BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), 160, 499–510

0007-1188/10
www.brjpharmacol.org

THEMED ISSUE: CANNABINOIDS

REVIEW

Animal models of cannabinoid reward

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The endogenous cannabinoid system is involved in numerous physiological and neuropsychological functions. Medications that target this system hold promise for the treatment of a wide variety of disorders. However, as reward is one of the most prominent of these functions, medications that activate this system must be evaluated for abuse potential. Meanwhile, cannabis is already being used chronically by millions of people, many of whom eventually seek treatment for cannabis dependence. Therefore, there is a need for procedures that can be used to: (i) better understand the mechanisms of cannabinoid reward; (ii) evaluate the abuse potential of new medications; and (iii) evaluate the effectiveness of medications developed for treating cannabis dependence. Animal models of cannabinoid reward provide a means of accomplishing these goals. In this review, we briefly describe and evaluate these models, their advantages and their shortcomings. Special emphasis is placed on intravenous cannabinoid self-administration in squirrel monkeys, a valid, reliable and flexible model that we have developed over the past decade. Although the conditions under which cannabinoid drugs have rewarding effects may be more restricted than with other drugs of abuse such as cocaine and heroin, work with these models indicates that cannabinoid reward involves similar brain mechanisms and produces the same kinds of reward-related behaviour. By continuing to use these animal models as tools in the development of new medications, it should be possible to take advantage of the potential benefits provided by the endocannabinoid system while minimizing its potential for harm.
British Journal of Pharmacology (2010) **160,** 499–510; doi:10.1111/j.1476-5381.2010.00775.x
This article is part of a themed issue on Cannabinoids. To view the editorial for this themed issue visit http://dx.doi.org/10.1111/j.1476-5381.2010.00831.x

Keywords: addiction; anandamide; conditioned place preference; drug discrimination; drug self-administration; electrical brain reward; FAAH; gateway hypothesis; microdialysis; rimonabant; withdrawal

Abbreviations: 2-AG, 2-arachidonoylglycerol; AM404, N-arachidonoylphenolamine; FAAH, fatty acid amide hydrolase; GPR55, G protein-coupled receptor 55; PPAR-α, alpha type peroxisome proliferator-activated receptor; THC, delta-9-tetrahydrocannabinol; TRPV1, transient receptor potential cation channel, subfamily V, member 1; URB597, [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate; WIN55212-2, (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanone

The endocannabinoid system as a target for medications development – a double-edged sword

Since the discovery of cannabinoid receptors in the 1980s and endogenous ligands for these receptors in the 1990s, it has become clear that the endocannabinoid system plays important roles in a diverse range of physiological functions. As each of these roles becomes better understood, opportunities arise for developing therapeutic treatments that target cannabinoid-related systems. This highly active area of biomedical research is likely to produce valuable new treatments for a number of disorders.

However, the fact that the endocannabinoid system has such important and diverse roles represents a double-edged sword. When targeting one function of the system to produce beneficial effects, it must be considered that other functions might be adversely affected, or that there might be unwanted repercussions of driving the targeted system farther than intended.

One function that must be considered when evaluating any potential cannabinoid-related medication is reward. Reward is

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Received 17 December 2009; revised 16 February 2010; accepted 16 February 2010

central to human behaviour, shaping virtually everything we do. This function is mediated by specific brain circuits, which are known to be modulated by the endocannabinoid system (Lupica *et al*., 2004; Gardner, 2005; Solinas *et al*., 2008). Cannabinoid-related treatments that provide opportunities for treating pain, anxiety and other disorders are likely to affect the reward system, possibly creating unwanted disturbances of the system. These disturbances can take two general forms, depending on the nature of the treatment: (i) drugs that enhance the functioning of the reward system have the potential to be abused and to produce dependence and addictive behaviour; and (ii) treatments that decrease the functioning of the reward system have the potential to produce depression-like symptoms.

Addictive effects of cannabis – the need for objective research

While intensive research is conducted in laboratories around the world to better understand the endocannabinoid system and develop new cannabinoid-related medications, marijuana and other illicit or semi-licit cannabis preparations are already widely used recreationally or as 'medical marijuana'. These uses are likely to become increasingly common as laws are relaxed and cannabis comes to be increasingly perceived by the public to be at least as safe as tobacco and alcohol. Thus, regardless of whether cannabis prohibition is advisable or effective, millions of people are already being exposed to the drug, and it is important to develop a better understanding of its effects on the brain and behaviour.

The question of whether marijuana is addictive is highly controversial. Unfortunately, strong opinions are often based on anecdotal evidence, personal observations or ideology, rather than objective scientific information. It should be remembered that as recently as the 1970s there was controversy concerning the addictiveness of nicotine (Tobacco Advisory Group of the Royal College of Physicians, 2000; Henningfield and Zeller, 2006) and even cocaine (Wesson and Smith, 1977; Musto, 1989; Das, 1993). In part because withdrawal from these drugs does not produce symptoms that resemble withdrawal from opiates, their use was considered 'social' or 'a habituation' rather than an addiction. However, researchers and clinicians in the drug abuse field came to recognize the 'preemptive significance' (Wikler, 1971) of behavioural symptoms, such as compulsive use and difficulty in achieving abstinence, over physical dependence. Recognition of the addictive potential of nicotine, in particular, was influenced by work with animal models of drug abuse (Goldberg *et al*., 1981; Goldberg and Henningfield, 1988).

