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Pharmacology, Biochemistry and Behavior 75 (2003) 1–7
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Lack of effect of $5HT_3$ antagonist in mediating subjective and behavioral responses to cotinine

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Received 22 July 2002; received in revised form 3 January 2003; accepted 22 January 2003

Abstract

Previous studies have shown that cotinine, a metabolite of nicotine, may antagonize some of the therapeutic effects of nicotine. The mechanisms underlying cotinine's effects are unclear, but cotinine has been observed to increase serotonin levels in the brain. Thus, it is possible that blocking serotonin effects may antagonize the actions of cotinine, thereby reducing its impact on responses to nicotine. This study determined whether granisetron, a $5HT_3$ receptor antagonist, would enhance the efficacy of the nicotine patch. Subjects were randomly assigned to one of the three granisetron conditions ($N=43$ for 2 mg/day; $N=43$ for 1 mg/day; $N=42$ for 0 mg/day) and asked to take the assigned medication daily during 15 days of tobacco abstinence. Because we were interested in interactions between cotinine and serotonin, all groups were also treated with a 21-mg nicotine patch. Assessments of withdrawal symptoms were made for 1 week during baseline smoking and several times during the experimental period. There was a near but nonsignificant difference among groups on a measure of tobacco withdrawal and no significant differences on global measures of drug effects or physiological measures. The data do not strongly support the hypothesis that $5HT_3$ agonism is the mechanism by which cotinine offsets the effects of nicotine.

Keywords: Nicotine withdrawal; $5HT_3$ antagonists; Nicotine patch; cotinine

1. Introduction

Recent human studies have suggested that cotinine, a metabolite of nicotine, may have effects opposite of nicotine or antagonize the effects of nicotine. In one study, oral cotinine fumarate in doses up to 200 mg dose-dependently increased ratings of restlessness and impatience among abstinent smokers (Schuh et al., 1996). In another study using a 2×2 placebo-controlled design with one factor associated with placebo vs. 80 mg of cotinine fumarate and the other factor nicotine vs. placebo patch, 80 mg of cotinine blocked the effects of the nicotine patch in reducing withdrawal symptoms (Hatsukami et al., 1998b). In a third study, administration of a high dose of cotinine fumarate (160 mg) increased serum nicotine blood levels compared to placebo or a low dose of cotinine during ad lib smoking, suggesting the occurrence of compensatory smoking resulting from antagonist activity (Hatsukami et al., 1998a). Moreover, studies conducted in laboratory animals have shown that cotinine blocks some of the physiological and hormonal biosynthesis responses from nicotine (Chahine et al., 1996, 1990; Kim et al., 1968).

High levels of cotinine are necessary to achieve antagonist or effects opposite of nicotine. For example, the cotinine levels attained during the nicotine patch and 80 mg cotinine study were three to four times higher than that attained during ad lib smoking. Similarly, the levels of cotinine attained to observe antagonist effects on smoking behavior were around nine times higher than observed during ad lib smoking. Nonetheless, further study is warranted even though the venous levels of cotinine observed in these studies are greater than levels observed during ad lib smoking. Brain levels of cotinine achieved during smoking

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0091-3057/03/$ – see front matter © 2003 Published by Elsevier Science Inc.
doi:10.1016/S0091-3057(03)00035-2

are likely to be significantly higher than reflected by venous levels of cotinine. Animal studies have suggested that high concentrations in the brain may be achieved with chronic smoking since cotinine does not appear to be metabolized in the brain and shows slower outflow to the periphery relative to nicotine (Crooks et al., 1997; Crooks and Dwoskin, 1997). As a result, cotinine, even at venous levels attained through the nicotine patch, may limit the efficacy of the nicotine patch in promoting abstinence from smoking. Conceivably, if the effects of cotinine could be minimized or blocked, the nicotine patch may be more efficacious.

