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Nicotine dependence
Studies with a laboratory model

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Abstract

Simple, rapid preclinical models of nicotine physical dependence and abstinence syndrome are needed to identify underlying neurobiological mechanisms and screen potential therapies. One such model induces dependence by 7 days of continuous subcutaneous nicotine infusion in the rat. Abstinence is initiated through termination of infusion or injection of nicotinic antagonist drugs. The result is an abstinence syndrome involving a pattern of behaviors somewhat resembling opiate abstinence in the rat as well as weight gain and depressed locomotor activity. The model has met a number of validity criteria and its essential features have been replicated in several laboratories. Several research groups have modified or extended the model by measuring emotional/motivational changes associated with nicotine abstinence such as conditioned aversion, intracranial self-stimulation (ICSS) thresholds and the startle response. Dependence models have been used to identify neurobiological systems that contribute to nicotine dependence, particularly endogenous opiate systems and the mesolimbic dopamine pathway. It is hypothesized that these different systems contribute to different behavioral aspects of nicotine abstinence syndrome. Increasingly used as a preclinical screening tool, the model has proved sensitive to various abstinence-alleviating therapeutic approaches, including some with already demonstrated clinical effectiveness.

Index terms: Nicotine dependence; Nicotine abstinence syndrome; Nicotine withdrawal; Physical dependence; Preclinical models; Nicotinic antagonists; Rat

1. Introduction

It has been estimated that 80% of all regular smokers want to quit smoking and a majority of them have tried to quit and failed (Schuckit et al., 1994). It has also been estimated that only 2.5% of unaided quit attempts are successful (Center for Disease Control, 1993). The primary reason for these dismal statistics is that tobacco products are addictive, with nicotine serving as the major addictive ingredient (US Department of Health and Human Sciences, 1988). The nature of this addiction appears to be complex. Nicotine is a positive reinforcer (Corrigall, 1999), and this property is probably central to the initial development of the tobacco habit. In addition, chronic tobacco use or chronic administration of nicotine alone generally results in a state of "physical dependence." The latter term will be used here to denote a state of the organism such that termination of drug administration will result in an abstinence syndrome. Thus, the occurrence of the abstinence syndrome serves as the operational definition of the state of physical dependence. It is important to note that the signs and symptoms of this syndrome may include behavioral abnormalities and psychological distress as well as physiological changes.

An abstinence syndrome during smoking cessation has been described by Shiffman and Jarvik (1976) and Hatsukami et al. (1984), among others. Typical signs and symptoms include irritability, anxiety, depression, increased hunger, restlessness, difficulty concentrating, sleep disturbances, weight gain, decreased heart rate and craving for tobacco (US Department of Health and Human Services, 1990). Abstinence syndromes are generally aversive, and smoking serves as a "negative reinforcer" by relieving this aversive state (Thompson and Hunter, 1998). In fact, the most commonly reported reason for relapsing to smoking during smoking cessation attempts is the desire to relieve the discomforts of smoking withdrawal (US Department of Health and Human Sciences, 1988). Thus, while positive reinforcement is undoubtedly the main factor in the acquisition and routine maintenance of the smoking habit, the abstinence syndrome and the ability of renewed smoking

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to provide negative reinforcement may become particularly critical during times of smoking cessation.

Evidence suggests that nicotine is the ingredient in tobacco products most responsible for inducing physical dependence in smokers. Reduction in the nicotine content of cigarettes can result in an abstinence syndrome (West et al., 1984). In addition, cessation of nicotine gum usage can initiate at least a moderate abstinence syndrome (West and Russel, 1985; Hughes et al., 1986). In view of these findings, it seemed desirable to analyze the neurobiology of nicotine dependence in laboratory animal studies. This raised the question: Can nicotine dependence and a quantifiable nicotine abstinence syndrome be induced in the laboratory rat?

