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Pharmacology, Biochemistry and Behavior 81 (2005) 879 – 886

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Effects of donepezil, nicotine and haloperidol on the central serotonergic system in mice: Implications for Tourette's syndrome

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Received 11 February 2005; received in revised form 8 June 2005; accepted 14 June 2005
Available online 19 July 2005

Abstract

We have previously reported that acute and chronic donepezil and nicotine administration significantly attenuate DOI-induced head twitch response (HTR) in mice. This behavior, primarily mediated by stimulation of 5-HT_{2A} receptors, has been proposed to model tic symptoms seen in Tourette's syndrome (TS). Haloperidol, a drug widely used to treat symptoms of TS, has also been reported to reduce DOI-induced head shakes in rodents when administered acutely. These findings suggest an inhibitory interaction of these drugs with 5-HT_{2A} receptors. To test this hypothesis, we evaluated the effects of chronic donepezil, nicotine and haloperidol on expression levels of 5-HT_{2A} mRNA and 5-HT_{2A} receptor density in select brain regions. Initially, we established a dose–response relationship for the acute and chronic haloperidol and DOI-induced HTR. Male ICR mice were treated twice daily with donepezil (0.1 mg/kg), nicotine (0.5 mg/kg), and once daily with haloperidol (0.4 mg/kg) for 14 days and were sacrificed 16–18 h after the last injection. These drug regimens were chosen because of their significant effects on DOI-induced HTR. Donepezil significantly increased 5-HT_{2A} mRNA level, but not the receptor density in the striatum. In the midbrain, donepezil significantly decreased the receptor density without affecting the 5-HT_{2A} mRNA level. In the frontal cortex, only haloperidol significantly reduced the 5-HT_{2A} receptor density. The cortex was the only area where donepezil, nicotine and haloperidol significantly reduced the 5-HT_{2A} receptor density. The results suggest that the anti-tic properties of donepezil, nicotine and haloperidol in this paradigm might be due to antagonism of cortical 5-HT_{2A} receptors. Thus, further investigation of involvement of cortical 5-HT_{2A} receptors in TS as well as evaluation of selective 5-HT_{2A} receptor antagonists in this disorder is warranted.

Keywords: Donepezil; Aricept; Nicotine; Haloperidol; 5-HT receptors; 5-HT mRNA; Cortex; Tourette's syndrome

1. Introduction

Tourette's syndrome (TS) is a neurological disorder characterized by chronic muscle movements and/or vocalizations called tics that occur in patients before eighteen years of age (Leckman, 2002). Many patients have reported that sensory symptoms often prompt tics, followed by feelings of relief after the performance of the tic (Bliss, 1980; Leckman et al., 1992; Cohen and Leckman, 1994; Leckman, 2003). The exact cause of TS is unknown. However, there are several theories proposing a neurological basis. Clinical, neuropathological and neuroimaging studies have suggested abnormalities of the basal ganglia and related thalamo-cortical circuits. It is also postulated that a developmental defect in cortico-striato-thalamo-cortical circuits is responsible for tic symptoms (Singer, 1997; Leckman et al., 1997; Singer and Minzer, 2003; Swerdlow and Sutherland, 2005). In addition, a genetic component and abnormalities of neurotransmitter systems may play a role in the pathophysiology of TS. Twin and family studies indicate that TS has strong genetic determinants (Price et al., 1985; Pauls et al., 1991; TSA International Consortium, 1999; Diaz-Anzaldua et al., 2004; Mercadante et al., 2004). Furthermore, an imbalance among neurotransmitter systems including the catecholamines and serotonin may contribute to the symptoms of the disorder (Baker et al., 1995; Leckman et al., 1995; Singer, 1997; Minzer et al., 2004).

Administration of the selective serotonin (5-HT) 2A/2C agonist (1-) 2,5-dimethoxy-4-iodophenyl-2-aminopropane

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doi:10.1016/j.pbb.2005.06.010

(DOI) in mice induces twitches of the head, and in rats, shakes of the shoulders; movements which have been proposed to model tics seen in TS (Handley and Dursun, 1992; Dursun and Handley, 1996; Gaynor and Handley, 2001). In mice, this behavior is termed the head twitch response (HTR). Although DOI has similar affinity for both 5-HT_{2A} and 5-HT_{2C} receptors, the head twitch behavior appears to be mediated primarily by 5-HT_{2A} receptors as this effect is blocked by selective 5-HT_{2A} but not 5-HT_{2C} antagonists (Kennett et al., 1994; Schreiber et al., 1995; Dursun and Handley, 1996; Kleven et al., 1997). Acute administration of haloperidol and pimozide, neuroleptics currently used to treat symptoms of TS, are also effective in reducing this DOI-induced behavior (Dursun and Handley, 1996; Yamada et al., 1995; Wettstein et al., 1999).