The opinion that marijuana is not addictive is contradicted by the high prevalence of users who seek treatment for dependence. For example, more people in the USA are treated for cannabis dependence than for cocaine dependence (1.2 million vs. 928 000 per year; (Substance Abuse and Mental Health Services Administration, 2007). In New York City, the percentage of those admitted to drug treatment programmes primarily for cannabis dependence increased from less than 5% in 1991 to nearly 28% in 2006 (Community Epidemiology Work Group, 2004, 2007). Thus, it is clear not only that cannabis dependence is a genuine phenomenon, but also that it would be valuable: (i) to improve our understanding of the brain mechanisms involved in cannabis reward and dependence; and (ii) to develop and test new treatments that might effectively aid in achieving and maintaining abstinence.

Animal models of drug abuse provide tools for achieving both of these goals. Behavioural and physiological animal research has demonstrated many commonalities, as well as some differences, between the addictive, rewarding effects of cannabinoids and those of other drugs such as cocaine, nicotine and opioids. The purpose of this review is to briefly describe and evaluate the methodology, advantages and shortcomings of this research concerning cannabinoids and reward.

Animal models of drug abuse – a valid and reliable approach

To be clinically useful, any theory of cannabinoid reward or any treatment for cannabis dependence must ultimately be tested in humans. Nonetheless, there are several advantages to performing basic research using animals to model human behaviour and physiology. With regard to studying behaviour, a high degree of experimental control can be achieved by precisely controlling the animal's life experiences and history of drug exposure. This allows clear inferences to be made concerning the causality of effects observed in the experiment. Although some behavioural procedures can be conducted in both humans and animals (e.g. see Haney, 2009), the experience and drug history of human research volunteers prior to entering the laboratory are difficult to assess and highly variable between individuals. Also, with regard to studying the brain mechanisms that underlie addictive behaviour, procedures that can be conducted in humans are severely limited compared with the manipulations and measures that can be performed in animals.

Drug self-administration is the 'gold standard' of animal models of drug abuse (Panlilio and Goldberg, 2007). In the typical drug self-administration procedure, animals are allowed to obtain a drug by performing a simple action, such as pressing a lever. Animals will readily self-administer virtually all of the same drugs that are abused by humans (Yokel, 1987). The main exceptions are drugs such as lysergic acid diethylamide, which are used recreationally by humans but in sporadic patterns that do not produce dependence. Thus, drug self-administration in animals is a valid and reliable predictor of whether a drug will have rewarding effects in humans. Another advantage of drug self-administration in animals is that it has a point-to-point correspondence with drug abuse in humans. This allows the basic procedure to be modified in various ways to focus on specific aspects of the behaviour, such as how the drug-taking response is initially acquired or learned, how it is maintained by cues in the environment that are associated with the drug's rewarding effects, and how various events can trigger relapse to drug use after a period of abstinence.

In the basic drug self-administration procedure used in our laboratory with squirrel monkeys (*Saimiri sciureus*), training sessions last about an hour each day. The monkey is seated in front of a panel that includes a lever that can be pressed and an array of coloured cue lights that are controlled by a computer. Pressing the lever causes a small bolus of drug solution to be delivered through a chronic venous catheter. Early in training, a single response on the lever is required to obtain each injection, which is accompanied by a change from green cue lights to amber cue lights for 2 s. To allow the drug from each injection to be distributed and begin to take effect before another injection is taken, there is a timeout period of 60 s during which the coloured lights are extinguished and lever responding has no programmed effect. Then, the green light is presented again to signal that the next injection is available. Once the lever-pressing response becomes reliable under these training conditions, the requirements for obtaining the drug can be manipulated to focus on various aspects of the monkey's behaviour that correspond to specific aspects of drug abuse in humans. These manipulations can include: (i) varying the number of responses required for each injection to manipulate the 'cost' of the drug; (ii) varying the amount of drug delivered in each injection to asses the dose dependency of the drug's effects; (iii) requiring that a certain interval of time must pass between successive injections, so that accelerated responding near the end of the interval indicates that the monkey anticipates and values the reward; and (iv) consistently presenting a brief cue light for responding, but only delivering the drug along with the cue intermittently. The latter manipulation, known as a second-order schedule, is used to model the effects of drug-associated environmental cues (Schindler *et al*., 2002). In the human drug abuse environment, these cues come to have signalling and rewarding effects of their own that motivate and guide the long sequences of behaviour that are typically required to obtain, prepare and ingest a drug of abuse.

Development of a procedure for obtaining robust cannabinoid self-administration in animals

Laboratory research using drug self-administration procedures in human volunteers confirms that smoked marijuana has robust rewarding effects that are mainly attributable to its delta-9-tetrahydrocannabinol (THC) content (see reviews by Justinova *et al*., 2005a; Cooper and Haney, 2008). But, many studies of THC self-administration in animals, mostly conducted in the 1970s, did not obtain robust rewarding effects, which we define as persistent, dose-related self-administration responding that ceases when vehicle is substituted for THC solution. These results seemed like an anomalous exception to the otherwise consistent finding that drugs abused by humans also have rewarding effects in animals. However, researchers in our laboratory subsequently developed procedures that produce robust cannabinoid self-administration in squirrel monkeys (Tanda *et al*., 2000). These procedures have been used successfully in many experiments over the past decade. The results of these studies support the validity of the animal model and indicate that, under appropriate conditions, cannabinoids have rewarding effects comparable to those of other drugs of abuse.

