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Neurobiology of the nicotine withdrawal syndrome

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Abstract

The aversive aspects of withdrawal from chronic nicotine exposure are thought to be an important motivational factor contributing to the maintenance of the tobacco habit in human smokers. Much emphasis has been placed on delineating the underlying neurobiological mechanisms mediating different components of the nicotine withdrawal syndrome. Recent studies have shown that both central and peripheral populations of nicotinic acetylcholine receptors (nAChRs) are involved in mediating somatic signs of nicotine withdrawal as measured by the rodent nicotine abstinence scale. However, only central populations of nAChRs are involved in mediating affective aspects of nicotine withdrawal, as measured by elevations in brain-stimulation reward thresholds and conditioned place aversion. Nicotine interacts with several neurotransmitter systems, including acetylcholine, dopamine, opioid peptides, serotonin, and glutamate systems. Evidence so far suggests that these neurotransmitters play a role in nicotine dependence and withdrawal processes. The available evidence also suggests that different underlying neurochemical deficits mediate somatic and affective components of nicotine withdrawal. The aim of the present review is to discuss preclinical findings concerning the neuroanatomical and neurochemical substrates involved in these different aspects of nicotine withdrawal.

Keywords: Nicotine; Drug withdrawal; Conditioned place aversion; Intracranial self-stimulation; Reward; Somatic signs; Depression; Acetylcholine; Dopamine; Opioid; Serotonin; Glutamate

1. Introduction

The burden of disease and negative economic impact of tobacco addiction on society is considerable. It has been projected that by 2020 tobacco will become the largest single health problem worldwide, causing approximately 8.4 million deaths annually (Murray and Lopez, 1997). Furthermore, the World Bank estimates that in high-income countries, smoking-related healthcare accounts for 6–15% of all annual healthcare costs. In fact, approximately £1.5 billion was spent between 1996–1997 on the care and treatment of patients suffering from tobacco-related diseases in England alone (Parrott et al., 1998). Therefore, there is much incentive to develop interventions designed to reduce and prevent tobacco use. To achieve this goal, it is necessary to understand the mechanisms by which tobacco addiction occurs. Evidence suggests that nicotine, which acts at neuronal nicotinic acetylcholine receptors (nAChRs), is one of the active components in tobacco smoke responsible for tobacco addiction (see Stolerman and Jarvis, 1995; Crooks and Dwoskin, 1997). Thus, there is considerable interest in elucidating the neurobiological mechanisms that give rise to nicotine dependence and withdrawal symptoms, processes thought to be crucial in the development and maintenance of the tobacco habit (Watkins et al., 2000a). The aim of the present review is to briefly describe the aversive behavioral consequences associated with nicotine withdrawal and discuss preclinical findings concerning the underlying neurobiology of this phenomenon.

2. Neuronal nAChRs

Nicotinic receptors are expressed on mature skeletal muscle, in autonomic ganglia and within the central nervous system (CNS) (Holladay et al., 1997). Not surprisingly, most interest in the behavioral actions of nicotine has focused on the role of nAChRs located within the CNS. Neuronal nAChRs are members of the superfamily of ligand-gated ion channels that include γ-aminobutyric acid$_A$ ($GABA_A$), $GABA_C$, glycine, and serotonin$_3$ (5-HT_3) receptors that are

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derived from a number of closely related genes. nAChRs, like other members of the superfamily of ligand-gated ion channels, are composed of five membrane spanning subunits that combine to form a functional receptor (Albuquerque et al., 1997; Dani, 2001; Lindstrom et al., 1996; Lena and Changeux, 1998; Role and Berg, 1996). Individual neuronal nAChR subunits arrange in different combinations to form individual nAChRs with distinct pharmacological and kinetic properties. The neuronal α subunit exists in nine isoforms ($\alpha2-\alpha10$) whereas the neuronal β subunit exists in three isoforms ($\beta2-\beta4$) (Elgoyhen et al., 2001; Arneric et al., 1995; Wonnacott, 1997). Oocyte expression systems injected with pairwise combinations of different neuronal α and β subunits have provided evidence that these subunits combine with a stoichiometry of $2\alpha:3\beta$ to produce a functional neuronal nicotinic hetero-oligomeric receptor (Deneris et al., 1991; Conroy and Berg, 1995; Colquhoun and Patrick, 1997). In contrast, $\alpha7$, $\alpha8$, and $\alpha9$ subunits form homo-oligomeric complexes composed of five α subunits and lacking β subunits (Chen et al., 1998), with only the $\alpha7$ pentamer being expressed in the CNS.

Based on radioligand binding studies, neuronal nAChRs have been broadly divided into three classes within the rat brain: those with a high affinity binding site for [^{3}H]-nicotine which correspond to $\alpha4$-containing nAChRs, the $\alpha4\beta2$ combination being the most abundant (Flores et al., 1992; Picciotto et al., 1995), those with high affinity for [^{125}I]-α-bungarotoxin which correspond to $\alpha7$ nAChRs (Clarke, 1992), and those with high affinity for neuronal bungarotoxin which correspond to $\alpha3$-containing nAChRs (Schulz et al., 1991). It should be noted that the precise combinations of nAChR subunits that constitute active nAChRs within the CNS *in vivo* are unknown and have so far only been inferred by their pharmacological profile (Kaiser et al., 1998; Luo et al., 1998; Sershen et al., 1997; Sharples et al., 2000). However, with the advent of more sophisticated tools it is becoming possible to identify the nAChR subunits expressed by individual neurons within specific brain regions (Sheffield et al., 2000; Lena and Changeux, 1999; Sudweeks and Yakel, 2000). For example, one brain region that is of particular interest in relation to the rewarding actions of acute nicotine and in mediating nicotine dependence and withdrawal is the ventral tegmental area (VTA), a midbrain dopaminergic nucleus. A recent study by Klink et al. (2001) attempted to characterize nAChRs located within the VTA by means of single-cell reverse transcription polymerase chain reaction (RT-PCR) and patch-clamp recordings. Based on these findings, it was proposed that most dopamine neurons in the VTA express putative $\alpha4\alpha6\alpha5(\beta2)_2$ and $\alpha4\alpha5(\beta2)_2$ nAChR subtypes, whereas approximately 50% express the $\alpha7$ homomeric receptor (Klink et al., 2001). Furthermore, GABA neurons in the VTA appear to express the $(\alpha4)_2(\beta2)_3$ subtype (Klink et al., 2001). Dopamine neurons in the VTA are heterogenous in terms of their projections to different terminal fields and have fundamentally different physiological characteristics (e.g., Takahata and Moghaddam, 2000). It is possible that the complex expression patterns of nAChRs within the VTA may represent differential expression of nAChR subtypes on different populations of dopamine neurons, which may contribute to the heterogeneity of these neurons. Such investigations offer the intriguing possibility that different nAChR subtypes involved in various aspects of nicotine reward, dependence, and withdrawal processes may eventually be identified and targeted.

The function of nAChRs within the brain also has been investigated. Nicotinic receptors within the CNS are situated mainly on presynaptic terminals (Wonnacott, 1997) but also are found at somatodendritic, axonal, and postsynaptic locations (for review see Sargent, 1993). It has been proposed that the exclusive or predominant role of nAChRs in the CNS is the modulation of neurotransmitter release (Wonnacott, 1997). Accordingly, by an action at nAChRs, nicotine has been shown to stimulate the release of most neurotransmitters in regions throughout the brain (Araujo et al., 1988; Toide and Arima, 1989; McGehee et al., 1995; McGehee and Role, 1995; Gray et al., 1996; Role and Berg, 1996; Wilkie et al., 1996; Albuquerque et al., 1997; Alkondon et al., 1997; Kenny et al., 2000a; Grady et al., 2001). Therefore, as discussed in detail below, it is likely that various neurotransmitter systems are involved in the adaptations that occur in response to chronic nicotine exposure that give rise to dependence and withdrawal responses.

3. The nicotine withdrawal syndrome in humans

There are over 4000 chemicals in cigarette smoke, many of which potentially contribute to the reinforcing properties of tobacco. However, in light of the myriad preclinical studies demonstrating nicotine's reinforcing properties across many species (Goldberg et al., 1981; Risner and Goldberg, 1983; Henningfield and Goldberg, 1983; Fudala et al., 1985; Goldberg and Henningfield, 1988; Corrigall and Coen, 1991; Huston-Lyons and Kornetsky, 1992; Donny et al., 1995, 1999, 2000; Corrigall, 1999; Watkins et al., 1999; Irvine et al., 2001; Markou and Paterson, in press), it generally has been accepted that nicotine is a major component in tobacco smoke responsible for addiction (see Stolerman and Jarvis, 1995). Therefore, the present review will focus on the role of nicotine in tobacco dependence processes. Nevertheless, before proceeding with a discussion of nicotine dependence and withdrawal, it is important to point out that obtaining nicotine is probably not the exclusive reason for maintaining the tobacco habit in smokers. For example, nicotine-containing and denicotinized cigarettes had similar measures of reinforcing efficacy in smokers when presented alone, although there was a preference for nicotine-containing cigarettes when smokers were offered a choice (Shahan et al., 1999). This observation suggests that conditioned stimuli associated with smoking may contribute to the

reinforcing properties of tobacco (Paterson and Markou, 2001; Donny et al., 1999; Shahan et al., 1999). Furthermore, it is possible that of the many constituents of cigarette smoke, there are other chemicals with reinforcing properties. For example, nornicotine, which is present in cigarette smoke (Crooks and Dwoskin, 1997; Jacob et al., 1999), is self-administered by rats (Bardo et al., 1999). Taken together, these observations suggest that in addition to nicotine, sensory and conditioned reinforcing effects of smoking and possibly other reinforcing ingredients also play a role in maintenance of the tobacco habit in smokers.

