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# Extracellular Hypothalamic γ-Aminobutyric Acid (GABA) and L-Glutamic Acid Concentrations in Response to Bicuculline in a Genetic Absence Epilepsy Rat Model

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**Abstract.** The posterior part of the hypothalamus plays a vital role in the homeostatic processes of the internal environment, including blood pressure and heart rate regulation, by means of γ-aminobutyric acid (GABA)ergic and glutamatergic neurotransmission. In this study we measured the extracellular levels of GABA and L-glutamic acid in the dorsomedial hypothalamic nucleus (DMH) and posterior hypothalamus (PH), following intracerebroventricular (i.c.v.) administration of bicuculline, a $GABA_A$-receptor antagonist, in genetic absence epileptic rats from Strasbourg (GAERS), where heart rate, blood pressure, and EEG recordings were also collected simultaneously. The i.c.v. injection of bicuculline (0.3 nmol) produced no response in non-epileptic Wistar rats but caused an increase in mean arterial pressure in GAERS ($P<0.01$). Microdialysis experiments showed that L-glutamic acid increased in the DMH in GAERS after bicuculline administration ($P<0.01$). Additionally, extracellular GABA concentration decreased in the PH ($P<0.05$). Bicuculline suppressed the spike-and-wave discharges, the characteristic sign of absence seizures. All these results suggest that the bicuculline-induced blood pressure response is accompanied by changes in L-glutamic acid levels in the DMH and GABA levels in the PH, indicating a bicuculline hypersensitivity in the DMH and PH of GAERS that may make the GAERS display an altered mode of central cardiovascular regulation. These results suggest that the circuits affected in GAERS are not only restricted to the regions responsible for seizure generation but also present in the hypothalamus.

***Keywords***: microdialysis, dorsomedial hypothalamic nucleus (DMH), posterior hypothalamus (PH), genetic absence epileptic rats from Strasbourg (GAERS), central cardiovascular regulation

## Introduction

The hypothalamus plays a key role in a variety of autonomic, endocrine, and behavioral responses that maintain homeostatic control of the internal environment (1). More specifically, the dorsomedial hypothalamic nucleus (DMH) and the posterior hypothalamus (PH) have been shown to control autonomic activity (2, 3). Disruption of γ-aminobutyric acid (GABA)-mediated neurotransmission in the DMH evokes increases in arterial blood pressure, heart rate, peripheral sympathetic nerve activity, and plasma catecholamine levels in both conscious and anesthetized rats, suggesting that the GABAergic system in the DMH exerts a tonic inhibitory influence over the sympathetic nervous system (4 – 6). In addition, the activation of NMDA receptors in the DMH evokes significant increases in blood pressure and heart rate (7, 8). These results provide evidence for the involvement of the DMH in the central regulation of cardiovascular functions. It was shown that PH receives tonic inhibition from GABAergic pathways and injection of $GABA_A$-receptor

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antagonists into this region of rats can increase arterial blood pressure and heart rate (4). This response is also produced by the inhibition of GABA synthesis in the posterior hypothalamus of normotensive rats (9). In a recent study, we have shown that intracerebroventricular (i.c.v.) administration of bicuculline, a $GABA_A$-receptor antagonist, produces greater increases in blood pressure in a genetically determined rat model of absence epilepsy, compared to non-epileptic controls (10). We further examined the role of the DMH and PH in $GABA_A$ receptor–mediated cardiovascular regulation in genetic absence epileptic rats from Strasbourg (GAERS; ref. 11). Bicuculline injections into the hypothalamus produced twofold increases in blood pressure and heart rate relative to control rats. These results indicate that the $GABA_A$ receptor–mediated cardiovascular response is altered in the dorsal region of the hypothalamus in conscious rats with absence epilepsy. Although the thalamus and cortex have previously been implicated in absence epilepsy mechanisms, this study provided the first evidence that the DMH and PH are other affected brain regions in the rat model of genetic absence epilepsy.

Based on the above findings, to explain whether the cardiovascular responses to bicuculline is associated with changes in the levels of GABA and L-glutamic acid in GAERS, the present study was designed to demonstrate basal and bicuculline-induced GABA and L-glutamic acid levels in the DMH and PH of the hypothalamus of GAERS.

## Materials and Methods

Of the 51 rats used in this study, 26 were of GAERS and 25 were of the Wistar strain. GAERS and Wistar rats of both sexes, weighing 250 – 275 g, 4 – 12 months of age, supplied by Marmara University, Experimental Research and Animal Laboratory were used in the experiments. The approval of Marmara University Ethical Committee for Experimental Animals was obtained before the experiments (Mar 12, 2003). The rats were kept in a temperature-controlled room with a 12-h light and 12-h dark cycle and fed with standard rat chow and water ad libitum.

*Stereotaxic interventions, cannulae, and microdialysis probes*

The rats were anesthetized i.p. with a mixture of ketamine (75 mg/kg) and chlorpromazine (1 mg/kg) and placed in a stereotaxic frame (Model 51600; Stoelting, Wood Dale, IL, USA). The scalp skin was incised and the periosteum was separated from the cranium. The bregma was used as the reference point.

