関（minus0.542, p=0.017）を認めた（Fig.5）。

Multivariate stepwise linear regression analyses により後部帯状回および左右側頭葉の MRS の比の関係を調べた。後部帯状回の NAA/Cr は Logical Memory I に 18%、Logical Memory II に 20.3%、年齢に 16.3%の寄与率であった。

D. 考察

我々は、認知機能障害を有する統合失調症では後部帯状回 NAA/Cr が低下していることを見出した。その一方、左右の内側側頭葉ではこの比に健常者との差はなかった。さらに我々は NAA/Cr は健常者で加齢に伴い低下し、統合失調症患者では一貫して低値であることを見出した。健常者でみられる加齢に伴う低下が統合失調症でみられないのは、疾患に関連した、後部帯状回の神経系の病理に基くものと考えられる。今回の全被験者で見出された後部帯状回 NAA/Cr と言語記憶スコアの正の相関も、後部帯状回の NAA/Cr が言語エピソード記憶機能と関係していることを示唆している。慢性統合失調症では内側側頭葉よりも、後部帯状回の NAA/Cr 減少が言語エピソード記憶機能障害を反映しているものと思われた。

統合失調症患者での後部帯状回における NAA/Cr の低下はこれが最初の報告である。多くのプロトン MRS 研究では内側側頭葉における NAA/Cr の低下である（Nasrallah et al., 1994; Maier et al., 1995; Bertolino et al., 1996, 1998; Fukuzako et al., 1996; Yurgelun-Todd et al., 1996; Deicken et al., 1998）が、否定する結果も報告されている（Buckley et al., 1994; Heimberg et al., 1998; Delamillieure et al., 2002）。我々の内側側頭葉についての結果は後者の変化がないとするものである。この薬物療法中の統合失調症患者における NAA 変化が一定していないのは、抗精神病薬の影響の違いかもしれない。Bertolino ら（2001）は、抗精神病薬治療により、統合失調症患者では背外側前頭前野における NAA が上昇するとしている。Fannon ら(2003)は未治療患者では海馬の NAA 低下は３ヶ月の薬物療法により消失し、また Heimberg ら(1999)は抗精神病薬治療により左前頭葉の NAA が上昇すると報告している。今回の研究では 19 人中 16 人が非定型抗精神病薬を内服していた。クロールプロマジン換算で右内側側頭葉の NAA/Cr は抗精神病薬用量と有意な正の相関が見られた。このことから、抗精神病薬は内側側頭葉においては、未治療では低下している NAA/Cr を回復させるのかも知れない。

E. 結語

統合失調症患者と健常者を対象にMRS検査を行い、後部帯状回と内側側頭葉の代謝比を算出し、認知機能検査結果や年齢、抗精神病薬用量との関連を調べた。その結果、統合失調症患者では後部帯状回の NAA/Cr および認知機能に低下みられた。健常者では加齢に伴う後部帯状回の NAA/Cr が低下がみられたが、統合失調症ではそのような相関は見られなかった。また後部帯状回 NAA/Cr は言語記憶スコアの間に正の相関があった。これらから、統合失調症では後部帯状回の NAA 代謝が病的に低下し、その低下は認知機能障害にも関与している可能性が示唆された。

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G. 研究発表

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2. 学会発表

無し

H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし。

3. その他

特記すべきことなし。

平成１７年度　刊行物に関する一覧表

研究成果の刊行に関する一覧表

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Regular Article

An open trial of outpatient group therapy for bulimic disorders: Combination program of cognitive behavioral therapy with assertive training and self-esteem enhancement

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Abstract The purposes of this study were to examine the therapeutic efficacy of combined group cognitive behavioral therapy (CGCBT) and to explore the characteristics of the patients who failed to complete it. Our group cognitive behavioral therapy combined with assertiveness training for alexithymia and self-esteem enhancement therapy were attended over a 10-week period. Twenty-five participants were enrolled in the study. The clinical symptoms were assessed before and after treatment, using rating scales including the Eating Disorder Inventory-2, the Bulimic Investigatory Test, Edinburgh, the Toronto Alexithymia Scale, the Rosenberg Self-Esteem Scale, and Global Assessment of Functioning. Sixteen participants (64%) completed the CGCBT program. Completion of the CGCBT resulted in significant improvements in reducing binge-eating behavior and improving social functioning. Eight patients (32%) significantly improved using the Clinical Global Impression Change (CGI-C). Stepwise logistic regression analysis of the results indicated that a lower age ($P = 0.04$) and psychiatric comorbidity ($P = 0.06$) were predictors of dropout from the CGCBT program. Our CGCBT program is a promising first-line treatment for bulimic outpatients. Lower age and the presence of comorbidity had effects on dropout rates.

Key words alexithymia, comorbidity, dropout, eating disorder, group cognitive behavioral therapy, self-esteem.

INTRODUCTION

Eating disorders represent a major and growing problem in community health all over the world. They have been divided into three types using the *Diagnostic and Statistical Manual of Mental Disorders* fourth edition (DSM-IV):[1] bulimia nervosa (BN), anorexia nervosa binge-eating/purging type (ANB/P) as well as anorexia nervosa restricting type (ANR), and eating disorders not otherwise specified (EDnos).

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Recent studies suggested that the number of recorded eating disorder cases is increasing around the world and across a wide range of ethnicities and cultures, including Japan.[2,3] Previous studies suggested that there has been at least a sixfold increase in the number of eating disorders over the past 25 years.[4,5] From adolescence through adulthood, the presence of dysregulation in the eating behavior among Japanese women has been documented at rates comparable to the rates among Western countries. The public is becoming concerned about this increase.[6]

According to the DSM-IV, preoccupation with weight and excessive self-evaluation of body shape are primary symptoms of the disorder. In addition to core psychopathology of eating disorders, they also have other specific psychopathologies such as low self-

esteem and alexithymic characteristics. These tendencies sometimes create difficulties in the treatment of such patients.[7] Fairburn noted that abnormal eating behavior and the related core cognitive distortion may be associated with lower self-esteem.[8] It has also been noted that some patients with an eating disorder have alexithymic characteristics.[9,10] It has been suggested that multidimensional therapeutic approaches are required to treat eating disorders. In particular, these approaches focus on assertiveness to deal with alexithymic characteristics, as well as dealing with low self-esteem.[11,12]

Many studies have examined the treatment of patients with eating disorders. Among them, cognitive behavioral therapy (CBT) is generally considered to be the treatment of choice, especially for binge-eating behaviors.[13,14] CBT has been used not only for individuals, but also in a group setting, combined with psychoeducation or training in problem-solving.[15,16] While it has been demonstrated that group CBT is highly cost-effective,[17–19] its effectiveness, patient completion rate, and predictors of the response to treatment has been inconsistent.[20] Garner *et al.* argued that the average dropout rates were lower when CBT was used to treat individuals (15.3%) compared with its use in a group setting (29.3%).[21] Conversely, Cox and Merkel concluded in their review that abstinence from bulimic episodes was equivalent in patients after group (40.4%) and individual CBT treatment (47.4%).[22] Given this lack of consistency, further development of newer group CBT programs focusing on maladaptive emotional problems, problems with low self-esteem, and quantitative verification of their efficacy are needed. In the study presented here, we used the original group CBT strategy and combined it with training in assertiveness and self-esteem enhancement to treat bulimic outpatients. This program will be referred to as combined group CBT (CGCBT). The purposes of this study were to examine the effectiveness of our CGCBT for bulimic outpatients and to explore the characteristics of those patients who failed to complete the program.

