

Development of Immunopharmacotherapy Against Drugs of Abuse

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Abstract: Drug addiction is a major worldwide medical and social problem that continues to escalate. The addiction syndrome is remarkably similar between different drugs of abuse, and can be characterized as a chronic relapsing brain disorder with neurobiological changes that lead to a compulsion to take a drug with loss of control over drug intake. Presently used medications for the treatment of dependence disorders are based on drugs that are either agonists or antagonists of drugs of abuse, and have yielded only limited success. Immunopharmacotherapy is based on the generation or administration of antibodies that are capable of binding the targeted drug before it can reach the brain, whereas replacement strategies based on agonists or antagonists of these drugs generally cause many undesired side effects. A large amount of data has been gathered in recent years on the effects of active and passive immunization against cocaine, nicotine, PCP and methamphetamine in animal models, suggesting potential efficacy of these treatments in humans; and clinical trials are currently underway for vaccines against cocaine and nicotine.



1. INTRODUCTION

Drug addiction is a major worldwide medical and social problem that continues to escalate. The addiction syndrome is remarkably similar between different drugs of abuse and can be characterized as a chronic relapsing brain disorder with neurobiological changes that lead to a compulsion to take a drug with loss of control over drug intake [1]. The final common pathway of addiction, the dopamine hypothesis of reward, has recently been evolving, with the mesocorticolimbic dopaminergic system now viewed as central both to natural rewards and drug-seeking behavior, though perhaps having less of a role in the maintenance of such behavior [2]. In its essence, drug taking subjective effects are euphoric and stimulating, and absence of the drug results in dysphoria and depression. Although drug dependence and the transition from "use" to "abuse" is ultimately complex, involving the interplay both of positive reinforcement and negative reinforcement/withdrawal phenomena, interestingly, the substances most sought and abused constitute a small subset of naturally occurring compounds and some related synthetic derivatives. In particular, the effects derived from cocaine, nicotine, amphetamines, and opiates trigger mechanisms in the brain as primitive and fundamental as those activated by food, water, and sex. Hence, although complete eradication of drug addiction in society may be an ideal, therapeutic interventions to alleviate addiction problems will nonetheless have an enormous impact on improving the quality of our lives. Presently used medications for the treatment of dependence disorders are based on drugs that are either agonists or antagonists of drugs of abuse, and have yielded

only limited success. Immunopharmacotherapy is based on the generation or administration of antibodies that are capable of binding the targeted drug before it can reach the brain. A number of reviews on the development of immunization methods to treat drug addiction have appeared in recent years [3, 4], and in this review we present a comprehensive summary of the current immunopharmacotherapeutic programs to treat addiction to cocaine, nicotine, PCP and methamphetamine.

Cocaine is a tropane alkaloid extracted from the leaves of the native South American plant *Erythroxylon coca* (Fig. (1)). Human use has likely taken place for thousands of years with substantial problems emerging within the context of modern civilization [5]. Even though restricted by the Drug Enforcement Agency as a Schedule II agent, recent surveys for cocaine abuse in the United States indicated that more than 23 million people have tried cocaine, nearly 400,000 use it daily and that 5,000 new users are added each day [6]. Although abuse appears to be stabilizing, as much as 0.3% of the population may be dependent on the drug [7]. A myriad of medical problems, including death, often accompany cocaine use and the association of the drug with the spread of AIDS is of concern [8]. Furthermore, the detrimental effects are especially tragic for pregnant women where "crack" is the most abused illicit drug [9]. From the behavioral pharmacology viewpoint, it has been evident that cocaine is highly addictive and may be the most reinforcing of all drugs [10].

Despite intensive efforts, there is no proven pharmacotherapy for the cocaine problem and the development of effective therapies for cocaine craving and addiction remain elusive [11]. A number of medications acting as agonists, antagonists, or antidepressants, which include amantidine [12], mazindol [13], bromocriptine [14], buprenorphine [15], desipramine [16], phentermine with fenfluramine [17], and

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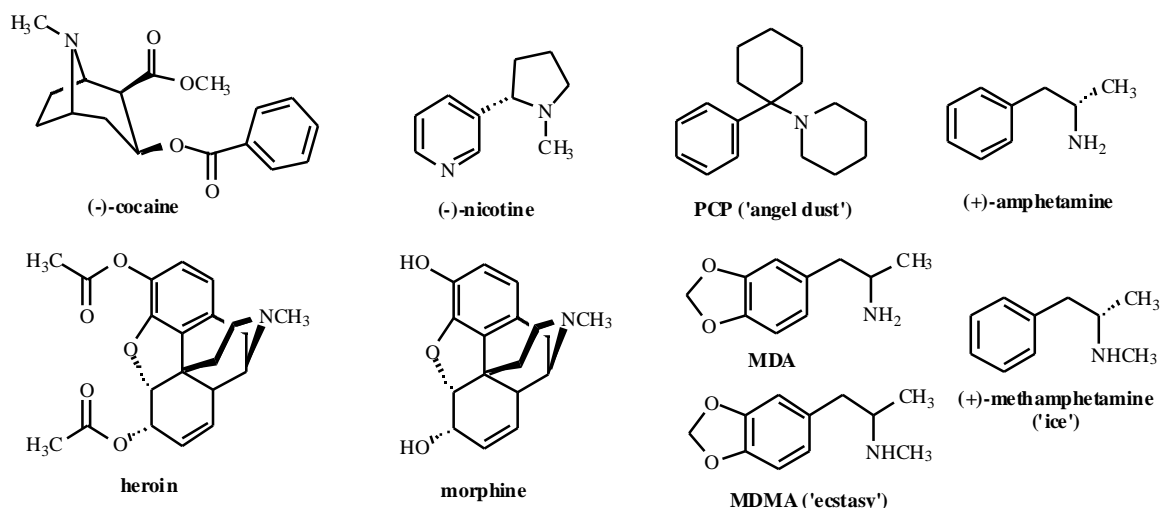


Fig. (1). Structures of widespread drugs of abuse.

-opioid [18] and α_2 -agonists [19], have been tried with limited success in both animal models and human clinical studies. Perhaps most promising to date, the partial D₃-receptor agonist BP897 was found to reduce cocaine-seeking behavior and had no reinforcing properties [20]. Yet, in the absence of a "magic bullet" drug, available pharmacological agents must be part of a comprehensive approach toward treatment. A number of biopsychosocial models have been proposed and evaluated to address addiction and relapse prevention [21]. Unquestionably, an improved pharmacotherapy would increase the effectiveness of such programs.

