receptors involves the control of nociception, and their agonists block the nociceptive responses to mechanical, thermal and chemical stimulation (see Dhawan et al., 1996).

**Morphine and endorphins**

As mentioned above, μ receptors initially were defined by their affinity for morphine. The analgesic effects of morphine are believed to be mediated mainly by OP3 (μ). When morphine is administered, the binding of the drug to the opioid receptors alters the normal series of biochemical events controlled by the opioid receptor-endorphin system, resulting in pharmacological effects such as analgesia (O'Brien, 1996; Dhawan et al., 1996).

Of the three families of endogenous opioid peptides, the enkephalins show higher affinity for the OP1 (δ) receptor binding sites, and the dynorphins for the

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Structure</th>
<th>Binding to OP3 (μ) receptors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>See Fig. 1</td>
<td>Ki (nM) 2.66 δ μ 42 κ μ 13</td>
<td>Emmerson et al., 1994</td>
</tr>
<tr>
<td>Etonitazene</td>
<td>nonpeptides</td>
<td>0.02 8.800 11.650</td>
<td>Emmerson et al., 1994</td>
</tr>
<tr>
<td>Endomorphin-1</td>
<td>YPF-W-NH2</td>
<td>0.36 4.183 15.077</td>
<td>Zadina et al., 1997</td>
</tr>
<tr>
<td>Endomorphin-2</td>
<td>YPF-F-NH2</td>
<td>0.69 13.381 7.594</td>
<td>Zadina et al., 1997</td>
</tr>
<tr>
<td>DAMGO</td>
<td>YDAC(NMF)NH(CH2)2OH</td>
<td>0.34 5.59 3.824</td>
<td>Zadina et al., 1997</td>
</tr>
<tr>
<td>β-Endorphin</td>
<td>YGGFMTEKQSTPLVTLF-KNAIKKNAYKGGQ</td>
<td>1.23 515 434</td>
<td>Emmerson et al., 1994</td>
</tr>
<tr>
<td>Met-encephalin</td>
<td>YGGFIM</td>
<td>4.4 1 46</td>
<td>Zadina et al., 1997</td>
</tr>
<tr>
<td>Dynorphin</td>
<td>YGGFLRRPPLKWDQ</td>
<td>9.5 468</td>
<td>Zadina et al., 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.73 3 0</td>
<td>Zadina et al., 1997</td>
</tr>
</tbody>
</table>

Ki is the concentration of agonist at which it inhibits 50% of the binding of [3H]DAMGO. Abbreviations:

A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; I, isoleucine; K, lysin; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.
OP₂ (κ) receptor, although β-endorphin binds with equal affinity to both OP₁ (μ) and OP₂ receptors. Recently, endogenous ligands to the OP₂ receptor, endomorphin-1 and -2 were identified in bovine and human brains by Zadina et al. (1997*, 1997†).

In contrast to the enkephalins, dynorphins and β-endorphin, all endogenous opioid peptides have the amino-terminal amino-acid sequence Tyr-Gly-Gly-Phe, endomorphin-2 and -2 consist of Tyr-Pro-Trp-Phe-NH₂ and Tyr-Pro-Phe-Phe-NH₂, respectively (Zadina et al., 1997†).

The pharmacological effects of the endorphins were examined in vitro with the peptide with Phe in the fourth position (Phe-4-Tyr-W-MIF-1, Phe-4 peptide) (Zadina et al., 1997†). Endorphins were proved to be more selective for the OP₂ (μ) receptors than DAMGO (Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol), which is known to be the most potent and OP₂ selective compound available. This in vitro effect was blocked and reversed by the antagonists naloxone and OP₂ (μ)-selective CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂), but not by the OP₂ (κ) selective antagonist, nornaltporphinoline (Zadina et al., 1997†).

The in vivo effects of the endorphins were also examined with the Phe-4 peptide (Zadina et al., 1997†). The in vivo activity of endorphin, which was injected intracerebroventricularly or intrathecally, was as potent as that of morphine in producing analgesia. The analgesia induced by endorphin (Phe-4 peptide) was reversed by a low dose of naloxone or by pretreatment for 24 hr with the irreversible OP₂ (μ)-selective antagonist β-funaltrexamine. Stone et al. (1997) suggested that endomorphin-1 and -2 are potent spinal antinociceptive and anti-allodynic agents and that they or related compounds may prove therapeutically useful as spinal analgesics.