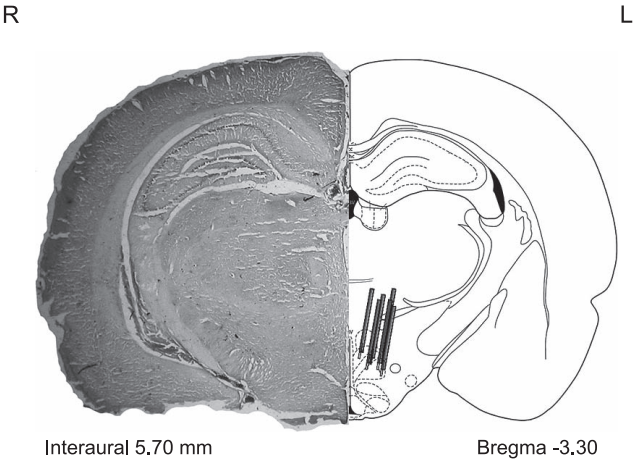


**Fig. 1.** Photomicrograph of 40-$\mu$m-thick coronal section of DMH region and the schematic representation of the serial section corresponding to bregma −3.30 mm (simplified from rat brain atlas). The letters R and L indicate the right and the left side of the rat.

A stainless-steel guide cannula was placed 1-mm above the right lateral ventricle to prevent any mechanical damage to that area. I.c.v. coordinates (−1.0 mm posterior and −1.5 mm lateral from the bregma, and −2.8 mm ventral from the surface of the scalp) were calculated according to the rat brain atlas of Paxinos and Watson (12). The i.c.v. cannula was covered with dental cement by preserving the bregma. As the inflammatory reaction prevents the microdialysis procedure, the microdialysis probe was implanted at a 2nd stereotaxic surgical procedure 2 days apart (Fig. 1).

Concentric microdialysis probes were made from 15-mm-long, 24 G stainless steel tubing (Cooper's Needle Works, UK). Inlet and outlet tubes were made of fused silica (OD: 0.19 mm; ID: 0.075 mm; SGE, UK). The fused silica tubes were placed under a surgical microscope and the inlet that emerges from the tip of the stainless tubing was trimmed to a length of 2 mm. Cuprophan dialysis membrane (OD: 0.216 mm; ID: 0.2 mm; Gambro, Ltd., UK) was passed over the inlet silica tubing. All the joints of the probes were sealed with epoxy resin. The time lag between the dialysis and its collection (calculated from the dead space of the collecting system at the flow rate used) was 20 min.

The microdialysis probe was inserted in the left DMH or the PH (the actual coordinates) in different groups of animals with an angle of 10° to the vertical plane. The coordinates for the DMH are −3.3 mm posterior and −2.1 mm lateral from the bregma and −9.1 mm ventral from the surface of the scalp. The coordinates of PH are −4.1 mm posterior and −1.6 mm lateral from the bregma and −8.1 mm ventral from the surface of the scalp. Four stainless-steel screw electrodes

to record EEG were implanted bilaterally onto the skull over frontal and parietal cortices, and the cannula, micro-connector, microdialysis probe, and screws were fixed to the skull with dental acrylic cement.

*Drugs and external standard solutions*

Bicuculline methiodide obtained from Sigma Chemical Company (St. Louis, MO, USA) was dissolved in artificial cerebrospinal fluid (aCSF). Ketamine was provided by Eczacıbaşı AŞ, Turkey. GABA and L-glutamic acid (both from Sigma) were used as external standards. The standards were dissolved in 0.1 M HCl and aliquots were stored at −20°C; and fresh dilutions of 0.25, 0.5, 1, 2.5 $\mu$M were prepared on each day of the experiments. The composition of aCSF was 2.5 mM KCl, 125 mM NaCl, 1.26 mM $CaCl_2 2H_2O$, 1.18 mM $MgCl_2 6H_2O$, and 0.2 mM $NaH_2PO_4 2H_2O$, and the pH was set to 7. The aCSF was filtered through 0.4-$\mu$m nylon membrane filters.

*Measurements of blood pressure and heart rate in conscious rats*

A polyethylene catheter (PE-10 fused to PE-50) filled with heparinized saline was inserted into the iliac artery and routed subcutaneously to exit at the back of the neck under brief ether anesthesia. An injection stylet extending 1-mm below the tip of the guide cannula was placed for i.c.v. administration of aCSF or bicuculline. The animals were transferred to Plexiglas cages (25 × 25 × 30 cm) and allowed to rest quietly for 2 – 4 h prior to the experiment. The extension tubing of the iliac catheter was attached to a pressure transducer to record blood pressure on a polygraph (Model 7; Grass, Quincy, MA, USA). Heart rates (beats/min) were obtained via a tachograph (Model 7P44, Grass). Following a 2-h stabilization period, basal blood pressure and heart rate were measured. Blood pressure, heart rate, observable behavioral changes, and EEG were recorded while the microdialysis samples were being collected simultaneously.

*Microdialysis procedures*

The day after the placement of microdialysis probes, polyethylene tubing was attached to the inlet of the microdialysis probes to collect the samples in conscious rats in a plexyglass cage (42 × 42 × 20 cm). aCSF was delivered continuously via a 250-$\mu$l Hamilton syringe that was connected to a microinfusion pump (KD Scientific, Holliston, MA, USA). Two basal samples were collected at 0.5 $\mu$l/min-flow rate every 20 min in 0.5-ml Ependorf tubes from rats after an equilibration period of 1 h. After collection of basal samples, i.c.v. injection of aCSF or 0.3 nmol bicuculline were given to GAERS and Wistar rats as a single dose for each animal via the internal cannula connected to the extension tubing. All injections were given over 1 min via a Hamilton microsyringe placed on an infusion pump and connected to the extension tubing of the internal cannula. After injections of aCSF or bicuculline, four more samples were collected. The dialysates accumulated in 200-$\mu$l ependorf tubes and the tubes were replaced every 20 min. The dialysates were kept at −80°C until analysis.