2. Early studies suggesting a nicotine abstinence state in rodents

There were a number of early indications that termination of chronic nicotine exposure in rodents resulted in an altered behavioral or physiological state. For example, withdrawal from chronic subcutaneous nicotine injections resulted in a modest increase in serum corticosterone, suggesting a stress response (Benwell and Balfour, 1979). In a drug discrimination paradigm, withdrawal from chronic subcutaneous nicotine injections showed some stimulus generalization to an anxiogenic pentylenetetrazol (PTZ) cue (Harris et al., 1986). Withdrawal from chronic intraperitoneal nicotine injections altered light/dark preferences in mice (Costall et al., 1989). In rats trained on various operant schedules, nicotine withdrawal disrupted ongoing patterns of appetitive operant responding (Carroll et al., 1989; Corrigall et al., 1989; Ford and Balster, 1976). Withdrawal from chronic subcutaneous nicotine injections also disrupted a previously trained pattern of unsignalled Sidman avoidance responding (Balfour, 1990). Termination of continuous subcutaneous nicotine infusion also resulted in weight gain and increased food consumption in female rats (Grunberg et al., 1986; Levin et al., 1987). Nevertheless, Emmett-Oglesby et al. stated in 1990 that there existed few robust, well-accepted models for evaluating nicotine dependence and withdrawal in rodents.

Around this same period, our research group at the University of Houston-Clear Lake began a systematic attempt to discover such a model. The aim was to develop and validate a laboratory model using the rat that could be used to test numerous hypotheses about the neurobiological mechanisms of nicotine dependence and to efficiently screen potential therapies for nicotine dependence and abstinence syndrome. Our laboratory had earlier introduced continuous subcutaneous infusion of morphine by osmotic minipump to induce morphine dependence (Malin et al., 1987). In the process, it was discovered that, compared with intermittent injections, continuous infusion resulted in measurable dependence in a shorter time with less total drug administered. Therefore, nicotine also was administered via subcutaneous Alzet osmotic minipump. In the analysis of morphine dependence, some of the most widespread and useful types of models were those where rats spontaneously exhibited countable unusual behaviors during abstinence (Gianutsos et al., 1975; Malin et al., 1988). In extensive pilot studies, our research group took various physiological measures and recorded all countable behavioral events before, during and after nicotine infusion. Certain behaviors were identified as being selectively elevated during the withdrawal phase. The same was true for weight gain, while locomotor activity was selectively reduced during this phase.

In the resulting model (Malin et al., 1992), dependence is induced by 7 days of continuous subcutaneous infusion of 3- or 9-mg/kg/day nicotine hydrogen tartrate (1.05 or 3.15 mg/kg/day expressed as the base). Alzet 2ML1 minipumps containing saline or nicotine hydrogen tartrate are inserted in the scapular region and removed under brief halothane anesthesia. Rats are placed in a clear plastic observation chamber and observed under "blind" conditions over a 15-, 20- or 30-min period. In the original study, they were observed beginning at 16-h post pump removal. However, our research group now believes that abstinence signs are more likely to peak around 18–22 h. A standard checklist of abstinence signs is employed, including teeth chattering, chewing, gasping, writhing, head shakes, body shakes, tremors (particularly cheek tremors), ptosis and miscellaneous less frequent signs including seminal ejaculation, hind foot scratches and licking the bare area of the hind foot. The number of occurrences of each sign is counted. The subject's overall abstinence score is the number of signs cumulated across all categories. In the original study (Malin et al., 1992), weight gain and locomotor activity differed significantly from saline control rats only following the 9-mg/kg/day infusion, while overall behavioral abstinence signs differed significantly from controls after both 3- and 9-mg/kg/day infusions. Thus, the behavioral abstinence signs appear to constitute a more sensitive measure. These have recently been termed "somatic abstinence signs" to distinguish them from other behavioral changes, particularly changes in trained or conditioned behavior (Epping-Jordan et al., 1998). This term might be somewhat open to misinterpretation, since a sign such as vacuous chewing or a wet dog shake is not a change in somatic state so much as an actual somatically expressed behavior coordinated by the central nervous system. Therefore, a term such as "somatic behavioral signs" might be preferable.

3. Validation and replication of the model

For the procedure to be an etiologically valid model of nicotine dependence, the observed abstinence behaviors must be shown to result from a period of nicotinic receptor overstimulation followed by reduced nicotinic stimulation.