Nicotine, in the form of gum (McConville et al., 1991), transdermal patch (Dursun et al., 1994; Shytle et al., 1996; Sanberg et al., 1997) or a single dose (Orth et al., 2005) has shown clinical efficacy in treating tic symptoms. In addition, combination of nicotine and a neuroleptic may further reduce the tic severity (McConville et al., 1991; Silver et al., 1996, 2001; Shytle et al., 1996; Sanberg et al., 1997; Howson et al., 2004). Previous studies have demonstrated that nicotine treatment significantly attenuates DOI-induced head twitches (Tizabi et al., 2001) or head shakes (Gaynor and Handley, 2001) in mice. Donepezil (Aricept, Eisai/Pfizer, Teaneck, NJ) is an acetylcholinesterase inhibitor that is approved for use in mild to moderate Alzheimer's disease. We have recently reported that acute and chronic administration of donepezil also significantly attenuated DOI-induced HTR (Hayslett and Tizabi, 2003). The acute inhibitory effects of donepezil and nicotine on the DOI-induced HTR could not be blocked by the nicotinic receptor antagonist mecamylamine, suggesting that the actions of donepezil and nicotine in this paradigm are not directly mediated by nicotinic receptors (Tizabi et al., 2001; Hayslett and Tizabi, 2003).

The current study was conducted to determine whether the actions of the drugs that attenuate DOI-induced HTR might be due to their interaction with 5-HT_{2A} receptors. Specifically, we hypothesized that chronic administration of donepezil, nicotine or haloperidol will reduce the 5-HT_{2A} mRNA and/or the receptor density in selective brain regions. In order to test this hypothesis, we first carried out behavioral studies to establish an optimal haloperidol dose and used chronic dosing of donepezil and nicotine based on our prior results (Hayslett and Tizabi, 2003).

2. Methods

2.1. Animals and drugs

Adult male albino ICR mice, weighing 22–25 g, were used throughout the study. The animals were housed four per cage and kept on a 12-h light/dark cycle (lights on at 7:00 A.M.), in a temperature-controlled room (24–26 °C). The animals had ad libitum access to food and water. Experimental procedures were conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication NO. 85-23, revised 1985). Donepezil HCl was supplied by Eisai Pharmaceutical Co. (Ibaraki, Japan). Nicotine hydrogen tartrate salt, mecamylamine HCl and DOI HCl were purchased from Sigma-Aldrich Co. (St. Louis, MO). Haloperidol was purchased from Tocris (Ellisville, MO). All drugs were dissolved in saline and were injected intraperitoneally (i.p.) in a volume of 10 ml/kg. Nicotine dose was calculated as the base weight. Control animals were injected with saline.

2.2. Behavioral studies

A dose of 1 mg/kg of DOI was used to induce HTR. This dose has been shown to produce robust frequencies of HTR in mice (Hayslett and Tizabi, 2003; Tizabi et al., 2001; Darmani et al., 1996; Darmani and Gerdes, 1995). Mice were randomly assigned to treatment groups (7–8 mice/group). Various doses of a single administration and repeated administration of haloperidol were tested. For acute experiments, haloperidol was injected (i.p.) 10 min prior to DOI injection. For chronic experiments, haloperidol was administered once daily for 14 days. 16 to 18 h after the last injection, mice were injected with DOI. This paradigm was chosen to distinguish possible neuroadaptive changes that might occur following chronic administration from those of acute effects. Each mouse was tested in a clear plastic cage lined with bedding. Animals were allowed to habituate to the test environment for 30 min prior to experiments. The test was carried out immediately after DOI injection and lasted for 30 min, during which the number of head twitches was recorded. As described previously in detail, the head twitch response is a robust behavior that cannot be mistaken for spontaneous behaviors such as head shakes and head jerks. Indeed, identical scoring was obtained when we carried out the behavioral scoring in blind or non-blind manner.

2.3. Dosing regimen for molecular and neurochemical studies

Mice were randomly assigned to treatment groups: saline, donepezil, nicotine, and haloperidol (N=4/group for RT–PCR and N=7–8/group for binding assay). Mice received twice daily administration of saline, donepezil (0.1 mg/kg) or nicotine (0.5 mg/kg) and once daily administration of haloperidol (0.4 mg/kg) for 14 days. These chronic dosing regimens were based on their significant reduction of DOI-induced HTR in the behavioral studies as determined previously for donepezil and nicotine (Hayslett and Tizabi, 2003) and in this study for haloperidol. Approximately 16–18 h after the last injection, the mice were decapitated and their brains were rapidly removed. For

RT–PCR the brains were placed in RNAlater (Ambion, Austin, TX) and stored at 4 °C until RNA isolation and assays. For binding assay, brains were frozen on dry ice and stored at −80 °C until assays were conducted.

