ALTERATION OF 5-HIAA LEVELS IN FRONTAL CORTEX AND DORSAL RAPHE NUCLEUS IN RATS TREATED WITH COMBINED ADMINISTRATION OF TRYPTOPHAN AND ETHANOL

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ABSTRACT — The present studies sought to investigate the effect of tryptophan alone or coadministration of tryptophan and ethanol on the interaction of central frontal cortex and dorsal raphe nucleus serotonergic functional activities by utilizing in vivo microdialysis. Tryptophan (50 mg/kg, i.p.) led to a significant increase in the levels of 5-HIAA, a metabolite of serotonin (5-HT), in the dorsal raphe nucleus, but not in the frontal cortex. Coadministration of tryptophan and ethanol caused very marked increases in 5-hydroxyindoleacetic acid (5-HIAA) levels in both the frontal cortex and the dorsal raphe nucleus, although ethanol (1.25 g/kg) did not change 5-HIAA levels in both areas. Moreover, the application of WAY100635 (10 μM), 5-HT₁A antagonist, into the frontal cortex after coadministration caused a marked increase in 5-HIAA levels in the frontal cortex and a decrease in the levels in the dorsal raphe nucleus, although WAY100635 alone had no effect on these levels. This may suggest that WAY100635-induced increase of 5-HIAA levels in the frontal cortex resulted from negative feedback following the blockade of serotonergic 5-HT₁A autoreceptors, and that this increase in 5-HIAA levels decreased 5-HIAA levels in the dorsal raphe nucleus by preventing the activation of dorsal raphe 5-HT₁A autoreceptors. WAY100635 into the dorsal raphe nucleus did not significantly change 5-HIAA levels in both areas. This may indicate that the blockade of dorsal raphe 5-HT₁A autoreceptors by WAY100635 resulted in unchanged 5-HIAA levels in the frontal cortex. Behavioral sign of teeth-chattering was markedly observed following the coadministration and in combination with WAY100635.

These results may suggest that the increased 5-HIAA levels in both areas after coadministration are indicative of the interrelation via activation of serotonergic neurons, and that the increased levels are partly responsible for behavioral activation of rats.

KEY WORDS: Frontal cortex, Dorsal raphe nucleus, Microdialysis, 5-HIAA (5-hydroxyindoleacetic acid), Tryptophan, Ethanol

INTRODUCTION

Tryptophan is the dietary precursor of serotonin (5-hydroxytryptamine, or 5-HT), and a deficiency in either tryptophan intake or its metabolism could result in behavioral disorders (Tiihonen et al., 2001). Dietary tryptophan augmentation is known to increase brain 5-HT synthesis and content (Stancampiano et al., 1997; Fadda et al., 2000) and to reduce ethanol consumption (Yamane et al., 2003). On the other hand, in laboratory animals, ingestion of a tryptophan-free diet alters behavioral indices of serotonergic function, increasing pain sensitivity, acoustic startle, motor activity, and aggression and reducing rapid eye movement in sleep (Schaechter and Wurtman, 1989; Bel and Artigas, 1996; Stancampiano et al., 1997; Williams et al., 1999). Alcohol intake has been suggested to be directly affected by the status of serotonergic neurotransmission (LeMarquand et al., 1994). The anxiolytic effect of ethanol is generally involved in the development of...
alcohol dependence, and a neurotransmitter that is suggested to be involved in both alcohol abuse and anxiety is 5-HT (Langen et al., 2002). An acute alcohol dose in healthy individuals lowers blood tryptophan and cerebrospinal fluid (CSF) tryptophan and decreased central 5-HT levels (LeMarquand et al., 1994; Morgan and Badawy, 2001). CSF 5-HIAA, the principal metabolite of 5-HT, reflects central 5-HT turnover and correlates with brain 5-HIAA in humans (Stanley et al., 1985; Williams et al., 1999). In addition, CSF 5-HIAA has been proposed as a valid indicator of general changes in 5-HT metabolism in the central nervous system (CNS) (Banki and Molnar, 1981; LeMarquand et al., 1994). Furthermore, CSF 5-HIAA levels also correlate with 5-HIAA levels in the cerebral cortex of humans at autopsy (Stanley et al., 1985). 5-HT excess in laboratory animals and humans had abnormalities in cognitive and behavioral functions, autonomic nervous system function, and neuromuscular function (Mills, 1997). Conversely, a deficiency in 5-HT neurotransmission, such as lesions of serotonergic neurons and decrease of 5-HT synthesis, has been implicated in several neuropsychiatric disorders, including an increase in alcohol intake (Badawy, 1998), aggression (LeMarquand et al., 1994) and depression (Meltzer, 1990).