*Chromatographic system and HPLC analysis of the samples*

The chromatographic system consisted of a gradient pump (Model 1100; Agilent, Germany) with four solvent bottles; degasser module; C18 reverse phase nucleosil column (15-cm and 3.9-cm length, 4.6-mm diameter, and 5-$\mu$m pore size); autosampler unit; fluorescence detector with excitation and emission wave lengths of 360 and 410 nm, respectively; and a computer. The mobile phase consisted of 250 mM Na acetate (pH 6.9), deionized HPLC grade water, and methanol, where 0.5% (v/v) tetrahydrofurane was added in all solutions. A gradient flow with an equilibration period of 10 min was delivered at a flow rate of 0.5 ml/min. The temperature of the column was set at 25°C. Pre-column derivatization was performed with *o*-phthaldialdehyde and 3-mercaptopropionic acid (both reagents from Sigma, San Antonio, TX, USA). Injections were given within a volume of 12 $\mu$l with the aid of an autosampler unit using injection software. The retention times of L-glutamic acid and GABA were 5.96 and 29.94 min, respectively. The chromatographic analysis was carried out with the aid of software (Chemstation).

*EEG recording*

EEG activity of the cortex in the non-epileptic Wistar and GAERS groups was amplified (through BioAmp ML 136) and recorded with a PowerLab 8S System running Chart v.5, (ADI Instruments, UK) continuously for 20 min before and 90 min after the injection. A spike-and-wave discharge (SWD) complex was identified as such if its duration was at least 1 s with a train of sharp spikes and slow waves (7.5 – 9 Hz) and amplitude of at least twice the background amplitude of the EEG. The cumulative total duration of SWDs was measured over 20-min periods.

*Histological examination*

Upon completion of the experiments, the rats were anesthetized with urethane (1.2 g/kg, i.p.). Methylene blue was injected for the verification of the i.c.v. cannula

and the DMH or PH probe placements. The rats were then decapitated and the brains were removed and kept in a 20% sucrose–formalin solution for 1 week. Coronal sections (40 $\mu$m) were taken using a cryostat (Microm, Germany), and the sections were stained with thionine dye for light microscopic examination. The data collected from properly implanted cannulae and probes were included in the data analysis.

*Experimental protocols*

Mean arterial pressure, heart rate, EEG, L-glutamic acid, and GABA levels in the DMH or the PH were examined in non-epileptic Wistar rats and GAERS after the injection of aCSF or bicuculline in the lateral ventricle. The drugs were administered through the i.c.v. cannula in a volume of 10 $\mu$l; the experimental groups were as follows: group 1: aCSF injection and probe implant in the DMH of non-epileptic Wistar (n = 6) or GAERS (n = 7), group 2: bicuculline injection and probe implant in the DMH of non-epileptic Wistar (n = 7) or GAERS (n = 7), group 3: aCSF injection and probe implant in the PH of non-epileptic Wistar (n = 6) or GAERS (n = 6), group 4: bicuculline injection and probe implant in the PH of non-epileptic Wistar (n = 6) or GAERS (n = 6).

*Data analysis*

The results were each expressed as a mean ± S.E.M. Mean arterial pressure was calculated as '1/3 pulse pressure + diastolic blood pressure'. Microdialysis and cardiovascular data were statistically evaluated using analysis of variance (ANOVA) for repeated measures followed by Dunnet's multiple comparison test. The basal mean arterial pressure and heart rate values were compared by Student's *t*-test. The criterion for statistical significance was set at $P<0.05$

## Results

*Analysis of EEG*

All of the non-epileptic control Wistar rats exhibited a normal EEG pattern and showed no SWDs, which are EEG findings indicating the absence of epilepsy, in the pre-injection period. No significant effect was observed following bicuculline injection in the EEG of Wistar rats (data not shown). Evaluation of EEG data collected from GAERS showed that i.c.v. injections of aCSF did not produce any significant change in the duration of SWDs, but injections of 0.3 nmol of bicuculline caused a significant decrease in the duration of SWDs within 40 min compared to the baseline values ($P<0.05$, Fig. 2). The basal SWD durations in the two groups were not statistically different ($P = 0.14$).

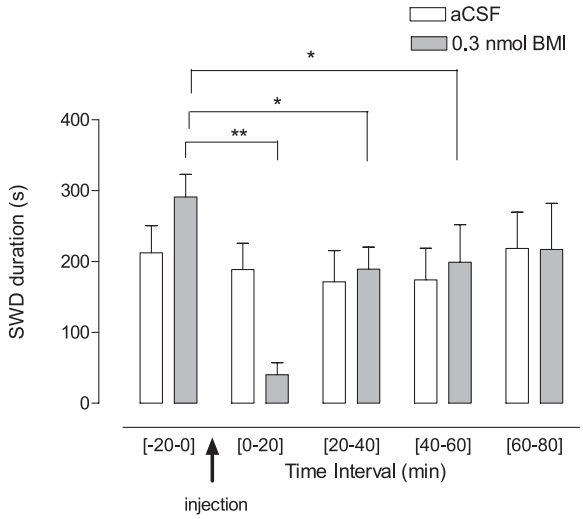


**Fig. 2.** The effect of i.c.v. injection of aCSF or bicuculline (BMI) on SWD duration in GAERS. *$P<0.05$, **$P<0.01$, compared to the baseline ([−20 – 0] min data) values.

