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Acute Effect of Alcohol and Nicotine on 5-Hydroxytryptamine Synthesis and Tryptophan Hydroxylase Expression in Dorsal and Median Raphe of Rats

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Alcohol abuse and cigarette smoking have been on the rise worldwide and it has been reported that alcohol and nicotine influence serotonergic neuronal activity in the dorsal raphe. Serotonin (5-hydroxytryptamine, 5-HT) has been implicated in the pathophysiology of various neuropsychiatric disorders. In the present study, the effects of alcohol and nicotine on the synthesis of 5-HT and the expression of tryptophan hydroxylase (TPH), the rate limiting enzyme of 5-HT synthesis, in the dorsal and median raphe of young rats were investigated *via* immunohistochemistry. The numbers of the 5-HT-positive and TPH-positive cells in raphe nuclei were reduced by alcohol and nicotine treatment, and these numbers were reduced more potently by co-administration of alcohol and nicotine. Based on the results, it can be suggested that the pathogenesis of alcohol- and nicotine-induced neuropsychological disorders involves alcohol- and nicotine-induced suppression of 5-HT synthesis and TPH expression in raphe, and that this may be of particular relevance in the consumption of alcohol and nicotine during adolescence.

Key Words: Alcohol, Nicotine, 5-Hydroxytryptamine, Tryptophan hydroxylase, Dorsal raphe nuclei, Median raphe nuclei, Adolescence

INTRODUCTION

Alcohol abuse and cigarette smoking have been on the rise worldwide. It is generally accepted that alcohol and nicotine affect learning and memory functions, and disrupt brain development during adolescence (Trauth et al, 2000; Kuperman et al, 2001).

Alcohol abuse is known to cause substantial neuronal loss in several regions of the brain, and it has also been reported that alcohol induces apoptotic neurodegeneration in the developing rat brain (Ikonomidou et al, 2000). Acute alcohol intoxication is known to adversely affect performance in tasks involving short-term memory functions in rodents and humans (Ryabinin, 1998). In addition, it has been reported that alcohol influences serotonergic neuronal activity in the dorsal raphe (Morzorati & Johnson, 1999; Berggren et al, 2001). Furthermore, alcohol abuse during the adolescent stage has been known to induce behavioral disorders (Kuperman et al, 2001).

Nicotine is a key neuroregulatory component of cigarette smoke. Numerous studies in animals and humans have shown that smoking during pregnancy causes developmental deficits, and several lines of evidence from recent studies indicate a strong correlation between gestational nicotine exposure and learning disability and disruptive behavior (Slotkin et al, 1997; Roy et al, 1998). It has been confirmed that nicotine itself is a neuroteratogen, damaging developing brain cells, altering cell differentiation and thus affecting synaptic maturation, and evoking permanent changes in synaptic activity and cell signaling (Slotkin et al, 1997; Roy et al, 1998). Recently, Breitinger et al. (2001) have shown that nicotine inhibits the activation of 5-HT_3 receptors, and Xu et al. (2001) suggested that nicotine might play a role in the subsequent onset of depression by suppressing 5-HT transporters.

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter and/or neuromodulator in the mammalian central nervous system (CNS); it has been implicated in the pathophysiology of many neuropsychiatric disorders such as schizophrenia, depression, generalized anxiety disorder, and panic disorder (Graeff, 1997). It is known to modulate emotion, cognition, endocrine activity, appetite, sexual behavior, motor function, and pain (Zhou et al, 2001). Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step of serotonin biosynthesis in serotonergic neurons of the raphe nuclei, and TPH enzyme activity in the brain is known to regulate 5-HT synthesis during development (Rind et al, 2000).

Although evidences suggest that alcohol and nicotine affect learning and memory, especially during adolescence,

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; TPH, tryptophan hydroxylase; DRN, dorsal raphe nuclei; MRN, median raphe nuclei; PBS, phosphate-buffered saline; PB, phosphate buffer; DAB, 3,3'-diaminobenzidine.

these effects of alcohol and nicotine have not been investigated in relation to the synthesis of 5-HT and the expression of TPH in the raphe nuclei. In the present study, the effects of alcohol and nicotine on the synthesis of 5-HT and expression of TPH in the dorsal raphe nuclei (DRN) and median raphe nuclei (MRN) of young rats were investigated *via* immunohistochemistry.