The model has met many validity criteria for these requirements. There are significantly more signs on the day or days immediately following termination of drug infusion than before infusion, during infusion or following a subsequent recovery period (Malin et al., 1992). There are more abstinence signs following nicotine infusion than saline infusion using identical pumps and surgical procedures (Malin et al., 1992). There are more abstinence signs after higher rates of infusion (Malin et al., 1992). Preliminary data from our laboratory strongly suggest that the dependence induced by nicotine infusion can be prevented by coinfusion with the nicotinic receptor antagonist mecamylamine. Nicotine abstinence can be potently and promptly reversed by injection of nicotine (Malin et al., 1992). Finally, an abstinence syndrome can be promptly precipitated in nicotine-infused rats by blocking nicotinic receptors with the competitive antagonist dihydro-β-erythroidine (DHBE; Epping-Jordan et al., 1998; Malin et al., 1998a,b) or by inactivating them with noncompetitive antagonists mecamylamine (Malin et al., 1994), hexamethonium (Malin et al., 1997) or chlorisondamine (Hildebrand et al., 1997). Precipitated abstinence can be convenient for certain research purposes, as a large number of abstinence signs are concentrated in a short time frame (around 30 min). In fact, a majority of experiments using the continuous infusion model of dependence have involved some sort of precipitated abstinence.

Whether spontaneous or precipitated abstinence is employed, there are many practical advantages of the continuous infusion model of nicotine dependence in the rat. The method is simple and rapid, involving no major surgery, no complicated equipment and no prolonged training of the subject. It is thus suited for massively repeated use in dissecting the mechanisms of nicotine dependence or screening potential therapies for smoking cessation. The model has also proved to be reliable in that it has produced reasonably consistent results in a series of experiments within the author's laboratory (Malin et al., 1993b, 1994, 1996a,b, 1998a,b, 2001) and it has been reproducible in other laboratories. A number of independent research groups have now confirmed the basic phenomena of the continuous infusion model (Malin et al., 1992, 1994): elevated behavioral abstinence signs following the termination of nicotine infusion or the administration of a nicotinic antagonist to nicotine-infused rats (Carboni et al., 2000; Epping-Jordan et al., 1998; Hildebrand et al., 1997, 1998; Watkins et al., 2000).

Of course, there remain many issues of external validity, that is, relevance to the clinical situation experienced by actual smokers. As with most laboratory models of clinical phenomena, there is limited "face validity,"—many obvious differences between laboratory procedures and real-world practices. For reasons of practicality, the period of nicotine exposure is shortened to 1–2 weeks, compared with years of smoking. To compensate for this, the rate of nicotine exposure is quite high. At the 1.05-mg/kg/day rate (expressed as the base), the amount of nicotine administered on a per weight basis is comparable to the amount ingested by a 70-kg smoker smoking three packs per day of average-yield cigarettes. At the 3.15-mg/kg/day rate, the amount administered is comparable to that ingested by the heaviest (five packs per day) smoker of high-yield cigarettes (Armitage et al., 1975). However, in comparing human and rat doses, one must consider the generally much higher metabolic and drug clearance rates in the rat, as well as the much shorter duration of exposure. Perhaps, blood nicotine concentration provides a more meaningful basis of comparison. A venous nicotine concentration of 40 ng/ml was recently measured in rats infused with 3.15-mg/kg/day nicotine for 7 days (LeSage et al., in press). This is virtually identical to the concentration steadily maintained during waking hours by human subjects who smoked 30 high-yield cigarettes per day (Benowitz et al., 1982). The amount of nicotine infused did not appear to debilitate the rats during the infusion period in terms of locomotor activity, open-field behavioral observations, weight gain, food consumption (Malin et al., 1992) and operant performance for food reward (LeSage et al., in press). Another difference is the continuous drug administration in the laboratory model, as opposed to intermittent administration in smoking. However, it is well established that many heavy smokers "titrate" their smoking consumption to maintain relatively stable levels of serum nicotine during waking hours. Thus, a more important distinction might involve drug cessation during sleep in even the steadiest smoker. Other differences involve the route of drug administration and the presence of nonnicotine ingredients in cigarette smoke. In view of these extensive differences, any phenomenon discovered through the use of the laboratory model cannot be assumed to occur in human smokers. However, it would at least be reasonable to look for that phenomenon in the human clinical situation. The preclinical usefulness of the model will ultimately depend on its cross-species predictive validity—upon the number of phenomena found to occur in both the clinical and laboratory situation. For example, as described below, the clinically observed abstinence-alleviating effects of nicotine replacement, bupropion and acetyl-L-carnitine have also been observed in the laboratory model. In fact, the model has already predicted one nonobvious phenomenon that was later observed in human smokers: the induction of an abstinence syndrome by injection of the opiate antagonist naloxone in smokers but not in nonsmokers (Krishnan-Sarin et al., 1999).