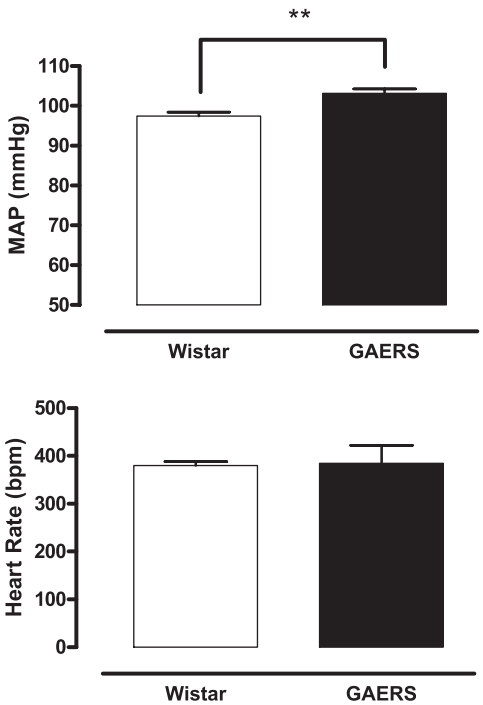


**Fig. 3.** The basal values of the mean arterial pressure (MAP) and heart rate in non-epileptic Wistar rats and GAERS. **$P<0.01$, compared with the control group.

*Effect of i.c.v. bicuculline injection on mean arterial pressure and heart rate in Wistar rats and GAERS*

In Wistar rats, the basal mean arterial pressure was recorded as 97.4 ± 1.0 mmHg and heart rate as 403.8 ± 16.3 beat/min. No changes were observed in these values after either aCSF or 0.3 nmol bicuculline injec-

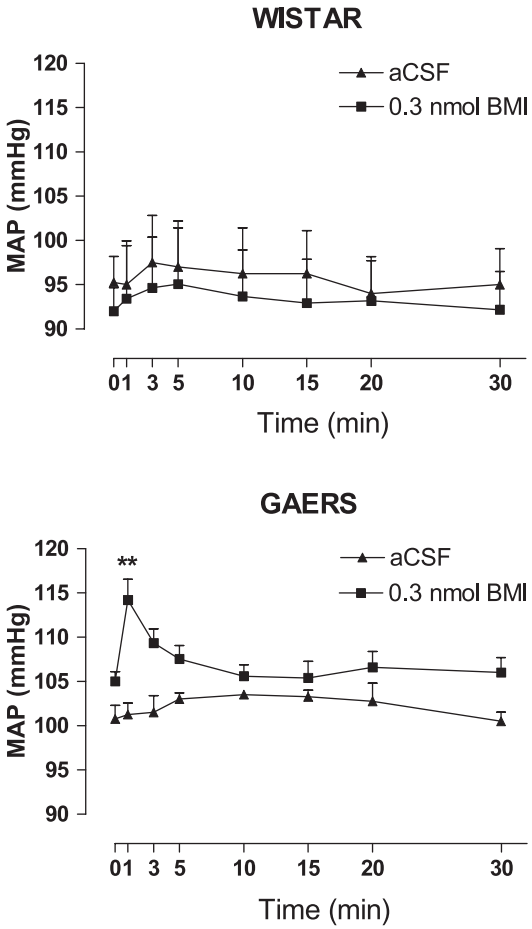


**Fig. 4.** The effects of i.c.v. aCSF or bicuculline (BMI) on mean arterial pressure (MAP) in non-epileptic Wistar rats and GAERS. **$P$<0.01, compared with the control group.

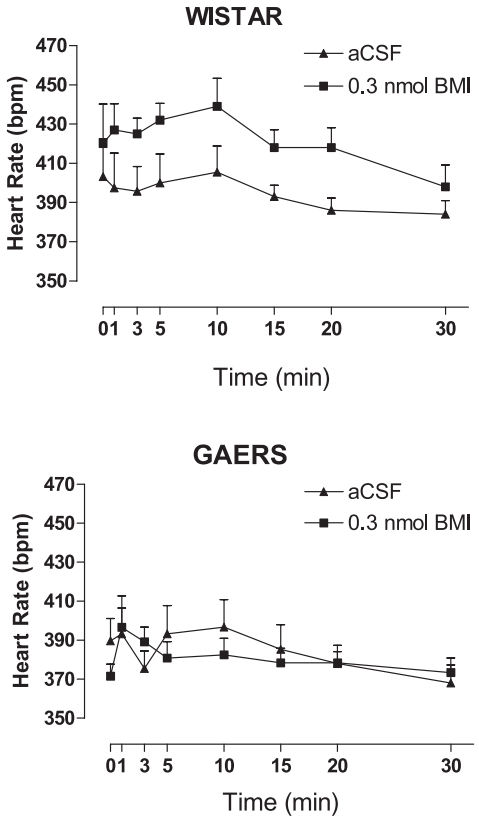


**Fig. 5.** The effects of i.c.v. aCSF or bicuculline (BMI) on heart rate in non-epileptic Wistar rats and GAERS.

tion in Wistar rats. In GAERS, the basal mean arterial pressure was 103 ± 1.0 mmHg and heart rate 378.8 ± 6.8 beat/min. Basal mean arterial pressure values in GAERS differed significantly from the values for the Wistar animals ($P$<0.01, Fig. 3), but heart rate values were not significantly different in the two groups. In GAERS, the mean arterial pressure values increased to 116 ± 2 mmHg within 1 min following i.c.v. injection of 0.3 nmol bicuculline and returned to basal level in post-injection 10 min. This increase was statistically significant when analyzed by ANOVA ($P$<0.01, Fig. 4). No change in mean arterial pressure and heart rate was observed following aCSF injections in GAERS. Bicuculline injections showed a tendency to decrease the heart rate in Wistar rats ($P$ = 0.060), but it was found to be non-significant when analyzed by ANOVA (Fig. 5).