METHODS

Subjects

The participants in this study were selected according to the following criteria: (i) meeting the DSM-IV criteria for BN, ANB/P, or EDnos with binge-eating behavior; (ii) with a body mass index (BMI)[23] >13.5; (iii) without incontrollable self- or other-injuring behavior.

A total of 31 outpatients were recruited from an eating disorders service at Chiba University Hospital, Chiba, Japan, from April 2002 to August 2003. All of the subjects met the criteria listed above. Six subjects were subsequently excluded from the study because they either decided not to take up the treatment that was offered or they failed to attend without notice after the assessment session. Twenty-five outpatients (24 females and one male), were enrolled in this study. All of the subjects provided informed consent to participate.

Measures

Prior to the first session of the program, the participants received a systematic medical interview for diagnosis using DSM-IV[1] and a number of questionnaires designed to assess quantitatively their clinical symptoms. The questionnaires include Eating Disorder Inventory-2 (EDI-2)[24] and the Bulimic Investigatory Test, Edinburgh (BITE),[25] which are used as measures of eating psychopathology, the Toronto Alexithymia Scale (TAS-20),[26] which measures the degree of alexithymia, and the Rosenberg Self-Esteem Scale (RSES),[27] which measures self-esteem. We also assessed their level of depression using the Hamilton Depression Rating Scale (HDRS),[28] and social functioning with the Global Assessment of Functioning (GAF).[1] The severity of their disease was assessed by the Clinical Global Impression of Disease Severity (CGI-S) and the responses to the treatment were indicated to either be much improved or very much improved, as assessed by the Clinical Global Impression Change (CGI-C).[20,29]

Treatment

Our CGCBT program consisted of 1-h sessions that were attended by the patients once a week over a 10-week period. The program was based on an existing cognitive behavioral model.[8] We also added assertiveness training and a self-esteem enhancement session into the program. Each therapy group comprised three to six patients, and the membership of each group was fixed throughout the program. During the CGCBT period participants were encouraged to write an eating-behavior diary. The contents of each session were determined prior to its commencement and adhered to strictly. They included psychoeducation about diet and eating behavior, cognitive therapy for restructuring their cognition about dieting and body shape, behavioral therapy for reacquiring a healthy diet and lifestyle, assertiveness training for coping with interpersonal problems, social skills training based on problem-solving therapy, and self-esteem enhancement.

In order to improve alexithymic characteristics, we conducted two sessions, using role plays, in which the patients were expected to recognize their own emotions and try to express them to the other participants by their attitude and speech. We also added sessions designed to enhance self-esteem, focusing on the self-efficacy of each group member from the past through to the present, and into the future.

The sessions regarding alexithymic characteristics and self-esteem enhancement were set up in the last half of the 10 sessions, in order that the group members could be more familiar with each other and would be able to talk freely.

At treatment discharge, we reassessed the participants' GAF and they were required to complete again the following questionnaires: EDI-2, BITE, TAS-20, and RSES.

Statistical analysis

We analyzed the clinical data and scores of each rating scale statistically. The data are presented as the mean ± SD.

The data were found to have a normal distribution, and so the pre- to post-treatment differences were examined using the paired *t*-test. The Wilcoxon non-parametric test was also used to compare the pre- and post-treatment data of those participants who completed the program. The level of statistical significance was set at $P < 0.05$.

In order to examine predictors of dropout from the treatment program, comparisons of initial psychopathology and clinical characteristics between those who completed and those who dropped out from the CGCBT were analyzed using logistic regression analysis. Calculations were performed using the statistical software package SPSS for windows (SPSS, Chicago, IL, USA). The level of statistical significance for this analysis was also set at $P < 0.05$.

RESULTS

A total of 25 patients entered the CGCBT. Table 1 shows the clinical characteristics of the participants. The mean age at treatment entry was 23.8 years (SD = 5.9). The mean BMI was 20.7 kg/m^2 (SD = 4.5). According to DSM-IV criteria, 15 of the participants were suffering from BN purging type (BNP), four were suffering from BN non-purging type (BNNP), five were suffering from ANB/P, and one received a diagnosis of EDnos.

Six participants were psychiatrically comorbid. Of those six, four had major depressive disorders, one had an impulse control disorder not otherwise specified, and one had a schizoid personality disorder.

Table 1. Profiles of participants

Sex	
Male	1
Female	24
Age	23.8 ± 5.9†
Duration of illness (years)	5.0 ± 4.2†
BMI (kg/m^2)	20.7 ± 4.5†
Diagnosis‡	
Bulimia nervosa purging type	15
Bulimia nervosa non-purging type	4
Anorexia nervosa binge-eating/purging type	5
Eating disorder not other specified	1
Medication	
Supplied	8
Free	17
Comorbidity‡	
Major depressive disorders	4
Impulsive control disorder	1
Schizoid personality disorder	1
Free	19

†Mean ± SD.
‡Diagnosed with DSM-IV-TR.

Table 2 gives the characteristics of all of the participants in the CGCBT program using the rating scales at treatment entry. At that time, the CGI-S scores revealed that two of the participants were considered to be mildly ill, nine were moderately ill, eight were markedly ill, and six were severely ill. Of the original 25 participants, 16 (64%) completed the CGCBT program and nine (36%) dropped out. Table 2 also gives the data of the 16 participants who completed the CGCBT, at the cessation of the program.

For those who completed the program, the mean post-treatment EDI-2 ($P = 0.01$), BITE (symptom assessment scale, $P = 0.01$), BITE (severity scale, $P = 0.01$), RSES ($P = 0.02$), and GAF ($P = 0.01$) scores improved significantly compared to the pretreatment scores. The mean TAS-20 scores showed a tendency toward improvement ($P = 0.06$). The CGI-C scores of those who completed the program were as follows: two unchanged, six minimally improved, four much improved, four very much improved, and none deteriorated. Therefore, by using the CGI-C, we saw that eight patients (32%; four much improved, and four very much improved patients), responded to the treatment.