Smoking cessation is known to produce an aversive withdrawal syndrome in humans (Hughes et al., 1991; Shiffman and Jarvik, 1976), components of which may be manifest for between 1–10 weeks (Hughes, 1992). This syndrome arises, at least in part, because of the reduction in nicotine intake in nicotine-dependent individuals. Accordingly, nicotine replacement therapy, of which sublingual nicotine tablets (Molander et al., 2000), nicotine gum (Schneider et al., 1984), and nicotine patch (Rose et al., 2001; Fagerstrom et al., 1993) are three examples, has been shown to reduce the occurrence of withdrawal symptoms in abstinent smokers. Conversely, reduction of the nicotine content in smoked tobacco induced a withdrawal syndrome in smokers that was accompanied by a significant reduction in plasma nicotine levels (West et al., 1984). The aversive aspects of the nicotine withdrawal syndrome are thought to be powerful motivational factors contributing to the main-

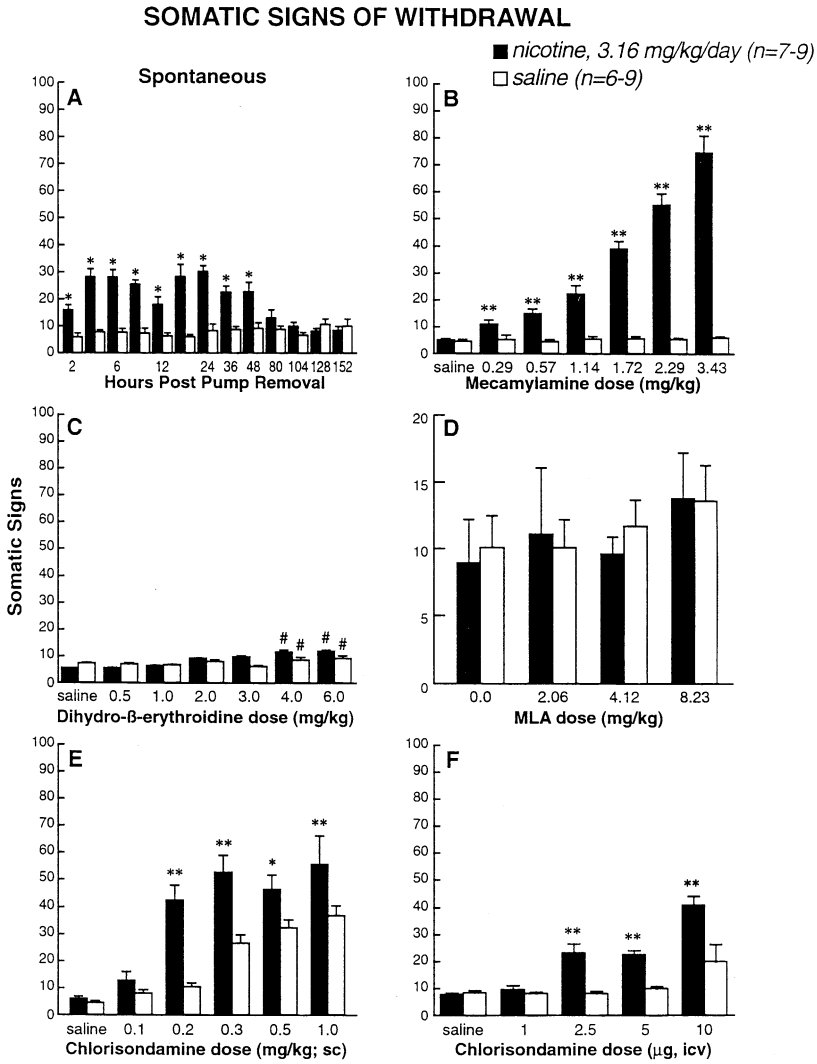

Fig. 1. During nicotine withdrawal rats exhibit increased number of somatic signs of withdrawal. (A) Somatic withdrawal signs in rats undergoing spontaneous nicotine withdrawal after removal of osmotic minipump delivering nicotine (3.16 mg/kg/day free base, 7 days) and in vehicle-treated control rats. (B) Effect of mecamylamine (sc) on somatic signs in nicotine- and vehicle-treated rats. (C) Effect of dihydro-β-erythroidine (DHβE, sc) on somatic signs in nicotine- and vehicle-treated rats. (D) Effect of methyllycaconitine (MLA) (sc) on somatic signs in nicotine- and vehicle-treated rats. (E) Effect of chlorisondamine (sc) on somatic signs in nicotine- and vehicle-treated rats. (F) Effect of chlorisondamine (icv) on somatic signs in nicotine- and vehicle-treated rats. Asterisks indicate statistically significant differences between nicotine- and saline-treated rats ($*P<.05$, $**P<.01$). Hash symbols indicate statistically significant difference in overall somatic withdrawal signs compared to 0.0 mg/kg DHβE ($^{\#}P<.05$). All data are expressed as mean (±S.E.M.) somatic signs of withdrawal at each time point or antagonist dose. Reproduced with permission from Epping-Jordan et al. (1998) and Watkins et al. (2000b).

tenance of the tobacco habit. Indeed, withdrawal duration and severity have been shown to predict smoking relapse (Piasecki et al., 1998, 2000). Further, the efficacy of nicotine replacement therapy in smoking cessation trials, at least in certain individuals (Fagerstrom, 1988; Fagerstrom et al., 1992; Sachs and Leischow, 1991), is related to its ability to prevent the onset and reduce the duration of nicotine withdrawal. Ironically, it appears that tobacco companies were amongst the first to recognize the importance of withdrawal in maintaining smoking behavior: *"Why do people smoke…to relax; for the taste; to fill the time; something to do with my hands… But, for the most part, people continue to smoke because they find it too uncomfortable to quit"* (Philip Morris, 1984).

This nicotine withdrawal syndrome is comprised of 'physical' or somatic, and affective components. The most common somatic symptoms include bradycardia, gastrointestinal discomfort, and increased appetite. Affective symptoms primarily include craving, depressed mood, dysphoria, anxiety, irritability, and difficulty concentrating (American Psychiatric Association, 1994; Hughes et al., 1991; West et al., 1991; Glassman et al., 1990; Parrott, 1993). Recently, attempts have been made to develop animal models of the somatic and affective aspects of nicotine withdrawal in order to investigate the underlying neurobiological substrates involved in these processes.

4. Somatic symptoms of nicotine withdrawal in rats

A somatic nicotine withdrawal syndrome analogous to that observed in humans also has been observed in rodents (Fig. 1). Malin and colleagues first identified and characterized this somatic nicotine withdrawal syndrome in rats (described in detail by David Malin in this issue), an observation that has since been replicated by other groups (e.g., Hildebrand et al., 1997; Epping-Jordan et al., 1998; Carboni et al., 2000). Most recently, a similar somatic nicotine withdrawal syndrome has been observed also in mice (Isola et al., 1999). The most prominent signs of this rodent withdrawal syndrome include abdominal constrictions (writhes), facial fasciculation, eyeblinks, ptosis, and gasps along with miscellaneous other signs including escape attempts, foot licks, genital grooming, shakes, scratches, and yawns (Watkins et al., 2000b; Malin et al., 1998; Hildebrand et al., 1997).