The brain 5-HT_{1A} receptor is implicated in numerous neurophysiological processes, and drugs acting on it have potential clinical value in the treatment of a wide variety of CNS disorders (Fletcher et al., 1993; Sharp et al., 1996). Acute ethanol may facilitate 5-HT reuptake in the hippocampus and decrease 5-HT_{1A} receptor functioning in the cortex (Dillon et al., 1991; LeMarquand et al., 1994). The activity of the dorsal raphe nucleus 5-HT neurons is regulated by the medial prefrontal cortex (mPFC), and 5-HT_{1A} autoreceptors in the dorsal raphe nucleus play a pivotal role in the physiological control of ascending 5-HT pathways, attenuating excessive activation of 5-HT neurons by excitatory afferents from the mPFC (Celada et al., 2001). On the contrary, the mPFC receives serotonergic afferents from the raphe nucleus via serotonergic 5-HT_{1A} receptors and contains a large density of various 5-HT receptors (Azmitia and Segal, 1978; Celada et al., 2001). As described above, there are electrophysiological findings on the serotonergic neuronal system (Wright et al., 1990; Tao et al., 2000; Celada et al., 2001). However, in vivo evidence on the neurochemical and functional interaction between the frontal cortex and the dorsal raphe nucleus is not provided as yet.

Therefore, to investigate relationships between the frontal cortex and the dorsal raphe nucleus, we performed two experimental approaches in vivo. In the first one, we examined alterations in 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in both the dorsal raphe nucleus and the frontal cortex after acute coadministration of tryptophan plus ethanol to rats. In the second one, we investigated how WAY100635 (10 μM), 5-HT_{1A} antagonist, or 8-OH-DPAT (10 μM), 5-HT_{1A} agonist perfused into the frontal cortex or the dorsal raphe nucleus after coadministration influences 5-HT release in both areas (Routledge et al., 1995; Kishitake and Yamanouchi, 2005). In addition, there are findings that the cerebral 5-HT system is associated with anxiety-related behavior (Gonzalez et al., 2000; Langen et al., 2002). Therefore, behavioral signs during the treatment with each drug were also observed to examine the relationship between 5-HIAA in both areas as well as behaviors.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats (260-330 g, SLC Japan Co., Hamamatsu, Japan) were purchased and maintained under conditions of 23 ± 1°C with a 12:12-hr light-dark cycle for one week prior to surgery. All procedures involving rats were performed using protocols approved by our Institutional Animal Care and Use Committee.

**Drugs**

L-Tryptophan, serotonin (5-hydroxytryptamine, 5-HT), 5-hydroxyindoleacetic acid (5-HIAA), N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-2-pyridinyl-cyclohexanecarboxamide maleate salt (WAY-100635) and R-(+)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydroanaphthalene hydrobromide (R-(+)-8-OH-DPAT) were purchased from Sigma Chemical Co. (St. Louis, MO). Ethanol was purchased from Wako Pure Chem. Co. (Osaka, Japan). Other reagents were of analytical grade.

**Surgical procedures**

As previously described (Hayashi et al., 2003, 2004), rats were anesthetized and a CMA/11 guide cannula (Bioanalytical Systems Inc., West Lafayette, IN) employed for penetration of a microdialysis probe into the frontal cortex and the dorsal raphe nucleus. A guide cannula was stereotaxically implanted in the frontal cortex +3.2 mm anterior to the bregma, 3.5 mm
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lateral, and 1.5 mm below the skull surface. On the other hand, a stainless steel guide cannula (outer diameter 0.35 mm, inner diameter 0.15 mm) with its stylet was stereotaxically inserted until the tip of the cannula was 2 mm above the dorsal raphe nucleus. Coordinates were (in mm) AP 7.8 posterior to bregma; L 0.0; V 5.0 from the skull surface, according to the atlas of Paxinos and Watson (1986). After surgery, rats were given 300,000 units of benzyl penicillin G subcutaneously and were allowed 1 week to recover from implantation. Upon completion of the experimental sequence, each brain was removed and fixed at least two days in formalin. The cannula track was verified visually by cutting vertically through the cannula marks on the cortex surface. The brain fragment was frozen and 14-μm sections were cut through the frontal cortex or the dorsal raphe nucleus using a microtome-cryostat (Ames Lab-Tek Inc., Westmont, IL). The probes were located within the frontal cortex and the dorsal raphe nucleus area.