*The effects of i.c.v. aCSF or bicuculline injections on amino acid levels from the DMH of two groups*

In Wistar rats, no change in the L-glutamic acid and GABA levels was detected after either aCSF or bicuculline injection in the samples collected from the DMH as shown in Fig. 6. Similarly, aCSF injection did not affect the L-glutamic acid or GABA levels in GAERS, but an increase in the concentration of L-glutamic acid was observed in the [0 – 20] min sample after 0.3 nmol bicuculline injection in GAERS ($P$<0.05, Fig. 6B). The GABA level in the DMH of GAERS showed a tendency to decrease in the [20 – 40] min sample following 0.3 nmol bicuculline injection, but this decrease was not found to generate a statistically significant difference ($P$ = 0.059, Fig. 5D).

*The effects of i.c.v. aCSF or bicuculline injections on amino acid levels from the PH of two groups*

The analysis of microdialysis samples collected from the PH region of Wistar rats and GAERS following i.c.v. aCSF injections showed no statistically significant changes in L-glutamic acid or GABA levels compared to the basal values (Fig. 7). Similarly, i.c.v. injections of bicuculline in Wistar rats failed to affect the levels of

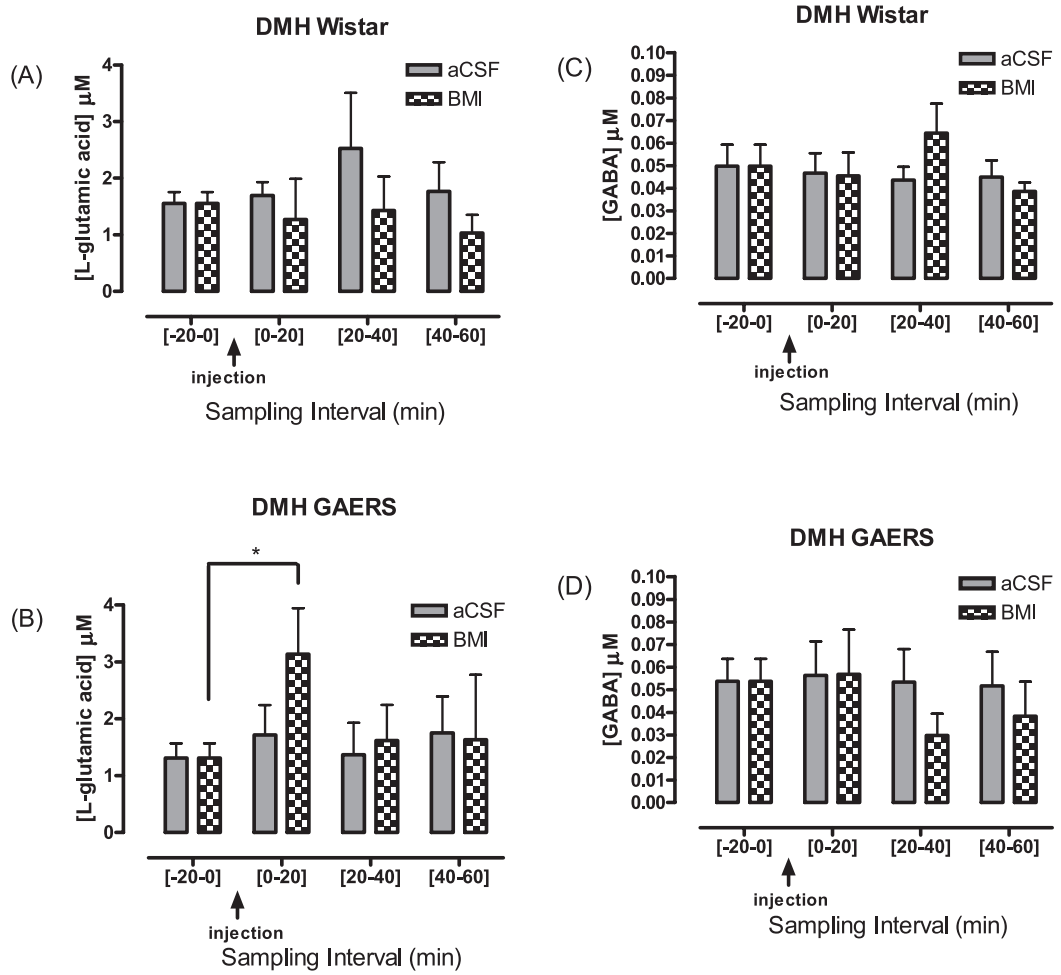


**Fig. 6.** The analysis of extracellular L-glutamic acid (A and B) or GABA (C and D) levels in response to aCSF or bicuculline (BMI) in the DMH of non-epileptic Wistar rats or GAERS. The time lag was 20 min. **P*<0.05, compared to the basal ([–20 – 0] min sample) value.

these two amino acids. However, in the samples collected from the PH region of GAERS, L-glutamic acid levels tended to be increased in the [0 – 20] min sample after 0.3 nmol bicuculline injection. This increase was not statistically significant ($P = 0.2$, Fig. 7B). Meanwhile, a statistically significant decrease in the GABA levels was observed in the [0 – 20] min sample ($P<0.05$, Fig. 7D).