Nicotine is the most widely used addictive drug in the world. As an alkaloid [(S)-(-)-1-methyl-2-(3-pyridyl)pyrrolidine] derived from tobacco leaves, nicotine is legally available in many forms such as cigarettes, cigars, pipe tobacco, and chewing tobacco. Hence, the drug is intimately linked with cigarette smoking, the leading preventable cause of death in the United States [22, 23]. Nicotine itself is known to have numerous cardiovascular, endocrine, and metabolic effects and is a neuroteratogen [24, 25]. Smoking contributes to coronary heart disease, stroke, vascular disease, peptic ulcers, chronic lung diseases and lung cancer, and fetal brain damage and morbidity. It is estimated that 51 million Americans smoke and that smoking causes approximately 419,000 deaths per year in the United States [22]. Although the dangers of smoking are well known, people continue to smoke [26]. A great deal of evidence supports the view that people continue to smoke because of the addictive effects of nicotine [24, 27, 28]. The effects on the central nervous system, connected with the action of smoking, makes an ideal situation for behavioral reinforcement. Many characteristics of tobacco use are strikingly similar to those of other drugs, so nicotine use through tobacco products fits the criteria of drug dependence or addiction.

Since nicotine is legally and widely available there is relatively little stigma associated with its use, unlike cocaine. Although a large percentage of addicted smokers have expressed a desire to stop smoking and most who quit do so

without treatment, less than 5% of unaided attempts lead to successful long-term abstinence [29]. The high rate of relapse in smokers who try to quit is indicative of the strong effect of nicotine dependence [30]. Significantly, relatively little is known about the specific neuropharmacologic mechanisms underlying nicotine addiction or the response to smoking cessation treatment. Currently, the two most popular therapies are nicotine gum and transdermal nicotine patches. These replacement medications act to deliver low amounts of nicotine to the user over a period of time to slowly wean the user off the drug. Based on several placebo-controlled studies, nicotine chewing gum increased the rates of smoking abstinence 0-20% [31]. Nicotine patches have typically afforded long-term success rates of 10-30% with minimal interventional support [32]. With both methods the results have been inadequate, and still involve dosing of the user with nicotine and its concomitant adverse physiological effects. Other pharmacotherapies such as mecamylamine and clonidine, serotonergic agents such as buspirone, and antidepressants such as bupropion have also been used [28]. Notably, bupropion has been perhaps the most effective in aiding smoking cessation efforts although the results are limited [33]. Moreover, all such pharmacotherapies act on the central nervous system (CNS) with attendant side effects that possibly outweigh the level of efficacy.

Drugs of abuse like PCP (phencyclidine) and methamphetamine produce a number of adverse effects that involve several sites in the CNS [34]. This has particularly hindered approaches to develop antagonists of selective binding sites for these drugs. Although PCP was first abused in oral form over a decade ago, it is only in recent years as a smoked or snorted drug that it has become a more serious problem involving significant numbers of users [35]. PCP in pharmaceutically pure form is a white powder which readily dissolves in water. In "street" form PCP is often adulterated and quite often misrepresented as a variety of other drugs. It is highly variable in appearance, being sold in liquid, powder, and tablet form; the latter two in many colors. As a powder or liquid, it is often placed on parsley or on other leaf mixtures to be smoked as cigarettes [36]. The medical

and psychological effects of PCP can vary greatly among people and the dosage of intake [36]. PCP is highly addictive and can cause extreme violence, psychotic and self-destructive behavior, and in some cases long-lasting schizophrenia and even death [34, 37]. The treatment of PCP abuse is difficult. This drug has a large volume of distribution in humans and, due to its lipophilic nature, large amounts can remain in fat stores for days, which makes traditional detoxification methods like dialysis ineffective.

Amphetamines in use today have their origins early in antiquity. Plants of the species *Ephedra* found in Asia yield the centuries-old drug Ma Huang which consists of <1% of the stereoisomers of the alkaloid ephedrine [38]. At present, dietary supplements that contain Ma Huang or synthetic equivalents are widely promoted and used in the United States as a means of losing weight and increasing energy [39]. Recently, the Food and Drug Administration requested an independent review of adverse events related to the use of supplements that contained ephedra alkaloids to assess causation and to estimate the risk posed to consumers [40]. Events related to the use of such supplements involved hypertension, heart palpitations, tachycardia, stroke, seizures, and death. Clearly, the use of dietary supplements that contain ephedra alkaloids are a health risk to some persons.

The hazards are generally increased when synthetic amphetamines are used in pure form. Amphetamine itself, the parent of the family of phenyl-substituted isopropylamines to which ephedrine is structurally related, has been marketed as a decongestant/bronchodilator for over 70 years and was legally available as tablets for much of this time [41]. Even though recognized early on as a potent central nervous system stimulant, oral consumption in the 1960's was not perceived as a problem. This position changed soon after when intravenous administration became popular, as well as the smoking of the highly addictive, long-acting substance methamphetamine, both of which created the intensely euphoric initial "rush." Psychotic behavior resulting from chronic amphetamine abuse of this type led to the "speed freak" era of drug addiction [42]. In the late 1980's, very pure crystalline methamphetamine called "ice" entered the recreational drug scene and was readily abused [43]. Besides many harmful physiological reactions, human amphetamine abusers display stereotyped motor patterns, such as dismantling and reassembling mechanical devices, report delusional thoughts, and have been described as being in a state of cognitive inflexibility [44]. Now listed as Schedule II controlled agents, it became apparent that toxic symptoms were more likely to occur with chronic and heavy amphetamine use than with cocaine use. As a result, the medical use of various amphetamines has also been curtailed. Yet, ephedrine is not controlled, despite the effects noted previously and the possibility that it may also induce toxic psychosis which has recently received renewed attention [45].

Although many compounds belong to the amphetamine family of stimulants, their effects on behavior are qualitatively similar, differing primarily with regard to potency and pharmacodynamics. Somewhat exceptional have been the "designer drugs" of the past two decades, the

most widely used being the 3,4-methylenedioxy derivative of methamphetamine (MDMA, "ecstasy"), which have serotonergic activity and seem to possess both stimulant and hallucinogenic properties (entactogen) [46, 47]. Initial effects include an increase in heart rate, sweating and dry mouth, and euphoria typically followed by feelings of serenity, calm, and emotional closeness. Hallucinogenic effects, though rare, can result in dangerous behavior leading to death. Chronic use may cause psychosis and the evidence is substantial that long-term neurotoxicity may ensue [47, 48]. Again, although primarily first used by clinicians in the 1970's for psychotherapy, MDMA became legally recreational until its potential for abuse and toxicity warranted placement as a Schedule I substance in 1985 [49]. Ecstasy is currently a fashionable drug among youth at club parties where individuals may orally consume only a single tablet or multiple doses depending on tolerance. It is likely that more than 10% of all adolescents and college students have used the drug with millions of doses sold each year [47, 50]. Methamphetamine and MDMA have high abuse potential and several other amphetamine analogs, such as 3,4-methylenedioxyamphetamine (MDA) and paramethoxyamphetamine (PMA), sometimes enter the illegal market and may be even more acutely and chronically toxic [46, 47, 51]. Ultimately, treatment for amphetamine dependence-related disorders is limited and usually includes psychosocial approaches and antidepressants.