Aspects of the procedure used in our laboratory that most likely contribute to its effectiveness include using: (i) a clear THC solution; (ii) doses of THC that are lower than in previous animal studies but comparable to the doses consumed by human marijuana smokers; (iii) rapid intravenous delivery of the drug, comparable to the rapid delivery of THC from smoked marijuana; and (iv) exposure to various doses of THC, along with frequent substitution of vehicle for THC, early in training to encourage sensitivity to changes in dose. The fact that most cannabinoid drugs are not water soluble can make them difficult to deliver effectively. A clear solution is obtained using a saline vehicle with 0.4–1.0% each of Tween-80 and ethanol. This solution remains stable in the syringe, is efficiently absorbed into the brain (Mantilla-Plata and Harbison, 1975) and contains only a negligible amount of ethanol (0.0008–0.002 $g \cdot kg^{-1}$ per injection, about 15–40-fold less than the intravenous dose of ethanol required to maintain self-administration responding in rhesus monkeys; Broadbear *et al*., 2005).

High doses of THC can produce adverse effects such as anxiety (Ilan *et al*., 2005; Grotenhermen, 2007) that might counteract the drug's rewarding effects. High doses also produce longer-lasting rewarding effects and sedative effects that depress the overall rate of responding during the session, making it difficult to determine whether the response is infrequent but still maintained by reward, or whether the response is simply occurring at a chance level. The dose of THC received by one of our squirrel monkeys is typically 2–4 $\mu g \cdot kg^{-1}$ in each injection, about the same dose received by a human from a puff of marijuana smoke (2.9 to 4.3 $\mu g \cdot kg^{-1}$; Tanda and Goldberg, 2003). Also like smoked marijuana, a rapid intravenous injection of THC has a fast onset of action. Rapid intravenous drug injections are known to be more rewarding than slow injections in humans (Abreu *et al*., 2001) and non-human primates (Balster and Schuster, 1973; Panlilio *et al*., 1998), presumably because a slow onset of drug effect creates a delay between the response and the reward.

The importance of early exposure to a variety of doses, including a zero dose (i.e. vehicle alone), relates to the phenomenon of well-learned behaviour becoming habitual and insensitive to its consequences. This phenomenon is highly relevant to addiction, which by definition involves behaviour that persists despite adverse consequences, and is worthy of studying in its own right. However, the development of this compulsive behaviour is not always the focus of the study. When the goal is to determine whether a specific drug has rewarding effects or to determine whether a potential treatment alters the rewarding effects of an abused drug, it is beneficial to have subjects that show abrupt changes in response rate when the value of the reward is manipulated. For this reason, it is our general practice when determining dose–response curves for a self-administered drug to offer a specific dose during consecutive sessions until response rates and patterns stabilize, then to offer vehicle until responding stabilizes at a low level before offering another dose of the drug.

Findings obtained with cannabinoid self-administration in squirrel monkeys

Self-administration of THC and other cannabinoid receptor ligands

Under the conditions described above, THC functions as a reward in squirrel monkeys, maintaining lever-pressing behaviour in a manner comparable to that maintained by other drugs (cocaine, nicotine) and non-drug rewards (food). Although our initial demonstration of THC self-administration was performed in squirrel monkeys that had learned to self-administer cocaine prior to being trained with THC (Tanda *et al*., 2000), monkeys in subsequent studies have been drug-naive prior to training with THC (Justinova *et al*., 2003; 2005b; 2008b). THC self-administration in these monkeys has been no less robust than in monkeys that had a history of cocaine self-administration. Most of our cannabinoid self-administration experiments have involved a fixed-ratio 10 schedule, in which 10 lever responses are required for each injection. THC dose–response curves obtained with this schedule are quite similar to the typical inverted *U*-shaped curves obtained in animals with most other drugs of abuse (see Panlilio *et al*., 2008). At low doses, or when vehicle alone is offered, the injections are not rewarding and response rates are very low. At intermediate doses, response rates are highest because the drug has rewarding effects but does not produce the sedative or satiation-like effects that suppress responding at higher doses. As with other self-administered drugs, the total drug intake per session increases monotonically with dose, despite the fact that response rates decrease at the higher doses (Tanda *et al*., 2000; Justinova *et al*., 2003; 2008b).

Several endogenous cannabinoid ligands have been identified in the brain, but the best characterized of these is anandamide (Freund *et al*., 2003; Di Marzo *et al*., 2004; Piomelli, 2004). Presumably, THC from smoked marijuana creates rewarding effects by 'hijacking' the brain circuitry that normally involves these natural ligands (Lupica *et al*., 2004; Gardner, 2005; Maldonado *et al*., 2006; Solinas *et al*., 2008). Consistent with this hypothesis, squirrel monkeys will readily self-administer anandamide intravenously (Justinova *et al*., 2005b). Anandamide in the brain is synthesized by neurons on demand, rather than being stored, and it is rapidly deactivated by the enzyme, fatty acid amide hydrolase (FAAH). When the FAAH inhibitor URB597 {[3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate} is administered, it enhances and prolongs the actions of anandamide (Piomelli *et al*., 2006). Treatment with URB597 shifts the anandamide self-administration dose–response curve to the left, such that anandamide has rewarding effects at lower doses (Justinova *et al*., 2008a). Methanandamide, a FAAH-resistant synthetic analogue of anandamide is also self-administered by squirrel monkeys (Justinova *et al*., 2005b).