Several lines of evidence support the notion that the somatic signs observed in rats after cessation of a period of chronic nicotine exposure constitutes a nicotine withdrawal syndrome. First, rats chronically treated and then withdrawn from nicotine administration display more somatic signs than when these same subjects were nicotine naïve, just prior to the termination of nicotine administration, after the recovery from withdrawal or compared to saline-treated control rats (Malin et al., 1992). Second, the severity of the withdrawal signs was proportional to the amount of prior nicotine exposure, i.e., animals exposed to higher concentrations of nicotine demonstrated more somatic signs when compared to animals treated with lower nicotine concentrations (Malin et al., 1992). Interestingly, the amount of nicotine consumed in the form of tobacco does not appear to be an accurate predictor of withdrawal severity in human smokers (Hughes et al., 1990). Third, nicotine reverses withdrawal signs in rats undergoing nicotine withdrawal, thereby demonstrating that tonic activation of nAChRs is crucial in preventing the onset of these somatic symptoms (Malin et al., 1992). This conclusion is further supported by the fact that various nAChR antagonists (see Fig. 1 and Table 1), such as chlorisondamine (Watkins et al., 2000b; Hildebrand et al., 1997), mecamylamine (Malin et al., 1994; Hildebrand et al., 1997; Watkins et al., 2000b), and hexamethonium (Malin et al., 1997) also precipitated withdrawal signs in nicotine-dependent rats. It should be noted, however, that in the case of human cigarette smokers administration of mecamylamine did not precipitate a withdrawal syndrome (Eissenberg et al., 1996; Stolerman, 1986; Nemeth-Coslett et al., 1986; Rose et al., 2001). Indeed, even a relatively high dose of mecamylamine (10 mg) did not precipitate withdrawal signs in smokers (Rose et al., 2001). It is likely that this dose of mecamylamine is sufficient to block nAChRs because similar doses (5–20 mg) were sufficient to block the subjective effects of nicotine after intravenous administration in cigarette smokers (Lundahl et al., 2000). One possible explanation for this discrepancy is that withdrawal signs were abated by the compensatory increase in tobacco consumption in smokers that was observed after mecamylamine administration (Eissenberg et al., 1996; Stolerman, 1986; Nemeth-Coslett et al., 1986; Rose et al., 2001). However, it

Table 1
Minimal dose (mg/kg) of nicotinic and opioid receptor antagonists that precipitated statistically significant increases in somatic abstinence signs, elevated brain reward thresholds or induced conditioned place aversions in nicotine-dependent rats compared to saline-treated controls

Somatic signs	Reward thresholds	Conditioned place aversion
Mecamylamine (0.29 mg/kg)	Mecamylamine (0.57 mg/kg)	Dihydro-β-erythroidine (10 mg/kg)
Chlorisondamine (0.2 mg/kg sc)	Dihydro-β-erythroidine (2 mg/kg)	Naloxone (0.12 mg/kg)
Chlorisondamine (2.5 μg icv)	Chlorisondamine (5 μg icv)	Mecamylamine[a]
Naloxone[a]	Naloxone[a]	
Dihydro-β-erythroidine[a]	Chlorisondamine[a] (sc)	
methyllycaconitine[a]	methyllycaconitine[a]	

[a] Indicates that these receptor antagonists either had no effect or did not induce differential effects on somatic signs of withdrawal, reward threshold elevations, or conditioned place aversions at any dose tested (data taken from Epping-Jordan et al., 1998; Watkins et al., 2000b; Markou and Paterson, in press).

is possible that administration of a higher dose of mecamylamine which would block an even greater number of nAChRs may precipitate withdrawal signs in nicotine-dependent smokers.

5. Affective symptoms of nicotine withdrawal in rats

Although the somatic components of withdrawal from drugs of abuse are certainly unpleasant, it has been hypothesized that avoidance of the affective components of withdrawal plays a more important role in the maintenance of dependence to drugs of abuse, including nicotine, than the somatic aspects of withdrawal (Koob et al., 1993; Markou et al., 1998; Watkins et al., 2000a). This hypothesis has generated interest in identifying behavioral procedures that can model affective aspects of nicotine withdrawal. One of the main affective symptoms associated with withdrawal from drugs of abuse is a 'diminished interest or pleasure' in rewarding stimuli (American Psychiatric Association, 1994; Covey et al., 1998). Interestingly, 'diminished interest or pleasure' in rewarding stimuli (i.e., 'anhedonia') is also one of the core symptoms of depression (American Psychiatric Association, 1994; Markou et al., 1998). Brain-stimulation reward threshold elevations in rats have been proposed as an operational measure of this core symptom of depression and drug withdrawal. Indeed, withdrawal from drugs of abuse such as cocaine, amphetamine, opiates, and alcohol (Markou and Koob, 1991; Baldo et al., 1999; Paterson et al., 2000; Wise and Munn, 1995; Kokkinidis et al., 1986; Schulteis et al., 1994, 1995; Lin et al., 1999) all have been shown to significantly elevate brain-stimulation reward thresholds, reflecting diminished interest in the rewarding electrical stimuli. In light of the numerous observations that smoking cessation precipitates depressive symptoms, even in individuals without a prior history of depression (Bock et al., 1996; Stage et al., 1996; Borrelli et al., 1996; Covey et al., 1997; Covey et al., 1998), the effect of nicotine withdrawal on brain-stimulation reward thresholds in rats was investigated. It was found that spontaneous withdrawal (Epping-Jordan et al., 1998; Harrison et al., 2001) or systemic administration of nAChR antagonists such as DHβE (Epping-Jordan et al., 1998) and mecamylamine (Watkins et al., 2000b) precipitated robust elevations in brain reward thresholds in rats chronically treated with nicotine (Fig. 2 and Table 1). Similarly, direct intracerebroventricular (icv), but not systemic, administration (Fig. 2) of the nAChR antagonist chlorisondamine at doses that do not cross the blood–brain barrier (Gosling and Lu, 1969) also elevated brain reward thresholds (Watkins et al., 2000b). Therefore, elevations in brain reward thresholds provide a useful tool to investigate the affective aspects of nicotine withdrawal.

A second procedure that can be used to investigate the affective aspects of nicotine withdrawal is the conditioned place aversion paradigm. Clinical and preclinical observations have demonstrated that negative affective states experienced during drug withdrawal can become associated with previously neutral stimuli and that these conditioned stimuli gain motivational significance. That is, drug-dependent rats are treated with an antagonist to precipitate withdrawal and are confined to one compartment of the conditioned place aversion apparatus. On a different day, the same subjects are confined in a different compartment following a saline injection. This procedure leads to an association of the withdrawal-paired compartment with a negative affective state. Thus, during subsequent exposures to the apparatus, subjects tend to avoid the compartment associated with the withdrawal effects. For example, precipitated opiate withdrawal in rats has been shown to produce an aversive motivational state that becomes associated with environmental cues and leads to a conditioned place aversion (Hand et al., 1988; Stinus et al., 1990; Schulteis et al., 1994, 1998; Spanagel et al., 1994). These conditioned effects appear to play a significant role in nicotine dependence (Butschky et al., 1995; Baldinger et al., 1995). The effect of antagonist-precipitated nicotine withdrawal on conditioned place aversion also has been recently investigated (Table 1). DHβE produced a conditioned place aversion after systemic administration in rats chronically treated with nicotine (Ise et al., 2000; Watkins et al., 2000b; Suzuki et al., 1996, 1997, 1999) (Fig. 3). This conditioned place aversion was demonstrated by a significant reduction in the time spent in the compartment paired with DHβE administration in nicotine-dependent but not control rats (Watkins et al., 2000b). However, a relatively high dose of DHβE (10 mg/kg) (Watkins et al., 2000b) was required to produce this conditioned place aversion compared to the low dose (2–4 mg/kg) (Epping-Jordan et al., 1998; Watkins et al., 2000a) required to precipitate elevations in brain reward thresholds (see Table 1). Watkins et al. (2000b) reported that mecamylamine (0.5–6 mg/kg) did not produce a conditioned place aversion (Fig. 3) but did precipitate elevations in brain reward function (Fig. 2) in nicotine-dependent compared to control rats. These observations taken together suggest that a dissociation may exist in the underlying mechanisms mediating conditioned place aversions compared to those mediating elevations in brain reward thresholds observed during nicotine withdrawal. More specifically, the fact that relatively low does of mecamylamine (0.57 mg/kg) or DHβE (2 mg/kg) (see Table 1) induced statistically significant threshold elevations in nicotine-dependent rats, whereas a much higher dose of DHβE (10 mg/kg) was required to produce a conditioned place aversion, with no differential effects of even high mecamylamine doses (4–6 mg/kg) in nicotine- versus saline-treated rats, indicates possible differences in the neurobiological substrates mediating various aspects of nicotine withdrawal. Interestingly, Suzuki et al. (1997) found that mecamylamine (1 mg/kg) did produce a conditioned place aversion in nicotine-dependent rats (Suzuki