4. Extending and varying the model: additional abstinence measures and another species

A number of research groups have discovered additional behavioral correlates of nicotine abstinence following continuous subcutaneous nicotine infusion in the rat. Helton et al. (1993) observed an increased startle response, sug-

gestive of heightened anxiety. It would be of interest in the future to determine whether nicotine abstinence similarly affects other measures of anxiety such as elevated plus maze and open-field behavior. Epping-Jordan et al. (1998) measured higher electrical thresholds for intracranial self-stimulation (ICSS), suggesting hypoactivity of brain reward pathways and thus a depression-like state. This phenomenon resembled those seen during abstinence from other habit-forming drugs. In fact, this motivational impairment could be precipitated by a lower dose of the nicotinic competitive antagonist DHBE than the somatic behavioral abstinence signs. Thus, the ICSS effect may be a particularly sensitive indicator of mild nicotine abstinence.

Suzuki et al. (1996) determined whether nicotine abstinence following continuous subcutaneous infusion was aversive enough to cause avoidance behavior. Using the conditioned place preference paradigm, they found that rats would avoid a chamber associated with mecamylamine-precipitated nicotine abstinence. The extent of this effect varied with the genetic strain of rat (Suzuki et al., 1999). Taken together, the above results suggest that nicotine abstinence in the rat continuous infusion model results not only in abnormal behaviors but also in major negative emotional and motivational changes.

There have been recent reports of somatic behavioral abstinence signs in mice following a 14-day series of nicotine injections (Isola et al., 1999) and continuous subcutaneous nicotine infusion (Damaj, 2000). This raises the future possibility of using knockout or other genetically engineered mouse strains to dissect the neurobiological mechanisms of nicotine dependence.

5. Mechanisms of dependence: central vs. peripheral systems

The introduction of a laboratory model of nicotine dependence made it possible to address basic questions of underlying biological mechanisms. For example, are nicotine dependence and abstinence mediated by the central nervous system? The nicotinic antagonist hexamethonium, which does not readily cross the blood–brain barrier, precipitated a nicotine abstinence syndrome with extraordinary potency through third ventricle administration in nicotine-infused but not saline-infused rats. By peripheral administration, it had no selective effect on nicotine-infused rats. This strongly suggests a central site of action. On the other hand, peripherally administered chlorisondamine, which also does not readily cross the blood–brain barrier, has been shown to precipitate somatic nicotine abstinence signs selectively in nicotine-infused rats (Hildebrand et al., 1997; Watkins et al., 2000). However, Watkins et al. (2000) also precipitated abstinence signs by intracerebroventricular (lateral ventricle) infusion of chlorisondamine. It required a far higher dose to precipitate an abstinence syndrome by the peripheral route than by central administration. This would not be the case if the site of action were only peripheral. Also, only intracerebroventricular, not peripheral, injection produced a significant elevation of ICSS thresholds. Taken as a whole, the data clearly suggest a major central nervous system component in nicotine dependence and abstinence syndrome, while the existence of an additional peripheral component remains a marked possibility.

6. Mechanisms of dependence: dopaminergic

Much evidence suggests that nicotine-induced dopamine release in the mesolimbic reinforcement pathway is essential to nicotine reinforcement (Balfour et al., 2000; Corrigall and Coen, 1991). Might it also contribute to inducing a state of nicotine dependence? Might subsequent reduction of dopamine release during nicotine withdrawal contribute to certain features of nicotine abstinence, such as increased self-stimulation thresholds (Epping-Jordan et al., 1998)?