At treatment discharge, four patients (i.e. 16% of all participants) were completely abstinent from bulimic episodes.

Table 2. Rating scales of completers for group cognitive behavioral therapy at pretreatment, post-treatment

Rating scales	Mean scores at the entering of all participants ($n = 25$) (mean ± SD)	Mean scores of completers ($n = 16$) Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value[†]
Eating Disorder Inventory-2	127.0 ± 35.4	124.7 ± 35.7	92.3 ± 45.3	0.01*
Bulimic Investigatory Test, Edinburgh (SAS)	23.1 ± 3.7	23.3 ± 2.5	17.3 ± 7.8	0.01*
Bulimic Investigatory Test, Edinburgh (SS)	11.2 ± 4.8	11.1 ± 4.9	8.9 ± 5.6	0.01*
Toronto Alexithymia Scale-20	61.1 ± 8.9	60.9 ± 8.6	54.7 ± 14.0	0.06
Rosenberg Self-Esteem Scale	16.3 ± 6.5	16.3 ± 2.2	18.9 ± 4.7	0.02*
Global Assessment Factor	48.8 ± 9.5	50.6 ± 8.3	61.9 ± 12.0	<0.01*

*$P < 0.05$.
[†]Paired-t-test, Wilcoxon non-parametric test.

Table 3. Comparison of initial psychopathology, clinical characteristics between completers and drop-outs to group cognitive behavioral therapy

Variable		Completers ($n = 16$)	Drop-outs ($n = 9$)	P	Odds ratio	95% Confidence Interval
Age (years)		26 ± 6.2	20 ± 2.5	0.04*	1.9	0.3–3.6
Diagnosis	BNP	10	5			
(DSM-IV)	BNNP	3	1			
	ANBP	2	3			
	EDNOS	1	0			
Comorbidity	Depressive disorders	1	3			
	Schizoid personality disorder	1				
	Impulsive control disorder NOS		1			
	Total	2	4	0.06	0.04	0.002–1.2
Illness duration (years)	5.9 ± 4.8	3.3 ± 2.0	NS			
BMI (kg/m^2)		21.4 ± 5.0	19.4 ± 3.0	NS		
Medication		5	3	NS		
Rating scales (at pretreatment)						
EDI-2		124.7 ± 35.7	133 ± 29.3	NS		
BITE (SAS)		23.3 ± 2.5	22.8 ± 5	NS		
BITE (SS)		11.1 ± 4.9	11.5 ± 4	NS		
HDRS		9.3 ± 5	9.6 ± 5.3	NS		
TAS-20		60.9 ± 8.6	61.8 ± 8.3	NS		
RSES		16.3 ± 2.2	16.5 ± 4.6	NS		
GAF		50.6 ± 8.3	45.7 ± 11.1	NS		

*$P < 0.05$.
ANBP, Anorexia Nervosa Binge-eating/Purging type; BITE, Bulimic Investigatory Test, Edinburgh; BMI, Body Mass Index; BNP, Bulimia Nervosa Purging type; BNNP, Bulimia Nervosa Non-Purging type; EDNOS, Eating Disorder Not Other Specified; EDI-2, Eating Disorder Inventory-2; GAF, Global Assessment Factor; HDRS, Hamilton Depression Rating Scale; NS, not significant; RSES, Rosenberg Self-Esteem Scale; SAS, Symptom Assessment Scale; SS, Severity Scale; TAS-20, Toronto Alexithymia Scale-20.

Completion and dropout from CGCBT

Of the 25 participants, 16 (64%) completed the CGCBT program and nine (36%) dropped out. Table 3 gives a comparison of the initial psychopathology and clinical characteristics between those who completed the CGCBT and those who dropped out. We entered all of the variables explored using logistic regression analysis in order to determine the independent predictors of dropout from the CGCBT program. Only two variables

entered and remained in the final regression equation: age ($P = 0.04$) and psychiatric comorbidity ($P = 0.06$) (Table 3). The odds ratio for age indicated that a lower age was predictive of dropouts from CGCBT. There was also a trend toward a positive correlation between psychiatric comorbidity and dropping out.

DISCUSSION

In the study presented here, we utilized the original strategy of group CBT combined with assertiveness training and a self-esteem enhancement for bulimic outpatients: the CGCBT. The purpose of this study was to examine the effectiveness of our CGCBT and to establish the predictors of dropout from the program.

A total of 25 patients participated in the CGCBT. All of the patients had bulimic symptoms. Of those 25, 16 (64%) completed the CGCBT program. Of those 16, bulimic behavior and psychopathology of eating disorder, as evaluated by EDI-2 and BITE (the symptom assessment scale and severity scale), respectively, were significantly improved after the CGCBT. The average RSES ratings of the patients at treatment entry were lower than that of average Japanese healthy controls.[30] The TAS-20 scores showed that the participants' alexithymic characteristics tended to be improved, and the GAF scores indicated a significant improvement of social function with the program. Therefore, our CGCBT was effective not only for improving bulimic symptoms, but also for improving alexithymic characteristics, low self-esteem, and the social function of those who completed the program. It was shown that by reducing binge-eating behaviors, alexithymic characteristics and low self-esteem favorably improved in these patients.

It has been suggested that CBT, when directed specifically at eating behavior and the underlying cognition of patients, is the most effective way of treating BN.[13,31] Our CGCBT program was based on an existing cognitive behavioral model.[8]

However, it is known that the characteristics of alexithymia and low self-esteem may be associated with this eating disorder.[8–10] Indeed, the patients who took part in our study were highly alexithymic, as indicated by their mean TAS-20 score (a score of >60 is considered to indicate alexithymia). We therefore added assertiveness training to attempt to modify this behavior.

Regarding alexithymic characteristics and low self-esteem

We conducted two sessions, including role plays, in order to improve alexithymic characteristics. The result was that the mean TAS-20 score of those who completed the program was significantly higher than it was before the program.

We also added sessions designed to raise self-esteem, focusing on the self-efficacy of each group member. With regard to low self-esteem, Silverstone *et al.*[32] suggested that this characteristic is one of the main factors responsible for maintaining the binge-eating behavior of eating-disordered patients.[26] In addition, Silverstone *et al.* suggested that the presence of major depressive disorders as well as eating disorders tended to show low self-esteem.[32] In this study, four participants (16%) had comorbid depressive symptoms, which would contribute to low self-esteem of the participants. Indeed, the RSES results of our participants suggest low self-esteem at treatment entry compared to the average Japanese individual.[30]

It has been suggested that group dynamics have an extremely favorable affect on interpersonal relationships among group members, as well as changes in distorted self-images. This led to the improvement of skills on how to recognize and express ones own feelings. By using RSES, furthermore, it was shown that by recognizing and expressing each members range of emotions, self-esteem increased by reflection on one's own accomplishments and progress.

