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ROLE OF DOPAMINE D_3 RECEPTORS IN THE ADDICTIVE PROPERTIES OF ETHANOL

Christian A. Heidbreder[1], Michela Andreoli[1], Clara Marcon[1], Panayotis K. Thanos[2], Charles R. Ashby, Jr.[3] and Eliot L. Gardner[4]

[1]Center of Excellence for Drug Discovery in Psychiatry, GlaxoSmithKline Pharmaceuticals, Verona, Italy;
[2]Medical Department, Brookhaven National Laboratory, Upton, New York, USA;
[3]Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, Saint John's University, Jamaica, New York, USA;
[4]Intramural Research Program, National Institute on Drug Abuse, Baltimore, Maryland, USA

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Summary

After the cloning of the dopamine D_1 and D_2 receptors (1–3), several additional dopamine receptors were identified. These new subtypes included the D_3 and D_4 receptors, which are similar to D_2, and the D_5 receptor, which is similar to D_1 (4–6). Although most studies have focused on the role of dopamine D_1 and D_2 receptors in mediating the addictive liability of drugs, the lack of selective pharmacological tools has significantly hampered this particular field of research. In contrast, recent studies using selective competitive antagonists have shown that the dopamine D_3 receptor is involved in drug-seeking behavior. The present review is intended to highlight a new, promising area in alcohol research that focuses on the role of the dopamine D_3 receptor in the addictive properties of ethanol.

Correspondence: Christian A. Heidbreder, Department of Biology, Center of Excellence for Drug Discovery in Psychiatry, GlaxoSmithKline Pharmaceuticals, Via A. Fleming 4, 37135 Verona, Italy. Tel.: +39-045-921-9769; FAX: +39-045-921-8047. E-mail: Christian_A_Heidbreder@gsk.com

Introduction

Alcohol dependence is characterized by progressive stages, including initiation or acquisition, compulsion, alcohol taking with a narrowing of the behavioral repertoire, excessive alcohol intake, loss of control in limiting intake and vulnerability to relapse. Although psychosocial therapies are an important component of treatment strategies, up to 40–70% of patients resume drinking behavior within one year of detoxification, psychosocial or behavioral treatment (7). Similarly, pharmacological agents have shown limited efficacy and consistency in the treatment of alcoholism. Hence, the development of new medications with improved long-term efficacy and reduced side effects should be given a high priority given the societal costs associated with alcoholism and alcohol-related pathologies. The continued elucidation of the mechanisms underlying withdrawal symptoms, alcohol intake, relapse and comorbid psychiatric associations has significantly boosted the development of new pharmacotherapies for the treatment of alcoholism. For example, the role of dopamine in reinforcement processes clearly points towards the potential use of dopamine receptor ligands as candidate medications to reduce alcohol consumption and craving. Recently, a growing body of evidence has strengthened the likelihood that the dopamine D_3 receptor is significantly involved in mechanisms of drug dependence and abuse. In the present review, we will first examine the extent of the alcohol dependence problem, and then briefly discuss the lack of effective agents for pharmacotherapeutic management of alcoholism. This will be followed by an introduction of the involvement of the mesolimbic dopamine system in the reinforcing properties of alcohol, with particular attention to the link between the selective distribution of the dopamine D_3 receptor in the mesolimbic dopamine system and the rewarding properties of alcohol. Finally, associations between gene variants at the dopamine D_3 receptor and alcoholism, as well as functional pharmacological aspects of different dopamine D_3 and mixed D_3/D_2 agonists and antagonists will be summarized in the context of alcohol intake and relapse.