Treatment of animals

Tryptophan was dissolved in 0.25 N KOH solution and administered 0.5 ml per 100 g weight. Rats were intraperitoneally given a single dose of tryptophan (50 mg/kg), ethanol (1.25 g/kg) or tryptophan plus ethanol (50 mg/kg plus 1.25 g/kg). The control animals received an equivalent volume of saline. To examine the effect of WAY-100635 or 8-OH-DPAT on the alteration of 5-HIAA levels in the frontal cortex or the dorsal raphe nucleus, we performed the perfusion of WAY100635 (10 μM) or 8-OH-DPAT (10 μM) into the frontal cortex or the dorsal raphe nucleus via the dialysis probe at 1 h after tryptophan (50 mg/kg) alone or combined administration of tryptophan plus ethanol (50 mg/kg plus 1.25 g/kg).

Measurement of behavioral signs elicited by the drugs

After administration of tryptophan, ethanol and both drugs combined, behavioral signs (teeth-chattering, wet-dog shakes, penis-licking, locomotion, stretching, scratching, salivation, sniffing and rearing) were scored for 180 min. For statistical purposes, a simple scoring system (1 point for each sign and summation of the 9 signs to give a total score for each rat) was used to compare responses between the control group receiving vehicle and the tryptophan, ethanol alone, or tryptophan plus ethanol groups.

Sampling for microdialysis and analysis of 5-HT and 5-HIAA

The microdialysis probes (CMA/11, 2 mm tip) and guide cannula were purchased from Bioanalytical System (BAS, Tokyo, Japan) and used within 3 months. The dialysis membrane tip of the probe had an outer diameter of 240 μm and inner diameter of 210 μm, a dead volume of 1 μl, and a molecular weight cut-off of 20,000 Daltons. The in vitro recovery of 5-HT and 5-HIAA was determined by immersing the probes in Ringer's solution containing 100 μM each of 5-HT and 5-HIAA at room temperature. Probes were dialyzed with Ringer's solution, and dialysate samples were analyzed by high-performance liquid chromatography (HPLC; BAS LC-4C Detector) with electrochemical detection using a Waters Spherisorb Column (150 × 4.6 mm; 5 μm ODS2) with electrochemical detection. The mobile phase consisted of 0.1 M citric acid monohydrate, 0.1 M sodium acetate, 5 mg/l EDTA-2Na, 15% methanol and 160 mg/l octanesulfonic acid sodium salt. The pH was adjusted to 3.9 with concentrated phosphoric acid; the solution was degassed and pumped at a flow rate of 2 μl/min. The system was calibrated using an external standard and the retention time for 5-HT and 5-HIAA was approximately 8.9 and 7.2 min, respectively. The sensitivity of the assay and the detection limits for those compounds was 0.03 and 0.02 ng/30 μl samples, respectively. The recovery of 5-HIAA was 9.6 ± 0.4% of the external concentration. Peak of 5-HT was not detected. Due to the variability of probe recovery, the extracellular fluid level of 5-HIAA was individually calculated for each animal.

General experimental procedures

One week following stereotaxic surgery and the day before beginning the experiment, a freshly calibrated microdialysis probe was placed into the frontal cortex and the dorsal raphe nucleus guide cannula. The probe was then perfused with filtered Ringer's solution at a low rate (0.2 μl/min) overnight. The morning of the following day, the flow rate was increased to 2 μl/min. Collection of consecutive 15-min samples for determination of basal values was begun after 2-3 h of equilibration. Following collection of 3 consecutive stable (not more than 20% of inter-sample variation) basal samples, a single i.p. injection of tryptophan (50 mg/kg), ethanol (1.25 g/kg) or tryptophan plus ethanol (50 mg/kg + 1.25 g/kg) was given, and consecutive 15-min samples were collected for 2 h. Similarly, rats were treated with tryptophan (50 mg/kg) or tryptophan...
plus ethanol 1 hr prior to perfusion of WAY100635 (10 μM) or 8-OH-DPAT into the frontal cortex or the dorsal raphe nucleus.