Little is known about the mechanisms by which cotinine may attenuate responses to nicotine. As a first step in evaluating these mechanisms, the present study explored interactions between cotinine and serotonin. Several studies support the notion that serotonin may mediate, at least in part, the effects of cotinine (DeClercq and Truhaut, 1963; Essman, 1973; Fuxe et al., 1979). In one study, chronic oral cotinine administration in rats produced significant increases in daily urinary excretion of the major serotonin metabolite, 5-hyroxyindoleacetic acid (5-HIAA; DeClercq and Truhaut, 1963). Essman (1973) found elevated serotonin and 5-HIAA levels in the mesencephalon and diencephalon in the rat without significantly affecting activity in the cerebral cortex. In another study, cotinine was found to release serotonin in rat brain and to weakly inhibit serotonin uptake, thereby increasing synaptic serotonin levels (Fuxe et al., 1979). Taken together, these studies suggest that cotinine's effects may be mediated by serotonergic neurotransmission.

Based on these findings, we hypothesized that blocking 5HT neurotransmission would attenuate the effects of cotinine on responses to nicotine. If the effects of cotinine were antagonized, the efficacy of the nicotine patch should be increased. To evaluate this issue, this study examined the effects of a $5HT_3$ antagonist (granisetron) in combination with the nicotine patch, on nicotine withdrawal symptoms in humans. A $5HT_3$ antagonist was chosen for several reasons. First, few medications are available that selectively target specific serotonin receptors, except for $5HT_3$ antagonists. For example, ondansetron, granisetron, and tropisetron are reported to have a selectivity ratio of 1000:1 for the $5HT_3$ receptor compared to any other serotonin receptor subtypes and dopamine and α_1 and μ opioid receptors (Freeman et al., 1992). Therefore, as a first step in understanding the role of serotonin in mediating the effects of cotinine, the $5HT_3$ receptor site was targeted. Second, a $5HT_3$ antagonist, ondansetron, has been found to be effective in reducing other addictive behaviors such as alcohol use (Sellers et al., 1994), particularly among early age of onset alcoholics (Johnson et al., 2000) and bulimic episodes (Faris et al., 2000). Third, although studies with humans indicate that $5HT_3$ antagonists do not reliably alter the reinforcing and behavioral effects of nicotine per se (e.g., Arnold et al., 1995; Corrigall and Coen, 1994; Zacny et al., 1993), studies with animals suggest that these drugs may attenuate the effects of nicotine withdrawal (Costall et al., 1990; Suzuki et al., 1997). Prior studies show that $5HT_3$ antagonists release suppressed behavior in animal models of anxiety, enhance cognitive performance, and modulate appetite (Barnes et al., 1992; Costall et al., 1990). Given these effects, it is conceivable that $5HT_3$ may modify withdrawal symptomatology indirectly by affecting mood or cognition. Studies in humans have found no effect of the $5HT_3$ antagonist alone in reducing tobacco withdrawal symptoms (West and Hajek, 1996), but perhaps greater attention to withdrawal symptoms that may be mediated by the serotonergic system, such as mood and appetite, is necessary. Furthermore, this pattern of results parallels those observed from our cotinine studies (Hatsukami et al., 1998a,b). That is, cotinine had minimal effect on nicotine self-administration or withdrawal, but modulated nicotine withdrawal symptoms only when given in conjunction with the nicotine patch. Therefore, the examination of a combination of the nicotine patch and $5HT_3$ antagonist was particularly important.

The goal of this study was to characterize the dose effects of granisetron in combination with the nicotine patch on tobacco withdrawal symptoms. Although most human studies have used ondansetron as the $5HT_3$ antagonist, granisetron was chosen because in a prior pilot study we conducted, ondansetron produced an unacceptably high prevalence of constipation as a side effect. Moreover, although granisetron and ondansetron have fairly similar receptor profiles (Freeman et al., 1992), granisetron is longer acting (Upward et al., 1990; Roila and Del Favero, 1995) and has better penetration into the brain than ondansetron (Simpson et al., 1992). We therefore felt that granisetron was a more appropriate choice for this study.