Opiates are compounds that exert pharmacological effects through interaction with specific μ -, κ -, and δ -opioid receptors primarily in the brain. Natural opiate alkaloids, such as morphine, are the active components found in the juice of the opium poppy seed from *Papaver somniferum* that has been used as a medicinal for thousands of years [52]. The most important property of opium alkaloids and other agonists of the μ -receptor subtype is their ability to relieve pain [53]. However, morphine, and synthetic derivatives, notably heroin, are highly addictive and have served as the most classic and well-studied examples of drugs with the capacity to promote self-administration, tolerance, withdrawal, and drug-seeking behavior [54]. In this regard, the results from both animal models (rodents, primates) and humans afford an excellent correlation. Humans typically self-administer opiates orally or intravenously, although the latter is preferred by abusers because an intensely euphoric intoxication state can be achieved, especially with the most potent opiate heroin. Heroin is also now increasingly abused intranasally and by smoking, given the reduced risk for HIV transmission and the recent greater availability of high-purity street heroin (70% versus 30%) [55]. Even a limited exposure to heroin or morphine, such as a single injection, in a nondependent individual can produce acute dependence in which there is some tolerance to additional drug and withdrawal symptoms provoked by a μ -receptor antagonist such as naltrexone [52, 56, 57]. Withdrawal from chronic heroin is a traumatic syndrome of severe physiological reactions lasting up to three days and so abstinence is difficult [52, 56, 57]. A heroin addict generally takes heroin up to four times a day to avoid the withdrawal phase. Recent trends indicate a steady increase of opiate abuse, especially of heroin by adolescents, with levels the highest in the past 30 years [52, 57]. It is estimated that three million persons in

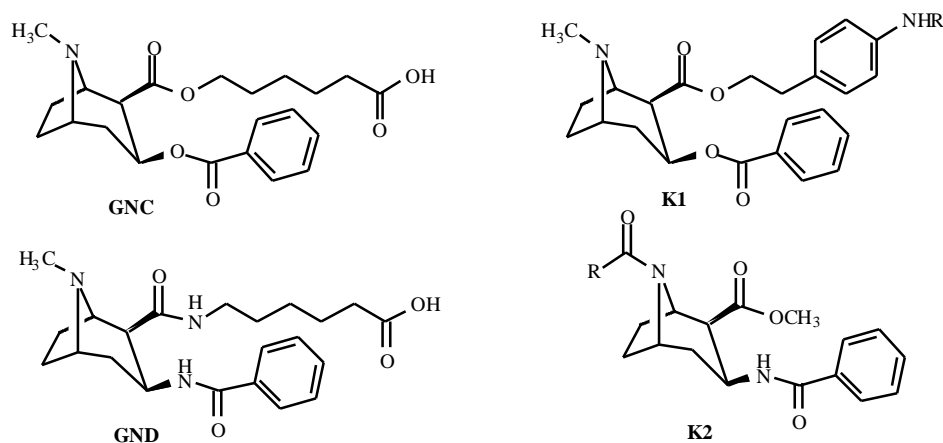


Fig. (2). Cocaine haptens.

the United States have used heroin at some time, perhaps 11 million have used other opiates for nonmedical purposes, and that as many as one million are currently addicted to heroin/opiates as defined by criteria for a treatment program [52, 57, 58].

Heroin addicts will enter and re-enter programs in an attempt to become free of the drug or reduce dependence. Detoxification "cold turkey" by elimination of opiate intake invariably fails and results in relapse [52]. Naltrexone antagonist maintenance following detoxification has had some efficacy in motivated individuals such as healthcare professionals with opiate dependence [52], and buprenorphine-naloxone (partial agonist-antagonist) treatment has shown promising results in clinical trials and has recently received FDA approval [58]. The most successful treatment has been long-term maintenance with the μ -opioid agonists methadone, and more recently L- -acetylmethadol (LAAM), where voluntary retention in programs can be 60-85% [58]. These compounds can be orally administered and have a duration of action of more than a day as compared to a few hours for heroin. Overall, μ -agonist maintenance treatment decreases illicit opiate abuse and associated criminal behavior and improves the health, well-being and productivity of the patient. The major drawbacks are that these drugs maintain physical dependence, produce many of the same pharmacological effects as heroin/morphine, and are likely to necessitate life-long use on a daily basis (methadone) or several times a week (LAAM). Heroin addiction is a chronic, life-long, relapsing disease with a high fatality rate [59]. Detoxification of an addict to a drug-free and abstinent state remains an ideal goal.

More than 30 years ago, a vaccine concept was described for the potential treatment of morphine and heroin addiction [60, 61] and digoxin toxicity [62]. Perhaps most notable was the 1974 report in which Bonese *et al.* used rhesus monkeys that were trained to self-administer heroin, and then treated them with an immunoconjugate consisting of morphine coupled to bovine serum albumin (BSA) [61]. The induction of antibodies capable of binding morphine and heroin significantly reduced their self-administration patterns, an effect that was overcome by increasing the dose of administered heroin. Even though this approach showed

promise, it was not extended to human subjects at the time, since treatment of addicts with methadone or naltrexone was assumed to be sufficiently effective. Also, such therapy may not have gained broad acceptance with the medical establishment and society of that era. Only recently has the strategy of anti-drug vaccines and/or monoclonal antibodies (mAbs) (termed "immunopharmacotherapy") evolved and gained interest for targeting health problems such as drug abuse [3, 4].

The far-reaching impact of addiction to drugs such as cocaine, nicotine, PCP, amphetamines, and opiates, together with the paucity of effective therapies, necessitates the development of alternative strategies. There is increasing evidence suggesting that immunopharmacotherapy offers a potentially useful approach. Herein, we review recent published work with respect to cocaine, nicotine, PCP, and amphetamines in which there are ongoing research programs. Reports of immunopharmacotherapy for opiates and other drugs of abuse have not appeared in the current literature and will not be further addressed.

2. Cocaine – Active Immunization

Various methodologies for the preparation of anti-cocaine vaccines have been pursued. Most notable, are the potential differences in immunoconjugate structures with regard to the structure of the cocaine hapten and the position of conjugation (e.g. tropane amine, methyl ester or benzoyl ester positions) to proteins and in the length and chemical composition of the linker.

In the first published study, male Fisher rats were with immunized with either cocaine emulsification in complete Freund's adjuvant (CFA) or with cocaine conjugated with keyhole limpet hemocyanin (KLH) as carrier plus CFA. The average analgesic effect of cocaine was significantly reduced in animals immunized with cocaine-KLH as compared to saline controls [63]. Certain doubts were raised however with respect to the character and the efficacy of the conjugate used in this study [64].

