Effect of Oral Administration of GABA on Temperature Regulation in Humans during Rest and Exercise at High Ambient Temperature

TAIKI MIYAZAWAI, TAKASHI KAWABATAI, TAKASHI SUZUKI, DAIKI IMAII, TAKESHI HAMAMOTO, TAKAHIRO YOSHIKAWA, and TOSHIAKI MIYAGAWA

Departments of Environmental Physiology for Exercise and Sports Medicine, Osaka City University, Graduate School of Medicine; and Food R&D Center, Japan Tobacco Inc.

Abstract

Background

Centric administration of gamma-aminobutyric acid (GABA) has been implicated to affect temperature regulation in animals during rest or under anesthesia. However, there are few reports concerning the effects of the oral administration of GABA on temperature regulation in humans during rest and exercise. In order to clarify the effects and underlying mechanisms, we measured several parameters related to temperature regulation of humans during rest and exercise at high ambient temperature (35°C).

Methods

On two occasions, eight endurance-trained men rested for 20 min and cycled at 65% VO\textsubscript{2peak} for 30 min. In control trial (trial-C), subjects drank the sample which was a sports drink of 200 mL (placebo) before the rest period. In another trial (trial-G), subjects drank the sample which was a sports drink containing 1000 mg of GABA (GABA drink) before the rest period.

Results

In trial-G, the plasma GABA concentrations were maintained higher than those in trial-C during the experiment. An increase of esophageal temperature during rest and exercise was inhibited in trial-G. Sweat rate, and plasma catecholamine concentrations during exercise were inhibited in trial-G.

Conclusions

Esophageal temperature inhibition is suggested to be induced by the suppression of cold-sensitive neurons during rest, and the inhibition of plasma catecholamine concentrations caused by the GABA-induced attenuation of the sympathetic nervous system during exercise.
Introduction

Gamma-aminobutyric acid (GABA), an amino acid, exists widely in nature. It is present in a variety of foods such as vegetables, fruits, and fermented foods, and is also usually a part of normal diet.

Recent developments in technology have enabled the extraction of high-purity GABA from foods, and it has been possible to orally ingest certain amounts of GABA on purpose. Moreover, there has been great progress in research concerning the physiological effects of oral administration of GABA. Several reports have shown that the oral administration of GABA induces various physiological effects\(^{1,2}\). GABA has become the center of attention as a functional food material and drug.

In mammals, GABA is generated by the decarboxylation of glutamic acid, and widely distributed in the central nervous system (CNS) as the most important depressive neurotransmitter\(^3\). Moreover, it is particularly more abundant in the preoptic area and anterior hypothalamus (PO/AH), which is regarded as the center of temperature regulation and is considered to be the primary locus for integration of thermal signals originating from different parts of the body, than in other brain regions\(^{4,5}\). Therefore, numerous researches concerning a relationship between GABA and temperature regulation have shown positive results\(^6\) and gradually clarified underlying mechanisms\(^7,12\).

However, most of the abovementioned studies concerning the effects of GABA on temperature regulation have involved animals as their subjects or have focused on centric administration of GABA, its agonists, or its antagonists; therefore, there are few reports concerning the effects of the oral administration of GABA on temperature regulation in humans. Furthermore, these reports have only involved the consequences of GABA administration during rest or under anesthesia. Therefore, the present study aims to determine the effects of the oral administration of GABA on thermoregulatory responses in humans during rest and exercise at high ambient temperature (35°C) and to clarify the mechanisms underlying these effects.

Methods

Subjects

Eight healthy non-smoking males who usually underwent exercise training 3-5 days a week participated in this study. The subjects had a mean (± SD) age of 22.8±3.7 years, height of 174.0±5.1 cm, weight of 64.2±5.1 kg, body mass index of 21.2±1.4, and peak oxygen uptake (\(\text{Vo}_{2\text{peak}}\)) of 59.7±7.8 mL·min\(^{-1}\)·kg\(^{-1}\). Written informed consent, which conformed to the guidelines in the Declaration of Helsinki, was obtained from all subjects before participation in this study. The study protocol and informed consent forms were approved by the Ethical Committee of Osaka City University, Graduate School of Medicine.

Experimental design

This study was performed in two trials. One was a control trial (trial-C), in which the sample was a sports drink of 200 mL (placebo); the other was trial-G in which the sample was a sports drink containing 1000 mg of GABA (GABA drink). Both the placebo and the GABA drink were
maintained at a temperature of 10°C. The sports drink sample (200 mL) provided 30 kcal of energy, 7.4 g carbohydrate, 0 g protein, and 0 g fat. The subjects were randomized to receive either a placebo or GABA drink on their first experimental visit. On their subsequent visit, they received the other drink. Each trial was conducted at the same time of the day separated by at least three days from the preceding trial to avoid any effects of circadian rhythm or thermal adaptation by exercise on the experiments.