Alcoholism: The magnitude of the problem

Alcohol dependence impacts 32 million adults in the top seven markets and has a higher societal cost than even nicotine dependence in the USA (US$ 148 billion *vs.* US$ 100 billion, respectively). Recent surveys from the National Household Survey on Drug Abuse (8) revealed that almost half of Americans aged 12 or older (approximately 109 million people) are current users of alcohol (at least one drink in the past 30 days, including binge and heavy use). Of that same population, 20.5% participated in binge drinking (five or more drinks on the same occasion at least once in the 30 days prior to survey, including heavy use). Finally, heavy drinking (five or more drinks on the same occasion on at least 5 different days in the past 30 days) was reported by 5.7% of the population aged 12 or older, or 12.9 million people. Although several drugs have been widely available for some years, the options for effective long-term treatment of alcohol abuse are limited. For example, the long-term efficacy of these agents is poor, although the optimal use of pharmacotherapy in relation to psychosocial treatment remains to be clarified. In addition, the safety of long-term use remains to be determined. Currently available agents have both gastrointestinal and central nervous system side effects and may cause liver or kidney toxicity. Useful efficacy measures include continuous quit rate and quit rate at the end of treatment, relapse prevention, decreased alcohol consumption (number of drinking days or number of drinks) and effect on craving. Although the development of pharmacological agents that successfully surmount these issues would resolve important aspects in the treatment of alcohol abuse, one additional problem that can elude direct intervention is treatment compliance.

Effect of alcohol on dopamine function in the mesolimbic reward system

Alcohol and other drugs that are voluntarily self-administered by laboratory animals have the commonality of enhancing basal neuronal firing and/or basal neurotransmitter release in the mesolimbic dopamine system, which originates from the ventral tegmental area and innervates various limbic and telencephalic structures. Evidence for a role of dopamine in the reinforcing actions of ethanol has been provided by studies showing that the microinjection of ethanol into the ventral tegmental area promotes ethanol self-administration. In addition, ethanol- or alcohol-related cues can increase dopamine release in the nucleus accumbens (NAc) of rats previously trained to self-administer ethanol. Finally, administration of dopamine agonists or antagonists into the ventral tegmental area or the NAc can alter ethanol-reinforced responding (reviewed in ref. 9). The administration of rewarding doses of alcohol preferentially increases dopamine levels in

the NAc compared with the dorsal striatum (10). Furthermore, alcohol can produce a dose-dependent increase in the firing rate of A10 dopamine neurons located in the ventral tegmental area, whereas activation of A9 dopamine neurons in the substantia nigra pars compacta requires doses of alcohol that are fivefold higher (11). Congruent with these findings, the alcohol-induced enhancing effects on dopamine have been recently linked to actions at dopamine cell bodies in the ventral tegmental area rather than at dopamine terminals in the NAc (12, 13). Interestingly, selective lesioning of dopamine neurons of the NAc with 6-hydroxydopamine (6-OHDA in the presence of desipramine) consistently shows that dopamine denervation of this region does not interfere with ethanol consumption or maintenance of ethanol-reinforced responding (14, 15). Furthermore, sustained increases in dopamine levels in the NAc by a selective dopamine reuptake inhibitor fail to alter ethanol self-administration (16). The reasons for the discrepancies between the direct effects of alcohol on dopamine transmission and the outcome of lesion studies remain unknown. It is possible that compensatory adaptations occurring after 6-OHDA lesioning may substitute for the loss of dopamine input to the NAc. Such compensatory mechanisms would then be sufficient to support ethanol-reinforced behavior *per se*.

There is evidence in humans indicating that intoxicating doses of ethanol can affect dopaminergic mechanisms. Recent neuroimaging studies (17) have shown that an acute oral dose of alcohol can produce a significant decrease in [^{11}C]raclopride binding potential bilaterally in the ventral striatum/NAc, indicative of increased extracellular levels of dopamine. In the same study, the magnitude of the change in [^{11}C]raclopride binding was associated with alcohol-induced increases in heart rate and with impulsiveness as assessed by the Tridimensional Personality Questionnaire (TPQ) (18). Thus, the selective increase in dopamine release in the human NAc following the oral ingestion of an intoxicating dose of alcohol matches the results from preclinical studies and seems to suggest that there is a relationship between enhanced dopamine neurotransmission into the NAc and the addictive properties of alcohol. Furthermore, the association between this increased dopamine transmission in the NAc and the "impulsiveness" personality trait suggests that levels of dopamine responsiveness in the NAc may account, at least in part, for the individual vulnerability to alcohol dependence.