The Janda laboratory, in collaboration with Koob and colleagues, reported the use of a new, stable cocaine immunoconjugate, GNC-KLH (hapten GNC (Fig. (2))

conjugated to KLH), that was used to immunize Male Wistar rats. High titers (~1:24,000) of anti-cocaine antibodies were measured and competitive binding studies demonstrated that the immune response was highly specific for cocaine (K_d -avg ~ 1 μ M compared to ~ 1 mM for benzoylecgonine). This approach resulted in suppressed locomotor activity and stereotyped behavior in rats challenged with cocaine. Furthermore, following acute injection of cocaine, levels of cocaine in the striatum and cerebellum of the immunized animals were significantly lower than those of control animals [65]. In a follow-up study, an animal model of relapse was used where rats were trained to self-administer cocaine [66]. The rats were subjected to a period of extinction by substituting the drug for saline, vaccinated, and re-exposed to cocaine. Compared with controls, animals immunized with GNC-KLH did not reinstate cocaine self-administration behavior when given a noncontingent cocaine infusion on two consecutive days, and an eight-fold increase in cocaine dosage (0.25 mg/infusion) was needed to surmount cocaine binding by the high serum titer (>1:25,000) of anti-cocaine antibodies.

Further studies compared the effects of a second-generation vaccine, based on the GND hapten, in terms of duration of action [67]. An increase in stability was expected for GND-KLH by replacing the relatively labile C-2/C-3 ester bonds with amides. Indeed, a greater and longer-lasting suppression of 80% reduction in locomotor activity after 12 days and 3 challenges of cocaine was observed.

Additional studies using different methods of conjugation have corroborated this approach of active immunization. Ettinger *et al.* [68] immunized Long-Evans rats with a cocaine-KLH conjugate, prepared using a photoactivated linker, and showed significant changes in cocaine-seeking behavior among immunized animals. However, the cocaine binding effects seemed to be surmounted by administration of larger doses (20 mg/kg) of cocaine [69].

In a different approach to address problems associated with hapten instability and examine efficacy, Schabacker *et al.* [70] explored the feasibility of immunization with an anti-idiotypic cocaine antibody. This elegant approach is based on the premise that an antibody (Ab1) specific for an antigen can elicit different sets of anti-idiotypic antibodies (Ab2). One of these sets (Ab2) bound an idiotope within the antigen-recognizing site of the Ab1, presenting an internal image of the antigen. This Ab2 antibody can thus elicit antibodies similar to the Ab1 type. Following this approach, two KLH conjugates were prepared, based on haptens K1 and K2, that were used to immunize mice and obtain mAbs specific for cocaine. These mAbs in turn were conjugated to KLH and immunization resulted in a selection of four Ab2 mAbs. Of these anti-idiotypic mAbs, one resulted in an anti-cocaine response upon immunization that was sufficient to significantly reduce the level of cocaine reaching the brain upon administration of the drug.

Koetzner *et al.* [71] performed the only cocaine vaccination study reported on rhesus monkeys. Three monkeys were immunized with a norcocaine-BSA conjugate and behavioral effects induced by cocaine were measured over time after immunization. A correlation was reported between anti-cocaine antibody titers and inhibition of the response

rate-decreasing effect of cocaine. Plasma cocaine concentrations increased in proportion to antibody titer. The reported data suggest that the antibody response to the cocaine conjugate can produce a specific pharmacokinetic shift in cocaine distribution sufficient to significantly inhibit behavioral effects induced by the drug, and no side effects were observed upon immunization.

An anti-cocaine vaccine that is currently in phase II clinical trials (TA-CD, Xenova (Cambridge, UK)[7]) is based on the initial studies of Fox and colleagues [72, 73]. Norcocaine was acylated using succinic anhydride and conjugated to BSA, resulting in an immunconjugate similar to K2-BSA. Mice that were immunized using this immunogen displayed very high titers (>1:80,000) with a moderate average K_d -avg ~ 8.5 μ M). Using both injections and intranasal administration of [³H]-labeled cocaine, it was shown that most of the cocaine was bound by the anti-cocaine antibodies. Brain levels of cocaine were shown to be significantly reduced within the immunized population of mice compared to the control group. In follow-up studies, the same hapten was used, but the carrier protein was replaced with recombinant cholera toxin B (conjugate IPC-1010, later renamed TA-CD) in order to make the vaccination strategy more amenable to studies with human subjects [74, 75]. Male Wistar rats were immunized and serum anti-cocaine antibody levels ranged from 0.008 to 0.709 mg/mL after several boosts. Self-administration behavior was shown to be affected significantly by immunization, but only for those animals that reached serum antibody concentrations greater than 0.05 mg/mL. Further studies confirmed these results and showed that the observed decrease in self-administration was not the result of side effects caused by the vaccine, which could cause a reduced physical ability to press the lever that triggers an injection with cocaine.

A randomized, double blind, placebo controlled phase I clinical trial (with 34 subjects) showed that TA-CD was well tolerated upon three intramuscular injections in two months, both locally and systemically [76]. Anti-cocaine antibody levels peaked after three months and gradually declined to baseline levels within one year. The serum showed good average binding affinity, but low specific anti-cocaine antibody levels were reported (0.003 mg/mL), much below the level needed in animal studies to reduce drug-seeking behavior significantly. Currently, phase II clinical trials are in progress.

2.1. Cocaine – Passive Immunization

In an approach that is conceptually different from active immunization, in which an injected antigen causes generation of polyclonal antibodies, studies have shown beneficial effects resulting from passive immunization as well, in which high affinity anti-cocaine mAbs are administered. Fox and coworkers described the use of mAb MO240 to demonstrate a straight correlation between dosage of administered mAb and cocaine self-administration in rats [72, 74]. The Janda laboratory reported persistent cocaine antagonizing effects for over 11 days upon administration of mAb GNC92H2 [72, 74]. While anti-cocaine mAbs would most likely be useful in reversing the toxic effects of cocaine