## Discussion

In this study, we observed a significant increase in mean arterial pressure values in response to i.c.v. administration of the $GABA_A$-receptor antagonist bicuculline in GAERS. DiMicco et al. have demonstrated that either the administration of bicuculline into the lateral ventricle or restriction of the agent to the lateral and third ventricles by cannulating the Aqueduct of Sylvius produced pronounced increases in arterial pressure (13). The onset of the effect bicuculline administered through i.c.v. cannula was reported to occur within seconds, suggesting the site of drug action being located superficially with respect to the ventricular space (14). Moreover, data from a study by Share and Melville indicated that the sites accessing $GABA_A$-antagonist drugs appear to be placed adjacent to the third ventricle rather than the lateral ventricle (15). Furthermore, it has been proposed that a forebrain periventricular GABAergic system exerts a tonic inhibitory influence on the sympathetic nervous system, and the GABAergic site is located within the hypothalamus and particularly in the posterior region (16, 17). In our laboratory, blood pressure changes evoked by i.c.v. administration of bicuculline or muscimol, a $GABA_A$-receptor agonist, were shown to be abolished following electrolytic lesions of the DMH, indicating that these responses are mediated through the DMH (3, 18). In the present study, we aimed to examine extracellular L-

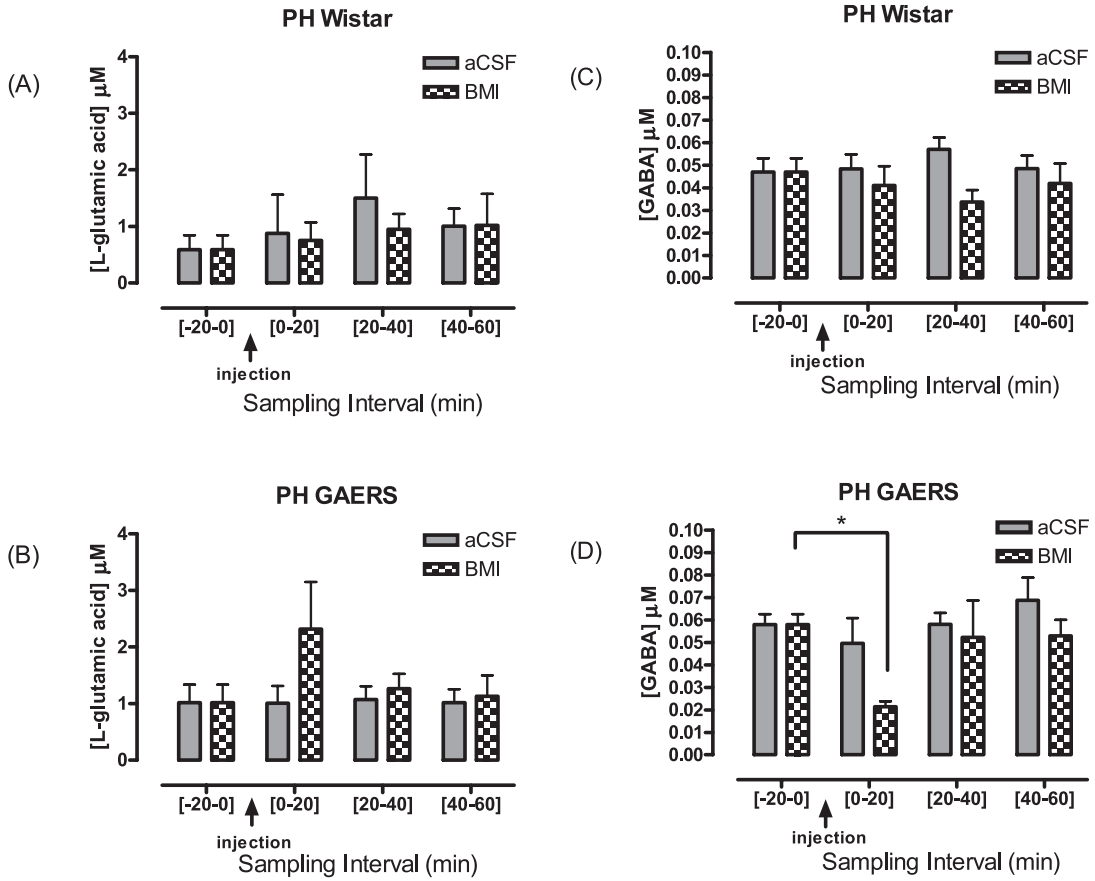


**Fig. 7.** The analysis of extracellular L-glutamic acid (A and B) or GABA (C and D) levels in response to aCSF or bicuculline (BMI) in the PH of non-epileptic Wistar rats or GAERS. The time lag was 20 min. **P*<0.05, compared to the basal ([–20 – 0] min sample) value.