In contrast with the acute effects of alcohol, its repeated administration typically leads to physiological adaptations (also referred to as counteradaptations) which tend to reduce the initial sedating effects produced by alcohol (19). Among these counteradaptations, it has been suggested that persistent dysregulations in dopamine function in the NAc prolong vulnerability to craving long after the acute symptoms of withdrawal have subsided. For example, withdrawal from chronic ethanol can lead to substantial decrements in dopamine neuron activity in the ventral tegmental area (20, 21) and in extracellular NAc dopamine levels (22, 23). Thus, these results indicate that, in contrast to the acute effects of alcohol, chronic ethanol exposure causes mesolimbic dopamine hypofunction. Such long-term neuroadaptations in dopamine systems may account, at least in part, for the high rate of relapse to alcohol-taking and -seeking behaviors even years after alcohol drinking has stopped (19). In fact, deficits in dopamine transmission in animals with a history of dependence seem to be long lasting, as suggested by reduced activity of dopamine neurons in the ventral tegmental area 3 days after withdrawal (24). Similarly, decreased dopamine release in the NAc is present as long as 2 months after ethanol withdrawal (25). Furthermore, a slow rate of recovery of dopamine receptor function predicts relapse and poor treatment outcome in clinical settings (26).

Altogether, these findings suggest that mesolimbic dopamine neurotransmission mediates, at least in part, the positive reinforcing properties of ethanol. Furthermore, counteradaptive changes in mesolimbic dopamine function may be the substrate for the negative reinforcing aspects of ethanol associated with both the acute and protracted withdrawal from ethanol.

Localization of the dopamine D_3 receptor: Relevance to the mesolimbic dopamine systems and the rewarding properties of alcohol

In contrast with dopamine D_1 and D_2 receptors, dopamine D_3 receptors in the rat brain are expressed preferentially in medium-sized spiny neurons of the rostral and ventromedial shell of the NAc and in granule cells of the islands of Calleja, regions in which dopamine D_2 receptors are scarcely expressed (4, 27–32). High densities of dopamine D_3 receptors are also observed in the medial and ventral lateral geniculate nuclei, the mammillary nucleus, the magnocellular preoptic nucleus, the lateral sub-

stantia nigra pars compacta, the dorsal cochlear nuclei, the Purkinje cell layer of the vestibulocerebellum, the paracentral thalamic nucleus, the bed nucleus of the stria terminalis and the vertical limb of the diagonal band of Broca (27, 29). Only moderate levels of D_3 mRNA are detected in the amygdala, the ventral pallidum, the thalamic and hypothalamic nuclei, the superior colliculus, the inferior olivary nucleus and the nucleus of the horizontal limb of the diagonal band of Broca (27, 29).

In the human brain, dopamine D_3 receptor mRNA is expressed in high levels in the islands of Calleja, nucleus accumbens, dentate gyrus and cortex (31, 33–35). Low to moderate densities are also present in the caudate-putamen, anterior and medial thalamic nuclei, amygdala, hippocampal CA region, anterior cingulate cortex, subcallosal gyrus, lateral geniculate body, substantia nigra pars compacta, locus coeruleus and median raphe (31, 33–35).

Expression studies clearly demonstrate that the dopamine D_3 receptor is expressed in brain regions that play a key role in the rewarding effects of drugs of abuse. For example, the density of dopamine D_3 receptors is elevated one- to threefold in the NAc and ventromedial subregions of the caudate-putamen in the brains of cocaine overdose fatalities compared with both age-matched, drug-free control subjects and cocaine overdose victims presenting preterminal excited delirium (36, 37). Furthermore, the expression of dopamine D_3 receptor mRNA in the human NAc is increased sixfold in cocaine overdose victims (38), although another study reported no significant changes in human cocaine abusers (39). It is also worth noting that a human postmortem study comparing dopamine receptor density between smokers and nonsmokers revealed no significant differences in dopamine D_3 receptor (40). In preclinical models, it was shown that dopamine D_3 receptor binding and D_3 receptor mRNA are both increased in cocaine-related cue-induced conditioned locomotion (41). In addition, nicotine-induced conditioned locomotion (42) and nicotine-induced behavioral sensitization (43) were shown to produce a significant increase in D_3 receptor binding and mRNA levels in the shell of the NAc, without any alterations in either D_1 or D_2 receptor mRNA in the shell and core subregions of the NAc. Finally, the twice-daily administration of morphine over 8 consecutive days with an escalating dosing regimen starting at 10 mg/kg was recently shown to produce a significant increase in D_3 receptor mRNA in the caudate-putamen and ventral midbrain, including the substantia nigra and ventral tegmental area (44).