URB597 inhibits the breakdown of not only self-administered anandamide, but also endogenously released anandamide. Because intravenous anandamide has robust rewarding effects and URB597 substantially increases endogenous levels of anandamide in the brain, it might be expected that intravenous URB597 would have rewarding effects. However, squirrel monkeys did not self-administer URB597, even though they already had experience with intravenous self-administration of THC, anandamide, or cocaine (Justinova *et al*., 2008a). Possibly, URB597 does not increase anandamide levels quickly enough to produce cannabinoid reward. This finding is consistent with findings that URB597 does not have THC-like subjective effects in rats (Solinas *et al*., 2007b), does not increase extracellular dopamine levels in the nucleus accumbens (an important part of the brain's reward circuitry) in rats (Solinas *et al*., 2006) and does not potentiate the rewarding effects of THC or cocaine in squirrel monkeys (Justinova *et al*., 2008a). In contrast to URB597, rewarding effects are produced by AM404 (N-arachidonoylphenolamine), a drug that increases anandamide levels by preventing reuptake of anandamide into the cell and by inhibiting FAAH (Zhang *et al*., 2007). These finding are important because FAAH inhibitors have been proposed as medications for treatment of cannabinoid dependence (Clapper *et al*., 2009) as well as a number of other disorders, including pain, inflammation, anxiety and depression. Before these drugs can be used clinically, it is essential to determine whether they have the potential to be abused.

Effects of treatment with a cannabinoid antagonist

The cannabinoid CB1 receptor antagonist/inverse agonist, rimonabant, can block the rewarding effects of THC, anandamide and methanandamide in squirrel monkeys (Tanda *et al*., 2000; Justinova *et al*., 2005b; 2008b). These findings verify that the rewarding effects of these drugs are mediated by cannabinoid CB1 receptors, and they indicate that cannabinoid antagonists might have value as a treatment for cannabis dependence.

More importantly, there is also evidence that cannabinoid antagonists have a *general* ability to counteract the effects of environmental cues associated with drugs of abuse (see review by De Vries and Schoffelmeer, 2005; Le Foll and Goldberg, 2005). Consistent with this hypothesis, rimonabant decreased lever pressing under a second-order schedule where every 10th response produced only a cue light throughout the session and THC was only delivered in association with the cue light at the end of the 30 min session (Justinova *et al*., 2008b). This kind of schedule – where drug is only delivered at the end of the session – is used to focus on drug-seeking behaviour (see Everitt and Robbins, 2000). As with other drugs of abuse that have been studied with this procedure, the drug-associated environmental cues were critical to the maintenance of long sequences of drug-seeking behaviour. High response rates were maintained when the cues were presented, but responding decreased immediately when the cues were discontinued, even though the drug was still delivered at the end of each session. In contrast, when THC delivery was discontinued, responding did not decrease until the next session. Thus, responding in this study was maintained by: (i) the rewarding effects of THC; and (ii) the rewarding effects of the THC-associated cues. Rimonabant was able to block both of these effects.

Rimonabant was also able to prevent THC-associated cues from reinstating the drug-seeking response in an animal model of relapse (see Shaham *et al*., 2003). In this procedure, the cues and THC delivery were both discontinued for monkeys trained under the second-order schedule described

above. Low rates of responding were maintained under these conditions for many days. Then, to model relapse during a test session, either: (i) the monkeys were given a free injection of THC at the beginning of the session; or (ii) presentation of the response-produced cues was reinstituted throughout the session. Responding produced only vehicle injections, not THC, during these tests. Rimonabant effectively blocked reinstatement of responding in both of these tests, which model drug-induced relapse and cue-induced relapse respectively. Taken together, all of these results obtained with rimonabant support the hypothesis that manipulating the endocannabinoid system can modulate the effects of drug-associated cues. Furthermore, these results suggest that cannabinoid antagonists could be particularly effective as a treatment for cannabis dependence because it can prevent the immediate rewarding effects of THC as well as the relapse induced by re-exposure to either THC or THC-associated cues.

Unfortunately, although rimonabant showed promise as a treatment for nicotine dependence and obesity in animals and humans, it was taken off the market in Europe and never approved for human use in the USA because of adverse side effects (Le Foll *et al*., 2009). These depression-like effects might be a consequence of blocking reward-related processes in the endocannabinoid system. But, it is possible that these adverse effects are due to rimonabant's inverse agonist properties, which not only block the effects of endogenous cannabinoid agonists such as anandamide, but have opposite effects. It remains to be seen whether cannabinoid antagonists that do not have inverse agonist properties can produce the anti-addiction effects of rimonabant without the depressive side effects.

Effects of treatment with non-cannabinoid drugs

There is much evidence, mostly from studies in rodents, that there are reciprocal interactions between the endogenous cannabinoid and opioid systems, including reward-related effects (see review by Robledo *et al*., 2008). For example, the opioid antagonist naloxone can block THC-induced enhancements of electrical brain stimulation reward (Gardner *et al*., 1989) and THC-induced increases in extracellular dopamine levels in the nucleus accumbens (Chen *et al*., 1990; Tanda *et al*., 1997). In rats, naloxone decreases self-administration of synthetic cannabinoid agonists (Navarro *et al*., 2001). Research with the opioid antagonist naltrexone in humans shows that it can decrease the intoxicating effects of marijuana (Haney, 2007). In squirrel monkeys, we found that naloxone treatment decreased THC self-administration by about 50% under a fixed-ratio 10 schedule (Justinova *et al*., 2004), a procedure that primarily measures the direct rewarding effects of the drug, as opposed to the effects of drug-associated cues. Under these conditions, naltrexone caused THC self-administration to decrease and stabilize at a low level. In contrast, under a second-order schedule designed to incorporate the effects of THC-associated cues, naltrexone only decreased cue-maintained THC seeking during the first session of treatment (Justinova *et al*., 2008b).