2. Materials and methods

2.1. Subjects

Males and females between the ages of 18 and 55 years were recruited with newspaper advertisements soliciting people who were interested in participating in a study on new smoking cessation medications. Interested cigarette smokers telephoned the clinic and were asked to answer a brief tobacco use history and medical screening questionnaire. Individuals who passed the initial screening came to the clinic for a full screening evaluation. After obtaining informed consent, subjects completed several comprehensive demographic history and tobacco use questionnaires (including the Fagerstrom Test for Nicotine Dependence [FTND], a measure of level of dependence; Heatherton et al., 1991) and underwent a complete physical examination, which also involved an electrocardiogram (EKG), routine laboratory screening, and a serum pregnancy screen for women. Subjects met inclusion criteria for the study if they smoked between 20 and 49 cigarettes daily for at least 1 year, had a history of experiencing DSM-IV criteria (American Psychiatric Association, 1994) for nicotine withdrawal

syndrome, were in good physical and mental health, did not use any forms of psychotropic medications (antidepressants, antipsychotics, or anxiolytics), or any other tobacco or nicotine products.

2.2. Procedure

Subjects were assessed during 1 week of ad lib smoking and 2 weeks of abstinence. All subjects used the active nicotine patch, under open-label conditions, on a daily basis during the abstinence period. They were also randomly assigned, under double-blind conditions, to either placebo b.i.d., 1 mg granisetron b.i.d. (1 mg, morning, and placebo, afternoon) or 2 mg of granisetron b.i.d. (1 mg, morning and afternoon). These doses are known to be pharmacologically active based on clinical data from patients who are treated for chemotherapy-induced nausea and emesis (Physicians' Desk Reference, 2002). SmithKlineBeecham (now Glaxo-SmithKline) supplied the granisetron and matching placebo pills, as well as the 21-mg 24-h nicotine patch (Nicoderm). No granisetron-only condition was included in the study design because prior research has suggested that the effects of cotinine on smoking withdrawal symptoms are only evident in the presence of nicotine.

During week 1, subjects were required to come into the laboratory two times (Monday and Thursday) for assessment during baseline ad lib smoking. On the second visit of the first week, subjects were given the assigned medications. Subjects were carefully and thoroughly instructed on how to use the medications (e.g., 8 h between pill doses) and told to quit smoking and begin using the medications on Monday morning of the following week. Subjects received a telephone call on Monday to verify that they started the medications. Subjects were seen three times during the second week (Tuesday, Wednesday, and Friday), two times during the third week (Monday and Thursday), and had a final visit during the fourth week (Monday). The periods of assessment during abstinence and medication use were therefore 2, 3, 5, 8, 11, and 15 days post-quit. During each of these visits, measurements were obtained. All sessions were held in the late afternoon or early evening, with the time of the visits consistent within subjects. Each subject was given brief, standardized individual counseling (<10 min) regarding potential high-risk situations and ways to deal with them. No specific instructions were given to the subject on methods to handle withdrawal symptoms, nor were withdrawal symptoms discussed. The research counselors were fully trained in adherence to the study protocol, in addition to being experienced in smoking cessation counseling. In order to ensure the integrity of the behavioral counseling intervention, the counselor completed a checklist for each session.

At discharge from the study, subjects underwent a physical examination. In addition, all subjects were asked whether they thought they were randomized to active or placebo medication. Subjects were encouraged to continue using the nicotine patch on their own, if deemed necessary. Follow-ups were conducted by telephone at 7, 14, and 30 days posttreatment with offers of smoking cessation treatment referrals if needed.

This study was reviewed and approved by the Institutional Review Board at the University of Minnesota.