**Protocols**

The subjects were requested to refrain from taking food or drink for at least 12 h and avoid caffeinated beverages, alcohol, and strenuous physical activity for at least 24 h before each experiment. Drinking water was allowed as needed.

All experiments were conducted in the morning during the winter season (from January to March). The subjects reported to the laboratory at 10:00 hours. Then, the fluid balance was regulated by drinking water (200 mL) and urinating to maintain appropriate exercise performance and physiological responses. The subjects were then weighed in the nude. For the exercise trials, the subjects were given a pair of shorts, socks, and shoes. They were then made to swallow an esophageal thermistor. A 20-gauge intravenous catheter (BD Insyte-W; Becton Dickinson Infusion Therapy System Inc., Utah, USA) was placed into the left antecubital vein for blood sampling. Subsequently, the subjects entered the climatic chamber (TBR-6W2S2L2M; Espec Co., Osaka, Japan) controlled at 35°C ambient temperature ($T_a$) and 50% relative humidity.

The subjects sat in a semirecumbent position for 1 h on the contour chair of the electronic cycle ergometer (EC-C400R; CATEYE, Osaka, Japan) while the devices for measurements were positioned, and received either the placebo (trial-C) or the GABA drink (trial-G). There was a transient reduction in the esophageal temperature ($T_{es}$) while drinking the sample. The beginning of the rest period was determined as the time at which the $T_{es}$ achieved again the basal level, i.e., before sample ingestion.

We had investigated previously the transitory plasma GABA concentrations during rest for 60 min after the intake of a GABA drink. We had observed that plasma GABA concentrations reached the peak at 20-40 min after the oral administration of GABA. Accordingly, in the present study, we decided to provide a rest period of 20 min to maintain a high plasma GABA concentration during exercise. Following the rest period, the subjects continuously conducted 30 min of ergometric cycle exercises in the semirecumbent position at 65% $V_{O2peak}$ with a pedaling frequency of 60 rpm without fan cooling. The mean (± SD) intensity was 179±13 W. When the exercise period was completed, the monitoring instruments were promptly removed; the subjects exited the chamber, wiped themselves, and were weighed again in the nude to estimate the total sweat loss.

$V_{O2peak}$

As a pre-experimental study, the $V_{O2peak}$ was determined using graded ergometric cycle exercises in a semirecumbent position at a $T_a$ of 25 (24-26) °C and a relative humidity of 40-50% three days prior to the experiment. The subjects started pedaling at 60 cycles/min at an initial intensity of 0 W. The intensity was increased by 20 W every 1 min. The oxygen uptake rate was calculated every 20 sec by using the oxygen and carbon dioxide fractions in expired gas and ventilatory volume (Vmax29; SensorMedics co., CA, USA).
Measurements

Values of $T_{es}$, skin temperature, skin blood flow (SkBF), sweat rate (SR) and oxygen uptake ($\dot{V}O_2$) at specific time points in testing protocol were expressed as the mean of 1 min of data collected at intervals of 5 min.

$T_{es}$ was measured using an esophageal thermistor in a polyethylene tube (LT-ST08-11; Gram Co., Saitama, Japan). The tip of the tube was advanced to a distance of one-fourth of the subject's standing height from the external nares. Skin temperatures were measured with thermistors (LT-ST08-12; Gram Co., Saitama, Japan) at the chest ($T_{chest}$), upper arm ($T_{arm}$), thigh ($T_{thigh}$), and leg ($T_{leg}$) on the left side. Mean skin temperature ($T_{sk}$) was calculated from the body surface area distribution and thermal sensitivity of each skin area by using the formula described by Ramanathan as follows:

$$T_{sk} = 0.3(T_{chest} + T_{arm}) + 0.2(T_{thigh} + T_{leg})$$

SkBF was measured by laser Doppler flowmetry (ALF21D; Advance, Tokyo, Japan) on the left side forearm. SR values were measured on the left side forearm by using the ventricular-capsule method (SS-1001I; K and S, Aichi, Japan). The site of the probe and capsule placement on the skin was identical for each subject.

Data for $T_{es}$, skin temperature, SkBF, and SR were collected with a 16-channel computerized data-acquisition system (Intercross310; Intercross co., Tokyo, Japan) and stored in data files on a laboratory computer (JPA32301WP; Hewlett-Packard Japan Ltd., Tokyo, Japan).

$\dot{V}O_2$ was measured from the oxygen and carbon dioxide fractions in expired gas and ventilatory volume by using a metabolic gas analyzer system (Vmax29; SensorMedics co., CA, USA).

Total sweat loss was estimated using the change in dry body weight measured immediately before and after the experiment as $(BW_{before}) - (BW_{after}) + 200$ g (weight of the drinking sample) - $80$ g (weight of the blood samples).