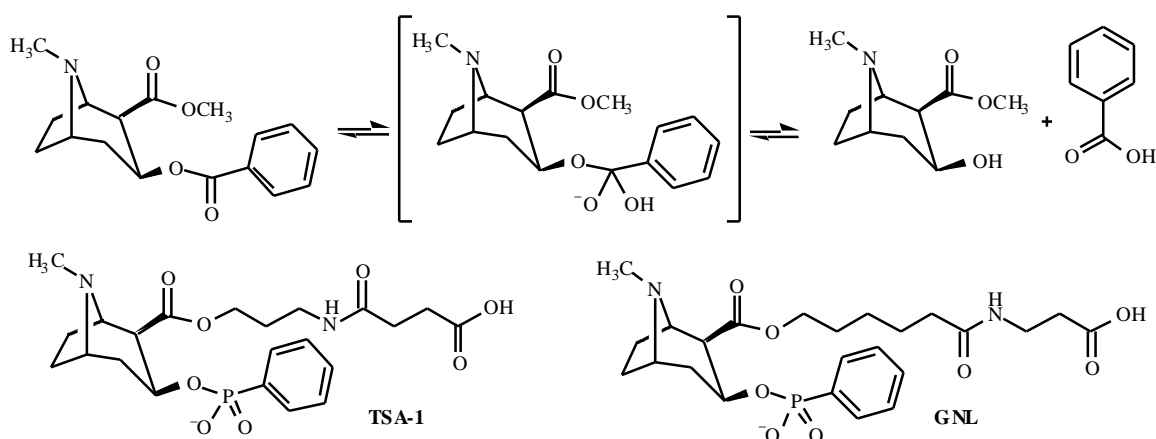


Fig. (3). Hydrolysis of cocaine; structures transition state analog haptens.

in cases of overdose, improvements upon presently available mAbs would be required for long-term immunization purposes. Recent advances in structure determination [77], structure-activity relationship modeling and humanization of murine mAbs specific for cocaine binding [78], as well as the successful generation of humanized Fab and single chain Fv antibodies based on the murine mAb GNC92H2 [79], show promise for the development and clinical use of high affinity anti-cocaine mAbs.

A related passive immunization approach invokes the use of catalytic antibodies, which have emerged as a powerful tool at the interface of chemistry and biology. An anti-cocaine catalytic mAb with sufficient kinetic properties would not only bind cocaine, but also metabolize the drug, thereby diminishing its impact on the CNS. The main advantage of a successful catalytic mAb that can catalyze the hydrolysis of the benzoate ester of cocaine would be a large decrease in the minimum required concentration of administered mAb for the clearance of cocaine from the serum, since one antibody molecule could degrade multiple cocaine molecules.

Landry and coworkers first reported the generation of anti-cocaine catalytic antibodies [80]. Stable phosphonate monoester analogs of cocaine (e.g. **TSA-1**) (Fig. (3)), resembling the transition state of the hydrolysis reaction of cocaine to ecgonine methyl ester, were synthesized and conjugated to a carrier protein. Using these haptens, murine mAbs were obtained that displayed a wide variety of catalytic activities, with one mAb (15A10) showing good activity ($k_{\text{cat}} \sim 2.3 \text{ min}^{-1}$, $K_m \sim 220 \mu\text{M}$). [81]. Rats that were pretreated with mAb 15A10 showed a significant dose-dependent increase in survival upon injection of a lethal amount of cocaine. Behavioral studies showed a dose-dependent effect on self-administration of cocaine upon pretreatment, indicating a relatively short *in vivo* half-life for the mAb. The recent elucidation of the crystal structure of mAb 15A10 [82] could aid in improving upon the present kinetic and pharmacodynamic properties.

The Janda laboratory has screened a large number of mAbs for cocaine hydrolysis, based on immunization with

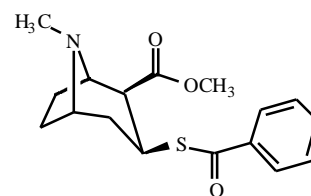


Fig. (4). Cocaine benzoyl thioester.

various transition-state analogs. Successful candidates were detected using the new hapten **GNL** [83]. Using hapten **GNL**, several catalytic mAbs were selected with moderate catalytic activity. The mAb GNL3A6 was found to be the most efficient (highest k_{cat}/K_m), with $k_{\text{cat}} \sim 0.03 \text{ min}^{-1}$ and $K_m \sim 55 \mu\text{M}$, $k_{\text{cat}}/K_m \sim 10 \text{ M}^{-1} \text{ s}^{-1}$. Yet, as with the Landry mAb 15A10, this antibody is still far short of what is required for therapeutic efficacy ($k_{\text{cat}}/K_m \sim 10^4 \text{ M}^{-1} \text{ s}^{-1}$). However, the screening of large numbers of antibodies using hybridoma or phage-display libraries could result in the selection of suitable catalysts. For this purpose, cocaine benzoyl thioester (Fig. (4)) having the natural cocaine configuration was synthesized, in order to carry out high-throughput screening for cocaine esterases by spectrophotometric methods [84].

Cashman *et al.* used a similar cocaine analog (nonnatural configuration at C-3) to select catalytic mAbs from a library of 450 hybridoma cell culture supernatants [85]. Using this high-throughput screening method, three mAbs with good catalytic activity were analyzed more thoroughly, and one mAb was found to efficiently hydrolyze cocaine ($k_{\text{cat}}/K_m \sim 10^4 \text{ M}^{-1} \text{ s}^{-1}$) but at the higher than physiological pH 8.4.

3. NICOTINE

Several research groups have been pursuing active immunization strategies for nicotine dependence in recent years. Over the past 30 years, a number of reports appeared in the literature which described nicotine haptens and immunoconjugates. The work was aimed primarily at the development of enzyme-linked immunosorbent assays (ELISA) for more convenient detection of nicotine in a variety of media such as blood, urine, and smoke residue.

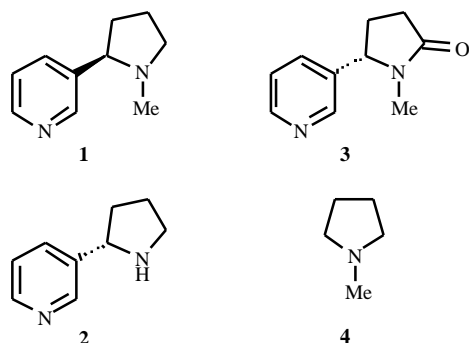


Fig. (5). Nicotine and several metabolites.

The majority of these studies utilized polyclonal immunoglobulins (antisera) from rabbits/goats and in a few examples murine mAb preparations were examined. While the data in most cases were acceptable with regard to antibody affinity and specificity, erratic and variable results were noted. In light of some of these observations, there is clearly room for improvement with regard to both hapten design and the quality of anti-nicotine immune responses and antibody preparations. Notably, even slight improvements would afford enhanced performance in immunopharmacotherapy protocols.

For optimum results, the design and synthesis of a nicotine hapten requires attention to stereochemistry, ionic/acid-base properties, and the site of attachment and characteristics of a linker moiety. It is instructive to examine (*S*)-(-)-nicotine and the structurally related compounds (*R*)-(+)-nicotine (**1**), nornicotine (**2**) present in tobacco and also a metabolite, cotinine (**3**) the major primary metabolite of nicotine, and *N*-methylpyrrolidine (**4**), a minor tobacco component (Fig. (5)).