glutamic acid and GABA levels of the posterior region of the hypothalamus after i.c.v. injection of bicuculline. In microdialysis procedures, we can measure the extracellular levels of substances. However, as far as glutamate is concerned, the glutamate derived from tissue injury and/or metabolism will make it difficult to differentiate the source of glutamate. In the experiments, we started collecting the microdialysis samples the day after the implantation of the probe to minimize the effects of tissue injury. As we also measured the time-course changes in glutamate levels in response to bicuculline, it is of no doubt that these changes are due to released glutamate not from other sources. It can be hypothesized that GABA or glutamate measured as a response usually diminishes due to compartmentalization. The neurotransmitter release is dependent on sodium channels and calcium-dependent. The responses to tetrodotoxin were shown to be strikingly slow and elevations were observed only if long infusions were performed (19, 20). It was also reviewed by Timmerman and Westerink (21) that calcium antagonism did not produce any better levels in microdialysis samples as shown by many investigators (19 – 22). We observed that the extracellular L-glutamic acid level was increased in the DMH and PH. This increase was statistically significant in DMH but not in PH. Comparably, GABA level decreased in the DMH and PH in response to i.c.v. administration of bicuculline in GAERS. This decrease was significant in PH but not in DMH. Thus, this finding suggests that the increase in L-glutamic acid level and the decrease in GABA level may be associated with the increase in blood pressure following bicuculline administration. Both accelerated glutamatergic transmission and reduced GABAergic inhibition can cause similar results. The drug failed to generate a cardiovascular and a neurochemical response in Wistar rats. This finding may indicate that excitatory and inhibitory mechanisms are affected in different proportions in these 2 nuclei since excitation and inhibition should be kept in equilibrium in order to maintain a normal brain function as stated previously (23). Our results may imply that $GABA_A$ receptors existing on glutamergic neurons

in the DMH controls the activity of DMH in cardiovascular regulation. Hypersensitivity to bicuculline may result from either a reduction of or a failure of GABAergic inhibition, but immunocytochemical and radioligand binding studies should be further performed to explain the mechanism underlying it in GAERS.

In the current study, although we demonstrated a significant increase in mean arterial pressure values following i.c.v. administration of the $GABA_A$-receptor antagonist in GAERS, this cardiovascular change was not observed in the Wistar strain with the same dose of bicuculline. The results of this study are also in agreement with the findings of the previous studies where cardiovascular responses to i.c.v. bicuculline were found to be increased in the WAG/Rij rat, which is one of the best characterized genetic rat models of absence epilepsy, where the smallest dose of bicuculline failed to generate a cardiovascular response in non-epileptic Wistar control rats (10). Moreover, the GAERS generate more prominent increases in blood pressure when bicuculline was given into the DMH of GAERS when compared to the non-epileptic control rats (11). We have previously demonstrated that BMI caused dose-dependent increases in both blood pressure and heart rate in conscious rats when injected intracerebroventricularly (5). Thus, even low doses of bicuculline injection led to a significant blood pressure response. It has been reported that the susceptibility to convulsions induced by threshold doses of $GABA_A$-receptor antagonists bicuculline and picrotoxin were found to be higher in GAERS compared to non-epileptic control animals (24). Another study showed that GAERS were more prone to develop a GABA-withdrawal syndrome than the non-epileptic inbred control rats do (25). These findings are in agreement with our previous results showing that GAERS are more sensitive to the bicuculline-induced blood pressure changes. Collectively, these results suggest that GAERS are hyperresponsive to agents related to GABAergic neurotransmission in the brain.

The present study also shows that the basal mean arterial pressure in GAERS is higher than the basal mean arterial pressure of Wistar animals. This difference may be due to a secondary adaptive change in response to environmental challenge or due to fragments of absence seizures observed in this genetic model. The reasons why GAERS have increased basal mean arterial pressure levels should be further assessed.

The i.c.v. injection of 0.3 nmol of bicuculline caused a decrease in the duration of SWDs in the EEG of the GAERS group. This finding is in agreement with the earlier data from this laboratory (11). Likewise, Peeters et al. have demonstrated that i.c.v. administration of bicuculline inhibits SWD activity, and in contrast, activation of $GABA_A$ receptors by muscimol causes a dose-dependent increase in the number of SWD complexes in the WAG/Rij model (26). A connection between posterior hypothalamic area and thalamus was reported previously (27). Based on the findings about the decrease in SWD activity in response to bicuculline injections, we can assume the involvement of the posterior hypothalamic regions in the generation of SWDs or an interaction between the hypothalamus and cortico-reticular system (or cortico-thalamic system) that generate SWDs. However, bicuculline causes an increase in locomotor activity (28), and the decrease in SWDs can also be related with the behavioral changes induced with bicuculline since it is well known that SWDs can be suppressed in epileptic rats with locomotor activity (29). Further studies are required to evaluate whether this decrease is related to the increased locomotor activity or to the direct connections present between these areas. This is a critical issue to understand the neuronal mechanism of absence seizures and autonomic symptoms observed in epileptic seizures.

In conclusion, we postulate that changes in extracellular glutamatergic and GABAergic neurotransmitter levels in GAERS are not only restricted to the regions that are responsible for seizure generation (18, 30) but also present in other areas related with central cardiovascular regulation, particularly within the hypothalamus.

## References


1 Swaab DF. Development of the human hypothalamus. Neurochem Res. 1995;20:509–519.

2 Bernardis LL, Bellinger LL. The dorsomedial hypothalamic nucleus revisited. Proc Soc Exp Biol Med. 1998;218:284–306.

3 Goren Z, Berkman K, Aslan N, Şan T, Oktay Ş, Onat F. Role of paraventricular and dorsomedial nuclei of hypothalamus and central nucleus of amygdala on muscimol-induced cardiovascular responses. Fundam Clin Pharmacol. 1997;11:408–415.

4 DiMicco JA, Abshire VM. Evidence for GABAergic inhibition of a hypothalamic sympathoexitatory mechanisms in anesthetized rats. Brain Res. 1987;402:1–10.