Taken together, these findings suggest that repeated exposure to either cocaine or nicotine may be associated with adaptive changes in dopamine D_3 receptors in key components of the mesolimbic dopamine system. Currently, however, no studies have examined the effect of prolonged or chronic ethanol administration on dopamine D_3 receptor number or mRNA levels.

Gene polymorphisms at the dopamine D_3 receptor and alcoholism

Alcoholism is a multifactorial disorder whose etiology can be found in both genetic and environmental factors. Recent molecular genetic approaches have made possible the identification of genetic variations that are associated with alcoholism. The mesolimbic dopamine system has been associated with novelty-seeking behavior (45, 46), a personality trait that may represent a risk factor for alcoholism.

In humans, a point mutation in the dopamine D_3 receptor gene, which consists of the substitution of a glycine (allele A2) for serine (allele A1) residue in the extracellular receptor *N*-terminal domain and results in the creation of a *Ba*/I restriction endonuclease site, has been reported (47). The *Ba*/I polymorphism of the dopamine D_3 receptor gene seems to be associated with alcoholism (48, 49). However, the great majority of studies failed to find a significant association between dopamine D_3 receptor gene variants and treatment outcome in alcohol dependence (50–57) and cocaine dependence (58). This also holds true for the low incidence of the *Ba*/I polymorphism of the dopamine D_3 receptor in the genetic risk associated with heroin abuse (59). Interestingly, however, an association between the *Ba*/I polymorphism of the dopamine D_3 receptor gene and measures of impulsiveness (60) and novelty seeking (49) has been found.

Most of the genetic studies reviewed above revealed either negative results or associations that are too weak to be vulnerability or risk factors for alcoholism. Polymorphism at the dopamine D_3 receptor gene does not seem to influence the risk of alcoholism directly, but rather appears to influence certain personality traits such as novelty seeking or impulsiveness, as assessed by scores obtained from the TPQ or the Eysenck's EIQ test, respectively. Importantly, the personality trait of novelty seeking, of which impulsiveness is one component, has been linked to dopamine function and addictive propensity (61). Thus, a high score on the nov-

elty-seeking scale of the TPQ is not only associated with polymorphism at the dopamine D_3 receptor gene, but also predicts later alcoholism (61) and relapse rate in detoxified alcoholics (62). Furthermore, amphetamine-induced dopamine release in the NAc also correlates with novelty-seeking scores on the TPQ (63). Thus, these findings indicate that there is a relationship between polymorphism at the dopamine D_3 receptor gene, novelty seeking, impulsiveness and dopamine release in the NAc. Clearly, additional studies and replications are needed in order to reveal the relevance of genetic variants in the dopamine D_3 receptor gene for novelty seeking and impulsiveness, which in turn may serve as vulnerability factors for alcoholism. Furthermore, clustering patients suffering from alcoholism in different subtypes according to biological and sociocultural criteria could lead to a multivariate typology of alcoholism that may refine the search for most relevant genes.

Functional pharmacological studies with dopamine D_3 ligands and animal models of alcohol-taking and -seeking behaviors

Nonselective dopamine receptor antagonists such as haloperidol, SCH 23390 and tiapride have been shown to attenuate low-dose alcohol-induced hyperactivity (64), to reduce alcohol-induced conditioned flavor (65) or to increase abstinence and/or attenuate measures of alcohol- and cue-induced craving in humans (66–68). However, the aforementioned dopamine receptor antagonists also have the potential to induce long-term neurological side-effects such as extrapyramidal syndrome, tardive dyskinesia and idiosyncratic reactions like neuroleptic malignant syndrome (69). Furthermore, tiapride has been reported to lower the seizure threshold and may therefore be associated with convulsions in recently abstinent alcoholics (70).