2.4. RNA isolation

Mice brains were removed from RNAlater solution. Frontal cortex, the rest of the cortex termed "cortex", striatum, midbrain and cerebellum were each dissected out on ice, under a magnifying glass as described previously (Tizabi et al., 2001). All instruments used were sterilized with RN-ase Erase (MP Biomedicals, Aurora, OH) to prevent contamination and degradation of RNA. Total RNA from each region was extracted using Trizol solution according to manufacturer's procedure (Invitrogen, Gaithersburg, MD). Briefly, tissue samples were homogenized in Trizol reagent using a Tekmar Tissumizer (Cincinnati, OH). Chloroform was added and the homogenate was centrifuged (Eppendorf Centrifuge 5415D) at 12,000×*g* for 10 min at 4 °C to allow for phase separation. The aqueous phase (containing RNA) was transferred to a fresh tube. The RNA was precipitated by mixture with isopropanolol. Samples were then centrifuged at 12,000×*g* for 10 min at 4 °C. The RNA precipitate formed a gel-like pellet on the side and bottom of the tube. The supernatant was discarded, the RNA pellet was washed with ethanol, vortexed and centrifuged again at 7500×*g* for 5 min at 4 °C. The RNA pellet was air-dried and dissolved in RN-ase- and DN-ase-free water (MP Biomedicals, Aurora, OH). The amount of total RNA was determined using a UV spectrophotometer (Ultrospec 2000, Pharmacia Biotech). Dissolved RNA has a 260:280 absorbance ratio of 1.7–1.9. Only RNA samples with purity between 1.7 and 1.9 were used for RT–PCR.

2.5. Reverse transcriptase–polymerase chain reaction (RT–PCR)

Total RNA (1 μg) was reverse transcribed into cDNAs using the Promega (Madison, WI) reverse transcription system. A 20 μl reaction was prepared per supplier's instructions using the following reagents supplied by the manufacturer: $MgCl_2$, reverse transcription buffer, deoxynucleotide triphosphates (dNTPs), recombinant RN-asin ribonuclease inhibitor, Avian Myeloblastosis Virus (AMV) reverse transcriptase, oligo(dT) primers, nuclease-free water and total RNA. The reaction was performed in a thermal cycler under the following conditions: incubation for 60 min at 42 °C, followed by 5 min incubation at 95 °C to inactivate the AMV reverse transcriptase. The cDNAs were stored at −20 °C until PCR amplification.

PCR reactions were performed using specific primer sets that were designed for mouse 5-HT_{2A} receptors and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the Primer Premier software program. GAPDH, expressed in all living cells, served as a housekeeping gene to exclude variations in the PCR amplification and quantification process (Vedder et al., 1999). The primers for 5-HT_{2A} receptors were as follows: forward/sense 5′–ATC CCA GTC TTC GGG CTA–3′ and reverse/antisense 5′–GGG CAC CAC ATT ACA. The primers produced products of approximately 386 base pairs (bp). The annealing (primer binding to cDNA) temperature was 54 °C. The primers for GAPDH were: forward/sense 5′–GAA GGG TGG AGC CAA ACG–3′ and reverse/antisense 5′–AAG GTG GAA GAG TGG GAG–3′. The primers produced products of approximately 550 bp. The annealing temperature was 53 °C. The primers were purchased from Invitrogen (Gaithersburg, MD) and were reconstituted in DN-ase and RN-ase free water to make a final concentration of 20 μM. The PCR reactions (20 μl) used *Taq* polymerase and PCR nucleotide mix purchased from Promega (Madison, WI). The reactions were conducted with the following reagents: reaction buffer, $MgCl_2$, dNTPs, forward and reverse primers, *Taq* polymerase, nuclease-free water and the cDNA of interest. Samples were denatured for 5 min at 94 °C, followed by 35 cycles of 30-s denaturing at 94 °C, 30 s at the annealing temperatures and a 30-s extension at 72 °C. A final extension of 7 min at 72 °C concluded the reactions. The PCR products were separated by electrophoresis on 1% agarose gels. The intensity of each band was analyzed using the NIH ImageJ Densitometry program.

Because no effect on GAPDH was detected, the intensity of the bands in the 5-HT_{2A} receptor gels was used as a direct indication of 5-HT_{2A} receptor mRNA levels. No product was observed when cDNAs were omitted from the assay, indicating that the PCR products were not due to DNA contamination. The results are expressed as optical density/μg total RNA.