5 Goren Z, Berkman K, Aslan N, Oktay Ş, Onat F. The role of amygdala and hypothalamus in GABAA antagonist bicuculline-induced cardiovascular responses in conscious rats. Brain Res. 1996;722:118–124.

6 Soltis RP, DiMicco JA. $GABA_A$ and excitatory amino acid receptors in dorsomedial hypothalamus and heart rate in rats. Am J Physiol. 1991;260:13–20.

7 Goren MZ, Akıcı A, Berkman K, Onat F. Cardiovascular responses to NMDA injected into nuclei of hypothalamus or amygdala in conscious rats. Pharmacology. 2000;61:257–262.

8 Goren MZ, Yananli HR, Berkman K, Onat F, Aker R. The influence of dorsomedial hypothalamic nucleus on contralateral paraventricular nucleus in NMDA-mediated cardiovascular responses. Brain Res. 2003;968:219–226.

9 Abshire VM, Hankins KD, Roehr KE, DiMicco JA. Injection of L-allylglycine into the posterior hypothalamus in rats causes decreases in local GABA which correlate with increases in heart rate. Neuropharmacology. 1988:27:1171–1177.
10 Karson AB, Aker R, Ateş N, Onat F. Cardiovascular effects of intracerebroventricular bicuculline in rats with absence seizures. Epilepsy Res. 1999;34:231–239.
11 Aker R, Onat FY. Cardiovascular regulation through hypothalamic $GABA_A$ receptors in a genetic absence epilepsy model in rat. Epilepsia. 2002;43:107–114.
12 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. London: Academic Press; 1986.
13 DiMicco JA, Gillis RA. Neuro-cardiovascular effects produced by bicuculline in the cat. J Pharmacol Exp Ther. 1979;210:1–6.
14 DiMicco JA. Blockade of forebrain gamma-aminobutyric acid (GABA) receptors and reflex activation of the cardiac vagus in anesthetized cats. J Pharmacol Exp Ther. 1982;223:654–661.
15 Share NN, Melville KI. Intraventricular injections of picrotoxin following central adrenergic blockade with phenoxybenzamine and dichloroisoproterenol. Int J Neuropharmacol. 1965;4:149–156.
16 Williford DJ, Hamilton BL, Souza JD, Williams TP, DiMicco JA, Gillis RA. Central nervous system mechanisms involving GABA influence arterial pressure and heart rate in the cat. Circ Res. 1980;47:80–88.
17 Martin DS, Segura T, Haywood JR. Cardiovascular responses to bicuculline in the paraventricular nucleus of the rat. Hypertension. 1991;18:48–55.
18 Goren MZ, Richards DA, Turner H, Bowery NG. Extracellular GABA levels are increased in brain regions associated with the generation of absence seizures. British Pharmacology Society Summer Meeting, Bristol. Br J Pharmacol. 1997;122:83.
19 Smith SE, Sharp T. An investigation of the origin of extracellular GABA in rat nucleus accumbens measured in vivo by microdialysis. J Neural Transm Gen Sect. 1994;97:161–171.
20 Bourdelais A, Kalivas PW. Apomorphine decreases extracellular GABA in the ventral pallidum of rats with 6-OHDA lesions in the nucleus accumbens. Brain Res. 1992;577:306–311.
21 Timmerman W, Westerink BH. Brain microdialysis of GABA and glutamate: what does it signify? Synapse. 1997;27:242–261.
22 Osborne PG, O'Connor WT, Drew KL, Ungerstedt U. An in vivo microdialysis characterization of extracellular dopamine and GABA in dorsolateral striatum of awake freely moving and halothane anaesthetised rats. J Neurosci Methods. 1990;34:99–105.
23 Cline H. Synaptogenesis: a balancing act between excitation and inhibition. Curr Biol. 2005;15:203–205.
24 Vergnes M, Boehrer A, Reibel S, Simler S, Marescaux C. Selective susceptibility to inhibitors of GABA synthesis and antagonists of GABA(A) receptor in rats with genetic absence epilepsy. Exp Neurol. 2000;161:714–723.
25 Brailowsky S, Montiel T, Boehrer A, Marescaux M, Vergnes M. Susceptibility to focal and generalized seizures in Wistar rats with genetic absence-like epilepsy. Neuroscience. 1999;93:1173–1177.
26 Peeters BWMM, van Rijn CM, Vossen JMH, Coenen AML. Effects of GABA-ergic agents on spontaneous non-convulsive epilepsy, EEG and behaviour in the WAG/Rij inbred strain of rats. Life Sci. 1989;45:1171–1176.
27 Aker RG, Ozyurt HB, Yananli HR, Cakmak YO, Ozkaynakçi AE, Sehirli U, et al. GABA(A) receptor mediated transmission in the thalamic reticular nucleus of rats with genetic absence epilepsy shows regional differences: functional implications. Brain Res. 2006;1111:213–221.
28 Shekhar A, Hingtgen JN, DiMicco JA. Selective enhancement of shock avoidance responding elicited by GABA blockade in the posterior hypothalamus of rats. Brain Res. 1987;420:118–128.
29 Vergnes M, Marescaux C, Boehrer A, Depaulis A. Are rats with genetic absence epilepsy behaviorally impaired. Epilepsy Res. 1991;9:97–104.
30 Terzioğlu B, Aypak C, Onat FY, Küçükibrahimoğlu E, Ozkaynakçi AE, Gören MZ. The effects of ethosuximide on amino acids in genetic absence epilepsy rat model. J Pharmacol Sci. 2006;100:227–233.