2.3. Outcome measures

Tobacco withdrawal symptoms were measured with two scales. The Minnesota Nicotine Withdrawal Scale (MNWS; Hughes and Hatsukami, 1986, 1998) is comprised of DSM-IV nicotine withdrawal symptoms and craving for cigarettes (American Psychiatric Association, 1994), which are rated on a 0–4 scale with 0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe (maximum score = 32). The Questionnaire on Smoking Urges (Tiffany and Drobes, 1991) measures two factors: Factor 1 reflecting intention, desire, and anticipation to smoke, and Factor 2 reflecting anticipation or relief from negative affect, nicotine withdrawal, and an urge or overwhelming desire to smoke. Other outcome measures included a Drug Effects Scale that assesses subjective responses on a 100-mm visual analogue scale to items measuring any, good or bad drug effects from the study drug, liking and desire for study drug, and effectiveness of the drug. An adverse events scale was completed to assess for nicotine toxicity symptoms (Keenan et al., 1994; Hatsukami et al., 1997) and other symptoms related to side effects from granisetron (e.g., constipation) and nicotine patch (e.g., erythema). In addition, tobacco use status was determined by a Tobacco Use Questionnaire, which inquired about any use of tobacco since the last visit, and by the daily tobacco use diary cards. These reports were verified by carbon monoxide (CO) samples. Levels of <8 ppm were considered confirmation of self-reported abstinence. The physiological measures included weight, sitting heart rate and blood pressure. All these measure were taken at each visit.

2.4. Drug accountability

Compliance with medication use was assessed by self-report on a daily diary. Pills were dispensed in containers that held the morning and afternoon doses separately for each day. A pill count was undertaken at each visit during the medication phase. Patch counts were also undertaken to verify daily use. Subjects were asked to return used and unused patches. Furthermore, serum and saliva samples were obtained twice during baseline and at 3, 8, and 15 days post-quit. The saliva samples were analyzed for nicotine and cotinine levels. Only the serum sample from day 8 was analyzed for granisetron to verify use of the oral medication.

2.5. Subject payment

Subjects were paid for compliance with abstinence because it was absolutely crucial to maintain abstinence in

order to reliably observe withdrawal symptoms. Subjects were paid $10.00 each time their carbon monoxide level was < 8 ppm, and a $90 bonus for compliance with the experimental procedures and total abstinence, for a potential total payment of $150.

2.6. Data analyses

Compliance with smoking abstinence instructions was determined for each group. Compliance was defined as CO < 8 at each clinic visit and self-report of five or fewer cigarette smoked. A previous study (Hughes and Hatsukami, 1986) would strongly suggest that withdrawal symptoms among individuals who provide a CO ≤ 8 ppm and self-report smoking five or fewer cigarettes are not significantly different from the withdrawal symptoms among individuals who provide a CO ≤ 8 and self-report no smoking. Because there were only three additional individuals when using the first criteria, we recorded those subjects as compliant.

Data were analyzed for study completion, patch and oral medication compliance, and protocol compliance (attendance at all visits and CO < 8 ppm). Fisher's exact test was used to make treatment comparisons on these measures. Levels of cotinine and granisetron were analyzed across groups to check for compliance with medication use.

Repeated-measures analysis of variance (RMAOV) was used to investigate the effects of granisetron administration on nicotine withdrawal symptoms. This analysis used treatment level as the between-subject factor along with other covariates (initial weight, age, gender, FTND score, compliance with patch administration, compliance with oral medication administration, compliance with both patch and oral medication administration, and compliance with smoking abstinence). Time was the within-subject factor. *F* tests were applied to judge the significance of treatment and time effects. Because regression analysis produced results similar to the RMAOV, only the RMAOV results are reported here. The primary analysis examined the total score for the MNWS and the factor scores from the Questionnaire on Smoking Urges. Similar methods were used to investigate the effects of granisetron administration on other subjective outcome measures (scores from the Drug Effects Scale) and physiological measures (blood pressure, heart rate, weight).