Significantly, nicotine contains two rings with an asymmetric center which occurs as the (*S*)-configuration in nature (e.g. tobacco). The hapten should incorporate a linker in such a way as to present both ring determinants when coupled as the immunoconjugate and have the proper stereochemistry and charge characteristics. The haptens employed by several groups were derived from 2-aminonicotine (**5**), 6-aminonicotine (**6**), and *recemic*-3'-hydroxymethylnicotine (**7**) (Fig. (6)). All these haptens were racemic compounds.

It was pointed out that when nicotine antibodies were prepared using **5** or **6** there was a strong cross-reaction with *N*-methylpyrrolidine (**4**), a constituent of tobacco smoke [86]. Using similar haptens, others observed only limited binding of **4** [87, 88]. Matsushita *et al.* believed that increased exposure of the pyrrolidine ring of a nicotine hapten would result via coupling at the pyridine nucleus [89]. These workers considered **5** as the most satisfactory structure and that the antibodies obtained were more specific than those elicited using **7** preferred by Langone *et al.* [86, 90] However, racemic **5** conjugated using a diazotized *p*-aminobenzoyl linker afforded antisera highly specific for natural (*S*)-(-)-nicotine with only 5% cross-reaction of the (*R*)-(+)-isomer [89]. While these data might be correct and even rather favorable, such a result is difficult to readily explain. Langone *et al.* [86, 90] prepared nicotine antisera

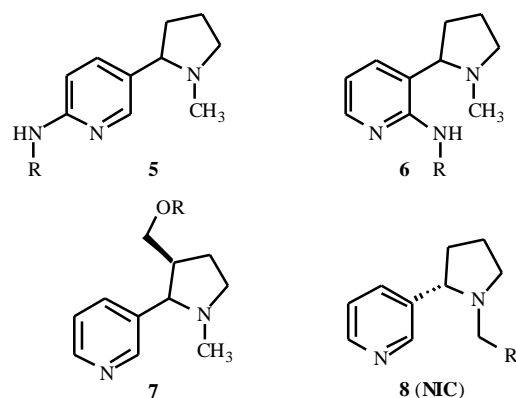


Fig. (6). Nicotine haptens.

and mAbs derived from **7**, perhaps the most widely recognized, studied and accepted hapten for anti-nicotine antibody production [90, 91]. Affinities on the order of $K_d \sim 10^{-8}$ M were reported for various mAbs. Yet, other workers using the same hapten isolated mAbs with only micromolar K_d values for nicotine and increased cross-reactivities versus some metabolites and tobacco components [92]. Castro and coworkers examined various linker lengths and compositions using **5** and **6** to assess effects on affinity and specificity, and somewhat surprisingly observed no significant differences [87].

Hieda *et al.* prepared a urea derivative of **5** as a hapten (**CMUNic**) that was coupled to KLH, and male Holtzman rats were immunized using this immunoconjugate. Although serum antibodies were shown to bind nicotine with good affinity, and plasma nicotine concentrations were elevated in immunized rats compared to nonimmunized animals upon nicotine administration, no significant alteration in brain nicotine concentration was observed [93]. The authors however measured brain nicotine levels 40 minutes after administration of the drug, and in follow-up studies [94] significant differences in brain nicotine levels were detected when measurements took place between 30 seconds and six minutes after injection. Rats that were immunized with **CMUNic**-KLH showed between 28 to 48% reduction in brain nicotine concentration compared to rats that were immunized with only KLH.

Pentel and coworkers then altered the immunoconjugate, in which nicotine was linked on the pyrrolidyl ring in hapten **7** to the carrier protein recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA). Rats were immunized with this immunoconjugate or treated with polyclonal antibodies obtained from rabbits immunized with the same immunoconjugate [95]. Active immunization resulted in a reduction of 64% in nicotine brain levels upon injection of nicotine compared to controls. However, this study mainly concentrated on the relation between administration of various dosages of anti-nicotine polyclonal antibodies (Nic-IgG) and their effect on nicotine distribution, and on behavioral and cardiovascular effects in rats. The reduction of nicotine brain levels was shown to be dependent on the concentration of administered Nic-IgG. Both the nicotine-induced locomotor activity and stimulation of blood pressure were shown to be affected by addition of Nic-IgG. A ratio of

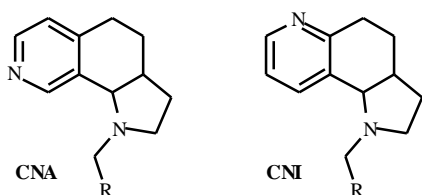


Fig. (7). Constrained nicotine haptens.

0.07 (Nic-IgG:nicotine) was sufficient to prevent the induction of locomotor activity by nicotine, while locomotor stimulation by cocaine was not attenuated, indicating good selectivity of these polyclonal antibodies. In a follow-up study, the effects of active immunization using the same immunoconjugate were examined in more detail [96], in which rats were vaccinated and the effects of continuous nicotine administration over 11 weeks were measured. At the end of this period, serum anti-nicotine antibody titers reached values of over 1:10,000 in all animals. A single dose of labeled nicotine was administered and vaccinated rats showed between 40 and 60 percent lower nicotine brain levels than rats that had received saline instead of the nicotine immunoconjugate. These data suggest that continuous exposure to nicotine, at concentrations comparable to those found in heavy smokers, does not saturate the capacity of induced anti-nicotine antibodies to bind nicotine. Further studies confirmed these results, and also showed that vaccinated rats were less prone to seizures induced by high doses of nicotine [97].

Two vaccines against nicotine dependence are currently in clinical trials. Phase I clinical trials were started in 2002 by Nabi Biopharmaceuticals (Rockville, MD, USA) to determine the safety and immunogenicity of the vaccine (termed NicVax™) developed by the group of Pentel. A different vaccine (TA-NIC, a nicotine-cholera toxin B immunoconjugate) is being developed by Xenova Research Ltd. [116], and a phase I clinical trial with 60 subjects has recently been completed [3]. Detailed results from both clinical trials have not yet been reported.

A number of other groups have developed nicotine vaccination strategies in recent years. Svensson and colleagues have immunized male Wistar rats with an immunoconjugate (IP18-KLH) that contains a hapten similar to the one used originally by the group of Pentel (hapten 5), in which a linker is attached to the pyridyl moiety of nicotine. Using *in vivo* voltammetry, the authors examined the effect of nicotine on dopamine release in a part of the brain that plays an important role in addiction to drugs, and they observed that immunized rats showed no significant dopamine release, whereas control animals showed dopamine release dependent on dosage of administered nicotine [98]. In further studies, the nicotine-seeking behavior among immunized rats was examined. Rats were trained to self-administer nicotine by pressing a lever for intravenous injection [99]. They were then extinguished from this nicotine-seeking behavior, and subsequently administered small amounts of nicotine that are known to induce nicotine-seeking. Rats that had developed high titers of anti-nicotine serum antibodies (>1:10,000) did not respond to this reinforcing primer, whereas control animals did show reinstatement of nicotine self-administration.