METHODS

Animals and treatments

Male Sprague-Dawley rats weighing 90±10 g (30 days postpartum) were used for the experiment. The experimental procedures were performed in accordance with the animal care guidelines of the Korean Academy of Medical Sciences and the National Institute of Health. Each animal was housed at a controlled temperature (20±2°C) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness. Animals were divided into four groups: the control group, the alcohol- treated group, the nicotine-treated group, and the alcohol- and nicotine-treated group (n=5 in each group). Rats of the alcohol-treated group were injected with alcohol (2 g kg^{-1}, i.p) twice per day for 3 days, and those of the nicotine- treated group were injected with nicotine (1 mg kg^{-1}, i.p.), also twice per day for 3 days. Those of the alcohol- and nicotine-treated group received both alcohol and nicotine at the same dosage and for the same duration of time. The control group received equivalent amounts of saline for the same duration of time. Animals were sacrificed 72 h after commencement of the experiment.

Blood alcohol concentration measurements

For analysis of serum alcohol concentration, blood was collected from animals *via* cardiac puncture 2 h after the last alcohol injection, and the blood alcohol concentration was measured using a Sigma Diagnostics® kit (Sigma Chemical Co., St. Louis, MO, USA) according to the manufacturer's protocol.

Histological procedures

For the sacrificial process, animals were first fully anesthetized with Zoletil 50® (10 mg kg^{-1}, i.p.; Vibac Laboratories, Carros, France), transcardially perfused with 50 mM phosphate-buffered saline (PBS), and then fixed with a freshly prepared solution consisting of 4% paraformaldehyde in 100 mM phosphate buffer (PB, pH 7.4). The brains were then removed, postfixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. Coronal sections of 40 μm thickness were made with a freezing microtome (Leica, Nussloch, Germany).

Eight sections on average were obtained from each brain for immunohistochemical processing. Free-floating tissue sections were incubated overnight with rabbit anti-5-HT antibody (Oncogene Reserch Product, Cambridge, UK) at a dilution of 1 : 1000 for visualization of 5-HT or with goat anti-TPH antibody (Oncogene Reserch Product, Cambridge, UK) at a dilution of 1 : 1000 for visualization of TPH expression. The sections were then incubated for 1 h with biotinylated anti-rabbit secondary antibody or with anti-mouse secondary antibody (Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with an avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3'-diaminobenzidine (DAB) and 0.01% H_2O_2 in 50 mM Tris-buffer (pH 7.6) for approximately 3 min.

Statistical analysis

Statistical differences were determined by one-way analysis of variance (ANOVA) followed by Scheffe's Post-hoc analysis, and results were expressed as mean±standard error mean (S.E.M.). Differences were considered significant for P<0.05.

RESULTS

Blood alcohol concentration

The serum alcohol concentration was 84.11±3.27 mg/dl in the alcohol-treated group, 78.74±2.89 mg/dl in the alcohol- and nicotine treated group, and 0 or negligible in all other groups.

5-HT-positive and TPH-positive cells in DRN of each group

5-HT-positive and TPH-positive cells in DRN are presented in Fig. 1. The number of 5-HT-positive cells in the DRN was 164.00±13.77 per section in the control group, 123.50±8.17 in the alcohol-treated group, 136.71±6.68 in the nicotine-treated group, and 102.88±9.66 in the alcohol- and nicotine-treated group. The number of TPH-positive cells in the DRN was 263.63±23.24 per section in the control group, 144.89±7.87 in the alcohol-treated group, 205.20±11.76 in the nicotine-treated group, and 128.30±7.40 in the alcohol- and nicotine-treated group (Fig. 2).

5-HT-positive and TPH-positive cells in MRN of each group

5-HT-positive and TPH-positive cells in MRN are presented in Fig. 3. The number of 5-HT-positive cells in the MRN was 63.20±7.05 in the control group, 39.00±3.09 in the alcohol-treated group, 48.14±4.36 in the nicotine-treated group, and 39.71±2.97 in the alcohol- and nicotine-treated group. The number of TPH-positive cells in the MRN was 72.63±7.38 in the control group, 49.75±5.84 in the alcohol-treated group, 39.00±5.37 in the nicotine-treated group, and 52.56±8.52 in the alcohol- and nicotine-treated group (Fig. 4).

5-HT levels in the DRN and MRN were decreased by both alcohol and nicotine treatment. The number of 5-HT-positive cells in the DRN was decreased more potently by co-administration of alcohol and nicotine than by treatment with nicotine alone. Expression of TPH in the DRN and MRN was decreased following treatment with alcohol and nicotine. The number of TPH-positive cells in the DRN was decreased more potently by co-administration of alcohol and nicotine than by treatment with nicotine alone.