3. Results

There were 1129 subjects who called to inquire about the study, of whom 672 were ineligible. Fifty-one percent of the subjects who met study criteria failed to come to the orientation session. Of the 164 subjects who were screened at the clinic, 128 subjects were randomized to treatment. (*N*=43 for 2 mg/day; *N*=43 for 1 mg/day; *N*=42 for 0 mg/day).

3.1. Demographics/baseline characteristics of the subjects randomized in the study

Randomized subjects were a mean age (S.D.) of 40.3 (8.3) years old. The study sample was 93.7% Caucasian, 4.7% African American, and 1.6% other. More than half the subjects (62.5%) were female. At baseline, the mean (S.D.) number of cigarettes smoked per day was 24.7 (8.1), the mean number of years of regular smoking was 22.9 (8.7), and the mean score on the FTND was 5.0 (1.5). No significant differences were observed on these variables across groups.

3.2. Compliance rates

Of the 128 subjects randomized to treatment, 120 subjects took the assigned medications and 112 subjects attended the last visit. Among all subjects who began taking the medication, the mean number of days the patch was applied was 13.9 (3.4) out of 15 and mean number of pills that were ingested was 27.1 (7.2) out of 30. No significant differences were observed across the treatment groups. Of all the subjects randomized to treatment, 63% were considered protocol compliant (e.g., attending all visits with CO < 8 ppm). No significant differences were observed across treatments (67% in the 2-mg group, 51% in the 1-mg group, and 69% in the placebo group).

3.3. Nicotine/cotinine and granisetron levels

An analysis of variance model at day 8 showed that serum granisetron concentrations did not change significantly based on cotinine levels at any dose (*P*=.538). Granisetron levels were analyzed on the log scale to satisfy normality and constant variance assumptions. There was a significant difference between the 2-mg and 1-mg granisetron groups based on a *t*-test (*P*=.006). Both the 2-mg and 1-mg granisetron groups were also significantly different from the level of detection based on one-sample *t*-tests (*P*<0.0001). The 2-mg group had a mean granisetron level of 3.46 ng/ml (S.D.=3.48) and the 1-mg group had a lower mean value of 1.96 ng/ml (S.D.=1.97). No significant differences were observed for levels of cotinine across the treatment conditions (2-mg group, mean = 262.34 ng/ml, S.D. = 75.23; 1-mg group, mean = 239.72 ng/ml, S.D. = 87.36; placebo group, mean = 259.19 ng/ml, S.D. = 94.01).

3.4. Minnesota Nicotine Withdrawal Scale

Baseline and treatment phases were analyzed separately. Average baseline MNWS score was added as a covariate when analyzing active treatment visits. The overall RMAOV showed no difference across the treatment groups with regard to baseline phase (*P*=.541) and a marginal difference during the treatment phase (*P*=.077; see Fig. 1).

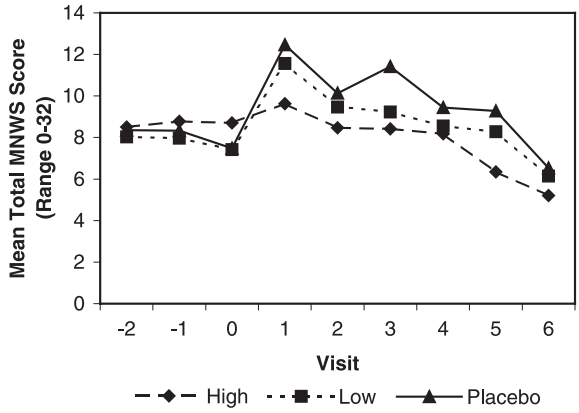

Fig. 1. Mean total Minnesota Nicotine Withdrawal Scale (MNWS) by treatment condition.

However, as expected, there was a significant time effect over the six treatment measurement days [$F(5,108)=17.57$, $P<.0001$], with total withdrawal symptoms increasing immediately after abstinence and decreasing thereafter. When examining individual withdrawal symptoms, no significant treatment effects were observed.