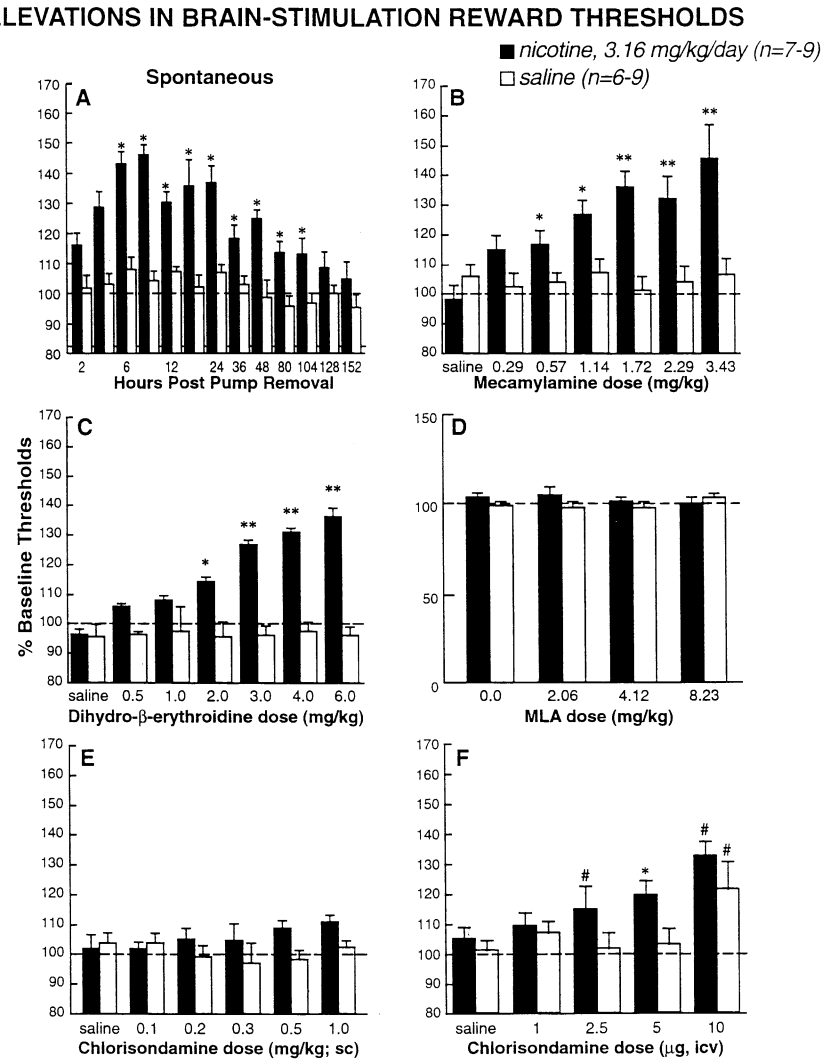

Fig. 2. Nicotine withdrawal in rats is associated with elevations in brain reward thresholds. (A) Percentage of baseline reward thresholds in rats tested 2–152 h after removal of osmotic minipump delivering nicotine (3.16 mg/kg/day free base, 7 days). (B) Percentage of baseline reward thresholds in nicotine- and vehicle-treated rats after mecamylamine (sc) administration. (C) Percentage of baseline reward thresholds in nicotine- and vehicle-treated rats after dihydro-β-erythroidine (DHβE, sc) administration. (D) Percentage of baseline reward thresholds in nicotine- and vehicle-treated rats after administration of methyllycaconitine (MLA) (sc). (E) Percentage of baseline reward thresholds in nicotine- and vehicle-treated rats after administration of chlorisondamine (sc). (F) Percentage of baseline reward thresholds in nicotine- and vehicle-treated rats after administration of chlorisondamine (icv). Asterisks indicate statistically significant differences between nicotine- and saline-treated rats ($*P<.05$, $**P<.01$). Hash symbols indicate statistically significant difference in overall somatic withdrawal signs compared to 0.0 mg/kg chlorisondamine ($^{\#}P<.05$). All data are expressed as mean (±S.E.M.) overall somatic withdrawal signs at each time point or antagonist dose. Reproduced with permission from Epping-Jordan et al. (1998) and Watkins et al. (2000b).

et al., 1997) contrary to the findings of Watkins et al. (2000b). However, Watkins et al. (2000b) used Wistar rats in their study whereas Suzuki et al. used Sprague–Dawley rats. These findings suggest that strain differences may play an important role in the expression of conditioned motivational states precipitated by mecamylamine in nicotine-dependent rats. This conclusion is supported by the recent observation that within the same study mecamylamine (1 mg/kg) produced a significant place aversion in Lewis, but not in Fischer 344 rats (Suzuki et al., 1999). Finally, naloxone also has been shown to produce a conditioned place aversion in nicotine-dependent rats (Fig. 3 and Table 1), suggesting that opioid systems may be particularly important in conditioned motivational states associated with nicotine withdrawal (see below for more detailed discussion).

6. Central versus peripheral location of nicotinic receptors involved in nicotine dependence

The precise location of the nAChRs involved in mediating the various aspects of nicotine withdrawal is unclear, although it is likely that both centrally and peripherally located nAChRs are involved. Systemic administration of the nAChR antagonist hexamethonium (0.5–10 mg/kg), which poorly penetrates the blood–brain barrier (Gosling

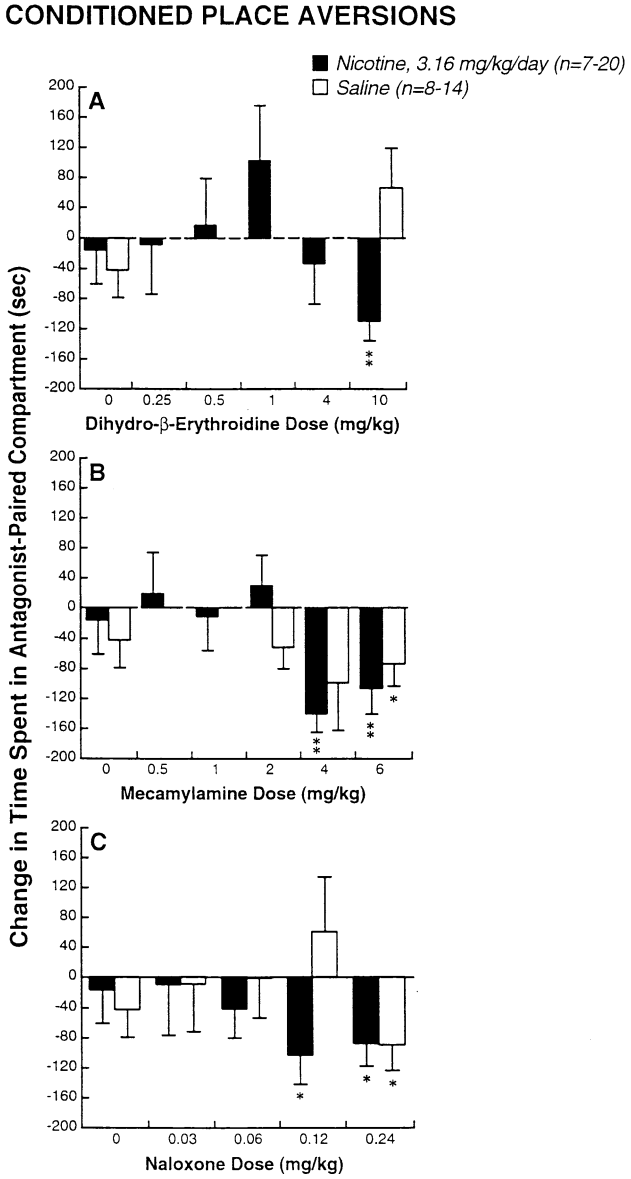

Fig. 3. Conditioned place aversion induced by sc administration of dihydro-β-erythroidine, mecamylamine, or naloxone in nicotine- (3.16 mg/kg/day free base) and vehicle-treated rats. All data are presented as mean (±S.E.M.) difference in time spent in the antagonist-paired compartment before conditioning versus after conditioning. Asterisks indicate statistically significant differences between the time spent in the antagonist-paired compartment compared before conditioning compared with after conditioning (* $P<.05$, ** $P<.01$). Reproduced with permission from Watkins et al. (2000b).

and Lu, 1969), precipitated few withdrawal signs in nicotine-dependent rats (Malin et al., 1997). Conversely, direct intracerebral hexamethonium injection precipitated somatic signs of withdrawal in nicotine-dependent rats (Malin et al., 1997). It was therefore concluded that somatic withdrawal signs are directly mediated by central but not peripheral populations of nAChRs (Malin et al., 1997). More recently, Watkins et al. (2000b) demonstrated that chlorisondamine at doses (0.1–1 mg/kg) that do not readily cross the blood–brain barrier (Gosling and Lu, 1969) precipitated significantly more somatic signs of withdrawal in nicotine-treated rats compared to saline-treated rats (Table 1). Similarly, Hildebrand et al. (1997) also showed that systemic administration of chlorisondamine (1 mg/kg) precipitated somatic withdrawal signs in nicotine-treated rats. Moreover, administration of either nicotine or the peripherally active nAChR agonist tetramethylammonium reversed somatic withdrawal signs (Hildebrand et al., 1997). Therefore, in contrast with the findings of Malin et al. (1997), these observations suggest that nAChRs located peripherally contribute to the expression of somatic signs in rats undergoing nicotine withdrawal. This discrepancy may be explained in part by the fact that hexamethonium is not very effective at blocking nAChRs compared to other nAChR antagonists (Marks et al., 1993) such as chlorisondamine, particularly at peripheral ganglia (Abdel-Rahman, 1989; Santajuliana et al., 1996). nAChRs located within the brain also likely play a role in mediating somatic withdrawal signs. For example, direct intraventral tegmental area injection of mecamylamine (Hildebrand et al., 1999) or icv administration of chlorisondamine (Watkins et al., 2000b; Fig. 1) both precipitated somatic withdrawal signs. Overall, it appears that centrally and peripherally located populations of nAChRs are involved in mediating somatic signs of nicotine withdrawal.