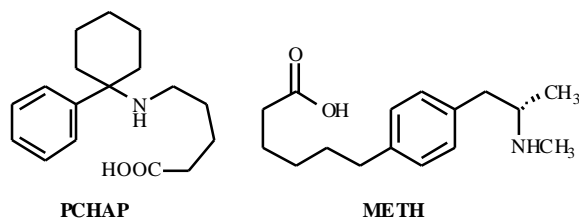


Fig. (8). PCP and (+)-methamphetamine haptens.

Cerny and coworkers [100] reported the preparation of an anti-nicotine vaccine based on the pyrrolidyl-modified hapten (7) that was used by the groups of Langone and Pentel. This hapten was linked to the carrier protein cholera toxin B, and mice were immunized using the immunoconjugate. Anti-nicotine serum antibody titers measured after one month were in the range of 1:1000-1:2000. Although these titers were relatively low, good average affinity for nicotine was reported (K_d -avg \sim 0.06 μ M) and brain nicotine levels after injection of 600 ng nicotine were shown to be reduced by 90% in vaccinated mice compared to their nonvaccinated counterparts.

The Janda laboratory has recently started contributing to the growing field of nicotine immunopharmacotherapy. A first study concentrated on the development of mAbs with high affinity and selectivity for nicotine and discrimination from its major metabolites, normicotine and cotinine [101]. Nicotine and cotinine based haptens were synthesized with alkyl linkers attached to the pyrrolidyl nitrogen in order to present correct mimics, in terms of stereochemistry, unaltered ring systems and the pyrrolidyl methyl moiety as part of the alkyl linker, of the naturally occurring molecules to the immune system. The nicotine hapten (8, NIC) was conjugated to KLH and immunization of mice with NIC-KLH yielded several mAbs with good affinity ($K_d \sim$ 0.2 μ M, measured by equilibrium dialysis) and selectivity for (S)-(-)-nicotine were obtained. No cross-reactivity with 1, 2, 3, or 4 was detected at 100-1000-fold molar excess of these compounds using competitive ELISA.

In a different study of Meijler *et al.*, the objective was to increase the immunogenicity of the nicotine immunoconjugate [102]. The previous investigations had indicated that NIC-KLH lacked immunogenicity, and it was postulated that constraint of the hapten could result in a more focused and thus greater specific immune response. This approach had been applied to the development of peptide-based vaccines, but was unprecedented for small-molecule haptens. Two constrained haptens, CNA and CNI (Fig. (7)), that mimic the energetically most stable conformations of nicotine at physiological pH were synthesized. The haptens utilized a linker for coupling to the carrier protein KLH that was identical to that of the first-generation immunoconjugate (NIC-KLH) which would allow a direct comparison.

Immunization of mice using the NIC-KLH immunoconjugate provided relatively low anti-nicotine serum antibody titers having a mean value of \sim 3,200. Competition ELISA and equilibrium dialysis measurements yielded the serum affinity for (S)-(-)-nicotine as a K_d -avg \sim 1.7 μ M \pm 0.20 μ M. On the other hand, immunizations using the

second-generation immunoconjugates CNA-KLH and CNI-KLH resulted in antisera with greatly increased titers of ~25,000. Significantly, the K_d -avg $\sim 1.0 \mu\text{M} \pm 0.10 \mu\text{M}$ and $0.60 \mu\text{M} \pm 0.10 \mu\text{M}$, respectively, were nearly two- and three-fold improved. Also, the antisera showed >10:1 specificity for (*S*)-nicotine versus the major metabolite (*S*)-cotinine, similar to NIC-KLH antiserum.

A novel approach has been described recently by Sanderson *et al.* [103], in which a peptide-based molecular adjuvant was incorporated in the hapten design. Part of this 19-residue peptide consists of a conformationally biased peptide that binds certain receptors on antigen presenting cells better than analogous receptors on inflammatory cells. A nicotine hapten similar to the compound employed by the groups of Pentel and Cerny (7) was conjugated to this peptide, and rats were immunized with relatively large amounts (200 μg + weekly boosts using 200 μg) of this immunoconjugate, but without added adjuvant. Serum anti-nicotine antibody titers were determined to be quite low (1:223 in vaccinated animals), but it must be noted that these values were measured only after the behavioral studies had finished (one month after the last immunization and after extensive administration of nicotine). Behavioral studies showed that immunized rats responded significantly less to high concentrations of nicotine than their nonvaccinated counterparts. All immunoconjugate vaccines described earlier were formulated with an adjuvant to enhance antibody titers. Obviating the need of an external adjuvant in order to create a sufficient immune response could be beneficial for use of this vaccine in human subjects, since any inflammatory side effects related to the adjuvant would be eliminated.

4. PCP AND METHAMPHETAMINE

Studies to combat addiction to the illicit drugs methamphetamine and PCP by way of immunization have been performed exclusively by Owens and coworkers. Over the past 20 years an impressive number of investigations have been reported by this group, concentrating largely on the treatment of PCP addiction using passive immunization strategies. Early studies focused on the development of anti-PCP mAbs for analytical purposes [104]. In an initial limited study in dogs, administration of Fab (antigen binding fragment of the IgG) specific for PCP was shown to affect the disposition of PCP significantly [105].

In a follow-up study in rats, the pharmacokinetic properties of a high affinity anti-PCP Fab ($K_d \sim 1.8 \text{ nM}$) were examined. This antibody fragment was prepared in large amounts from an anti-PCP whole mAb, obtained through immunization of mice with hapten PCHAP conjugated to BSA (Fig. (8)) [106]. The Fab was found to have about 80 times higher affinity for PCP than the highest affinity PCP-specific binding site in mammals. Combined with the pharmacokinetic data obtained using [^3H]-labeled Fab, this suggests that the high affinity PCP-specific fragment could be used in treating toxicity induced by PCP. Further studies confirmed that rats treated with anti-PCP Fab showed a substantial redistribution of PCP in several organs, compared to untreated animals. Brain, fat, heart, lung and testis levels decreased, whereas concentrations of PCP in the

liver markedly increased [107]. Furthermore, treatment of rats with the anti-PCP Fab significantly reduced the change in locomotor activity induced by PCP and with good selectivity [108]. The crystal structure of a complex between PCP and the anti-PCP Fab used in these studies was elucidated and the binding pocket was shown to be relatively deep and hydrophobic in nature [109]. A number of other studies using both Fab as well as whole IgG with high affinity for PCP strengthened the finding that administration of mAbs specific for PCP in rats can markedly inhibit the toxic and behavioral effects induced by PCP intake [110]. Of special interest were the observed effects upon administration of a single large dose of anti-PCP mAb. Rats were treated with 1.0 g/kg mAb (either nonspecific IgG or anti-PCP specific IgG) and large doses of PCP were administered every one to three days over two to four weeks. PCP effects were blocked significantly, and inhibition was remarkably persistent, in both behavioral studies and measurements of PCP brain levels, during the entire period of PCP administration, even though the dose of administered PCP already exceeded the measured binding capacity on the first day [111, 112]. These results corroborate the findings reported by Hieda *et al.* [96] on the absence of saturation of anti-nicotine antibodies generated by vaccination, after continuous nicotine administration for one month, and indicate that binding affinities and serum antibody titers as a measure of antibody efficacy might underestimate the *in vivo* binding capacity of antibodies generated by either active or passive immunization.