A different kind of interaction between cannabinoids and drugs from other classes involves the ability of a drug to trigger relapse to use of a drug from a different pharmacological class. For example, Spano *et al*. (2004) found that exposing rats to heroin, but not cocaine, reinstated drug seeking in rats that had previously self-administered the synthetic cannabinoid agonist WIN55212-2 [(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanone]. In squirrel monkeys, we found that cocaine did not reinstate THC seeking, even in monkeys with a prior history of cocaine self-administration. However, THC seeking was reinstated by exposure to either THC or the opioid agonist morphine (Justinova *et al*., 2008b). To further assess this cannabinoid–opioid interaction, we attempted to block these reinstatement effects with either the opioid antagonist naltrexone or the cannabinoid antagonist rimonabant. Although each antagonist blocked the effects of the agonist from the same class, the opioid antagonist did not block THC-induced reinstatement, and the cannabinoid antagonist did not block opioid-induced reinstatement. Taken together with the results obtained when naltrexone was studied with the FR10 and second-order schedules of THC self-administration, these reinstatement results are consistent with the hypothesis that the endogenous cannabinoid and opioid systems interact, and they suggest that opioid antagonists might have some utility for treating cannabis dependence in humans, but that they would probably be less effective than cannabinoid antagonists.

Some criticisms and remaining questions concerning cannabinoid self-administration in squirrel monkeys

The initial criticism of this research was that the monkeys had learned to self-administer cocaine before being trained with THC (Tanda *et al*., 2000). However, subsequent work has clearly shown that drug-naive squirrel monkeys will readily self-administer THC and other cannabinoid agonists (Justinova *et al*., 2003; Justinova *et al*., 2005b). In addition, as mentioned above, work with the reinstatement model of relapse has shown that even in monkeys that were trained to self-administer cocaine before THC, cocaine did not reinstate the THC-seeking responding; this indicates that the monkeys' behaviour was indeed rewarded by THC, rather than being maintained by memories of cocaine reward (Justinova *et al*., 2008b). The sensitivity of the self-administration response to changes in the cannabinoid dose, including cessation of the response when only the drug's vehicle is offered, further attests to the fact that cannabinoids have robust rewarding effects in this animal model.

A criticism of this research and drug self-administration research in general is that the animals might only self-administer drugs because they are raised in an impoverished environment. Although it is clear that housing conditions, stressors and other environmental factors can influence the susceptibility of an individual (whether human, non-human primate, or rodent) to developing and continuing use of a drug, this has not been the focus of these studies in squirrel monkeys. These monkeys' environment is quite different from the wild, but it is not impoverished. Through an enrichment programme, they are exposed to a variety of foods, toys and activities on a daily basis. They are closely monitored by veterinary staff to ensure their health and safety. Their housing conditions and all experimental procedures are approved and

monitored by an animal care and use committee with the primary goal of ensuring the welfare of the animals.

We expect that further application of the cannabinoid self-administration procedure in squirrel monkeys will continue to provide information concerning the nature of cannabinoid reward and how cannabinoid dependence can be treated. In particular, two basic kinds of question remain to be addressed concerning this model. First, as mentioned above, most previous studies of THC self-administration in animals failed to obtain robust behavioural effects. More recently, procedures have been developed to obtain intravenous self-administration of synthetic cannabinoids in rodents (see below). However, there is still a need to address the issue of species differences in cannabinoid reward. Do they truly differ? How and why? Are there fundamental differences between rodents and primates or between various types of primates that make certain species inappropriate for modelling the cannabinoid-related behaviour and physiology of humans?

The second kind of question relates to differences between the rewarding effects of cannabinoids and other classes of drugs. It appears that self-administration of cannabinoids occurs under more limited conditions than self-administration of some other drugs of abuse. Does this mean that cannabinoid dependence is less severe when it does develop? The value of drug rewards can be measured and compared using behavioural economics techniques (Hursh, 1993), but these have not yet been applied to cannabinoid self-administration in squirrel monkeys.

Other animal models of cannabinoid reward and related phenomena

We consider drug self-administration in non-human primates to be the most valid and flexible model of cannabinoid reward in humans. But, there are a number of rodent-based models and related procedures that also provide valuable information about cannabinoid reward. These procedures are briefly described below, along with their advantages and disadvantages.

Intravenous cannabinoid self-administration procedures in rodents

Intravenous cannabinoid self-administration procedures in rodents have been refined in recent years (Martellotta *et al*., 1998; Fattore *et al*., 2001; 2007; Fadda *et al*., 2006; Mendizabal *et al*., 2006). Most of these studies have involved self-administration of the synthetic cannabinoid CB1 receptor agonist WIN55212-2. Although intravenous catheters do not last as long in rodents as in squirrel monkeys, which limits the kind of tests that can be employed, the rodent model has certain advantages over primate models. For example, inbred strains of rodents allow the assessment of genetic and gender effects on the acquisition and maintenance of cannabinoid self-administration (Deiana *et al*., 2007; Fattore *et al*., 2009).