Fung et al. (1996) observed a reduction in nucleus accumbens and striatum dopamine content 24 h after terminating nicotine infusion. This corresponded to a reduction in locomotor activity. Rasmussen and Czachura (1995) detected changes in the firing rate of midbrain dopaminergic neurons as a function of nicotine infusion and its termination. Hildebrand et al. (1998) found reduced nucleus accumbens dopamine release during mecamylamine-precipitated abstinence. Dopamine release in prefrontal cortex was not affected. Carboni et al. (2000) also reported reduced dopamine release during mecamylamine-precipitated abstinence. However, in this case, a significant increase in prefrontal cortex dopamine release was also detected.

There are numerous nicotinic receptors at several levels of the mesolimbic pathway, notably the ventral tegmental area and the nucleus accumbens. It seems likely that reduced nicotinic stimulation at the tegmental level indirectly triggers the changes in the nucleus accumbens. Reduced nucleus accumbens dopamine outflow and some behavioral abstinence signs can be precipitated by local injection of mecamylamine in the ventral tegmentum of nicotine-dependent rats (Hildebrand et al., 1999) but not in the nucleus accumbens (Hildebrand and Svensson, 2000).

Nicotine abstinence precipitated by systemic mecamylamine resulted in reduced dopamine release in a second limbic region, the central nucleus of the amygdala. Concomitantly, this region was activated in an anatomically selective manner as determined by c-*fos* induction (Panagis et al., 2000). This observation may help account for the symptom of anxiety in smoking cessation, since the central nucleus of the amygdala is prominently associated with anxiety and stress responses that can be inhibited by dopaminergic activity (Glavin, 1992). In summary, many changes in dopaminergic stimulation occur as correlates of nicotine dependence and abstinence. These changes might help to explain certain behavioral phenomena, such as the depression often observed during smoking cessation and the

reduced locomotor activity observed during nicotine withdrawal in the rat (Malin et al., 1996a,b).

7. Mechanisms of dependence: opiate

Nicotinic receptor stimulation induces release of endogenous opioid peptides (Gilbert et al., 1992; Hexum and Russett, 1987; Jensen et al., 1990; Pomerleau et al., 1983; Rosecrans et al., 1985; Suh et al., 1995). It might be hypothesized that prolonged nicotine exposure would result in prolonged overstimulation of opiate receptors, which would, in turn, result in an opiate dependence-like state. The sudden cessation of nicotine exposure would then result in a sudden reduction of opiate receptor stimulation, leading to an opiate abstinence-like condition. A number of studies employing the continuous subcutaneous infusion model of nicotine dependence (Malin et al., 1992, 1994) have produced results consistent with this hypothesis.

Nicotine abstinence syndrome in the rat involves many of the same behavioral signs as morphine abstinence syndrome (Malin et al., 1988, 1992, 1993a,b, 1996a,b). Jumping is a major opiate abstinence sign in the mouse but not in the rat, so it is interesting that it was also a major nicotine abstinence sign in the mouse (Isola et al., 1999) but not in the rat (Malin et al., 1992). Nicotine abstinence signs are potently morphine reversible, and they can be precipitated by subcutaneous injection of the opiate antagonist naloxone (Adams and Cicero, 1998; Carboni et al., 2000; Malin et al., 1993b), although a fourth research group was unable to detect the latter effect (Watkins et al., 2000). In a human study, naloxone induction of an abstinence syndrome has been observed in chronic smokers but not in nonsmokers (Krishnan-Sarin et al., 1999). Naloxone also prevents nicotine alleviation of nicotine abstinence in the rat (Malin et al., 1996a,b), suggesting that nicotine relieves abstinence syndrome in large part through indirect activation of endogenous opioid pathways. Additionally, nicotine abstinence syndrome was precipitated by injection of a systemically active analog of neuropeptide FF (NPFF) (Malin et al., 1996b). NPFF has antiopiate effects and has been shown to contribute to opiate tolerance and dependence (Lake et al., 1991; Malin et al., 1990a,b, 1993a, 1995).