Owens and colleagues have also explored immunization strategies for treatment of (+)-methamphetamine abuse (MA) abuse in recent years [111, 113]. Rats were treated with high affinity anti-MA mAbs ($K_d \sim 11 \text{ nM}$), and effects on methamphetamine induced locomotor activity were compared between treated and nontreated animals [114]. A decrease in locomotor activity was observed in treated rats when the dose of administered methamphetamine was low (0.3–1.0 mg/kg), but at a higher dose (3 mg/kg) an increase was unexpectedly observed. Brain concentrations of the drug were markedly lower among immunized animals when 1.0 mg/kg of methamphetamine was administered.

In a different study, rats were immunized using an immunoconjugate based on the hapten (*S*)-(+)-4-(5-carboxypentyl)methamphetamine, (+)-METH, (Fig. (8)) that was coupled to KLH [115]. Serum anti-(+)-METH antibody titers reached maximum values after about one month, and though they were not very high (1:1000), they remained constant, irrespective of continuous administration of large doses (3 mg/kg) of methamphetamine to the rats. However, no significant inhibition of methamphetamine induced effects on locomotor activity was measured for vaccinated rats compared to their nonimmunized counterparts. The development of new haptens with altered design could aid in generating an improved immune response and possibly effect significant changes in MA-induced locomotor activity.

CONCLUSIONS

This review on the current status of immunopharmacotherapy for drug dependence shows that distinct progress in the field has been made in recent years. The promise for this

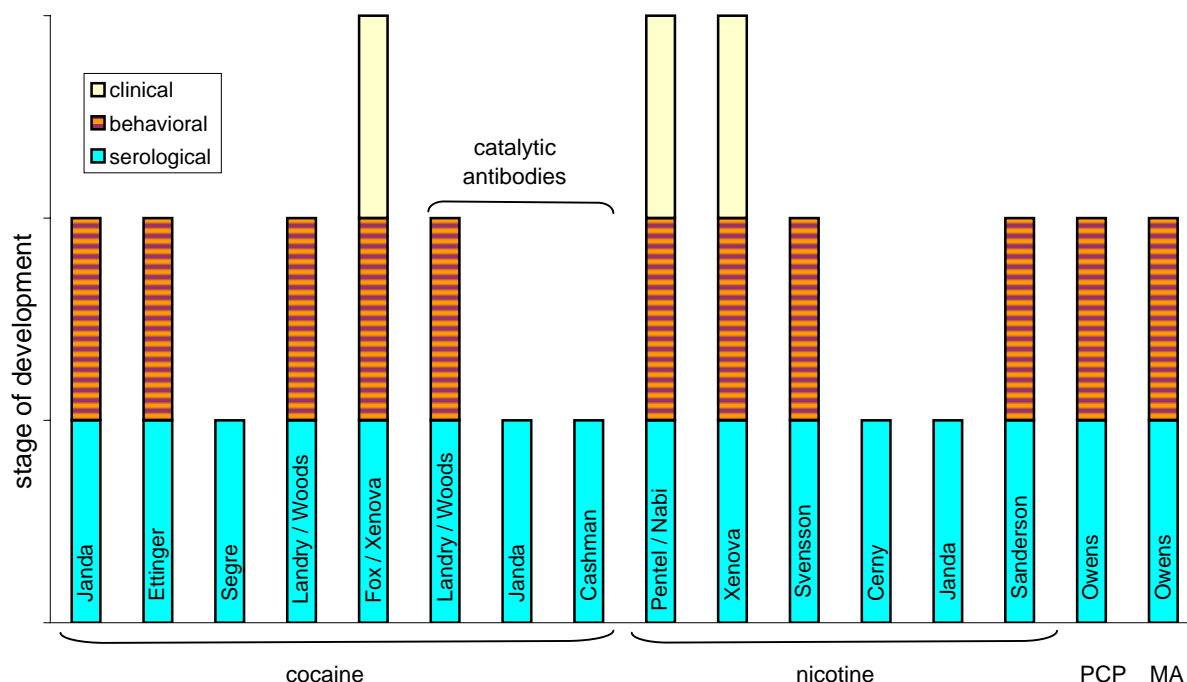


Fig. (9). Status of current immunopharmacological strategies against four drugs of abuse. The x-axis lists the programs in various research groups. The y-axis shows the current status of development for each program: - serological: only antibody titers and affinities are reported; - behavioral: effects of immunization on behavior in animals and/or brain levels of the targeted drug are reported; - clinical: human clinical trials are underway. PCP: phencyclidine; MA: (+)-methamphetamine.

strategy resides primarily in its mechanism of action. Whereas all presently used medications for the treatment of dependence disorders are based on drugs that interact with the CNS, antibodies act by binding the drug of abuse in the bloodstream before it can reach the brain. Hence, the forte of immunopharmacotherapy resides in its minimal side effects, while replacement strategies using agonists or antagonists of the abused drugs generally cause numerous adverse reactions. A large amount of data (see Fig. (9) for a summary) has been gathered on the effects of active and passive immunization against cocaine, nicotine, PCP and methamphetamine in animal models, suggesting potential efficacy of these treatments in humans, and clinical trials are currently underway for vaccines against cocaine and nicotine.

Due to the possibility that immunization against a drug of abuse can be overcome by large doses of the targeted drug, the efficacy of immunopharmacotherapy will be highest in individuals that are motivated to combat their addiction, and would ideally be combined with a psychosocial rehabilitative program. In a successful scenario, the antibody-mediated blockade of drug passage into the brain will attenuate reinforcing effects and promote recovery from dependence. The studies that we have reviewed demonstrate a proof-of-concept for immunopharmacotherapy in animal models with four drugs of abuse (cocaine, nicotine, PCP and MA). Results of the human clinical trials for cocaine and nicotine vaccinations are awaited with great interest.

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