Zadina and coworkers generated an antibody against the Phe-4 peptide to detect 1 pg peptide and separate no crossreactivity for over 40 opioid and non-opioid peptides and compounds (Zadina et al., 1997†). They succeeded in isolating and identifying Tyr-Pro-Trp-Phe-NH₂ (endomorphin-1) and Tyr-Pro-Phe-Phe-NH₂ (endomorphin-2) from bovine cortical brain extracts. Like the two endogenous ligands (Met-enkephalin and Leu-enkephalin) for OP₁ (δ) receptor, endomorphin-1 and endomorphin-2 differ by a single amino acid at a third position of the peptide, Trp and Phe as underlined above, respectively (Zadina et al., 1997†).

Both peptides were also purified from the frontoparietal cortex of human brain tissue (Zadina et al., 1997†). The isolated endorphins showed full biological activity and were found in the human brain in much higher amounts than in bovine frontal cortex. When sections of spinal cord were stained immunocytochemically with antisera raised against endomorphin-2, a dense plexus of fibers and varicosities was visualized in the superficial dorsal horn of rats and one monkey (Pierce et al., 1998). Labeling for endomorphin-2 after unilateral multiple dorsal rhizotomy was markedly reduced ipsilaterally to the lesion. These data suggest that endomorphin-2 occurs in small diameter primary afferent fibers in rodents and primates. It appears possible that the release of endomorphin-2 from the primary afferent terminal might regulate the release of neurotransmitters from nociceptive primary afferents (Pierce et al., 1998).

It was shown that both endomorphin-1 and -2 were anti-allodynic in the dynorphin-induced allodynia model (Stone et al., 1997). Acute tolerance develops rapidly against both endorphins, although endomorphin-1 requires a longer pretreatment time before tolerance is observed. These data suggest that the endorphins are potent spinal antinociceptive and anti-allodynic agents and that they or related compounds may prove therapeutically useful as spinal analgesics.

Dependence by morphine

The development of tolerance and physical dependence with repeated intake is a characteristic feature of opiate alkaloids. The tolerance necessitates the administration of increasing doses of the alkaloid to induce the same apparent biological response (Hirschhorn and Rosecrans, 1974; Emmett-Oglesby et al., 1988; Shannon and Holtzman, 1976; Sunnerud and Young, 1987; Lazarus et al., 1996). Although the endogenous opioids like endorphins will not be able to cause euphoria because of the large molecules for the blood-brain barrier, opioids such as morphine and heroin produce a pleasurable state of euphoria and then addiction in humans, which forces their abuse, leading to psychological and physical dependence. The involvement of OP₂ opioid receptor desensitization and/or down-regulation in opioid tolerance and dependence has been postulated. However, results from multiple studies examining the binding of the radioligand to opioid receptors in the brain from laboratory animals following chronic treatment with morphine are contradictory, and probably due to differences in experimental conditions such as exposure time, dose, route of administration and assay methods (Rogers and El-Fakahany, 1986; Werling et al., 1989; Collin and Cesselin, 1991).
Nicotine, tetrahydrocannabinol, or morphine, and their endogenous systems in CNS.

However, in brain regions from heroin addicts who had died by opiate overdose or other causes, the affinity (Kd) and density (Bmax) of OP₁ were similar to those in controls (Gabilondo et al., 1994) (Fig. 5 A, B).

Opiate addiction can be treated by gradually reducing the dose of opiates to the addict. However, subjects receiving morphine chronically show an opiod withdrawal syndrome when the administration is abruptly discontinued or the opioid antagonist naloxone is injected during chronic administration (Reisine and Pasternak, 1996). The withdrawal syndrome seen in humans includes both physical signs, such as vomiting and diarrhea, and motivational symptoms, such as dysphoria (Kumar and O'Brien, 1994).

Toxicology of dependence induced by morphine

The mechanisms by which chronic administration of opioids such as morphine produce tolerance, psychological and physical dependence, and the withdrawal syndrome including the phenomenon following antagonism with naloxone are not well understood. As mentioned above, opioids inhibit adenyl cyclase via the activation of G (Go/Gi) protein, which links to opiate receptors. G protein-linked receptors generally exhibit a diminished responsiveness (desensitization or tolerance) or a reduced density (down-regulation) in the continued presence of agonists by repeated administration. Chronic administration of morphine may induce such adaptations in signal transduction including G proteins and cAMP in various brain regions known to be involved in the chronic effects of opiates.