Statistical analysis

One-way analysis of variance (ANOVA) and the Newman-Keul's test (Winer, 1971) were used. Calculated values of p<0.05 were considered statistically significant. Data are expressed as percentage change from basal values. Mean values ± S.E.M. are reported.

RESULTS

Effects of tryptophan alone, tryptophan plus ethanol and in combination with WAY100635 on the levels of 5-HIAA in the frontal cortex and the dorsal raphe nucleus

Coadministration of tryptophan and ethanol (50 mg/kg plus 1.25 g/kg) caused a marked increase in 5-HIAA levels in the frontal cortex when compared to the tryptophan alone, although tryptophan (50 mg/kg) alone had no effect on these levels in the frontal cortex as compared to the control receiving normal saline (Fig. 1).

However, administration of tryptophan alone and in combination with ethanol led to a significant increase of the levels of 5-HIAA in the dorsal raphe nucleus, and these levels were respectively increased (p<0.05) to 178.5 and 165.7% of the control levels in the 75 min sample (Fig. 2). In tryptophan-treated rats, WAY100635 significantly increased 5-HIAA levels in the frontal cortex (Fig. 3), but not in the dorsal raphe nucleus. Moreover, the tryptophan plus ethanol-induced levels of 5-HIAA in the frontal cortex were markedly increased (p<0.05) with the perfusion of WAY100635 (10 μM) into the frontal cortex at 1 h after coadministration of tryptophan and ethanol to 237.1 and 221.0% of the control levels in the 75 and 90 min samples, respectively (Fig. 4). On the contrary, following the perfusion of WAY100635 (10 μM) into the frontal cortex at 1 h after combined administration, the levels of 5-HIAA in the dorsal raphe nucleus signifi-

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Fig. 1. Extracellular fluid levels of 5-HIAA in the frontal cortex induced by coadministration of tryptophan and ethanol.

Rats were i.p. given a single dose of tryptophan (○, 50 mg/kg; n= 6), ethanol (●, 1.25 g/kg; n= 5) alone or tryptophan plus ethanol (△, 50 mg/kg; 1.25 g/kg; n= 6), respectively. Rats were perfused WAY100635 (10 μM) into the frontal cortex (■, n= 4). The control animals received an equivalent volume of saline (●, 50 mg/kg; n= 5). Values are expressed as percentage change (mean ± S.E.M.) from basal values. * p < 0.05, control vs. tryptophan plus ethanol; # p < 0.05, tryptophan vs. tryptophan plus ethanol.

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...cantly decreased in the 90 min sample (Fig. 5). On the other hand, the application of WAY100635 into the dorsal raphe nucleus after concurrent administration did not observe a significant alteration in 5-HIAA levels in both the frontal cortex and the dorsal raphe nucleus. In addition, ethanol (1.25 g/kg) and WAY100635 (10 μM) alone had no effect on the levels of 5-HIAA in both areas (Figs. 1 and 2). In this study, the levels of 5-HT in both areas after treatment with tryptophan alone or tryptophan plus ethanol remained unchanged. Basal values of 5-HIAA in the frontal cortex averaged 25.2 ± 2.0, 28.7 ± 2.9, 27.6 ± 4.1 and 25.3 ± 4.3 nM, respectively, in the saline-control, tryptophan alone, tryptophan plus ethanol, and tryptophan plus ethanol plus WAY100635 groups. On the one hand, basal values of 5-HIAA in the dorsal raphe nucleus averaged 31.9 ± 2.6, 28.3 ± 5.7, 29.7 ± 5.42 and 33.0 ± 5.2 nM, respectively, in the tryptophan alone, tryptophan plus ethanol, tryptophan plus ethanol plus WAY100635 and control groups.

**Effects of tryptophan plus ethanol and in combination with 8-OH-DPAT on the levels of 5-HIAA in the frontal cortex and the dorsal raphe nucleus**

The perfusion of 8-OH-DPAT (10 μM) into the dorsal raphe nucleus at 1 hr after coadministration of tryptophan and ethanol resulted in a significant increase in 5-HIAA levels in the frontal cortex (Fig. 6), although 5-HIAA levels in the dorsal raphe nucleus remained unchanged. On the other hand, following the perfusion of 8-OH-DPAT (10 μM) into the frontal cortex at 1 hr after combined treatment, the levels of 5-HIAA in both areas remained unchanged.