3.5. Smoking Urges Questionnaire

Analyses were performed on each of the two factors of the Smoking Urges Questionnaire (see Figs. 2 and 3). Comparisons of treatment groups were adjusted for the same covariates as in the analysis of the withdrawal symptoms. Average baseline factor score was incorporated as an additional covariate when analyzing active treatment visits. The overall RMAOV showed no difference across the treatment groups for Factor 1 with regard to baseline (P=.062) or during treatment (P=.699). There was a significant time result for Factor 1 both for baseline [$F(2,111)=23.39$, $P<.0001$] and for treatment [$F(5,107)=3.04$, P=.013]. During baseline, the score for Factor 1 significantly decreased. During treatment, there appeared to be two distinct phases of Factor 1: the first from visit 1 to visit 3 (days 2–5) and the second from visit 4 to visit 6 (days 8–15). Factor 1 significantly decreased

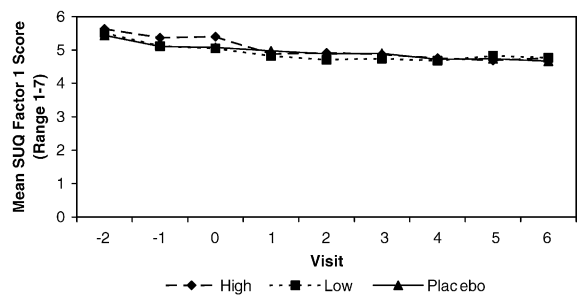

Fig. 2. Mean Smoking Urges Questionnaire (SUQ) Factor 1 by treatment condition.

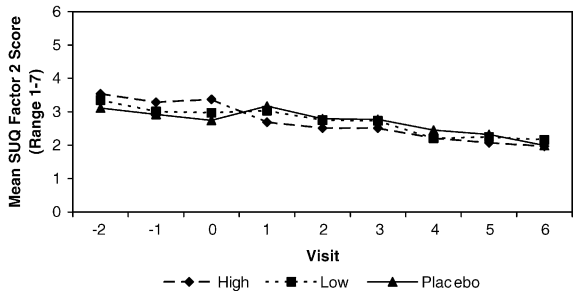

Fig. 3. Mean Smoking Urges Questionnaire (SUQ) Factor 2 by treatment condition.

between the two phases, but remained constant within each phase. The results were similar for Factor 2 with no significant treatment effect for baseline (P=.191) or during treatment (P=.191). There were again significant time effects both for baseline [$F(2,111)=7.61$, P=.0008] and for active treatment [$F(5,107)=11.21$, $P<.0001$]. For Factor 2, there was a general decrease both in the baseline and treatment phases with the treatment phase lower than the baseline phase. These results seem to indicate that neither factor score is affected by treatment, but both tend to decrease over time.

3.6. Drug effects scale

Similar analyses were conducted on each question of the drug effects scale. Neither treatment nor time effects were significant for any of the questions.

3.7. Physiological measures

No significant differences were observed for heart rate, diastolic and systolic blood pressure, or weight across treatment groups for baseline or active treatment measurements.

3.8. Adverse events

Relatively few side effects were observed. Fisher's exact test was used to look at differences in effect severity among the three treatment groups. Significant group differences were observed for constipation at day 15, with the data showing a greater number of patients in both granisetron groups with mild or moderate constipation compared to the placebo group (P=.003). No abnormal laboratory results were observed at the end of treatment and all EKGs were normal.

3.9. Integrity of the blind

At the end of treatment, subjects were asked whether they believed the medication they received was active or placebo. Subjects did not accurately identify their assigned treatment condition (P=.1289): 59.0.0 % [95% CI (42.1,74.4)] of 39

subjects on placebo thought they were on active medication and 73.0% [95% CI (61.4, 82.6)] of 74 subjects on active medication believed they were on active medication.