2.6. Binding assay

Mice brains were thawed on ice. Frontal cortex, the rest of the cortex "cortex", striatum and midbrain, were each dissected out under a magnifying glass, weighed and homogenized in ice-cold buffer solution (approximately 10 mg tissue/ml in 50 mM Tris–HCl, pH 7.4). The homogenate was centrifuged (Beckman Avanti J-25I) at 39,000×*g* for 10 min. The supernatant was discarded and the pellet was resuspended and homogenized again at 39,000×*g* for 10 min. The supernatant was discarded, the pellet was resuspended and incubated at 37 °C for 10 min, then subjected to centrifugation as stated above. The final pellet was resuspended in assay buffer (50 mM Tris–HCl, 10 μM pargyline, 4 mM $CaCl_2$, 0.1% ascorbic acid at pH 7.4) (Leysen et al., 1981; Kleven et al., 1997) and the amount of protein was determined using a BCA protein assay kit (Pierce, Rockford, IL) prior to receptor measurements.

The binding assays were performed in triplicate in a final volume of 250 μl with the following components: tissue homogenate (200 μl), radioligand (25 μl) and displacing ligand (25 μl) or buffer (25 μl). Approximately 4 nM of [^{3}H]

ketanserin, a selective 5-HT_{2A} receptor antagonist (specific activity 76.5 Ci/mmol, Perkin-Elmer, Boston, MA) was used to determine 5-HT_{2A} receptor binding. Ketanserin (10 μM) (Sigma, St. Louis, MO) was used to determine nonspecific binding. The tubes were incubated for 30 min at 37 °C, filtered under vacuum (Brandel, Gaithersburg, MD) and washed three times with 4 ml of buffer (Leysen et al., 1981; Kleven et al., 1997). The amount of [^{3}H] ketanserin trapped on Brandel GF/C glass filter papers (presoaked in 0.5% polyethylenimine, to reduce nonspecific binding) was measured in 5 ml of Econosafe scintillation cocktail fluid (Mt. Prospect, IL). The radioactivity (disintegrations per min) was determined using a beta counter (Beckman LS 7800). Specific binding (SB) was calculated as nonspecific binding (NSB) subtracted from total binding (TB). Specific binding was expressed per mg protein.

2.7. Statistical analysis

Behavioral data were analyzed by a repeated measure ANOVA using dose as a between group factor. The molecular and neurochemical data were analyzed using an overall analysis of variance with each region as a within factor. The ANOVAs were followed by Tukey's post hoc test. Differences were considered statistically significant when p values were less than 0.05. Data was analyzed using the SPSS software program.

3. Results

3.1. Behavioral studies

Fig. 1 illustrates the effects of a single administration of various doses of haloperidol on DOI-induced HTR. The doses of 0.2 mg/kg and 0.4 mg/kg significantly reduced DOI-induced HTR compared to control (saline group) by approximately 40% and 50%, respectively [$F(3,27)=8.59$, $p<0.05$, and $F(3,28)=4.86$, $p<0.05$]. There were no significant differences between the effects of these two doses. The lower doses had no effect on DOI-induced HTR compared to control.

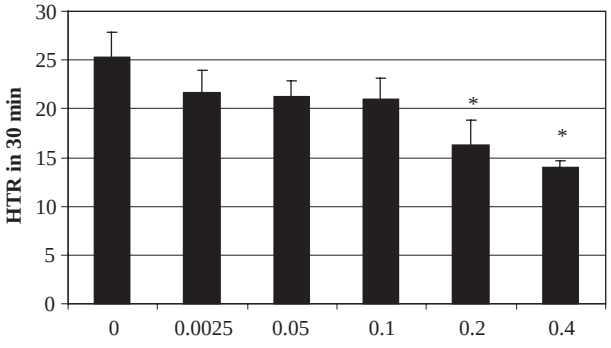

Fig. 1. The effects of a single administration of various doses of haloperidol (mg/kg) on DOI (1.0mg/kg)-induced HTR in male ICR mice. Haloperidol was injected 10 min prior to DOI. Immediately following DOI injection, mice were observed for HTR for 30 min. *$p<0.05$, compared to saline-treated group (0 mg/kg) ($N=7-8$/group).

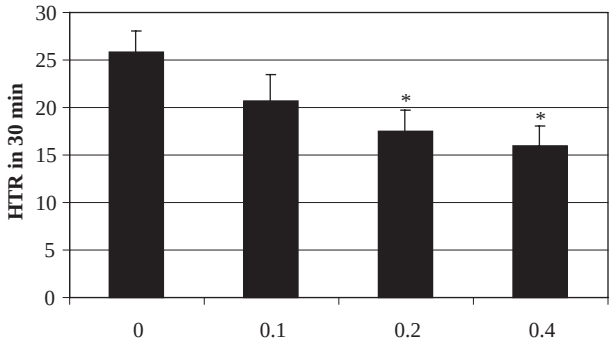

Fig. 2. The effects of various doses of chronic (14 days) haloperidol (mg/kg) on DOI (1.0mg/kg)-induced HTR in male ICR mice. Mice were treated with haloperidol once daily for 14 days. 16–18 h after the last injection, mice were injected with DOI and observed for HTR for 30 min. *$p<0.05$, compared to saline-treated group (0 mg/kg) ($N=7-8$/group).