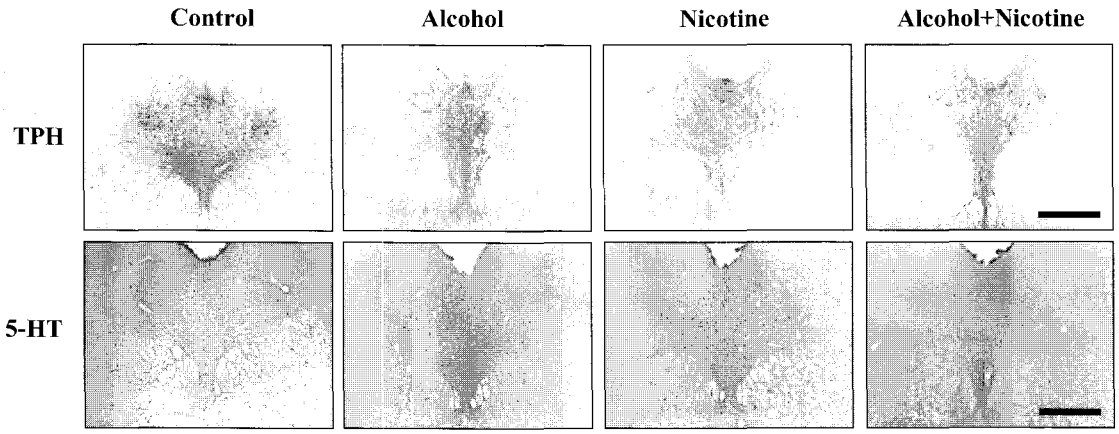

Fig. 1. Photomicrographs of 5- hydroxytryptamine (5-HT, serotonin)-positive and tryptophan hydroxylase (TPH)-positive cells in the dorsal raphe nuclei (DRN) of the midbrain. Sections were stained for 5-HT and TPH (reddish brown). Scale bar represents 250 μm.

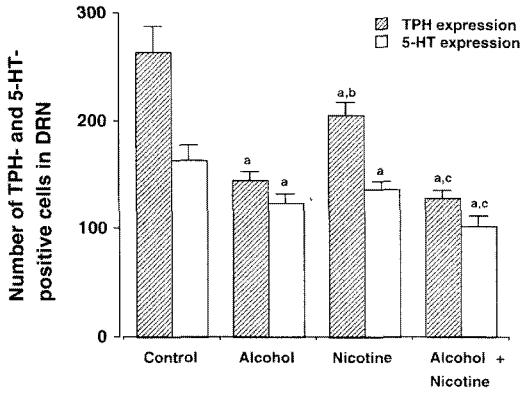

Fig. 2. Mean number of 5-hydroxytryptamine (5-HT)-positive and tryptophan hydroxylase (TPH)-positive cells in the dorsal raphe nuclei (DRN) in each group. Values are represented as mean ± S.E.M. [a] represents $P<0.05$ compared to the control group. [b] represents $P<0.05$ compared to the alcohol-treatedl group [c] represents $P<0.05$ compared to the nicotine-treated group.

DISCUSSION

Serotonin plays a role in structural formation of the brain both during development and in adulthood. Serotonin depletion leads to decreased synaptic density and learning deficits, and results in neurodevelopmental disorders with cognitive deficits (Mazer et al, 1997). Lalonde & Vikis-Freibergs (1985) showed that serotonin has been linked to learning and memory in rats, and Gould (1999) demonstrated that serotonin stimulates neurogenesis in hippocampal dentate gyrus.

In clinical studies, dysfunction of the central serotonergic system has been associated with pathogenesis of depressive mood disorders, and administration of tryptophan, a 5-HT precursor, was shown to produce anti-depressive effect (Middlemiss et al, 2002). During the adolescent stage, throughout which neuroproliferation continues (Bayer et al, 1982), alcohol and nicotine administration may result in neuropsychiatric disorders by bringing about alterations in the central serotonergic system (Morzorati & Johnson, 1999; Berggren et al, 2001; Breitinger et al, 2001; Xu et al, 2001).