Another contributor to opiate dependence is the activation of nitric oxide synthase (NOS) resulting from chronic opiate exposure (Leza et al., 1996). NOS inhibitors have attenuated the expression of morphine abstinence signs (Adams et al., 1993; Cappendijk et al., 1993; Kimes et al., 1993; Kolesnikov et al., 1993; Vaupel et al., 1995). In a similar fashion, the NOS inhibitor L-NNA significantly attenuated both spontaneous and mecamylamine-precipitated nicotine abstinence signs in the rat (Malin et al., 1998a,b). A different NOS inhibitor, L-NAME, likewise attenuated both mecamylamine- and naloxone-precipitated abstinence syndromes (Adams and Cicero, 1998). In summary, a considerable pattern of evidence is consistent with the hypothesis that some common mechanisms underlie opiate and nicotine dependence.

8. A dual-factor hypothesis of nicotine abstinence syndrome

Carboni et al. (2000) found that naloxone, like mecamylamine, precipitated somatic abstinence signs in nicotine-infused rats. Unlike mecamylamine, it did not induce a reduction in nucleus accumbens dopamine release. Dopaminergic activity in the nucleus accumbens plays a well-known role in reinforcement and maintaining high rates of activity. Thus, the data of Carboni et al. suggest the possibility that some depression-like aspects of nicotine abstinence, such as reduced locomotor activity and hypoactive reward pathways, may be dissociated from the actions of nicotine on endogenous opioid peptides and from somatic behavioral signs. However, the involvement of opiate mechanisms in the somatic behavioral signs suggests the possibility that these mechanisms may help mediate other dimensions of nicotine abstinence syndrome, perhaps those involving irritability and anxiety. In this connection, it is of great interest that naloxone administered to nicotine-infused rats results in an aversive state as determined by the conditioned place preference procedure (Ise et al., 2000; Watkins et al., 2000), while morphine and a δ-opiate receptor agonist potently attenuate the aversiveness of mecamylamine-precipitated nicotine abstinence (Ise et al., 2000). It is precisely the *aversiveness* of nicotine abstinence syndrome that confers on nicotine its ability to serve as a negative reinforcer (a reinforcer that functions by removing an aversive stimulus). Thus, the opiate component of nicotine abstinence syndrome may play a particularly critical role in maintaining smoking behavior under conditions of nicotine deprivation, such as attempted smoking cessation. These data might also suggest a hypothesis for future research concerning marked individual differences in behavioral responses to smoking cessation. Differences in abstinence symptoms such as depressive reactions as opposed to irritable, anxious reactions might conceivably derive from individual differences in the relative intensity of dopaminergic and opioid components of the nicotine abstinence process.

9. Mechanisms of dependence: cholinergic and serotonergic

Not surprisingly, understimulation of the nicotinic category of acetylcholine receptors seems to trigger nicotine abstinence syndrome in the nicotine-infused rat. In contrast to a variety of nicotinic antagonists, the muscarinic antagonist scopolamine failed to precipitate an abstinence syndrome (Malin et al., 1994). It would be of great interest to learn whether certain subtypes of nicotinic receptors play

a selective role in the induction of nicotine dependence and the expression of nicotine abstinence syndrome. This might ultimately permit the refinement of nicotine replacement therapy. Of course, such studies are currently somewhat limited by the availability of specific agonists and antagonists for the great variety of receptor subtypes. In one of the first studies of this sort, selective antagonism of α-7 receptors in the ventral tegmentum resulted in behavioral abstinence signs as well as decreased dopamine release in the nucleus accumbens (Nomikos et al., 1999).

It has been suggested that withdrawal from various dependence-inducing drugs, including nicotine, may result in activation of serotonergic neurons in the nucleus of Raphe, which project to the amygdala, resulting in overstimulation of amygdala $5HT_3$ receptors. This would hypothetically lead to heightened anxiety (Costall et al., 1990). Consistent with this hypothesis, the $5HT_3$ antagonist odansetron reduced the effect of withdrawal from a series of nicotine injections in the mouse on a light/dark preference test of anxiety (Costall et al., 1990). The same drug dose-dependently reduced the aversiveness of mecamylamine-precipitated nicotine abstinence in nicotine-infused rats (Suzuki et al., 1997). As with various subtypes of nicotinic cholinergic receptors, the role of numerous other serotonin receptor subtypes in nicotine dependence remains to be explored.