**Behavioral signs during tryptophan alone, tryptophan plus ethanol and in combination with WAY100635 or 8-OH-DPAT**

There was a slight increase in sniffing and rearing in the tryptophan-treated group as compared to control or ethanol groups, but these signs returned to the control levels in tryptophan plus ethanol-treated groups.

**Fig. 2.** Extracellular fluid levels of 5-HIAA in the dorsal raphe nucleus induced by coadministration of tryptophan and ethanol.

Rats were i.p. given a single dose of tryptophan (○, 50 mg/kg; n = 5), ethanol (□, 1.25 g/kg; n = 5) or tryptophan plus ethanol (△, 50 mg/kg + 1.25 g/kg; n = 5), respectively. Rats were perfused WAY100635 (10 μM) into the dorsal raphe nucleus(■, n = 4). The control animals received an equivalent volume of saline (●, n = 4). Values are expressed as percentage change (mean ± S.E.M.) from basal values. *p < 0.05, control vs. tryptophan alone or tryptophan plus ethanol.
We observed a significant difference between the tryptophan alone and tryptophan plus ethanol or tryptophan plus ethanol plus WAY100635 groups. The composite score for teeth-chattering was significantly (P<0.05) higher in the tryptophan plus ethanol- or the tryptophan plus ethanol plus WAY100635-treated rats (47.2 ± 4.1 or 45.0 ± 1.9; n=6), as compared to that for the tryptophan or ethanol alone groups (22.8 ± 6.5 or 24.2 ± 4.0; n=5), respectively (Table 1). However, no significant difference in teeth-chattering between the tryptophan plus ethanol and tryptophan plus ethanol plus WAY100635 groups was observed. Then, following the perfusion of 8-OH-DPAT (10 μM) into both areas after combined treatment, the behavioral signs were at the same levels as control groups.

DISCUSSION

Our results indicate that the extracellular fluid levels of 5-HIAA in the dorsal raphe nucleus, but not in the frontal cortex, were enhanced by tryptophan alone, and that 5-HIAA levels in the dorsal raphe nucleus and the frontal cortex were significantly increased by coadministration of tryptophan and ethanol. This study provides evidence to indicate that the increased 5-HIAA levels in the frontal cortex and the dorsal raphe nucleus after tryptophan plus ethanol are indicative of the interrelation via activation of serotonergic neurons.

Tryptophan is the dietary precursor of 5-HT, and a deficiency in either tryptophan intake or its metabolism could result in behavioral disorders (Tiihonen et al., 2001). Dietary tryptophan augmentation is known to increase brain 5-HT synthesis and content (Fernstrom, 1983; Stancampiano et al., 1997; Fadda et al., 2000). 5-HIAA, the principal metabolite of 5-HT, reflects central 5-HT turnover (Fernstrom and Wurtman, 1971; Williams et al., 1999). Moreover, when tryptophan is given to normal rats, there is a much greater accumulation of 5-HIAA than 5-HT (Williams et al., 1999; Hayashi et al., 2003, 2004).

![Graph showing extracellular fluid levels of 5-HIAA in the frontal cortex elicited by WAY100635 after administration of tryptophan.](image-url)

Fig. 3. Extracellular fluid levels of 5-HIAA in the frontal cortex elicited by WAY100635 after administration of tryptophan. Rats were i.p. given a single dose of tryptophan alone (○, 50 mg/kg; n=6). On the other hand, rats were perfused WAY100635 (10 μM) into the frontal cortex after administration of tryptophan (■, 50 mg/kg; n=4). Rats were perfused WAY100635 (10 μM) into the frontal cortex (■, n=4). The control animals received an equivalent volume of saline (●, 50 mg/kg; n=5). Values are expressed as percentage change (mean ± S.E.M.) from basal values. * p < 0.05, tryptophan vs. tryptophan plus WAY100635.
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Frontal cortex serotonergic neurons are descending projections to the dorsal raphe nucleus, and 5-HT concentration in the dorsal raphe nucleus is modulated by 5-HT$_{1A}$ autoreceptors located within the dorsal raphe (Hjorth et al., 1987; Hutson et al., 1989; Celada et al., 2001). Conversely, mPFC receives serotonergic afferents from the raphe nucleus via serotonergic 5-HT$_{1A}$ receptors and is dependent on the activation of 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus (Azmitia and Segal, 1978; Celada et al., 2001).