Intracranial microinjection

Intracranial microinjection is related to intravenous drug self-administration. By implanting a cannula into a discrete area of the brain and allowing a rat to press a lever that delivers miniscule amounts of a drug into that area, it is possible to map the brain areas where the drug has rewarding effects. Rats will self-administer cannabinoid agonists, including THC and anandamide, into the ventral tegmental area and the shell of the nucleus accumbens (Zangen *et al*., 2006). These critical areas of the brain's reward circuitry are also the sites of action of other rewarding drugs, including amphetamines, cocaine, heroin and nicotine. A potential drawback of this technique is that injecting cannabinoids directly into discrete areas might produce concentrations of the drug that are not reached when the drug is administered systemically. It is also possible that the effects observed by isolating an area might not be representative of the effects that occur when cannabinoids – such as THC from smoked marijuana – are distributed throughout the brain. But, when considered along with the results obtained with the other models described here, these findings lend additional support to the conclusion that cannabinoids produce rewarding effects through essentially the same reward-related brain circuitry as other drugs of abuse.

Microdialysis

Microdialysis procedures allow sampling of fluid from discrete brain regions. The fluid can then be analysed to measure extracellular levels of neurotransmitters and other neurochemicals. For studying reward-related functions, dopamine and its metabolites are usually measured in the mesolimbic dopaminergic pathway, including the ventral tegmental area and nucleus accumbens. Results obtained with microdialysis procedures show that cannabinoid agonists have effects on these areas that are comparable to those produced by all other drugs of abuse and that presumably form the basis for reward. For example, intravenous THC and WIN55212-2 cause the release of dopamine in the nucleus accumbens (Tanda *et al*., 1997; Fadda *et al*., 2006; Lecca *et al*., 2006). Microinjection of THC into the ventral tegmental area or nucleus accumbens also causes dopamine overflow in these areas, suggesting that cannabinoids produce their rewarding effects by acting directly on neurons in these areas (Chen *et al*., 1993).

Electrical brain stimulation

Electrical brain stimulation is a method that involves allowing a rat to press a lever that produces a brief electrical current in a discrete brain area. This stimulation has robust rewarding effects when the micro-electrodes are placed in the mesolimbic dopaminergic system. With regard to cannabinoid reward, this technique is of interest because it can be used to detect drug-induced changes in the sensitivity of the reward system. Increased sensitivity presumably reflects activation of the system, and decreased sensitivity presumably reflects inhibition of the system. Although there have been some inconsistent reports (see Solinas *et al*., 2008), there is evidence that cannabinoid agonists, like other drugs of abuse, increase sensitivity to electrical brain stimulation (Gardner *et al*., 1988; Lepore *et al*., 1996), while the antagonist/inverse agonist rimonabant can have an opposite effect (Xi *et al*., 2007). Thus, results obtained with intracranial microinjection, microdialysis and electrical brain stimulation all converge to indicate

that cannabinoid agonists have effects on the reward circuitry of the rodent brain that are consistent with those of other drugs of abuse.

Conditioned place preference

Conditioned place preference procedures provide another alternative for measuring the rewarding effects of cannabinoids. Although they do not have the strong face validity or flexibility of drug self-administration procedures, place preference procedures are easier to conduct because they do not require catheterization or extensive behavioural training of the animals. These procedures involve using a chamber with two distinctive compartments. The effects of a drug are associated with one compartment by giving a rat an intraperitoneal injection and confining it in the compartment for a short period of time (usually about 15 min). At other times, the rat is injected with vehicle and confined in the other compartment. After a few trials in each compartment (typically conducted one trial per day), a test can be performed by removing a barrier between the compartments and allowing the rats free access to both sides. A rewarding effect of the drug is indicated by a preference for the drug-associated side, and an aversive effect of the drug is indicated by a preference for the vehicle-associated side. THC and synthetic cannabinoid agonists have been reported to have rewarding effects in this model under some conditions (Lepore *et al*., 1995; Valjent and Maldonado, 2000; Ghozland *et al*., 2002; Braida *et al*., 2004) but aversive effects under others (Parker and Gillies, 1995; McGregor *et al*., 1996; Sanudo-Pena *et al*., 1997; Chaperon *et al*., 1998; Hutcheson *et al*., 1998; Mallet and Beninger, 1998; Cheer *et al*., 2000; Valjent and Maldonado, 2000). This is determined at least partly by dose, but it is not presently clear what other factors determine whether rewarding or aversive effects are obtained.

Drug discrimination

Drug discrimination studies with THC involve training rats to detect when they have been injected with the drug (Colpaert, 1999). On training days when the rat receives an injection of THC before the session, responding on one of two levers produces food pellets. On training days when the rat is only injected with vehicle, responding on the opposite lever produces food. After extensive training, the rat learns to respond exclusively on the appropriate lever during training sessions. Then, test sessions can be conducted to determine whether a drug produces subjective effects similar to those of THC. Drugs can also be administered in combination with THC to determine whether they alter its effects. Although a considerable amount of time (several months) is required to train rats to reliably detect the subjective effects of drugs, including THC, after this training period a large number of test compounds can be screened relatively easily with drug discrimination techniques. To the extent that drug detection by the rat is based on subjective effects related to reward, this procedure can be used to assess rewarding effects. However, it is difficult to ascertain what property of the drug is being detected, and this procedure is best used as a screen prior to more extensive testing with drug self-administration or other procedures. Nonetheless, there is a strong correlation between the drugs that have subjective effects similar to those of THC in rats and those that produce marijuana-like intoxication in humans (Balster and Prescott, 1992). Anandamide, which is rapidly inactivated in the body, produces THC-like subjective effects at high doses or when its breakdown is inhibited by the FAAH inhibitor URB597 (Solinas *et al*., 2007b). Methanandamide, the FAAH-resistant analogue of anandamide, also produces THC-like effects in this model. In mice, inhibiting both FAAH and the enzyme monoacylglycerol lipase – the latter of which degrades the endocannabinoid 2-arachidonoylglycerol (2-AG) in a manner analogous to the degradation of anandamide by FAAH – produced THC-like subjective effects (Long *et al*., 2009); as inhibiting either FAAH or monoacylglycerol lipase alone did not produce these effects, this suggests that activation of CB1 receptors in both the anandamide and 2-AG pathways might be important in cannabinoid reward.