In this study, four of the 25 patients had comorbid depressive disorders, which most likely influenced low self-esteem characteristics in this study. The improvement of low self-esteem characteristics can be attributed to the reduction of depressive symptoms in the patients who had comorbid depressive disorders.

In the present study, the psychological measures implemented demonstrate the efficacy of our CGCBT program for the bulimic behavior and disturbed social function of patients with eating disorders, although the program was completed in a short period of time. It would be interesting to observe the changes in their clinical course and the scores of the rating scales over a longer period of time. The dropout rate of our CGCBT was 36%. Blouin *et al.*[33] reported a dropout rate of 28.7% from their individual therapy program for eating disorders. Many reports have been published regarding the dropout rate from group therapy designed to treat eating disorders, but the results vary.[21] McKisack and Waller noted in their review that longer and intensively scheduled group therapies reduce the dropout rate.[34] Our CGCBT program comprised only 10 sessions, but well-structured multidimensional strategies were included in it, which may have contributed to the good completion rate that we achieved.

In the study presented here, a lower age and psychiatric comorbidity were predictive factors for dropouts from our CGCBT. According to logistic regression

analysis, there were no associations between the severity of the rating scales and dropout from the CGCBT. To the best of our knowledge, there are no reports in which it is suggested that age or comorbidity are associated with dropping out from group CBT. Wilson indicated that comorbid personality disorder is associated with a poorer response not only to CBT, but also to alternative therapies.[12]

In this study, nine patients (36%) dropped out from the group program. Four patients were psychiatric comorbid among the dropouts, of which, three patients (33%) had major depressive disorders. In contrast, only one patient had comorbid depressive disorders among those who completed the study. It was shown that the comorbid depressive disorders had some affect on increasing patient dropout. Further treatment strategies are needed to improve the treatment outcome for bulimic patients with psychiatric comorbidity.

Limitations in this study

As far as we know, this is the first report on group CBT including interventions to improve alexithymia and low self-esteem, and the evaluation of its efficacy for the treatment of eating disorders. Furthermore, this is the first report to indicate that a lower age and psychiatric comorbidity are associated with dropping out from group CBT. However, this study clearly has limitations. A control group was not enrolled, the sample size is not large enough, and long-term follow-up observation after treatment is required. Further studies are needed to confirm these results.

CONCLUSIONS

Our outpatient CGCBT, which includes CBT, assertiveness training, and self-esteem enhancement therapy is effective for the improvement of binge-eating behavior and social function of patients with eating disorders.

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ORIGINAL INVESTIGATION

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Tropisetron improves deficient inhibitory auditory processing in DBA/2 mice: role of α7 nicotinic acetylcholine receptors

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Abstract *Rationale:* Deficient inhibitory processing of the P50 auditory evoked potential is a pathophysiological feature of schizophrenia. Several lines of evidence suggest that α7 nicotinic receptors play a critical role in this phenomenon. Similar to schizophrenic patients, DBA/2 mice spontaneously exhibit a deficit in inhibitory processing of the P20–N40 auditory evoked potential, which is thought to be a rodent analog of the human P50 auditory evoked potential. *Objective:* The present study was undertaken to examine whether tropisetron, a partial agonist at α7 nicotinic receptors and an antagonist at 5-hydroxytryptamine-3 receptors, improves this deficit in DBA/2 mice. *Results:* Administration of tropisetron (1 mg/kg i.p.) significantly improved the deficient inhibitory processing of the P20–N40 auditory evoked potential in DBA/2 mice. Coadministration of methyllycaconitine (MLA; 3 mg/kg i.p.), a partially selective antagonist at α7 nicotinic receptors, significantly blocked the normalizing effect of tropisetron. Furthermore, MLA alone did not alter the deficient inhibitory processing of the P20–N40 auditory evoked potential in DBA/2 mice. *Conclusions:* The data suggest that tropisetron improves the deficient inhibitory processing of the P20–N40 auditory evoked potential in DBA/2 mice by effects on α7 and perhaps α4β2 nicotinic receptors. Tropisetron may be useful for the treatment of deficient inhibitory processing in schizophrenia.

Keywords Nicotinic receptors · Schizophrenia · Antipsychotic · Sensory gating

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Introduction

Deficient inhibition of response to repetitive auditory stimuli has been observed in schizophrenia patients and is thought to underlie some of the symptomatology of the disorder (Braff and Geyer 1990; Freedman et al. 1994; Braff and Freedman 2003). Inhibitory processing of the P50 auditory evoked potential has been studied in an attempt to identify the neuronal mechanism(s) of this phenomenon. Presentation of pairs of identical auditory stimuli, 500 ms apart, elicits responses 50 ms after stimulus onset. Schizophrenia patients generally show similar amplitude responses to both stimuli, whereas normal subjects have a significantly reduced response to the second stimulus compared to the first (Freedman et al. 1994, 1987; Braff and Geyer 1990; Braff and Freedman 2003). This reduction in the second response reflects inhibitory gating of the brain's response to stimuli, which may protect the individual from being overwhelmed by repetitive sensory information. Several lines of evidence suggest that the α7 subtype of the nicotinic acetylcholine receptor may be critically important to the inhibitory mechanism (Adler et al. 1998; Freedman et al. 1994, 1997, 2000; Leonard et al. 1996, 2002; Leonard 2003; Simosky et al. 2002; Martin et al. 2004).