Alcohol intake has been suggested to be directly affected by the status of serotonergic neurotransmission (LeMarquand et al., 1994). An acute alcohol dose in healthy individuals lowers blood tryptophan and CSF tryptophan and decreased central 5-HT levels (LeMarquand et al., 1994; Morgan and Badawy, 2001). Moreover, acute ethanol may facilitate 5-HT reuptake in the hippocampus and decrease 5-HT$_{1A}$ receptor functioning in the cortex (Dillon et al., 1991; LeMarquand et al., 1994).

In our study, the significantly increased 5-HIAA levels in the dorsal raphe nucleus by acute administration of tryptophan alone may have resulted in unchanged levels of 5-HIAA in the frontal cortex via the activation of dorsal raphe 5-HT$_{1A}$ autoreceptors (Fig. 1). In addition, from the results in Fig. 2, which are the markedly increased 5-HIAA levels in both areas after coadministration, the unchanged 5-HIAA levels after ethanol alone and the same increase in 5-HIAA levels in the dorsal raphe nucleus of both tryptophan plus ethanol- and tryptophan-treated rats, it may be suggested that the dorsal raphe serotonergic neurons were not directly affected by the ethanol dose used in this study, and that the combined treatment-induced increase of 5-HIAA levels in the frontal cortex was obtained in accord with the decrease of serotonergic 5-HT$_{1A}$ receptors by ethanol.

5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus play a pivotal role in the physiological control of ascending 5-HT pathways, attenuating excessive acti-

![Graph](image_url)

**Fig. 4.** Extracellular fluid levels of 5-HIAA in the frontal cortex elicited by WAY100635 after coadministration of tryptophan and ethanol.

Rats were i.p. given a single dose of tryptophan plus ethanol (Δ, 50 mg/kg + 1.25 g/kg; n = 6). On the other hand, rats were perfused WAY100635 (10μM) into the frontal cortex after coadministration of tryptophan plus ethanol ▲ (50 mg/kg + 1.25 g/kg; n = 4). Rats were perfused WAY100635 (10μM) into the frontal cortex ■ (n = 4). The control animals received an equivalent volume of saline (○, 50 mg/kg; n = 5). Values are expressed as percentage change (mean ± S.E.M.) from basal values. * p < 0.05, tryptophan plus ethanol vs. tryptophan plus ethanol plus WAY100635.
vation of 5-HT neurons by excitatory afferents from the mPFC. Moreover, postsynaptic 5-HT1A receptors in the mPFC exert a distal feedback control of serotonergic activity through the modulation of descending excitatory afferents to the dorsal raphe nucleus (Celada et al., 2001). Under the same condition as the other experiments in this study, the application of WAY100635 into the frontal cortex in tryptophan-treated rats significantly increased 5-HIAA levels in the frontal cortex (Fig. 3), but not in the dorsal raphe nucleus. Moreover, WAY100635 into the frontal cortex after coadministration of tryptophan and ethanol led to a significant increase in 5-HIAA levels in the frontal cortex, while it decreased these levels in the dorsal raphe nucleus (Figs. 4 and 5). This may suggest that WAY100635-induced increase of 5-HIAA levels in the frontal cortex after tryptophan alone and in combination with ethanol resulted from negative feedback following the blockade of serotonergic 5-HT1A autoreceptors achieved with WAY100635, and that this increase in 5-HIAA levels decreased the levels of 5-HIAA in the dorsal raphe nucleus by preventing the activation of 5-HT1A autoreceptors in the dorsal raphe nucleus via descending projections from the frontal cortex.