Chronic administration of morphine induce related adaptive responses through affecting G proteins, protein kinase activity and neurofilament proteins in numerous brain regions, such as caudate putamen and nucleus accumbens which have a high density of OP₁ receptors and receive major dopaminergic projections from the substantia nigra and ventral tegmental area (Busquets, et al., 1995; Higgins and Sellers, 1994; Lazarus et al., 1996; Reisine and Pasternak, 1996; Ozaita, et al., 1998). Since the administration of dopaminergic antagonists, however, does not consistently prevent the reinforcing effects of opioids, it is suggested that some nondopaminergic mechanisms may also play a role. The finding on the localization of the opioid-receptor mRNA and binding sites in the nigrostriatal loop suggest that opioid receptors are probably present on presynaptic terminals of dopaminergic or nondopaminergic nervous systems originating from the brain regions such as substantia nigra, striatum and ventral tegmental area (Mansour et al., 1995; Noble and Cox, 1997). Therefore, the behavioral effects of chronic administration of opioids observed as tolerance, psychological and physical dependence,

Fig. 5. Comparison of binding of [D-Ala²,MePhe⁷,Gly-ol⁹]enkephalin (DAGO) to µ opioid receptors (OP₁) in the frontal cortex (cortex), thalamus and caudate of heroin addicts and control subjects.

The apparent dissociation constant (Kd) and the numbers (Bmax) of OP₁ were estimated from the binding of [H]DAGO to an OP₁ fraction prepared from the cortex, thalamus and caudate obtained at autopsy from control subjects (average age, 24 ± 1 years, n=3-13) and addicts (average age, 28 ± 3 years, n=7-11) whose postmortem delay was 36 ± 5 and 44 ± 9 hr respectively. No statistical differences were found in the values of Kd and Bmax between controls and addicts every brain region.

This Fig. was prepared from the data in Table 2 in the report by Gabilondo et al. (1994).
withdrawal syndrome and the phenomenon induced by naloxone, possibly involve suppression or disturbances of dopaminergic or nondopaminergic mechanisms that associate with the activities of those transmitter receptors and opioid receptors, and also with the release of endogenous opioids such as endomorphin-1 and -2.

On the other hand, Colpaert insists that tolerance to opioids does not exist (see Colpaert, 1995). He examined whether tolerance develops to the discriminative stimulus (DS) properties of opiate drugs, because some early studies had concluded that tolerance does not develop to opiate drug discrimination (Colpaert et al., 1978), but the earliest (Hirschhorn and Rosecrans, 1974) and all other subsequent investigations claimed that tolerance does develop to the DS properties of opiates (e.g., Emmett-Oglesby et al., 1988; Shannon and Holtzman, 1976; Sannerud and Young, 1987). Colpaert (1995) interpreted no evidence to support the notion that the discriminative response to the training dose of an opiate can be diminished by repeatedly administering large doses.

Future directions

In this review we have focused on three naturally occurring abuse drugs which are contained originally in plants and have their own apparent receptors in the central nervous system of humans and animals, and mimic the effects of the endogenous agonists. Although recent findings in molecular biology and molecular pharmacology have provided us with a better knowledge of nicotine, tetrahydrocannabinol and morphine that are involved in the relationship of respective endogenous agonists—the corresponding receptors, i.e., ACh-nAChR, anandamide-CB1 receptors and endomorphins (1 and/or 2) -OP receptors, respectively, many crucial details are still missing, especially in the neurotoxicological aspects. To clarify the neurotoxicological characteristics, it will be important to identify the physiological roles of those endogenous agonists and their receptors that are specific and peculiar to expressions of homeostatic functions and psychological functions. In fact, we even do not know exactly the function and location of nAChR in the central nervous system. For example, it remains to be determined which subtypes of receptors and how their synaptic mechanisms act presynaptically or postsynaptically in various circuits to produce their behavioral effects in chronic dose, overdose or withdrawal of nicotine, Δ2-tetrahydrocannabinol and morphine.

REFERENCES


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