Pre-exposure studies

Pre-exposure studies with cannabinoids can be used to objectively evaluate the controversial 'gateway hypothesis', which states that cannabis use increases the likelihood of becoming addicted to other drugs. We have used this procedure to determine whether prior exposure to THC alters the rewarding effects of heroin (Solinas *et al*., 2004; see also Ellgren *et al*., 2007). Separate groups of rats were exposed to THC (with twice daily intraperitoneal injections escalating from 2 to 8 $mg \cdot kg^{-1}$ per injection) or vehicle over the course of 3 days. Then, starting 1 week after the last THC or vehicle injection, rats were allowed to self-administer heroin. A history of THC exposure did not increase the percentage of rats that developed heroin self-administration. It also did not alter the effectiveness of heroin as a reward, which was measured by increasing the cost (i.e. response requirement) until responding ceased. But, THC-exposed rats did take more heroin than non-exposed controls when the cost was low. This increased heroin intake is probably due to the fact that THC pre-exposure produces cross tolerance to the locomotor-depressant effects of heroin, allowing THC-exposed rats to recover more rapidly from each heroin injection and to self-administer the next injection sooner than vehicle-exposed rats.

A different profile of results was obtained when the same THC pre-exposure procedure was applied to cocaine self-administration (Panlilio *et al*., 2007). A history of THC exposure did not alter the likelihood of developing cocaine self-administration or the rate of self-administration when the cost of cocaine was low. However, THC pre-exposure actually decreased the effectiveness of cocaine as a reward when the cost was increased. Follow-up experiments with animal models of anxiety suggest that THC exposure sensitized the rats to aversive effects of cocaine. Like THC, cocaine is known to be capable of producing both rewarding effects and aversive effects. With cocaine, the aversive effects are believed to be delayed relative to the rewarding effects (Ettenberg, 2004), such that self-administration occurs despite the aversive effects. The time course of these effects is not known for THC. The aversive effects of cannabinoids, and their interaction

with the rewarding effects, represent an area that deserves to be studied in more detail. This might shed light on differences between rodents, non-human primates and humans with regard to cannabinoid self-administration. Overall, pre-exposure studies show that even brief exposure to cannabinoids can have lasting effects on the individual's response to other drugs, but they do not indicate that THC exposure makes heroin or cocaine more rewarding.

Withdrawal studies

Chronic exposure to THC causes a down-regulation and desensitization of cannabinoid receptors in the brain (McKinney *et al*., 2008). Consequently, during withdrawal from THC there may be a loss of normal, endogenous cannabinoid signalling. Discontinuing chronic marijuana use in humans or THC administration in non-human primates can produce withdrawal symptoms that include restlessness, insomnia and depression (Fredericks and Benowitz, 1980; Haney, 2002). These symptoms are mild compared with those produced by withdrawal from drugs such as heroin, ethanol and barbiturates, in part because THC is eliminated from the body slowly (Karschner *et al*., 2009). More intense effects occur if withdrawal is rapidly precipitated by administering a cannabinoid antagonist such as rimonabant (Maldonado, 2002; Gonzalez *et al*., 2005). However, simply discontinuing chronic use might be unpleasant enough to contribute to the persistence of cannabis use (Haney *et al*., 1999a,b; Lichtman and Martin, 2002; Clapper *et al*., 2009). Therefore, medications that alleviate withdrawal symptoms might be helpful for achieving abstinence. Some drugs (bupropion, divalproex, nefazodone, lofexidine and orally administered THC) that are already approved for other uses have been tested in the laboratory for this purpose in humans but have not undergone clinical trials (Haney, 2002; Vandrey and Haney, 2009). Animal models can also play a role in this endeavour, as a wider variety of test compounds can be tested and experimental conditions can be controlled more precisely. Most of this work has involved precipitated withdrawal in rodents (Sanudo-Pena *et al*., 1999; Lichtman *et al*., 2001; Dhawan *et al*., 2002; Celerier *et al*., 2006; Tourino *et al*., 2007). A sensitive animal model has also been developed for studying non-precipitated cannabinoid withdrawal by measuring disruptions in ongoing food-rewarded lever pressing in rhesus monkeys (Beardsley *et al*., 1986).