Fig. 2 represents the effects of 14 days' administration of various doses of haloperidol on DOI-induced HTR. Significant reductions in DOI-induced HTR were produced by doses of 0.2 mg/kg and 0.4 mg/kg by approximately 31% and 38%, respectively [$F(3,26)=3.39$, $p<0.05$, and $F(3,27)=3.19$, $p<0.05$] compared to control (saline group). There were no significant differences between the effects of these two doses.

3.2. RT–PCR

All agarose gels displayed cDNAs from discrete mouse brain regions, obtained by RT–PCR using the primers for mouse GAPDH (a gel representative is shown in Fig. 5A, upper panel). Similarly, all regions except cerebellum also displayed cDNAs for 5-HT_{2A} receptors (a gel representative is shown in Fig. 5A, bottom panel).

Fig. 3 revealed that repeated administration of donepezil, nicotine, and haloperidol had no significant effects on mRNA expression levels of 5-HT_{2A} receptors in mouse frontal cortex compared to control (saline group).

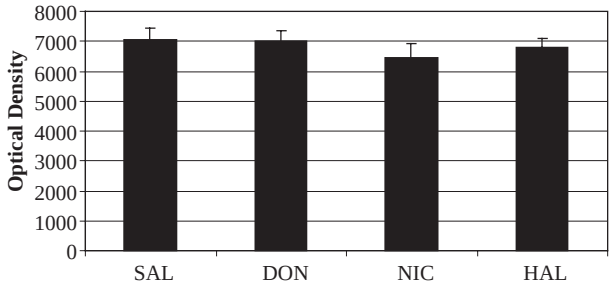

Fig. 3. 5-HT_{2A} receptor mRNA levels in the frontal cortex of male ICR mice following twice daily administration of donepezil (DON; 0.1 mg/kg), nicotine (NIC; 0.5 mg/kg) and once daily administration of haloperidol (HAL; 0.4 mg/kg) for 14 days. Animals were sacrificed 16–18 h after the last dose ($N=4$/group).

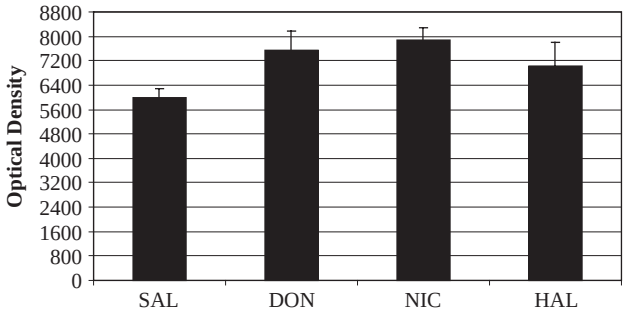

Fig. 4. 5-HT_{2A} receptor mRNA levels in the cortex of male ICR mice following twice daily administration of donepezil (DON; 0.1 mg/kg), nicotine (NIC; 0.5 mg/kg) and once daily administration of haloperidol (HAL; 0.4 mg/kg) for 14 days. Animals were sacrificed 16–18 h after the last dose (N=4/group).

Fig. 4 depicts that mRNA expression levels of 5-HT_{2A} receptors were slightly increased in the cortex by repeated administration of donepezil, nicotine and haloperidol compared to the saline group. However, these increases did not achieve statistical significance.

Fig. 5 shows that twice daily treatment of donepezil for two weeks significantly increased striatal 5-HT_{2A} mRNA levels by approximately 31% [$F(3,12)=7.83$, $p<0.05$]. Repeated administration of nicotine and haloperidol had no significant effects on 5-HT_{2A} mRNA levels in the striatum.

Fig. 6 reveals that repeated drug administrations had no significant effects on 5-HT_{2A} mRNA levels in the midbrain compared to control (saline group).