It is likely that centrally located populations of nAChRs are exclusively involved in mediating affective aspects of nicotine withdrawal. Systemic administration of the neuronal nAChR antagonist, DHβE, which selectively blocks centrally located high affinity neuronal nAChRs (Harvey et al., 1996), precipitated elevations in brain reward thresholds (Epping-Jordan et al., 1998; Fig. 2) and produced a conditioned place aversion (Watkins et al., 2000b; Fig. 3) in nicotine-treated rats (Table 1). However, DHβE produced only modest increases, and only at a high dose (4 mg/kg), in the number of somatic signs with no differences between nicotine-dependent and control rats (Epping-Jordan et al., 1998; Fig. 1), suggesting that nicotine-dependent animals are *not* more sensitive than saline-treated controls to the increases in somatic signs of withdrawal induced by DHβE (Table 1). Further, systemic administration of doses of chlorisondamine (0.1–1 mg/kg) that do not penetrate the blood–brain barrier had no effect on brain reward thresholds (Epping-Jordan et al., 1998; Watkins et al., 2000b) whereas direct icv administration of chlorisondamine precipitated elevations in reward thresholds (Fig. 2). Finally, systemic administration of hexamethonium (1–3 mg/kg) at doses that do not penetrate the blood–brain barrier had no effect on conditioned place aversion in rats chronically treated with nicotine (Ise et al., 2000). Overall, this pattern of results suggests that unlike somatic withdrawal signs that are peripherally and centrally mediated, affective aspects of nicotine withdrawal (conditioned place aversion and elevations in brain reward thresholds) are mediated exclusively by central populations of nAChRs. Furthermore, observations

demonstrating that somatic withdrawal signs can be precipitated in the absence of affective signs and vice versa supports the notion that a dissociation exists in the underlying mechanisms mediating somatic and affective aspects of nicotine withdrawal.

7. Subtypes of neuronal nAChRs involved in mediating nicotine withdrawal

At present it is unclear which class of neuronal nAChR subtypes are involved in mediating the symptoms of nicotine withdrawal and only recently have studies addressed this question. Mecamylamine, which precipitated both somatic and affective symptoms of nicotine withdrawal (Table 1), is a relatively nonspecific noncompetitive nAChR antagonist (Lindstrom et al., 1996; Varanda et al., 1985), but has nevertheless been shown to be slightly more selective for the α3-containing nAChRs compared to those containing α4 subunits, with the least activity at the α7 nAChRs (Gotti et al., 1997). Affective, but not somatic, signs of nicotine withdrawal also were precipitated with the competitive nAChR antagonist DHβE (Epping-Jordan et al., 1998). This nAChR antagonist is relatively selective for the α4- and β2-containing high-affinity nAChRs compared to other classes of nAChRs, although at higher concentrations DHβE E also will antagonize other classes of nAChRs (Harvey and Luetje, 1996; Harvey et al., 1996; Damaj et al., 1995; Wonnacott, 1997). Therefore, it is likely that α4-containing nAChRs are involved in mediating nicotine dependence, as measured by the ability of antagonists of this receptor to precipitate signs of withdrawal in rats chronically treated with nicotine. It should be recognized that firm conclusions concerning subtypes of nAChRs mediating nicotine dependence cannot be made based on the effects of DHβE because of its ability to block many different classes of nAChRs. Nevertheless, the observation that chronic nicotine exposure selectively up-regulates the expression (Flores et al., 1997; Fenster et al., 1999; Sparks and Pauly, 1999; Buisson and Bertrand, 2001) and possibly the function (Buisson and Bertrand, 2001) of α4-containing nicotinic receptors compared to other non-α4-containing nAChRs subtypes supports a role for α4-containing nAChRs in nicotine dependence.

The role of the α7 nAChR in nicotine dependence also has been investigated. This class of nAChR appears to play some role in mediating the reinforcing actions of acute nicotine. For example, direct administration of the α7-selective nAChR antagonist, methyllycaconitine (MLA) into the VTA reversed the potentiation in brain stimulation reward observed after acute systemic nicotine administration (Nomikos et al., 1999). Furthermore, MLA (3.9 and 7.8 mg/kg free base) significantly reduced nicotine self-administration in rats (Markou and Paterson, in press; however, see Grottick et al., 2000). Nevertheless, in nicotine-treated rats systemic administration of MLA (7.8 mg/kg) did not precipitate nicotine withdrawal as reflected by the absence of elevations in brain reward thresholds or somatic signs of withdrawal (Markou and Paterson, in press). Previously, it was shown that MLA primarily antagonized α7 nAChRs and does not act at other nAChRs until concentration levels are 10–30-fold higher than those obtained with 5 mg/kg MLA (dose expressed as salt) (Wonnacott et al., 1993). Further, nAChR antagonists such as mecamylamine and DHβE that act selectively at nAChRs other than the α7 nAChR precipitate nicotine withdrawal. If MLA were acting at nAChRs other than the α7 nAChR, it too would have been expected to precipitate nicotine withdrawal. Taken together, these observations suggest that MLA at the doses used (7.8 mg/kg) antagonized primarily α7 and not other subtypes of nAChRs. Therefore, even though α7 nAChRs appear to be involved in the rewarding actions of acute nicotine, these receptors do not appear to play a significant role in nicotine dependence as reflected by the lack of precipitation of the nicotine withdrawal syndrome in nicotine-dependent animals. One possible explanation for these data is that in drug naïve animals, nicotine activates α7 nAChRs that contribute to the rewarding effects of nicotine. However, in nicotine-dependent rats, α7 nAChRs are already in a desensitized state. Thus, antagonism of these receptors would have no effect on neuronal activity. Indeed, α7 nAChRs in different brain sites are known to undergo rapid desensitization in the presence of concentrations of nicotine achieved in the brains of smokers (Alkondon et al., 2000; Pidoplichko et al., 1997; Mansvelder and McGehee, 2000). Thus, inactivation of α7 nAChRs may occur in nicotine-dependent rats in response to chronic nicotine exposure. Interestingly, it has been hypothesized that smokers regulate their pattern of nicotine intake in order to either activate or desensitize populations of nAChRs, and thereby control the activity of these different populations of receptors (Dani and Heinemann, 1996). Thus, the α7 subtype may be a class of nAChR whose activity is regulated in rats and humans by careful titration of the level of nicotine intake. Similarly, nAChRs other than the α7 receptor subtype also undergo rapid nicotine-induced desensitization (Dani et al., 2000; Pidoplichko et al., 1997; Lena and Changeux, 1998; Fenster et al., 1997). Therefore, it is possible that by careful titration of nicotine intake, smokers modulate the activity of many classes of nAChRs in order to regulate the activity of nicotine in the brain.

8. Neurotransmitter systems involved in nicotine withdrawal

8.1. The role of acetylcholine in nicotine withdrawal

The cholinergic system arises within basal forebrain (medial septum, diagonal band nucleus, and substantia innominata) and pontine (pedunculopontine and laterodorsal tegmental nuclei) sites and projects throughout the

brain. The cholinergic system appears to play a significant role in mediating the rewarding actions of acute nicotine. For example, lesioning of the pedunculopontine tegmental nucleus reduced the rewarding effects of self-administered nicotine (Lanca et al., 2000). The cholinergic system probably also plays a role in mediating nicotine withdrawal. Blockade of nAChRs increased the occurrence of withdrawal-related behaviors in rats chronically treated with nicotine (see above; Epping-Jordan et al., 1998; Hildebrand et al., 1997; Watkins et al., 2000b; Malin et al., 1993). Moreover, administration of nicotinic receptor antagonists precipitated withdrawal-like responses in nicotine naïve animals (Epping-Jordan et al., 1998; Watkins et al., 2000b; see Table 1). This observation suggests that endogenous cholinergic tone, by an action at nAChRs, prevents the expression of somatic and affective signs usually associated with nicotine withdrawal, and that these withdrawal responses arise because of deficits in cholinergic transmission. Interestingly, direct infusion of nicotinic receptor antagonists into the VTA elevated brain-simulation reward thresholds (Yeomans and Baptista, 1997) by a similar magnitude to that observed in rats undergoing nicotine withdrawal (Epping-Jordan et al., 1998; Watkins et al., 2000b). Therefore, a reduction in endogenous cholinergic tone may be one neurochemical adaptation involved in mediating elevations in brain-stimulation reward thresholds observed in rats during withdrawal from chronic nicotine exposure.