4. Discussion

This study was a first step in attempts to understand the underlying mechanism for the antagonistic effects of cotinine on responses to nicotine replacement. The aim of this study was to explore the effects of granisetron, a $5HT_3$ antagonist, on the efficacy of the nicotine patch in reducing smoking withdrawal symptoms. If the efficacy of the patch was increased by granisetron, this could be taken as providing some support for hypothesis that cotinine may exert antagonist effects to nicotine by enhancing serotonin. The results from this study do not support our hypothesis. Granisetron did not alter smoking withdrawal symptoms at any dose, nor did it alter any other measures examined.

Several reasons may contribute to this lack of clearly significant findings. First, cotinine may not play a significant role in antagonizing the effects of nicotine at doses that are achieved by the nicotine patch. The studies that have been conducted to date have shown antagonist activities at venous levels of cotinine that are significantly higher than the venous levels attained by smoking or the nicotine patch. To date, animal studies would suggest that smoking leads to high levels of cotinine in the brain (Crooks et al., 1997); however, no human study has examined the actual arterial levels of cotinine attained during chronic smoking. Therefore, more research needs to be conducted in this area to determine whether examining the role of cotinine in nicotine dependence is a worthwhile pursuit.

Second, to date, few studies have been conducted to elucidate the neuropharmacology of cotinine. The results of previous studies have suggested that cotinine activates the serotonin system (DeClercq and Truhaut, 1963; Essman, 1973; Fuxe et al., 1979). Another study, however, found no increases in release of serotonin in the striatum (Toth et al., 1992). Not surprisingly, cotinine also affects other neurotransmitter systems. For example, in vivo studies show that cotinine stimulates nicotinic receptors to evoke the release of dopamine (Dwoskin et al., 1999). However, a role for nicotinic mechanisms in cotinine's effects is unlikely to be clinically significant, since cotinine has low affinity for nicotinic receptors (Abood et al., 1981). Moreover, a recent study has shown the existence of distinctive cotinine and nicotine receptors in mammals (Riah et al., 2000). Given the paucity of data on cotinine's effect on neurochemistry, it is possible that its effects on withdrawal symptoms could be independent of serotonin. Future research needs to be undertaken to further and more directly explore the role of cotinine on the serotonergic system, as well as other systems, since relatively few studies have been conducted in this area.

The lack of robust significant findings in this study could be related to several methodological issues. For example, the doses of granisetron may not have been sufficiently high. This seems unlikely in view of the behavioral activity of similar doses in a therapeutic context. Even if higher doses may have been warranted, safety concerns would have limited their use. It is equally likely that the dose of the nicotine patch may not have been high enough. However, to the extent that the nicotine dose used here is clinically relevant, use of a higher dose may not have been informative. The receptor profile of granisteron may not have been appropriate to alter the effects of cotinine. As discussed above, serotonin receptors other than $5HT_3$, or receptors in other neurotransmitter systems, may play a more significant role. The near but nonsignificant results could also be due to the heterogeneity of the smoking population, and perhaps a more targeted approach or a larger sample would have led to a more robust outcome. For example, some smokers have a serotonin transporter polymorphism that interacts with the personality trait of neuroticism and affects smoking practices (Lerman et al., 2000). It is possible that a $5HT_3$ antagonist would be effective for only this subset of smokers. In a study with alcoholics, ondansetron was found to be effective only in early onset alcoholics and not late onset alcoholics (Johnson et al., 2000). Early onset alcoholics are considered more likely to possess a polymorphic variant of the serotonin transporter (Schuckit et al., 1999; Ishiguro et al., 1999).

In summary, the results suggest that actions of serotonin at $5HT_3$ receptors may not play a significant or strong role in mediating the effects of cotinine on responses to nicotine. Much research remains to be done on this topic. For example, other serotonin receptors should be explored for potential modulatory effects on cotinine or even nicotine. In addition, few studies have been conducted that take into account different biological phenotype or genotype of smokers.

Acknowledgements

This study was funded by the NIH grant P50DA09259 with statistical support from P50DA13333. GlaxoSmithKline provided the nicotine patches and granisetron.

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