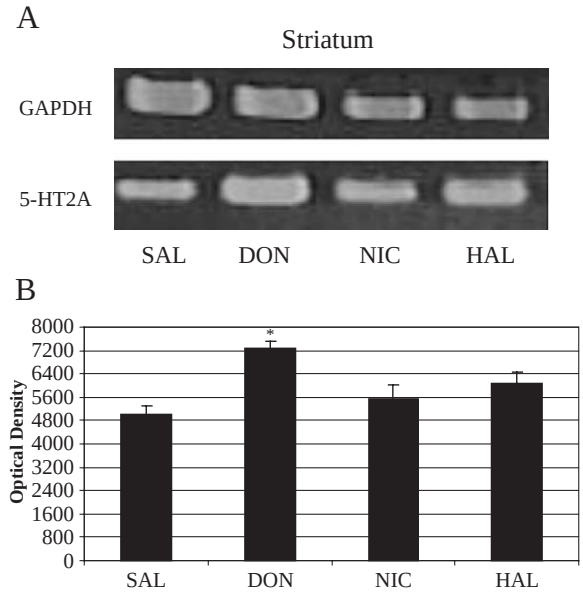

Fig. 5. Gel images (A) and levels (B) of 5-HT_{2A} receptor mRNA in the striatum of male ICR mice following twice daily administration of donepezil (DON; 0.1 mg/kg), nicotine (NIC; 0.5 mg/kg) and once daily administration of haloperidol (HAL; 0.4 mg/kg) for 14 days. Animals were sacrificed 16–18 h after the last dose. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. *$p<0.05$, compared to saline-treated group (SAL) (N=4/group).

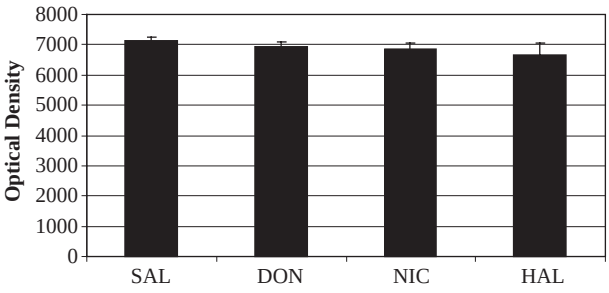

Fig. 6. 5-HT_{2A} receptor mRNA levels in the midbrain of male ICR mice following twice daily administration of donepezil (DON; 0.1 mg/kg), nicotine (NIC; 0.5 mg/kg) and once daily administration of haloperidol (HAL; 0.4 mg/kg) for 14 days. Animals were sacrificed 16–18 h after the last dose (N=4/group).

5-HT_{2A} mRNA was not detectable in the cerebellum. In areas where 5-HT_{2A} mRNA was detected, the levels did not differ significantly from each other.

3.3. Binding assay

Figs. 7–10 illustrate the effects of twice daily administration for 14 days of donepezil (0.1 mg/kg), nicotine (0.5 mg/kg) and once daily administration for 14 days of haloperidol (0.4 mg/kg) on 5-HT_{2A} receptor density in mouse frontal cortex, cortex, striatum and midbrain, respectively. Because no mRNA was detected in the cerebellum, the receptor assay was not carried out in this region. Significant regional differences were observed in receptor densities [$F(3,67)=8.12$, $p<0.001$]. The highest levels were present in the cortex and midbrain and lowest levels in the frontal cortex and striatum. The effect of treatment on 5-HT_{2A} receptor density differed between regions [$F(3,67)=5.31$, $p<0.01$].

In the frontal cortex, haloperidol significantly decreased 5-HT_{2A} receptor density by approximately 46% [$F(3,26)=5.35$, $p<0.05$] compared to saline group. Donepezil and nicotine produced no significant effects (Fig. 7).

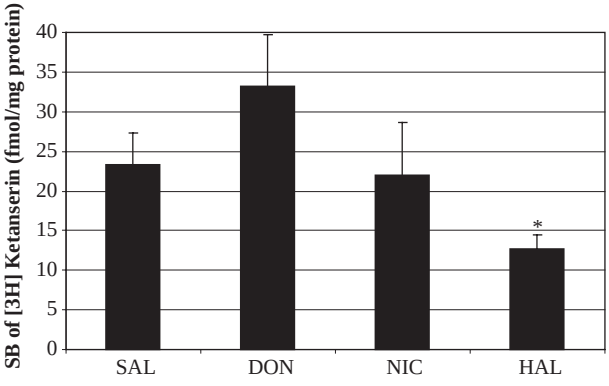

Fig. 7. The effects of twice daily administration of donepezil (DON; 0.1 mg/kg), nicotine (NIC; 0.5 mg/kg) and once daily administration of haloperidol (HAL; 0.4 mg/kg) for 14 days on 5-HT_{2A} receptor density in the frontal cortex of male ICR mice. Animals were sacrificed 16–18 h after the last dose. *$p<0.05$, compared to saline-treated group (SAL) (N=7–8/group).