In the rat, dopamine D_3 receptors are implicated in the modulation of cocaine-seeking behavior and cocaine-induced conditioned hyperactivity (41, 71). However, an understanding of the precise role of the dopamine D_3 receptor in drug dependence processes has been significantly hampered by the lack of pharmacological tools showing significant selectivity for dopamine D_3 over D_2 receptors. For example, it has been reported that rats' lever pressing for 10% ethanol decreases following the administration of a dose of 7-OH-DPAT that activates D_3 receptors. Furthermore, this study reported a significant correlation between the *in vitro* D_3 receptor potencies of various dopamine receptor agonists (assessed by stimulation of mitogenesis in CHO transfected with D_2 receptors and NG-108-15 cells expressing D_3 receptors) and their potency in decreasing oral ethanol self-administration *in vivo* (72). However, a number of factors may confound the interpretation of the results. For example, this study did not examine the effect of a selective dopamine D_3 receptor antagonist on the decrease in oral ethanol self-administration produced by the various dopamine receptor agonists. Furthermore, the R-(+) isomer of 7-OH-DPAT can induce amnesic effects in mice (73), and low doses of 7-OH-DPAT can produce conditioned place aversion (74). Although the dopamine agonists used did not significantly affect responding to water, the response rate was low and variable. Furthermore, the correlation with the potency at dopamine D_3 receptors in preparations that are not derived from normal brain tissue presents a number of interpretation problems. Finally, our preliminary data indicate that the highly selective D_3 receptor antagonist SB-277011A (see below) significantly attenuated ethanol self-administration in mice and rats, suggesting that agonism of the D_3 receptor is not required to decrease alcohol self-administration. It has also been reported that 7-OH-DPAT decreases ethanol self-administration in a two-bottle-choice procedure (75) and can also decrease alcohol consumption in rats in the dynamic phase of development of alcohol preference and in a drug discrimination paradigm. However, the same studies also showed that 7-OH-DPAT did not significantly alter the preference for a 3% solution of ethanol in rats that exhibited an established and stable preference (76). The dopamine D_3-preferring antagonist U-99194A can enhance the acquisition of ethanol preference in a mouse place conditioning paradigm (77, 78). However, this compound has only a 10-fold selectivity for dopamine D_3 over D_2 receptors (79, 80). Furthermore, the locomotor stimulating effects of both *l*-nafadotride and U-99194A have been observed in dopamine D_3 knockout mice, indicating that the stimulant properties of both compounds are unrelated to their D_3 receptor blocking properties (81). Interestingly, mice lacking the D_3 receptor showed significantly greater withdrawal signs following termination of chronic ethanol treatment (7%) compared to wild-type mice (82), and they also showed a decrease in ethanol intake. However, the withdrawal scores were significantly elevated only 3 and 6 hours after withdrawal. Furthermore, the interpretation of data from

knockout mice is complicated by the fact that compensatory changes, as well as various deficits, may occur in the absence of the dopamine D_3 receptor. For example, mice lacking D_3 receptors have spatial working memory deficits (83).