8.2. The role of dopamine in nicotine withdrawal

There is now considerable evidence suggesting that the dopamine fibers that arise within the VTA and project to the nucleus accumbens (NAcc), known as the mesolimbic dopamine system, play a major role in mediating the reinforcing properties of acute nicotine. For example, acute nicotine increased the firing rate of VTA dopamine neurons (Grenhoff et al., 1986; Pidoplichko et al., 1997) and elevated dialysate dopamine levels in the NAcc (Imperato et al., 1986; Benwell and Balfour, 1992; Nisell et al., 1997). Furthermore, direct injection of DHβE into the VTA (Corrigall et al., 1994), 6-hydroxydopamine lesions of the NAcc (Corrigall et al., 1994), or systemic administration of a selective D1 or D2 dopamine receptor antagonist (Corrigall and Coen, 1991; Corrigall et al., 1992) all attenuated nicotine self-administration in rats.

Spontaneous and antagonist-precipitated withdrawal from various drugs of abuse such as amphetamine, cocaine, morphine, and ethanol (see Rossetti et al., 1992; Weiss et al., 1992, 1996) has been shown to produce marked deficits in accumbal dopamine release. These observations are consistent with the notion that, in addition to mediating the rewarding properties of drugs of abuse like nicotine, the mesolimbic system also is involved in mediating aversive behavioral states associated with drug withdrawal (Stinus et al., 1990). Recently, Hildebrand et al. have shown that besides an increase in somatic withdrawal signs, mecamylamine also significantly decreased accumbal dopamine release in rats chronically exposed to nicotine compared with control rats (Hildebrand et al., 1999). Therefore, it is likely that deficits in dopamine transmission in the NAcc play a role in mediating nicotine withdrawal. However, somatic withdrawal signs in this study were measured for 30 min immediately after mecamylamine challenge, whereas the decreases in dopamine output first were observed approximately 45 min after injection. This temporal dissociation in the onset of somatic withdrawal signs and decreased dopamine output suggests that accumbal dopamine may not necessarily be involved in mediating the somatic aspects of nicotine withdrawal. Accordingly, Carboni et al. (2000) have recently shown that the opioid receptor antagonist naloxone (see below) increased somatic withdrawal signs in nicotine-dependent rats without affecting accumbal dopamine release. However, it should be noted that mecamylamine administered directly into the VTA precipitated somatic withdrawal signs (Hildebrand et al., 1999). Therefore, at present the precise role of the mesolimbic dopamine system in the mediation of somatic nicotine withdrawal signs is unclear. Interestingly, a dissociation in the role of accumbal dopamine levels and somatic withdrawal signs has been proposed to occur in the case of opiate withdrawal. For example, Diana et al. demonstrated that decreases in accumbal dopamine release during morphine withdrawal were not correlated with the onset or duration of somatic withdrawal signs (Diana et al., 1999). It has therefore been proposed that decreases in accumbal dopamine output observed during drug withdrawal are specifically related to reward and motivational deficits, such as elevations in brain-stimulation reward thresholds, but not somatic signs of withdrawal (Stinus et al., 1990; however, see Harris and Aston-Jones, 1994). Based on this hypothesis, it may be predicted that dopamine receptor agonists would reverse the affective, but not somatic, withdrawal signs in rats undergoing nicotine withdrawal.

In addition to the NAcc, dopamine fibers that arise within the VTA also terminate in the prefrontal cortex (PFC), a projection known as the mesocortical dopamine pathway. Enhanced dopamine transmission in the PFC has been observed during exposure to stressful (Thierry et al., 1976; Deutch and Roth, 1990; Inglis and Moghaddam, 1999) and aversive stimuli (Kawasaki et al., 2001) and has been implicated in mediating anxiety-related behaviors (Bradberry et al., 1991; Broersen et al., 2000). In contrast to the deficits in dopamine transmission observed in the NAcc, withdrawal from drugs of abuse typically increases dopamine release in the PFC in rats (Acquas and Di Chiara., 1992; Bassareo et al., 1995). Carboni et al. (2000) have shown that mecamylamine increased dopamine output in the PFC in rats chronically exposed to nicotine and have suggested that these increases in PFC dopamine release may be important in mediating

aversive aspects of nicotine withdrawal. It should be noted that Hildebrand et al. (1998) did not observe any difference in PFC dopamine output in rats undergoing mecamylamine-precipitated nicotine withdrawal. However, there were a number of methodological differences between these two studies that may account for this discrepancy. First, Carboni et al. used male Sprague–Dawley rats, whereas Hildebrand et al. used Wistar rats. Second, the same stereotaxic coordinates were not used in each case, giving rise to the possibility that subregions within the PFC respond differently during nicotine withdrawal. Third, slightly different amounts of nicotine were administered to rats in each study (Hildebrand: 3.61 mg/kg/day, Carboni: 3.16 mg/kg/day free base). Finally, there was a dramatic increase in PFC dopamine release in response to even a saline injection in the study by Hildebrand et al., possibly reflecting a mild stress response. This effect on PFC dopamine levels may have masked potential increases in dopamine levels that may have been associated with nicotine withdrawal. Consistent with this explanation is the observation that injection of vehicle had no effect on PFC dopamine release in the study by Carboni et al. in which an effect of nicotine withdrawal on PFC dopamine levels was observed.

The effect of nicotine withdrawal on dopamine transmission has also been examined in the central nucleus of the amygdala (CNA). Panagis et al. (2000) reported that mecamylamine-precipitated nicotine withdrawal significantly reduced dopamine overflow and increased c-*fos* expression in the CNA. There is tentative evidence suggesting that dopamine possibly may mediate an anxiolytic effect in this brain structure (Beaulieu et al., 1987; Coco et al., 1992; Ray et al., 1988; Glavin, 1992). Therefore, the reduction in dopamine output observed during nicotine withdrawal in the CNA may be involved in mediating the increase in anxiety associated with nicotine withdrawal. However, at present the precise role of CNA dopamine neurotransmission in mediating anxiety states is unclear and further studies are required before any firm conclusions can be drawn regarding the significance of this observation.

In conclusion, there appears to be some evidence suggesting that dopamine may play a role in mediating nicotine withdrawal, particularly in deficits in reward and motivational processes. It is noteworthy that the recently licensed smoking cessation aid, bupropion (Zyban™) acts, at least in part, by inhibiting neuronal uptake of dopamine and thereby enhancing dopamine transmission (Terry and Katz, 1997; Nomikos et al., 1992).

8.3. The role of opioid peptides in nicotine withdrawal

Opioid receptor antagonists such as naloxone and naltrexone have been reported to modulate cigarette consumption and have been used as smoking cessation aids (Karras and Kane, 1980; Wewers et al., 1998; Covey et al., 1999; but see Nemeth-Coslett and Griffith, 1986; Sutherland et al., 1995). Further, smoking status (nonsmoker, nondependent smoker, or dependent smoker) has been shown to provide a powerful predictor of opiate use among methadone-maintained opiate-dependent individuals (Frosch et al., 2000). Therefore, there may be an interaction between cholinergic and opioid receptor systems, with opioid receptors playing a role in mediating smoking behavior.

The opioid receptor agonist morphine reversed withdrawal signs in rats undergoing spontaneous nicotine withdrawal (Malin et al., 1993). Interestingly, nicotine significantly reduced naloxone-precipitated opiate withdrawal in rats (Zarrindast and Farzin, 1996), suggesting that common neurobiological substrates may mediate nicotine and opiate withdrawal. Accordingly, naloxone and an analog of the endogenous antiopiate, neuropeptide FF, have been shown to precipitate somatic withdrawal signs after chronic nicotine treatment (Malin et al., 1993, 1996; Carboni et al., 2000). However, it should be noted that the doses of naloxone (2–4.5 mg/kg) (Carboni et al., 2000; Malin et al., 1993) required to precipitate somatic withdrawal signs in nicotine-dependent rats were extremely high compared with those required to precipitate somatic signs in opiate-treated rats (>0.006 mg/kg) (Gellert and Sparber, 1977; Brady and Holtzman, 1981; Koob et al., 1989; Higgins and Sellers, 1994; Schulteis et al., 1994). Moreover, Watkins et al. (2000b) showed that a high dose of naloxone (8 mg/kg) increased somatic withdrawal signs by a similar magnitude in nicotine- and vehicle-treated rats, whereas lower doses (0.03–4 mg/kg) had no effect, suggesting no specific role of opioid receptors in mediating somatic nicotine withdrawal signs (Table 1). However, this discrepancy may be explained by the fact that Watkins et al. used Wistar rats whereas Sprague–Dawley rats were used in the other studies (Carboni et al., 2000; Malin et al., 1993). High naloxone doses (2–4 mg/kg) also have been shown to precipitate elevations in brain reward thresholds in nicotine-dependent rats (Watkins et al., 2000b). However, once again naloxone elevated thresholds by a similar magnitude in nicotine- and vehicle-treated rats, with lower doses (0.03–1 mg/kg) having no effect (Watkins et al., 2000b), although the effect of naloxone on reward thresholds in strains of rat other than Wistar has not so far been investigated. Overall, this pattern of results suggests that somatic withdrawal signs and brain reward thresholds are not particularly sensitive to alterations in opioid transmission. These observations also further support the hypothesis that differential substrates mediate various aspects of nicotine withdrawal. Interestingly, both naloxone (Tome et al., 2001) and naltrexone (Almeida et al., 2000) have been shown recently to antagonize nAChRs, suggesting that opioid receptor antagonists may precipitate nicotine withdrawal, at least in part, by directly blocking nAChRs.