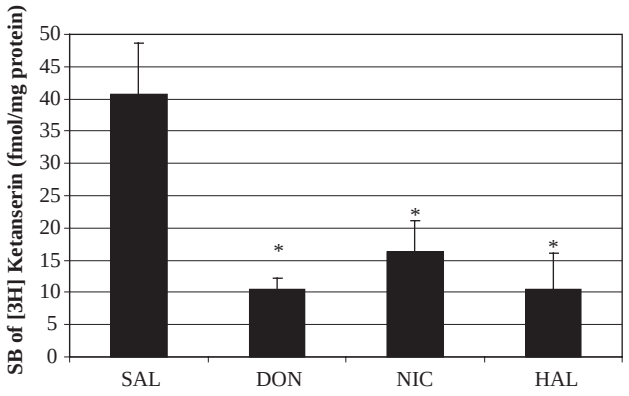

Fig. 8. The effects of twice daily administration of donepezil (DON; 0.1 mg/kg), nicotine (NIC; 0.5 mg/kg) and once daily administration of haloperidol (HAL; 0.4 mg/kg) for 14 days on 5-HT_{2A} receptor density in the cortex of male ICR mice. Animals were sacrificed 16–18 h after the last dose. *$p<0.05$, compared to saline-treated group (SAL) ($N=7–8$/group).

In the cortex, donepezil, nicotine and haloperidol all significantly reduced 5-HT_{2A} receptor density [$F(3,25)=6.808$, $p<0.05$] by approximately 74%, 60% and 74%, respectively, as compared to control (Fig. 8).

In the striatum, donepezil and nicotine appeared to increase whereas haloperidol appeared to decrease 5-HT_{2A} receptor density (Fig. 9). However, none of these changes were statistically significant.

In the midbrain, only donepezil significantly decreased 5-HT_{2A} receptor binding by approximately 60% [$F(3,26)=6.796$, $p<0.05$] compared to control. Nicotine and haloperidol resulted in approximately 20% and 45% decreases in 5-HT_{2A} receptor binding, respectively. However, neither one of these effects was statistically significant.

4. Discussion

It is believed that the head twitches induced by DOI are primarily mediated through activation of the 5-HT_{2A} receptors (Kennett et al., 1994; Schreiber et al., 1995; Dursun and Handley, 1996; Kleven et al., 1997), which are particularly abundant in the cortex (Lopez-Gimenez et al., 1997; Wu et al., 1998). A major finding in these studies is that chronic treatment with donepezil, nicotine or haloperidol in regimens that reduce DOI-induced HTR, also selectively reduced cortical 5-HT_{2A} receptor densities, suggesting that reductions in cortical 5-HT_{2A} receptors may be a shared mechanism for their anti-tic activity. This contention is consistent with several studies in rodents indicating that drug-induced decreases in 5-HT_{2A} mediated behaviors are correlated with decreases in 5-HT_{2A} receptor densities in the cortex (Blackshear and Sanders-Bush, 1982; Leysen et al., 1989; Smith et al., 1990, 1999; Aloyo et al., 2001).

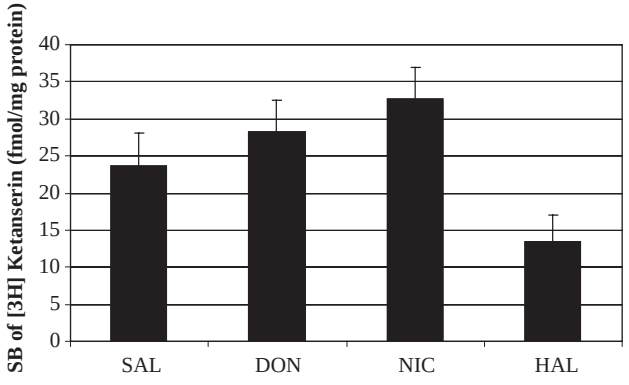

Fig. 9. The effects of twice daily administration of donepezil (DON; 0.1 mg/kg), nicotine (NIC; 0.5 mg/kg) and once daily administration of haloperidol (HAL; 0.4 mg/kg) for 14 days on 5-HT_{2A} receptor density in the striatum of male ICR mice. Animals were sacrificed 16–18 h after the last dose ($N=7–8$/group).

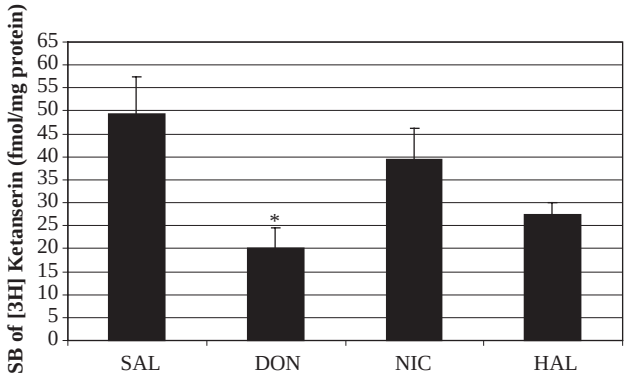

Fig. 10. The effects of twice daily administration of donepezil (DON; 0.1 mg/kg), nicotine (NIC; 0.5 mg/kg) and once daily administration of haloperidol (HAL; 0.4 mg/kg) for 14 days on 5-HT_{2A} receptor density in the midbrain of male ICR mice. Animals were sacrificed 16–18 h after the last dose. *$p<0.05$, compared to saline-treated group (SAL) ($N=7–8$/group).