The hippocampal P20–N40 auditory evoked potential in rodents is thought to be analogous to the human P50 potential (Adler et al. 1986, 1988; Stevens et al. 1996), though this opinion is not universally held (Connolly et al. 2003; Maxwell et al. 2004; Miyazato et al. 1999, 2000; Teneud et al. 2000). The rodent P20–N40 has been used to model the neurobiology and pharmacology of the human P50 processing deficit (Willot et al. 1982; Stevens and Wear 1997; O'Neill et al. 2003; Simosky et al. 2001, 2003; Stevens et al. 1996, 1998). Studies in both rats and inbred

mouse strains have demonstrated a role for the α7 nicotinic in the modulation of P20–N40 inhibitory processing. Specifically, blockade of the α7 but not the α4β2 nicotinic acetylcholine receptor or the muscarinic acetylcholine receptor disrupted normal sensory inhibition in rats (Luntz-Leybman et al. 1992). Assessment of sensory inhibition across several inbred mouse strains demonstrated a correlation between hippocampal α7 receptor density and the level of sensory inhibition, such that strains with reduced numbers of these receptors showed deficient sensory inhibition while strains with larger numbers had normal inhibition (Stevens et al. 1996). In that study, DBA/2 mice had deficient inhibition and about half the number of hippocampal α7 receptors as strains with normal inhibition. Subsequent studies with this mouse strain have demonstrated improvement in sensory inhibition with administration of nicotine or selective α7 nicotinic agonists (Simosky et al. 2001; Stevens and Wear 1997; Stevens et al. 1998). Recently, drugs which indirectly increase hippocampal acetylcholine levels have also been shown to improve sensory inhibition in the DBA/2 mouse (Simosky et al. 2003, 2004). Thus, drugs which are agonists at α7 nicotinic receptors or increase endogenous acetylcholine levels are drug candidates that may prove efficacious in normalizing deficient P50 processing in schizophrenic patients (Leonard 2003; Martin et al. 2004; Simosky et al. 2002; Hashimoto et al. 2005).

Both α7 nicotinic receptors and 5-hydroxytryptamine-3 (5-HT_3) receptors are members of the superfamily of ligand-gated ion channels. These two receptors demonstrate the greatest similarity within the family, displaying approximately 30% sequence homology (Maricq et al. 1991). 5-HT_3 receptors have been found on presynaptic cholinergic nerve terminals where they act to inhibit release of acetylcholine (Ramirez et al. 1996); thus, blockade of these receptors can increase release of acetylcholine. Tropisetron is a potent 5-HT_3 receptor antagonist that is widely used in the treatment of patients with chemotherapy-induced or postoperative nausea and vomiting (Simpson et al. 2000). It has also been reported to be a partial agonist at α7 nicotinic receptors with a high affinity (K_i=6.9 nM for α7 nicotinic receptors, K_i=5.3 nM for 5-HT_3 receptors; Macor et al. 2001; Papke et al. 2004). Recently, we have found that tropisetron improves deficits of human P50 suppression in schizophrenic patients (Koike et al. 2005). The present study sought to extend this work by assessing the effect of tropisetron in the DBA/2 mouse model of deficient sensory inhibition and determination of the role of α7 nicotinic receptors in any observed change through the administration of methyllycaconitine (MLA), a partially selective antagonist at α7 nicotinic receptors.

Methods

Animals

Male DBA/2 mice (18–25 g) were obtained from Harlan SD (Indianapolis, IN, USA) and group-housed until recorded. Food (Purina Rodent Chow) and water were available ad libitum, and lighting was cycled at 12-h intervals (lights on at 6:00 a.m.). *Principles of Laboratory Animal Care* (National Institutes of Health publication no. 85-23, revised 1985) were followed. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Denver Veterans Affairs Medical Center.

Drugs

Tropisetron hydrochloride was provided by Novartis Pharma AG (Basel, Switzerland). MLA citrate was purchased from Sigma-Aldrich Corporation (St. Louis, MI, USA). These drugs were solved in physiological saline.

Auditory evoked potential recordings

The mice were anesthetized with chloral hydrate (400 mg/kg i.p.) and pyrazole (400 mg/kg i.p.) to retard the metabolism of the chloral hydrate. Anesthesia was supplemented periodically to maintain a surgical plane of anesthesia (2.0 mg/kg i.p. each, chloral hydrate and pyrazole). The animal was placed in a mouse adapter (Neuroprobe, Cabin John, MD, USA) for a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA). Hollow ear bars, attached to miniature earphones that were connected to the sound amplifier, were placed adjacent to the externalization of the aural canal. Because the auditory evoked potentials are more consistent at a stable temperature of 35°C, body temperature was maintained at this level with a heating pad. The scalp was incised and a burr hole opened over the CA3 region of hippocampus (−1.8 mm anterior–posterior, +2.70 mm medial–lateral; Franklin and Paxinos 1997). A teflon-coated, stainless steel wire microelectrode was inserted into the CA3 pyramidal cell layer of the hippocampus (1.65–1.70 mm below the dorsal brain surface). Final electrode location was identified by the presence of complex action potentials typical of hippocampal pyramidal neurons (Miller et al. 1992). A reference electrode was placed on dura, anterior to bregma, contralateral to the recording electrode. The electrical activity was amplified 1,000 times with bandpass 1 to 500 Hz (Miller et al. 1992) and led to an analog to digital converter (RC Electronics, Bakersfield, CA, USA) for averaging by computer. Tones, 3,000 Hz, 10-ms duration, 72 dB, and sound pressure level generated as a sine wave, were presented in pairs with a 500-ms intrapair interval and 10 s between pairs (Miller et al. 1992). Although DBA/2 mice suffer hearing loss as they age, these tones were within the audible range for the mice (Willot et al. 1982). Responses to 16 pairs of tones were averaged at 5-min intervals. Each average was filtered digitally with bandpass between 10 and 250 Hz. The maximum negativity between 20 and 60 ms after the first stimulus was selected as the N40 wave and measured relative to the preceding positivity, a P20 wave. Each of the waves (P20 and N40) was also measured relative to the mean pre-

Table 1 Variance for different components of the conditioning and test evoked potentials

Wave component	Baseline conditioning (μV^2)	Baseline test (μV^2)	Drug conditioning (μV^2)	Drug test (μV^2)
P20	0.00312	0.00410	0.01620	0.00607
N40	0.00723	0.01805	0.01424	0.00734
P20–N40	0.00458	0.00441	0.00464	0.00632
N10–P20	0.00360	0.00515	0.01685	0.00903

stimulus activity, and the P20 wave was also measured relative to the preceding N10.

The ratio of the amplitudes of response to the second (test) stimulus and the first (conditioning) stimulus provides a measure of sensory inhibition; the ratio of the test to the conditioning amplitude (TC ratio) is 0.5 or less for most rodent strains and normal humans (Stevens et al. 1996). Four records were obtained before any drug injection to establish baseline sensory processing performance. Each mouse was drug naive at the time of experimentation. To determine the optimal agonist dose of tropisetron, we examined several doses in a preliminary experiment (0.3, 1, and 3 mg/kg). The 1- and 3-mg/kg doses improved the inhibition of P20–N40 responses in DBA/2 mice. Therefore, the lowest effective dose (1 mg/kg) was selected for further study in comparison with saline vehicle in groups of eight mice. In a subsequent group of studies, the antagonist MLA was administered peripherally (3 mg/kg in saline i.p.), a dose that results in brain levels adequate to antagonize $\alpha 7$ nicotinic receptors (Turek et al. 1995). Recordings were obtained at 5-min intervals for 20 min before and 60 min after injection of each substance.