Cannabinoid-induced alterations of the rewarding effects of other drugs of abuse

As mentioned above, cannabinoids and opioids can have interactive effects when combined, such as when the opioid antagonist naltrexone decreases THC self-administration in squirrel monkeys. A history of exposure to one class of drug can also alter the reward-related effects of the other, such as in the gateway hypothesis experiments described above and other studies showing that cannabinoid and opioid drugs can produce cross tolerance or cross sensitization to each others effects (Fattore *et al*., 2005; Robledo *et al*., 2008). Cannabinoids also interact with a number of non-opioid drugs of abuse. Due to the difficulties that have been encountered in the past with establishing models of cannabinoid reward in animals, most studies of interaction effects have examined the acute effects of cannabinoids on the rewarding effects of other drugs, such as nicotine (Merritt *et al*., 2008) and psychostimulants (Wiskerke *et al*., 2008).

For example, recent evidence shows that the FAAH inhibitor URB597, which increases and prolongs the effects of endogenous anandamide, might be useful as a treatment for nicotine dependence. An advantage of this approach is that URB597 alters the endocannabinoid system without having the abuse liability associated with direct cannabinoid agonists (Justinova *et al*., 2008a) or the depressant effects associated with the cannabinoid inverse agonist/antagonist rimonabant (Gaetani *et al*., 2009). URB597 was found to block the effects of nicotine on dopamine cell firing (Melis *et al*., 2008) and dopamine levels (Scherma *et al*., 2008) in the reward circuitry of the rat brain. URB597 also counteracted nicotine's rewarding behavioural effects in the drug self-administration, reinstatement and conditioned place preference models (Scherma *et al*., 2008). However, it is currently unclear whether these effects are due to URB597's effects on the endocannabinoid system or on endocannabinoid-related systems such as PPAR-α (alpha type peroxisome proliferator-activated receptor), TRPV1 (transient receptor potential cation channel, subfamily V, member 1) or GPR55 (G protein-coupled receptor 55) (Bradshaw and Walker, 2005; O'Sullivan, 2007; Ryberg *et al*., 2007). In addition, there are constituents of marijuana, such as cannabidiol, that can interact with the effects of THC (e.g. see Bhattacharyya *et al*., 2010) and might have beneficial effects for the treatment of opioid abuse (e.g. Ren *et al*., 2009). The roles of non-THC constituents of marijuana and of endocannabinoid-related systems in cannabinoid reward remain to be explored.

A combined approach

The main advantage of each of these animal models is that it provides information concerning a specific aspect of cannabinoid reward that is difficult or impossible to obtain by other means. Some are more appropriate for investigations of underlying mechanisms, and some are more appropriate for predicting the effects of a drug on human behaviour. Intracranial microinjection and microdialysis are valuable for examining the effects of cannabinoids on the reward circuitry of the brain. Electrical brain stimulation is an especially useful technique for studying the reward-related effects of cannabinoids due to the difficulties in obtaining cannabinoid self-administration in rodents. Conditioned place preference has the advantage of being able to detect aversive effects, which might be an important component of the overall effects of cannabinoids. Pre-exposure studies provide an objective and scientifically controlled means of examining the gateway hypothesis. Withdrawal studies address a potentially important issue, whether avoidance of withdrawal symptoms contributes to cannabis use. Studies of the cannabinoid-induced alterations of the effects of other drugs in animals can indicate how cannabinoid drugs might interact with other drugs in humans, and they also contribute to our understanding of the general role of the endocannabinoid system in reward.

The main disadvantage of each of these techniques also relates to the specificity of the information obtained. No model is sufficient by itself. To determine the generality and usefulness of a finding, it must be confirmed in complementary models. For example, a recent study combined THC discrimination, WIN55212-2 self-administration and microdialysis procedures in rats to examine the effects of the α7 nicotinic acetylcholine receptor antagonist methyllycaconitine on cannabinoid reward (Solinas *et al*., 2007a). The fact that the findings obtained with these three procedures are in agreement encourages further study of methyllycaconitine-like drugs with THC self-administration in non-human primates and eventually, if they continue to be promising, in human volunteers.

Conclusion

Cannabinoid drugs can activate the same reward circuits in the brain and produce the same kind of drug-seeking behaviour as other drugs of abuse. These effects provide the basis for recreational cannabis use, which can lead to dependence. As cannabis use and dependence continue to increase, there will be an increasing need for medications to treat this dependence and for valid, reliable ways to assess the effectiveness of these medications. At the same time, new cannabinoid-related medications are being developed to treat a wide variety of disorders, and there will be a need to assess their abuse liability. The animal models described here provide a means of meeting these needs. Ideally, medications should be developed that produce the beneficial effects of cannabinoids (e.g. anti-emetic, analgesic and antidepressant effects) without the adverse effects (e.g. addictive, psychotomimetic and amnestic effects). Toward this end, FAAH inhibitors appear to represent an effective way to enhance endocannabinoid function without producing cannabinoid reward. Cannabinoid antagonists have shown promise for the treatment of substance abuse and addiction, but ligands need to be developed that do not produce the depressive side effects associated with rimonabant.

Most of what we know about cannabinoid reward comes from animal research, but there is still much to be learned. For example, it is not clear to what extent and in what ways the underlying mechanisms of cannabinoid reward differ between, humans, non-human primates and rodents. The promise of safe and effective cannabinoid-based treatments for tobacco smoking, obesity and other disorders has not yet been fulfilled, nor have medications been specifically approved for the treatment of marijuana dependence. But, by continuing to use and improve the existing models, and by combining them in complementary ways, it should be possible to develop medications that take advantage of the extraordinary potential provided by the endocannabinoid system while minimizing its potential for harm.

Acknowledgements

The preparation of this manuscript was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services and Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, Maryland.

Conflicts of interest

None.

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