Measuring behaviors that are induced by stimulation of 5-HT_{2A} receptors can be used to assess the functional responsiveness of these receptors (Aloyo et al., 2001). It has been shown that administration of agonists and antagonists can cause down-regulation of 5-HT_{2A} receptors (Blackshear et al., 1983, 1986; Mikuni and Meltzer, 1984; Andree et al., 1986; Leysen et al., 1989; Wilmot and Szczepanik, 1989; O'Dell et al., 1990; Aloyo et al., 2001; Dean, 2003). Inhibition of the DOI-induced behavior and the reduction in 5-HT_{2A} receptor density in select brain regions suggest that donepezil, nicotine and haloperidol may be acting as antagonists at this receptor in our system. That haloperidol may act as an antagonist at 5-HT_2 receptors has been previously reported (Arnt et al., 1984; Yamada et al., 1995; Dursun and Handley, 1996). Our finding of a decrease in 5-HT_{2A} receptor density by nicotine is also consistent with reports that chronic administration of nicotine results in decreased 5-HT_2 receptor binding and function in select rat brain regions (Xu et al., 2002) and that withdrawal from nicotine results in supersensitivity of these receptors (Suemaru et al., 2001; Yasuda et al., 2002). Moreover, 5-HT_2 receptors have been implicated in the locomotor and rewarding effects of nicotine (Grottick et al., 2001;

Olausson et al., 2001). To our knowledge, this is the first study reporting an interaction between donepezil and 5-HT_2 receptors.

We also found that only donepezil affected 5-HT_{2A} mRNA where a 31% increase in mRNA levels was observed in the striatum. This increase in 5-HT_{2A} mRNA, however, was not translated into protein expression in this region, suggesting dissociation between mRNA and protein expression. Thus, it is unlikely that donepezil's effect on DOI-induced behavior is influenced by the change in 5-HT_{2A} mRNA in the striatum. We did not observe any effect of nicotine on 5-HT_{2A} mRNA. The lack of effect of haloperidol on 5-HT_{2A} mRNA in specific brain regions is consistent with previous reports (Burnet et al., 1996; Buckland et al., 1997; Steward et al., 2004). However, Buckland et al. (1997) found that haloperidol significantly decreased 5-HT_{2A} mRNA in the rat midbrain. Lack of haloperidol effect on 5-HT_{2A} mRNA in the midbrain in our study might be due to differences in species and dosing regimens between the two studies. Buckland et al. (1997) used rats and administered haloperidol at a dose of 3 mg/kg/day for 32 days whereas we used mice and administered 0.4 mg/kg haloperidol once daily for 14 days. The incongruity between the mRNA and protein results in our paradigm suggests that the effects of donepezil, nicotine or haloperidol on receptor expression are likely to be due to post-translational modifications.

Our behavioral findings with haloperidol confirm previous acute results (Yamada et al., 1995; Dursun and Handley, 1996; Wettstein et al., 1999) and extend them to include the effectiveness of chronic haloperidol in this paradigm. It is of interest to note that both acute and chronic haloperidol administration resulted in greater reduction of DOI-induced HTR (approximately 50% and 38%, respectively) compared to either donepezil or nicotine which were very close to one another (acute 30–32%; chronic 27–28%, Hayslett and Tizabi, 2003). The higher effectiveness of haloperidol in this paradigm may be due to its added effect in reducing the 5-HT_{2A} receptor densities in the frontal cortex where donepezil and nicotine were without a significant effect. However, this suggestion requires direct comparisons of the drugs in the animal model and perhaps in clinical trials as well. Moreover, the mechanism of action of these drugs may be quite different. Thus, each drug may operate through a unique mechanism which can indirectly result in reducing 5-HT_{2A} receptor density or function. For example, chronic administration of nicotine and donepezil may induce prolonged inactivation of the nicotinic cholinergic receptors that may indirectly reduce the 5-HT_{2A} receptor function whereas haloperidol may act through dopaminergic and/or serotonergic systems.

In summary, our findings suggest that the anti-tic properties of donepezil, nicotine and haloperidol might be due to antagonism of cortical 5-HT_{2A} receptors. Thus, further investigation of involvement of cortical 5-HT_{2A} receptors in TS as well as evaluation of selective 5-HT_{2A} receptor antagonists in this disorder is warranted.

Acknowledgment

This work was supported by an unrestricted grant from Eisai/Pfizer. The authors wish to thank Dr. Jonathan Wiest for his support and allowing the use of his facilities for the measurements of mRNA.

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