In contrast with most dopamine D_3 antagonists developed so far (79, 80, 84), the dopamine D_3 receptor antagonist SB-277011-A (trans-N-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide) shows high affinity and 100-fold selectivity for D_3 over D_2 receptors and ion channels (85, 86). Recently, SB-277011-A has shown efficacy against both cocaine- and nicotine-seeking behaviors in the rat (87–89). Efficacy of SB-277011-A has also been demonstrated against heroin-induced conditioned place preference in the rat (90). In preliminary studies (91), we recently investigated the effect of SB-277011-A on the intake of ethanol in alcohol-preferring and alcohol-nonpreferring rats using the two-bottle-choice (water and 15% v/v ethanol) self-administration paradigm. A single acute intraperitoneal administration of SB-277011-A (3 mg/kg) failed to alter ethanol intake in alcohol-preferring rats compared with vehicle-treated rats. However, the acute administration of higher intraperitoneal doses of SB-277011-A (10–30 mg/kg) produced a significant decrease in ethanol intake, percent ethanol preference and cumulative ethanol lick responses in alcohol-preferring rats. The highest dose of SB-277011-A (30 mg/kg) also significantly decreased the intake of ethanol in alcohol-nonpreferring rats. We also examined the effect of a single acute intraperitoneal administration of SB-277011-A (10, 20 and 30 mg/kg) and its vehicle, hydroxypropyl-beta-cyclodextrin, on oral ethanol self-administration in adult male C57BL/6N mice (92). Animals were initially trained to lever-press for water using an FR-1 schedule of reinforcement. Following stable responding, water was removed and substituted with increasing ethanol solutions (1%, 2%, 4% and 8% w/v). The ethanol concentration was maintained at 8% until mice reached a stable performance. Mice were then shifted to an FR-2 schedule. They were allowed to stabilize their intake of 8% ethanol for 15 days and meet an established criterion for ethanol intake. The acute administration of either 10 or 20 mg/kg of SB-277011-A did not significantly alter oral alcohol self-administration compared with vehicle-treated animals. However, 30 mg/kg of SB-277011-A significantly decreased both the number of reinforcements (71%) and the amount of ethanol consumed (72%) compared with vehicle-treated animals. The latter effects were significantly higher than those observed following treatment with reference compounds such as naltrexone, acamprosate and baclofen, previously shown to increase rates of abstinence and to decrease relapse rates in alcohol-dependent individuals who are in abstinence-oriented programs.

Altogether, these findings suggest that although dopamine D_1/D_5 or D_2/D_3 receptor antagonists may increase abstinence and/or attenuate measures of alcohol- and cue-induced craving in animals and humans, they also have side-effect liabilities that do not permit their use in daily clinical work. In contrast with dopamine D_2/D_3 antagonists, it has been shown that SB-277011-A does not produce any significant effect on spontaneous locomotion or on amphetamine- or phencyclidine-induced hyperactivity (85). SB-277011-A does not produce catalepsy when administered in doses of up to 78.8 mg/kg (85). Furthermore, again in contrast with dopamine D_2 receptor antagonists, SB-277011-A does not increase serum prolactin levels (85). The systemic administration of SB-277011-A does not produce a conditioned place preference response or reinstatement of cocaine use (89), and it does not maintain cocaine self-administration when substituted for cocaine in an FR-10 or PR schedule of reinforcement in adult male rats (93). Finally, SB-277011A does not produce conditioned place aversion (86), in contrast to the dopamine D_3 agonists 7-OH-DPAT and PD-128907 and the partial D_3 agonist BP-897 (90). Thus, these results support the suggestion that SB-277011-A can diminish alcohol-taking and-seeking behaviors through a primary action at dopamine D_3 receptors, while avoiding significant side effects.

Conclusions

The NAc has been the framework of theories exploring the chemoarchitectural substrates of reward and motivation, including aspects of drug addiction, because it has a prominent position in the ventral striatum and is one of the main targets of the mesolimbic dopamine system. As such, mesolimbic dopamine neurotransmission mediates, at least in part, the positive reinforcing properties of several drugs of abuse, including ethanol. Counteradaptive changes in the same mesolimbic dopamine system may also be the substrate for the negative reinforcing aspects of ethanol associated with both the acute and protracted withdrawal from etha-

nol. In the present review, we have shown that repeated exposure to drugs of abuse such as cocaine or nicotine is associated with adaptive changes in the dopamine D_3 receptor in the NAc and that there is a relationship between polymorphism at the dopamine D_3 receptor gene, novelty seeking, impulsiveness and dopamine release in the NAc in response to ethanol. Although significant neuroadaptive changes in the dopamine D_3 receptor have not yet been reported following the repeated exposure to ethanol, it is possible that levels of dopamine responsiveness and the status of the dopamine D_3 receptors in the NAc partially account for the individual vulnerability to alcohol dependence. Pharmacological studies aiming at investigating the role of dopamine D_3 receptors in alcohol dependence have been limited by the lack of selective compounds with significant selectivity for dopamine D_3 over D_2 receptors. However, recent studies clearly show that selective antagonism at dopamine D_3 receptors can significantly reduce alcohol-seeking and -taking behaviors in rodents. If these findings can be extended to humans, this may represent a potential new pharmacotherapeutic strategy devoid of the typical neurological side effects associated with dopamine D_2 receptor antagonists.

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