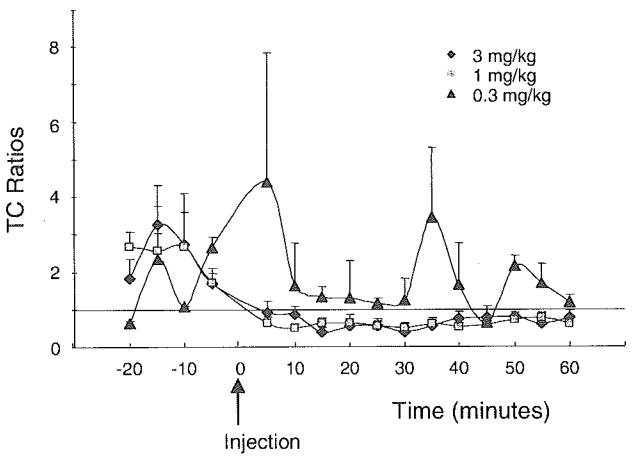

Fig. 1 TC ratios for three doses of tropisetron (0.3, 1, and 3 mg/kg i.p.). The 1-mg/kg dose was the lowest dose that produced a consistent decrease in TC ratio and was chosen for further study. Data were collected at 5-min intervals; injection of tropisetron occurred at the *arrow*. Data are mean±SEM; n=3 for 0.3 mg/kg, 8 for 1 mg/kg, and 4 for 3 mg/kg

Statistical analysis

P20–N40 auditory evoked potential amplitudes and the TC ratio in response to the drug treatments were analyzed by a 2×2 factorial repeated measures multivariate analysis of variance (MANOVA) with the 5-min records as the within-subjects variable, followed by Tukey's honestly significant difference (HSD) a posteriori analysis, as appropriate. The significance level was set at $p<0.05$.

Results

The variance (σ^2) of the different components of the evoked potential waveforms was compared in the baseline recordings and during the peak drug effect (20–30 min post tropisetron injection, as initially assessed with P20–N40

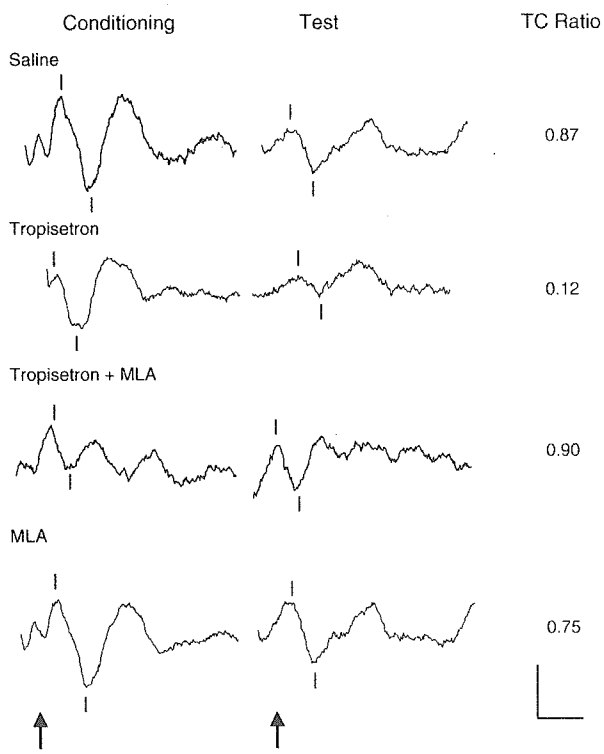

Fig. 2 Grand average P20–N40 waves from DBA/2 mice 20–30 min after the injection of saline (4 ml/kg i.p.), tropisetron (1 mg/kg i.p.), tropisetron + MLA (3 mg/kg i.p.), or MLA alone. The P20 and N40 peaks are denoted by *tick marks*; stimulus onset by the *arrows*. Calibration 25 μV/50 ms

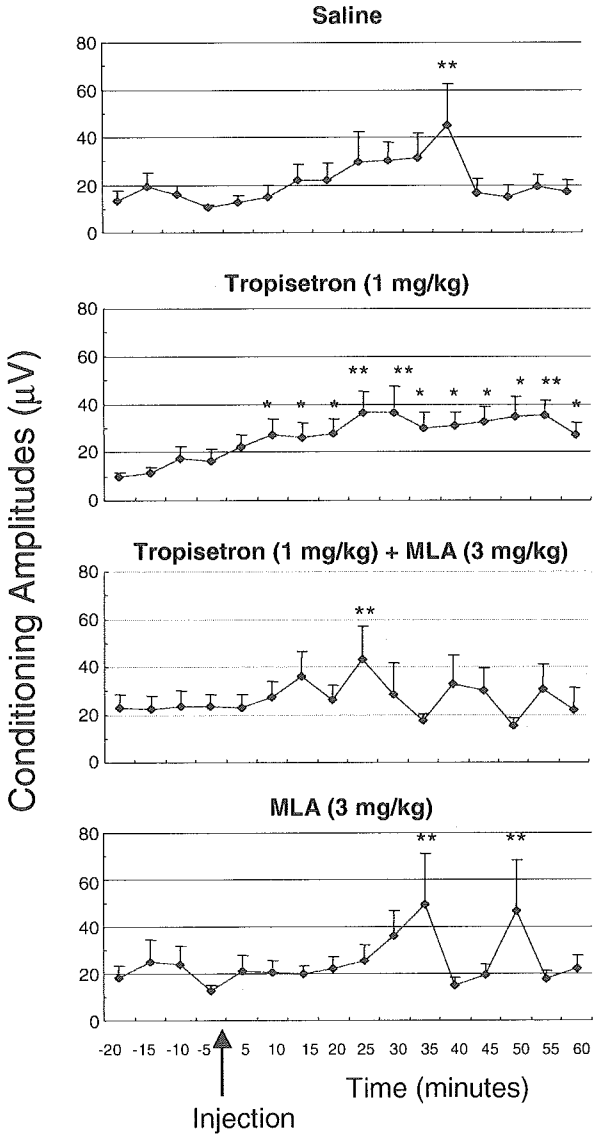

Fig. 3 Effects of tropisetron with or without MLA on P20–N40 conditioning amplitudes before and after drug administration. The first four time points (−20, −15, −10, and −5 min) are the baseline recordings prior to administration. *Asterisks* mark those postdrug time points at which the conditioning amplitude is significantly different from the average of the baseline conditioning amplitudes, as determined using Tukey's HSD (*p<0.05,). Data are mean±SEM of eight mice