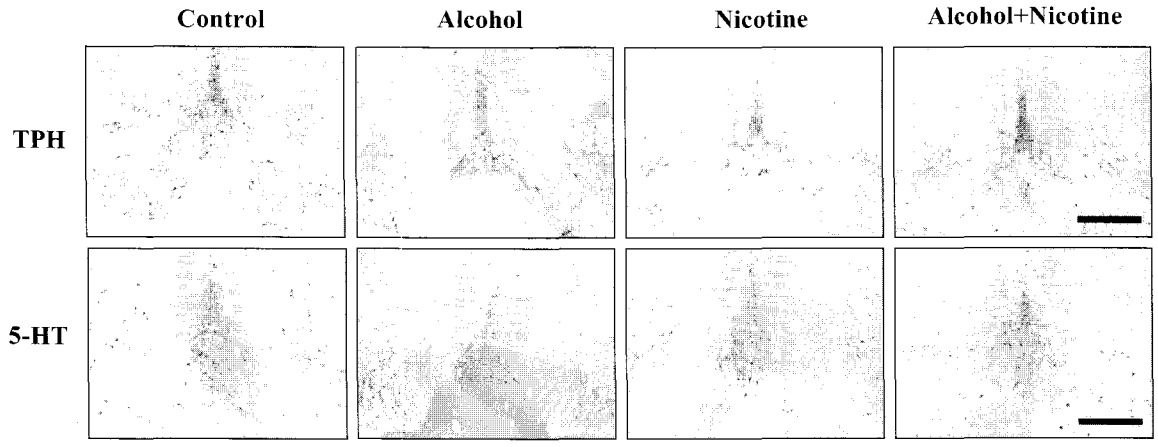

Fig. 3. Photomicrographs of 5- hydroxytryptamine (5-HT, serotonin)-positive and tryptophan hydroxylase (TPH)-positive cells in the median raphe nuclei (MRN) of the midbrain. Sections were stained for 5-HT and TPH (reddish brown). Scale bar represents 250 μm.

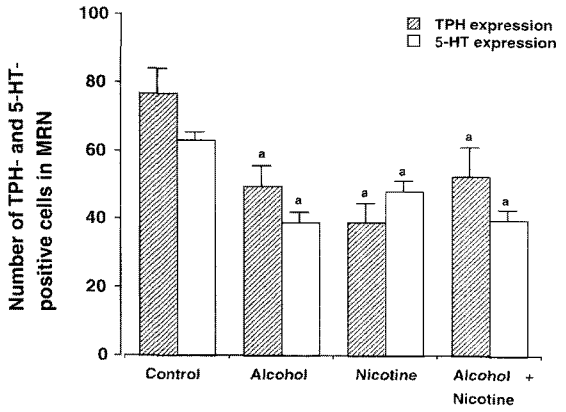

Fig. 4. Mean number of 5- hydroxytryptamine (5-HT)-positive and tryptophan hydroxylase (TPH)-positive cells in the median raphe nuclei (MRN) in each group. Values are represented as mean± S.E.M. [a] represents $P<0.05$ compared to the control group.

From the present results, it has been demonstrated that administration of alcohol suppresses the synthesis of 5-HT and the expression of TPH in the DRN and MRN. In several studies, it was reported that alcohol induces apoptotic damage in the brain of the developing rat *in vivo* (Ikonomidou et al, 2000) and that such apoptotic cell death is associated with dysfunction of the serotonergic system (Tajuddin & Druse, 1999).

In the present study, nicotine administration also reduced 5-HT and TPH levels in the DRN and MRN. It has been documented that nicotine exposure during adolescence stage has a deleterious effect on neural development, characterized by cell damage and loss that appears after a substantial delay period (Trauth et al, 1999; 2000). In numerous studies, it was shown that nicotine administration during adolescence upregulates nicotinic acetylcholine receptors (Trauth et al, 1999) and promotes apoptosis (Roy et al, 1998). Adolescent smoking is also associated with the subsequent development of psychological disorders (Fergusson et al, 1998; Wu et al, 1999). Close relationships between depression and nicotine exposure during adolescence have been suggested: this form of depression may be caused by reductions in neurogenesis, which is controlled in part by 5-HT administration (Jacobs et al, 2000).

Based on the results, it can be suggested that alcohol- and nicotine-related neuropsychiatric disorders originating from dysfunction of the serotonergic nervous system may be due to alcohol- and nicotine-induced suppression of the synthesis of 5-HT and the expression of TPH in the dorsal and median raphe, and that this may be of particular relevance in the consumption of alcohol and nicotine during adolescence.

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