There are electrophysiological findings that the firing activity of 5-HT neurons and the release of 5-HT in forebrain are dependent on the activation of 5-HT1A autoreceptors in the dorsal raphe, but that the activation of 8-OH-DPAT of postsynaptic 5-HT1A receptors on cortical pyramidal neurons reduces 5-HT cell firing in the dorsal raphe and 5-HT release in the dorsal raphe and mPFC, likely through the inhibition of the activity of excitatory inputs from the mPFC to the dorsal raphe (Celada et al., 2001). This may indicate that the blockade of dorsal raphe 5-HT1A autoreceptors by WAY100635 offset the combined treatment-induced increase of 5-HIAA levels in the dorsal raphe nucleus, and resulted in unchanged levels of 5-HIAA in the frontal cortex via ascending serotonergic neuronal

![Fig. 5. Extracellular fluid levels of 5-HIAA in the dorsal raphe nucleus elicited by WAY100635 after coadministration of tryptophan and ethanol.](image)

Rats were i.p. given a single dose of tryptophan plus ethanol $\Delta$ (50 mg/kg + 1.25 g/kg; n = 5). Rats were perfused WAY100635 (10 μM) into the frontal cortex ( ■, n = 4). Moreover, rats were perfused WAY100635 (10 μM) into the frontal cortex after coadministration of tryptophan plus ethanol ( ▲, 50 mg/kg + 1.25 g/kg; n = 5). The control animals received an equivalent volume of saline ( ○, 50 mg/kg; n = 5). Values are expressed as percentage change (mean ± S.E.M.) from basal values. * p < 0.05, tryptophan plus ethanol vs. tryptophan plus ethanol plus WAY100635.
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Fig. 6. Extracellular fluid levels of 5-HIAA in the frontal cortex elicited by 8-OH-DPAT after coadministration of tryptophan and ethanol. Rats were i.p. given a single dose of tryptophan (O, 50 mg/kg; n=6), ethanol (Δ, 1.25 g/kg; n=5), tryptophan plus ethanol (Δ, 50 mg/kg + 1.25 g/kg; n=6). Rats were perfused 8-OH-DPAT (10 μM) into the dorsal raphe nucleus (●, n=4). Moreover, rats were perfused 8-OH-DPAT (10 μM) into the dorsal raphe nucleus after coadministration of tryptophan plus ethanol (●, 50 mg/kg + 1.25 g/kg; n=4). The control animals received an equivalent volume of saline (●, 50 mg/kg; n=5). Values are expressed as percentage change (mean ± S.E.M.) from basal values. * p<0.05, tryptophan plus ethanol vs. tryptophan plus ethanol plus 8-OH-DPAT.

Table 1. Acute effect of each administration of tryptophan, ethanol, tryptophan plus ethanol and in combination with WAY100635 on behavior in rats.

<table>
<thead>
<tr>
<th>Behavioral signs</th>
<th>Control</th>
<th>Tryptophan</th>
<th>25% Ethanol</th>
<th>Tryptophan +25% Ethanol</th>
<th>Tryptophan +25% Ethanol + WAY100635</th>
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</thead>
<tbody>
<tr>
<td>Teeth-chattering</td>
<td>17.4 ± 4.9</td>
<td>22.8 ± 6.5</td>
<td>24.2 ± 4.0</td>
<td>47.2 ± 4.1 abc</td>
<td>45.0 ± 1.9 abcd</td>
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<td>Wet-dog shakes</td>
<td>1.6 ± 1.7</td>
<td>2.9 ± 2.1</td>
<td>2.6 ± 1.8</td>
<td>1.7 ± 1.1</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Penis-licking</td>
<td>2.1 ± 0.9</td>
<td>1.4 ± 0.7</td>
<td>0.9 ± 0.7</td>
<td>1.1 ± 0.7</td>
<td>2.5 ± 1.6</td>
</tr>
<tr>
<td>Stretching</td>
<td>0.9 ± 0.7</td>
<td>0.7 ± 0.5</td>
<td>0.9 ± 0.5</td>
<td>1.0 ± 0.9</td>
<td>0.3 ± 0.3</td>
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<tr>
<td>Locomotion</td>
<td>9.4 ± 4.2</td>
<td>13.1 ± 6.1</td>
<td>11.2 ± 3.4</td>
<td>8.2 ± 5.9</td>
<td>3.8 ± 1.5</td>
</tr>
<tr>
<td>Scratching</td>
<td>9.2 ± 6.9</td>
<td>0.4 ± 0.2</td>
<td>4.1 ± 2.9</td>
<td>6.0 ± 5.1</td>
<td>1.8 ± 1.4</td>
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<td>Sniffing</td>
<td>1.5 ± 1.1</td>
<td>12.7 ± 3.7 ab</td>
<td>3.6 ± 1.0</td>
<td>1.5 ± 0.4 c</td>
<td>4.8 ± 1.9</td>
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<tr>
<td>Rearing</td>
<td>1.6 ± 1.0</td>
<td>10.2 ± 3.2 b</td>
<td>3.3 ± 0.9</td>
<td>1.4 ± 0.5 c</td>
<td>4.2 ± 2.1</td>
</tr>
<tr>
<td>Salivation</td>
<td>2.2 ± 0.5</td>
<td>2.5 ± 0.7</td>
<td>2.4 ± 0.9</td>
<td>1.7 ± 1.4</td>
<td>3.3 ± 1.8</td>
</tr>
</tbody>
</table>