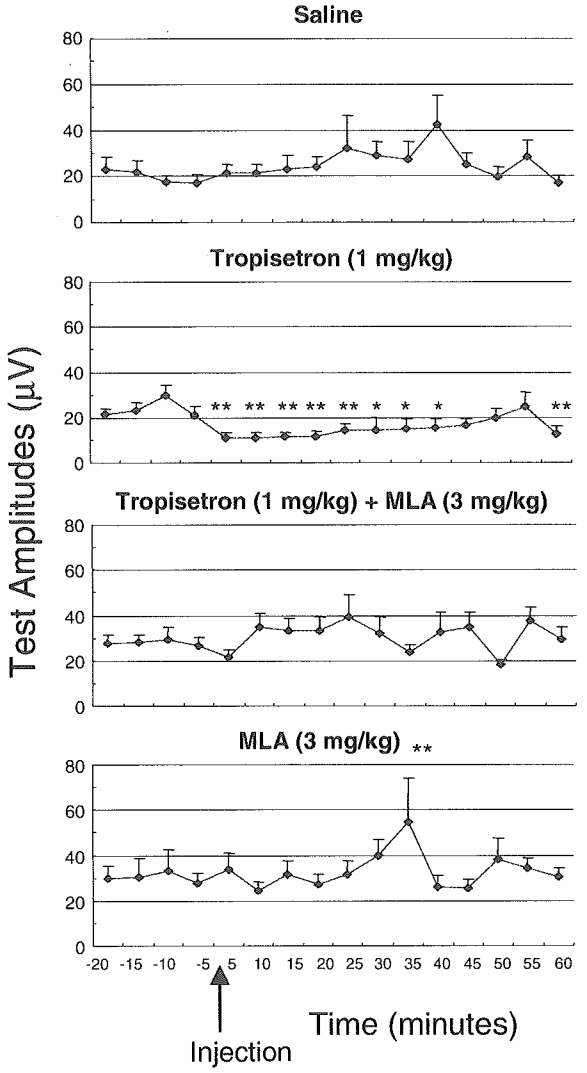

Fig. 4 Effects of tropisetron with or without MLA on the P20–N40 test amplitudes before and after drug administration. The first four time points (−20, −15, −10, and −5 min) are the baseline period of recording prior to drug administration. *Asterisks* mark those postdrug time points at which the test amplitude is significantly different from the average of the baseline test amplitudes, as determined using Tukey's HSD (*p<0.05, **p<0.01). Data are mean±SEM of eight mice

analysis). Table 1 shows the individual variances for the P20, N40, P20–N40, and N10–P20 components of the conditioning and test evoked potentials for the four baseline recordings and the 20-, 25-, and 30-min time points after tropisetron injection. The lowest overall variances were observed with the P20–N40 component of the evoked potentials, in agreement with previously published work (Cook et al. 1968). Therefore, the remaining analyses use the P20–N40 component of the auditory evoked potential as the measure of response.

As observed in previous studies, DBA/2 mice failed to show P20–N40 inhibition during the baseline period of recording; the TC ratios, which were all greater than 1, indicated that there was a greater response to the second stimulus than to the first stimulus. Three pilot doses of tropisetron (0.3, 1, and 3 mg/kg i.p.) were compared to choose an optimal dose (Fig. 1). Analysis of variance for TC ratio showed a significant difference among the doses [F(30,165)=1.61, p=0.033]. The 1-mg/kg dose was chosen for further study since it was the lowest dose that produced consistent improvement in sensory inhibition.

MANOVA for conditioning amplitude across the four test groups revealed a significant three-way interaction

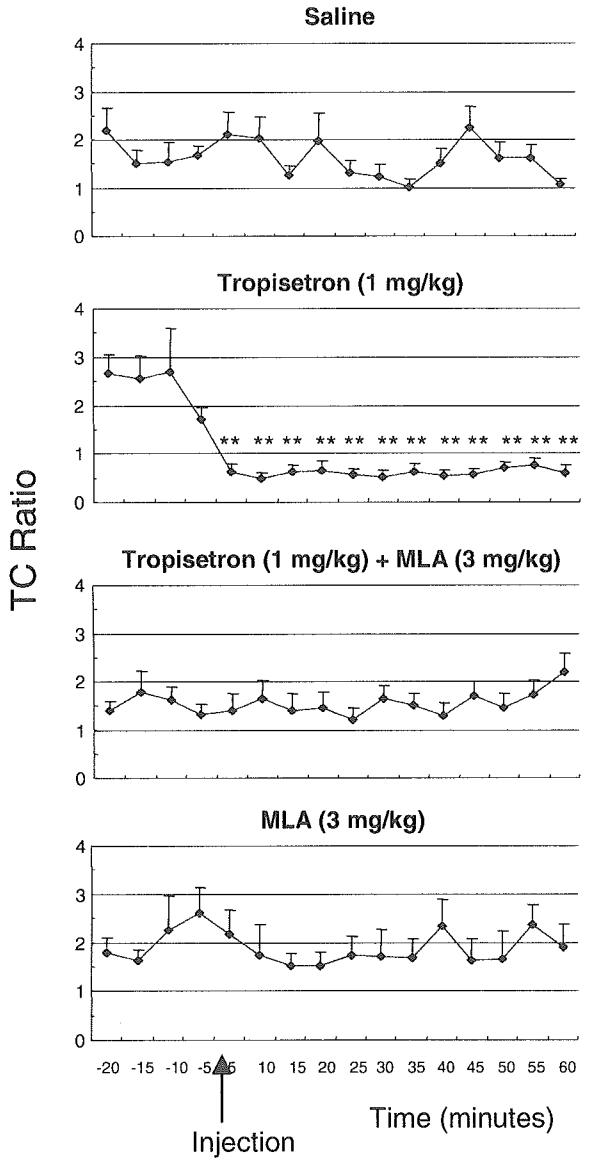

Fig. 5 Effects of tropisetron with or without MLA on TC ratios before and after drug administration. The TC ratio is significantly decreased compared to predrug baseline following tropisetron injection, while there are no significant changes following any other treatment. The first four time points (−20, −15, −10, and −5 min) are the baseline period of recording prior to drug administration. *Asterisks* mark those postdrug time points at which the TC ratio is significantly different from the average of the baseline TC ratio, as determined using Tukey's HSD (**p<0.01). Data are mean±SEM of eight mice

(two drug conditions and time) [F(15,420)=1.69, p=0.049]. Tukey's HSD a posteriori analyses found significant increases in conditioning amplitude at all time points, except the first 5 min, following tropisetron administration. There was one significantly increased time point each for saline (40 min) and tropisetron + MLA (25 min) and two significantly increased time points for MLA alone (35 and 50 min), but no consistent pattern of increase for saline, tropisetron + MLA, or MLA alone (Figs. 2 and 3). Similarly, a significant three-way interaction was found for test amplitude [F(15,420)=1.97, p=0.016]. HSD analysis showed significant decreases in test amplitude at most time points following tropisetron administration but not with any other drug condition. The overall pattern for the remaining three drug treatments was for an increase in test amplitude which reached significance for one time point (35 min) for MLA alone (Figs. 2 and 4).