In addition to its effects on somatic withdrawal signs and brain reward thresholds, naloxone administration appears to induce an aversive state in nicotine-dependent rats that can be associated with environmental stimuli and expressed as a conditioned place aversion (Ise et al., 2000;

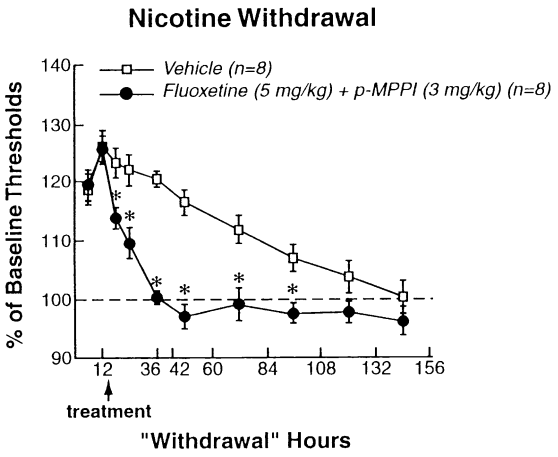

Fig. 4. Pretreatment with WAY-100635 reversed the enhanced startle response of nicotine withdrawing rats. Startle responses were measured daily for 3 days beginning 24 h after removal of nicotine- or vehicle-containing minipumps. Rats received either chronic vehicle in pumps and acute daily pretreatment during withdrawal with vehicle (Sal/Sal), or chronic nicotine in pumps and acute daily pretreatment during withdrawal with vehicle (Nic/Sal), chronic nicotine in pumps, and acute daily pretreatment during withdrawal with three doses of WAY-100635 (0.001–1 mg/kg). Asterisks indicate statistically significant differences between nicotine- and saline-treated rats ($*P<.05$). Reproduced with permission from Rasmussen et al. (1997).

Watkins et al., 2000b; see Fig. 3). Interestingly, the dose of naloxone (0.12 mg/kg) that induces a conditioned place aversion in nicotine-treated rats is relatively low when compared to doses required to precipitate somatic signs of nicotine withdrawal (Table 1). Indeed, nicotine-dependent rats appear more sensitive to opioid receptor antagonists than nicotinic receptor antagonists in the conditioned place aversion paradigm (Watkins et al., 2000b; Malin et al., 1993; Ise et al., 2000). Overall, these observations suggest that opioid receptors may play a role in nicotine dependence, particularly in relation to conditioned motivational states.

8.4. The role of serotonin in nicotine withdrawal

Evidence is accumulating that serotonin (5-HT), and the 5-HT_{1A} receptor in particular, plays a role in nicotine withdrawal (Benwell et al., 1990; Kenny et al., 2001). Clinically, the 5-HT_{1A} receptor partial agonist buspirone shows efficacy in smoking cessation trials and may reduce withdrawal severity in abstinent smokers (Hilleman et al., 1992, 1994; West et al., 1991; but see Schneider et al., 1996). Preclinical studies also have investigated the role of 5-HT and the 5-HT_{1A} receptor in nicotine withdrawal. Helton et al. (1993) have reported that nicotine withdrawal significantly increased the acoustic startle response in rats for approximately 4–5 days. It has been suggested that this increased startle reactivity perhaps most closely resembles the increased irritability observed in smokers undergoing nicotine withdrawal (Hughes and Hatsukami, 1992).

Systemic administration of 5-HT_{1A} receptor agonists such as 8-OH-DPAT exacerbates this response, whereas 5-HT_{1A} receptor antagonists, such as WAY-100635, alleviate this enhanced response (Rasmussen et al., 1997, 2000; see Fig. 4). Further, electrophysiological investigations have demonstrated that the responsiveness to 8-OH-DPAT of neurons in the dorsal raphe nucleus (DRN) was significantly increased during nicotine withdrawal (Rasmussen and Czachura, 1997). Therefore, one possibility is that nicotine withdrawal increases the inhibitory influence of somatodendritic 5-HT_{1A} autoreceptors located within the raphe nuclei and thereby decreases 5-HT release into forebrain and limbic brain sites (e.g., Benwell and Balfour, 1979, 1982; Ridley and Balfour, 1997) which contributes to nicotine withdrawal signs. This conclusion is supported by the observation that a serotonergic antidepressant treatment that combines the serotonin-selective re-uptake inhibitor fluoxetine and the 5-HT_{1A} receptor antagonist p-MPPI [4-(2′-methoxy-phenyl)-1-[2′-(n-2″pyridinyl)-p-iodobenzamidol]-ethyl-piperazine] rapidly reverses the elevation in brain-stimulation reward thresholds observed in rats undergoing nicotine withdrawal (Harrison et al., 2001; see Fig. 5). Interestingly, this same treatment did not block the increased expression of somatic signs in rats undergoing nicotine withdrawal (Harrison et al., 2001), providing further evidence for a dissociation of the mechanisms mediating affective and somatic aspects of nicotine withdrawal.

Contrary to the view propounded above that reduced serotonergic transmission contributes to nicotine withdrawal, Cheeta et al. (2001) have shown that administration of nicotine directly into the DRN, at a concentration that activates somatodendritic 5-HT_{1A} receptors, reversed the increase in anxiety observed in rats undergoing nicotine withdrawal as measured in the social interaction test. This observation suggests that there is enhanced serotonergic transmission during nicotine withdrawal that mediates the observed increases in anxiety. Taken together, these data suggest that serotonin and 5-HT_{1A} receptors are involved in

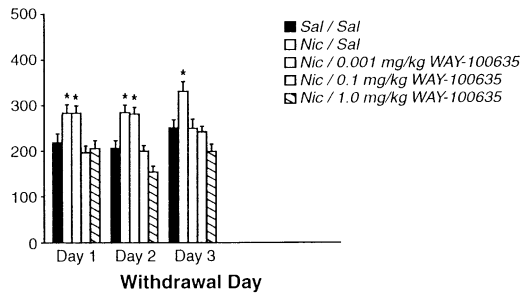

Fig. 5. Serotonergic treatment reversed the elevations in brain reward thresholds observed during nicotine withdrawal. Fluoxetine combined with p-MPPI lowered the threshold elevations of nicotine withdrawing rats. Arrow indicates the time-point at which fluoxetine and p-MPPI treatment was administered. All data are expressed as percent mean (±S.E.M.) baseline reward thresholds at each time point. Asterisks indicate statistically significant differences between nicotine- and saline-treated rats ($*P<.05$). Reproduced with permission from Harrison et al. (2001).

nicotine withdrawal, although at present it is unclear exactly what role they play.