a) p<0.05 Control vs. 25% Ethanol+Tryptophan
b) p<0.05 25% Ethanol vs. 25% Ethanol+Tryptophan
c) p<0.05 Tryptophan vs. 25% Ethanol+Tryptophan
d) p<0.05 Control vs. 25% Ethanol+Tryptophan+WAY100635
e) p<0.05 Tryptophan vs. 25% Ethanol+Tryptophan+WAY100635
f) p<0.05 25% Ethanol vs. 25% Ethanol+Tryptophan+WAY100635
g) p<0.05 Control vs. Tryptophan
h) p<0.05 Tryptophan vs. 25% Ethanol
pathways from dorsal raphe to frontal cortex. On the other hand, it may be suggested that the unchanged 5-HIAA levels in the dorsal raphe nucleus with the application of 8-OH-DPAT after coadministration resulted from the stimulation of serotonergic 5-HT1A autoreceptors achieved with 8-OH-DPAT, and that 8-OH-DPAT-induced activation of dorsal raphe 5-HT1A receptors caused the increase of frontal cortex 5-HIAA levels (Fig. 6) by preventing the activation of frontal cortex 5-HT1A autoreceptors via ascending projections from the dorsal raphe nucleus, which may be "feed back control". Conversely, following the perfusion of 8-OH-DPAT into the frontal cortex after combined treatment, the levels of 5-HIAA in both the frontal cortex and the dorsal raphe nucleus remained unchanged. This may suggest that the activation of frontal 5-HT1A receptors by 8-OH-DPAT reduced 5-HIAA levels in both areas, likely through the inhibition of activity of excitatory inputs from the frontal cortex to the dorsal raphe. Our in vivo results described above agree with the electrophysiological findings of Celada et al. (2001).

It is known that the neurotransmission in both the locus coeruleus neurons and the dorsal raphe nucleus is closely related to behavioral signs (Rasmussen et al., 1990; Aghajanian et al., 1994; Kaehler et al., 1999; Hoshi et al., 1997, 2000; Hayashi et al., 2003, 2004). In this connection, the cerebral 5-HT system is associated with anxiety-related behavior (Gonzalez et al., 2000; Langen et al., 2002) and the results of many animal studies indicate that increased 5-HT activity results in increased anxiety and that reducing 5-HT reduces anxiety (Langen et al., 2002). In our previous experiment, the increase in activity of the serotonergic systems in the concurrent administration of tryptophan and ethanol temporally correlated to behavioral signs (Hoshi et al., 1997, 2000; Hayashi et al., 2003, 2004; Bandoh et al., 2004). In the present study, the alteration of behaviors such as teeth-chattering, sniffing and rearing in Table 1 may result from combined treatment-induced neuronal activation in both the frontal cortex and the dorsal raphe serotonergic systems. In addition, the enhancement in 5-HIAA levels in the frontal cortex and the increased behavioral signs after coadministration and in combination with WAY100635 showed virtually identical time courses. Our experimental results may indicate that the anxiolytic-like effect of ethanol is accompanied by the stimulative effect on 5-HT release in the frontal cortex of rats, and that alterations in 5-HT turnover rates or serotonergic activity (firing rates) are responsible for behavioral signs. The coadministration of tryptophan diet with alcohol may elicit more 5-HIAA (5-HT) levels in the cerebral 5-HT system than tryptophan or alcohol alone (Gonzalez et al., 2000; Morgan and Badawy, 2001; Langen et al., 2002). This should indicate that novel pharmacological strategies may be exploited to prevent and/or manage psychiatric disorders such as anxiety and depression at alcohol intake with tryptophan diet.

REFERENCES


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diet markedly reduces frontocortical 5-HT release, but fails to modify ethanol preference in alcohol-preferring (sP) and non-preferring (sSNP) rats. Behav. Brain Res., 108, 127-132.


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