MANOVA for TC ratio across the four test groups showed a significant three-way interaction [F(15,720)= 2.07, p=0.010]. Tukey's HSD a posteriori analyses found significant reductions in TC ratio at all time points after administration of 1 mg/kg tropisetron but no significant changes for any of the other drug treatments (Figs. 2 and 5).

Discussion

The major findings of the present study are that tropisetron improves several aspects of P20–N40 auditory evoked response processing in DBA/2 mice. Increases were observed in the conditioning amplitude, which suggest increased hippocampal excitability, and decreases were found in the test amplitude, which demonstrates increased inhibition of response to the second stimulus. As would be expected, combined, they produced significant decreases in the TC ratio. The paired stimulus or conditioning–testing paradigm was designed for the study of monosynaptic feedback inhibition in the spinal cord (Eccles 1969). The initial response is taken as a constant, so that decreased amplitude of the second response can be used as a specific measure of inhibition. If there is a change in the overall excitability of the system as the result of a manipulation, such as a drug administration, it is reflected as change in the conditioning response. Therefore, the test response amplitude as a fraction of the conditioning response amplitude (TC ratio) is used as the primary parameter of inhibition, so that change in inhibition can be measured even if the overall excitability of the neuronal system under study has increased. In most forebrain systems, excitation and inhibition are polysynaptic, rather than monosynaptic, and thus, the variance in the conditioning response is larger than it would be in a simpler monosynaptic system. Therefore, the data in this study were analyzed for changes in both the conditioning and test responses as well as for their ratio.

The most significant changes in response to tropisetron were in the TC ratio, consistent with other studies of nicotinic agonists, such as dimethoxybenzylidene anabaseine, a specific α7 nicotinic receptor agonist, and nicotine itself (Stevens and Wear 1997; Stevens et al. 1998, 1999). This decrease in TC ratio was produced, in part, through a reduction in test amplitude, as has been previously seen with selective stimulation of α7 nicotinic receptors (Stevens et al. 1998), but there was also a significant increase in conditioning amplitude, which is now thought to be mediated through α4β2 nicotinic receptors (Miner et al. 2004). This phenomenon has also been observed in studies which employed mixed agonists, such as nicotine (Stevens and Wear 1997), and indirect agonists, such as clozapine, which in-

creases endogenous acetylcholine release (Simosky et al. 2003). In these studies, there were also both a decrease in test amplitude and an increase in conditioning amplitude.

The similar changes observed in the present study are not unexpected, given the pharmacology of tropisetron. As a 5-HT_3 antagonist (Simpson et al. 2000; Macor et al. 2001), it blocks presynaptic receptors on cholinergic neurons, thus disinhibiting release of acetylcholine (Ramirez et al. 1996) as well as having direct partial agonist activity at nicotinic $\alpha7$ receptors (Macor et al. 2001; Papke et al. 2004). The indirect stimulation of nicotinic receptors is thought to account for the effects of the 5-HT_3 antagonists ondansetron (Adler et al. 2005) and clozapine (Nagamoto et al. 1996, 1999; Simosky et al. 2003) on sensory inhibition. As noted earlier, studies have demonstrated modulation of the conditioning amplitude through the nicotinic $\alpha4\beta2$ receptor (Miner et al. 2004), while the effect on test amplitude is produced through the nicotinic $\alpha7$ receptor (Stevens et al. 1996, 1998). Thus, the increase in conditioning amplitude may have been produced by an increase in release of acetylcholine acting at the $\alpha4\beta2$ nicotinic receptor, and the decreased test amplitude is probably produced by a combination of the increased acetylcholine and the tropisetron itself acting at the $\alpha7$ nicotinic receptor.

The coadministration of MLA with tropisetron fully reversed both the increase in conditioning amplitude and the decrease in test amplitude elicited by tropisetron administration, producing TC ratios commensurate with baseline levels, while MLA alone had no significant effect. Previously, MLA was considered to be a selective antagonist for the nicotinic $\alpha7$ receptor (Ward et al. 1990). However, recent studies in brain synaptosomes and stable cell lines have called this specificity into question. These studies demonstrate that MLA can bind $\alpha4\beta2$ receptors in the low nanomolar range (Clarke and Rueben 1996; Grady et al. 1997; Karadsheh et al. 2004). The current data in whole mouse are in concert with this view. The study of Miner et al. (2004) suggests that the $\alpha4\beta2$ receptors mediate changes in conditioning amplitude in mice. Therefore, the blockade of the tropisetron-induced increase in conditioning amplitude by MLA may indicate MLA's activity at this receptor subtype as well as the blockade of $\alpha7$ receptors leading to the blockade of the decrease in test amplitude. Alternately, if MLA is indeed selective for $\alpha7$ receptors in vivo, the present data would suggest that both the conditioning and test amplitude changes were mediated through $\alpha7$ receptors. In hippocampus, $\alpha7$ nicotinic receptors are active postsynaptically on inhibitory interneurons (Frazier et al. 1998) and presynaptically on excitatory neurons (Gray et al. 1996), so that an $\alpha7$ nicotinic receptor agonist, such as tropisetron, could presumably activate both conditioning response excitation and test response inhibition.

In conclusion, the present study suggests that tropisetron improves the deficient inhibitory processing of the P20–N40 auditory evoked potential in DBA/2 mice through $\alpha7$, and perhaps $\alpha4\beta2$, nicotinic receptors. Tropisetron (Koike et al. 2005) thus joins clozapine as a medication that increases the inhibitory processing of auditory evoked responses in humans (Nagamoto et al. 1996, 1999) and animals (Simosky et al. 2003), and the two medications appear to share a common end effect on nicotinic receptors, especially the $\alpha7$ subtype. If the correlation between failure of sensory inhibition and neurophysiological measures of attentional dysfunction in schizophrenia (Cullum et al. 1993) reflects a common neurobiological mechanism, then 5-HT_3 antagonists, such as tropisetron and ondansetron, may offer a treatment option for attentional problems, and perhaps cognitive difficulties, often experienced by schizophrenia patients.

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