10. Screening for potential therapies

The nicotine infusion model of nicotine dependence in the rat (Malin et al., 1992, 1994) was designed to be rapid, simple and inexpensive so that it would be suitable for preliminary screening of numerous compounds as potential treatments for smoking cessation. So far, the model has proved sensitive to many types of abstinence-alleviating interventions ranging from nicotine replacement (Malin et al., 1992) to NOS inhibitors (Adams and Cicero, 1998; Malin et al., 1998a,b) to serotonergic compounds (Suzuki et al., 1997). Preliminary data from our laboratory strongly suggest that bupropion, a drug used clinically for smoking cessation, potently reduces abstinence signs in this model. Abstinence signs were also significantly reduced by acetyl-L-carnitine, an endogenous polyamine with cholinergic activity and reported clinical efficacy in smoking cessation (Persico et al., 1995).

Recently, the model has been used to evaluate the effects of nicotine immunization based on the immunogen *trans*-3′-aminomethyl nicotine conjugated to recombinant *Pseudomonas aeruginosa* exoprotein A (NicVax). Both active and passive immunization resulted in reduced free serum nicotine and reduced nicotine entry into brain following a nicotine injection. Passive immunization also interfered with some immediate locomotor and cardiovascular actions of nicotine (Pentel et al., 2000). Passive immunization prevented nicotine from relieving the spontaneous nicotine abstinence syndrome in the rat (Malin et al., 2001). This raises a possibility that an immunized smoker who relapses and smokes might fail to experience the expected relief from the discomforts and craving of smoking cessation. This might hypothetically aid the extinction of the smoking habit.

11. Directions for future research

Rodent laboratory models of nicotine dependence have only been in widespread use for a few years. Nevertheless, they have already exposed some basic outlines of the neurobiological processes leading to nicotine dependence and abstinence. Prolonged overstimulation followed by reduced stimulation of brain nicotinic receptors leads to changes in downstream neurotransmitter/receptor systems, including endogenous opioid systems and mesolimbic dopamine pathways. These various downstream alterations then lead to a corresponding spectrum of behavioral, emotional and motivational changes. This picture is, of course, highly incomplete. For example, there are very little published data on the differential role of acetylcholine, dopamine and opiate receptor subtypes. Also, few other transmitter/receptor systems have been explored, despite the fact that neurons with nicotinic receptors interact with a broad array of other transmitter and hormonal systems (Pomerleau et al., 1983). For example, one obvious candidate for exploration would be glutamic acid and its corresponding receptor subtypes, notably the NMDA receptors. NMDA receptors are prominently involved in dependence on other drugs (Trujillo, 2000; Tsuda et al., 1999). They are well situated to participate in nicotine dependence, since they appear to mediate the effect of ventral tegmental nicotinic receptor stimulation on nucleus accumbens dopamine release (Schilstrom et al., 1998).

The changes in transmitter function caused by chronic nicotine exposure probably reflect underlying changes in gene expression. There is a need to explore for such changes induced by the same parameters of nicotine exposure and withdrawal that are known to result in a behavioral abstinence syndrome. Microarray technology could be used in a wide-ranging search for the relevant genes.

Additional research is needed on the dependence model itself. For example, little is known about the effect of the duration of nicotine infusion on the induction of physical dependence. It might be possible to modify the model to increase its external validity — its relevance to actual smokers. For example, infusion could be interrupted during periods of sleep, and subjects could be infused at lower rates over longer time periods. Data are also needed on gender differences in the induction of dependence by nicotine infusion.

In recent years, rodent self-administration models have revealed a great deal about nicotine as a positive reinforcer (Balfour et al., 2000; Corrigall, 1999). It is now

desirable to investigate the relationship between self-administration processes on one hand and dependence and abstinence processes on the other hand. Specifically, does nicotine abstinence increase the incentive value of nicotine reinforcement (as suggested by the enormous number of relapses during the first few days of smoking cessation)? This might be best addressed through progressive ratio studies of nicotine self-administration in rats undergoing nicotine abstinence syndrome. Another approach would be to determine whether nicotine abstinence intensified conditioned place preference for nicotine-associated environments.

While current therapies for smoking cessation have proven helpful, none can claim a very high rate of long-term success. Therefore, there remains a great need to better understand the interacting behavioral and biological factors in physical and psychological nicotine dependence. It is hoped that laboratory models of dependence can help to identify those contributing factors that can serve as targets for future additional therapies.

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