8.5. The role of glutamate in nicotine withdrawal

Acute nicotine is thought to act at several loci within the mesolimbic system in order to increase dopamine release within the NAcc and thereby produce its rewarding effects (Corrigall and Coen, 1989; Corrigall et al., 1992). First, nicotine acts at nAChRs located on dopamine neurons in the VTA, and increases their firing rates (Pidoplichko et al., 1997). Nicotine also acts at presynaptic α7 nAChRs located upon glutamate efferents (Mansvelder and McGehee, 2000) that arise within the PFC (Kalivas et al., 1989; Suaud-Chagny et al., 1992; Taber and Fibiger, 1995) to increase glutamate release in the VTA. This enhanced glutamate release then acts at *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor sites on postsynaptic dopamine neurons and increases their firing rate. Finally, nicotine also acts at α7 nAChRs located on dopamine cell bodies in the VTA (Pidoplichko et al., 1997) and on presynaptic terminals in the NAcc (Fu et al., 2000b) to increase dopamine release. In addition to its role in mediating the rewarding effects of drugs like nicotine, there is also evidence for a role of glutamate in drug dependence and withdrawal states (Davidson et al., 1995; Manzoni and Williams, 1999). For example, coadministration of the NMDA receptor antagonist MK-801 blocked the development and/or expression of opiate (Gonzalez et al., 1997), ethanol (Liljequist, 1991), and benzodiazepine (Steppuhn and Turski, 1993) dependence. Further, NMDA receptor antagonists have been shown to block tolerance to the locomotor depressant effects of acute nicotine (Shoaib and Stolerman, 1992; Shoaib et al., 1994) and sensitization to the locomotor stimulant effects of chronic nicotine (Shoaib and Stolerman, 1992). Recently, the role of glutamate transmission in nicotine withdrawal has been investigated. Group II metabotropic glutamate receptors (mGluR), which include $mGluR_2$ and $mGluR_3$, are inhibitory receptors that are located at presynaptic and postsynaptic locations (for review see Cartmell and Schoepp, 2000). Stimulation of $mGluR_{2/3}$ decreased glutamate release throughout the hippocampus, striatum, and cortex (East et al., 1995; Di Iorio et al., 1996; Toth, 1996; Moghaddam and Adams, 1998; Cartmell and Schoepp, 2000). Interestingly, Helton et al. (1997) have shown that the Group II mGluR selective agonist LY354740 ameliorated the increase in acoustic startle response observed in rats undergoing nicotine withdrawal (Helton et al., 1997). In light of this observation, it was suggested that enhanced glutamate release may play a role in mediating the aversive aspects of nicotine withdrawal that were reflected by an increase in startle reactivity (Helton et al., 1997). It is interesting that acute nicotine administration increased the release of glutamate in various brain sites including the VTA (Mansvelder and McGehee, 2000; Fu et al., 2000a; Grillner and Svensson, 2000), NAcc (Reid et al., 2000), PFC (Gioanni et al., 1999), and hippocampus (Gray et al., 1996), whereas acute LY354740 decreased glutamate release (see Cartmell and Schoepp, 2000). In fact, because withdrawal effects are most often opposite in direction to acute drug actions (Koob and Bloom, 1988), it might be expected that nicotine withdrawal would be associated with deficits in glutamate transmission. It is therefore somewhat surprising that a drug that acts to decrease glutamate release would ameliorate nicotine withdrawal, particularly because activation of glutamate receptors plays a role in mediating the rewarding actions of nicotine (Nisell et al., 1994a,b; Schilstrom et al., 1998; Fu et al., 2000a). One possibility is that glutamate release is increased only in certain brain sites and not in others and that LY354740 selectively decreases glutamate release involved in facilitating enhanced startle reactivity. It is also possible that $mGluR_{2/3}$ may be expressed on presynaptic terminals that release a neurotransmitter other than glutamate that enhances startle reactivity during nicotine withdrawal, one such example being cholecystokinin (Rasmussen et al., 1996). Therefore, LY354740 may act at these putative $mGluR_{2/3}$ heteroreceptors to block this release and thereby block the enhanced startle reactivity observed during nicotine withdrawal.

9. Discussion

Evidence so far suggests that the negative affective aspects of nicotine withdrawal, which appear to be mediated exclusively by central populations of nAChRs, are regulated by a number of different neurotransmitter systems. For example, deficits in serotonergic neurotransmission are likely to be involved in mediating elevations in brain reward thresholds (Harrison et al., 2001) whereas decreased opioid receptor activity is likely to be involved in the conditioned aversive motivational states associated with nicotine withdrawal (Watkins et al., 2000b). Although it is clear that both central and peripheral populations of nAChRs are involved in mediating somatic aspects of nicotine withdrawal, it is unclear what other neurotransmitter systems besides the cholinergic system are also involved. One strong candidate is the noradrenergic system. There is now a considerable amount of evidence suggesting that noradrenaline plays a major role in mediating somatic signs in rats undergoing opiate withdrawal (Delfs et al., 2000; Maldonado, 1997). Moreover, clonidine, which acts to decrease noradrenergic neurotransmission, has shown efficacy in smoking cessation trials (for review see Gourlay et al., 2000). Therefore, it is possible that noradrenaline may play a role in mediating somatic signs in rats undergoing nicotine withdrawal, although further studies are required to address this possibility.

In addition to the classical neurotransmitters such as serotonin and glutamate discussed in the present review, it is likely that neuropeptides other than endogenous opiates play a role in nicotine dependence and withdrawal. For

example, the cholecystokinin-B (CCK-B) receptor antagonist LY288513 reversed the enhanced startle response in rats undergoing nicotine withdrawal (Rasmussen et al., 1996). Interestingly, withdrawal from various drugs of abuse, including ethanol (Pich et al., 1995), cocaine (Richter and Weiss, 1999), opiates (Milanes et al., 1998), and even cannabis (Rodriguez de Fonseca et al., 1997) has been shown to increase the release of corticotropin-releasing factor (CRF). CRF receptor antagonists alleviated symptoms of withdrawal associated with these drugs (Baldwin et al., 1991; Rassnick et al., 1993; Menzaghi et al., 1994; Heinrichs et al., 1995; Basso et al., 1999; Lu et al., 2000). Furthermore, similar to nicotine withdrawal, CRF and urocortin, a CRF receptor agonist, elevated brain-stimulation reward thresholds in rats (Macey et al., 2000). Taken together, these observations suggest that increased CRF also may play a role in the aversive aspects of nicotine withdrawal. Interestingly, Heinrichs et al. (1996) and Sarnyai et al. (2001) have reported that the CRF-binding protein ligand inhibitor CRF (6–33) that acts to increase levels of free CRF in the brain, suppressed the increase in weight gain observed in rats undergoing nicotine withdrawal, but had no effect in nicotine naïve rats. This observation suggests that the activity of CRF-binding protein may increase during nicotine withdrawal in order to compensate for an increase in the levels of free CRF. Another neuropeptide that potentially could play a role in nicotine withdrawal is substance P. This neuropeptide acts at the neurokinin-1 (NK-1) receptor. Blockade or genetic disruption of this receptor has been shown to have anxiolytic (File, 1997, 2000; Santarelli et al., 2001) and antidepressant (Kramer et al., 1998) effects. NK-1 receptor antagonists significantly decreased naloxone-precipitated withdrawal behaviors in opiate-dependent rats (Maldonado et al., 1993). Similarly, there was a significant reduction in opiate withdrawal in mice with genetic deletion of the NK-1 receptor (Murtra et al., 2000). Because there is such a close interaction between nicotinic and opioid receptors in mediating nicotine dependence, these observations suggest that substance P also may play a role in nicotine withdrawal.

As evidenced by the content of the present review, most investigations into the mechanisms underlying nicotine withdrawal have focused on particular neurotransmitter systems or receptors. However, it is likely that many of the neurochemical effects produced by chronic nicotine exposure converge at common molecular and cellular targets to give rise to the long-lasting changes in brain structure and function that ultimately lead to dependence. Therefore, investigation of molecular alterations associated with nicotine dependence and withdrawal offers a promising research target. There are a number of candidate molecular substrates that might play a role in mediating cellular adaptations to chronic nicotine exposure. One possibility is that chronic nicotine treatment may alter the expression of specialized intracellular signaling proteins like cyclic AMP response element-DNA-binding protein (CREB) and c-*fos* (Pandey et al., 1999) that are known to regulate the expression of many genes throughout the brain. Indeed, nicotine withdrawal decreased CREB expression in the medial and basolateral nucleus of the amygdala and hippocampus (Pandey et al., 2001) whereas increased c-*fos* expression was observed in the CNA (Panagis et al., 2000). Neurotrophic factors play a vital role in neuronal plasticity (McAllister et al., 1999) and survival (Ghosh et al., 1994) and represent another set of possible targets involved in the long term actions of nicotine on the brain. Brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and fibroblast growth factor-2 (FGF-2) are three examples of neurotrophic factors involved in neuronal plasticity. Acute nicotine treatment has been shown to decrease BDNF expression in rat dorsal hippocampus whereas chronic treatment increased its expression (Kenny et al., 2000b). Nicotine also has been shown to produce long-lasting increases in NGF in the hippocampus (French et al., 1999) and FGF-2 in the cerebral cortex, hippocampus, striatum, and substantia nigra (Roceri et al., 2001; Belluardo et al., 1998, 1999). Interestingly, deficits in neurotrophin expression, particularly BDNF, had been proposed to play a significant role in the etiology of depression (Duman et al., 1997; Altar, 1999), a major symptom of the nicotine withdrawal syndrome (e.g., Covey et al., 1997). Therefore, it is an intriguing possibility that nicotinic modulation of neurotrophic factor expression may represent a mechanism by which chronic nicotine treatment produces structural changes in the brain that give rise to dependence and which contributes to the negative affective symptoms observed during withdrawal from chronic nicotine treatment.

In conclusion, perhaps the most striking observation regarding the nicotine withdrawal syndrome is its complexity. Nicotine withdrawal is not characterized by any one single behavioral deficit, nor is it mediated by a change in any single neurotransmitter system. Instead, this syndrome comprises a plethora of characteristic behaviors each mediated by different underlying neuroanatomical and neurochemical substrates. Further research is required to identify the precise neurobiological substrates mediating the affective and somatic aspects of nicotine withdrawal. Such investigations eventually may lead to future strategies for treating nicotine dependence beyond nicotine replacement therapy.

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