We defined the threshold $T_{es}$ for cutaneous vasodilatation as the value of $T_{es}$ at which SkBF began to progressively increase and that for sweating as the value of $T_{es}$ at which SR began to progressively increase above the resting level.

For plasma concentrations, 20 mL of blood was sampled at the beginning of rest and exercise, and at 15 and 30 min after the beginning of exercise in each trial. The blood sample for the measurement of plasma GABA, adrenaline, and noradrenaline concentrations was immediately transferred into a tube containing heparin and centrifuged at 4°C. The separated plasma was stored in the freezer at $-80$°C until further use. Plasma GABA, adrenaline, and noradrenaline concentrations were measured using high performance liquid chromatography (HLC-725CA II; Tosoh Corporation, Tokyo, Japan).

Statistics

A two-way (trial-by-time) repeated measures analysis of variance (ANOVA) was performed to test for the effects of the GABA administration and time. Subsequent post-hoc tests to determine significant differences in the various pairwise comparisons were performed by Fisher's least squares difference test. A Student's $t$-test was used to assess differences in the total sweat loss and the threshold temperature for cutaneous vasodilatation and sweating between trial-C and trial-G. $P<0.05$ was considered to indicate statistical significance. The data are represented as mean±SD, unless stated otherwise.
Results

All subjects completed the entire submaximal exercise protocol in both trial-C and trial-G. \( \dot{V}O_2 \) during rest and 65% \( V_{O2\text{peak}} \) exercise were very similar for trial-C and trial-G. Subjects cycled at similar intensities in both the trials (trial-C, 64\( \pm \)12% \( V_{O2\text{peak}} \); trial-G, 64\( \pm \)11% \( V_{O2\text{peak}} \); \( p=0.81 \)).

The changes in plasma GABA concentration are shown in Figure 1. Plasma GABA concentration at -20 min was similar in trial-C and trial-G, averaging 0.17\( \pm \)0.02 and 0.15\( \pm \)0.02 nmol/mL, respectively. In trial-C, the plasma GABA concentration did not change during rest and exercise. In trial-G, plasma GABA levels rose significantly (\( p<0.05 \)) above those in trial-C as also above the -20 min values of trial-G to values of 0.8-1.5 nmol/mL during the 0-30 min period, i.e., the exercise period.

The time course of \( T_{es} \) during rest and exercise is shown in Figure 2. \( T_{es} \) at -20 min was almost identical in both trials. There was a tendency (\( p=0.09 \)) for \( T_{es} \) at 0 min in trial-C to increase in response to heat exposure (35\( ^\circ \)C) during the 20 min rest period as compared to the value at -20 min, while there were no changes in trial-G. During exercise, \( T_{es} \) rose significantly compared to the baseline value at 0 min in each trial. Because trial-G resulted in an attenuation of the increase in \( T_{es} \) as compared with trial-C, the differences in \( T_{es} \) values between trial-G and trial-C became significant by 15 min (\( p<0.05 \)). Furthermore, the differences grew progressively, and at 30 min, the \( T_{es} \) value in trial-G was 0.23\( ^\circ \)C lower than that in trial-C (i.e., 38.55\( \pm \)0.25 vs 38.78\( \pm \)0.21\( ^\circ \)C; \( p<0.05 \)).

Table 1 shows results of \( T_{sk} \), SkBF, SR, plasma adrenaline concentration, and plasma noradrenaline concentration. The values at the beginning of rest period, the beginning of exercise period, 15 min, 30 min after the beginning of exercise are presented in the table to simplify. The \( T_{sk} \) values were significantly increased by exercise in each trial. There were no significant differences between the trials for \( T_{sk} \). The SkBF did not change from the beginning of rest to the beginning of exercise. During the 0-15 min of exercise period in both trials, because of exercise, SkBF increased from the value at the beginning of exercise, and during the 15-30 min
period, SkBF was maintained at almost the same level as the 15 min value. There were no significant differences between trials for SkBF at any time point. A rapid increase in SR was observed during the first 15 min of exercise followed by a slower increase in the last 15 min. Although the patterns of the time course of SR were very similar in each trial, during exercise, the SR value in trial-G was significantly lower than that in trial-C at 15 min and 30 min of exercise. Plasma adrenaline and noradrenaline levels did not change during rest in either of the trials, and there were no significant differences between the trials. During exercise, plasma adrenaline levels in trial-C rose progressively from 0.04±0.02 ng/mL at the beginning of exercise to 0.30±0.15 ng/mL after exercise (p<0.05), and at 30 min, the noradrenaline level was also significantly elevated above the value at the beginning of exercise (i.e., 2.04±0.70 vs 0.19±0.06 ng/mL; p<0.05). In contrast, in trial-G, GABA appeared to have attenuated the increase in

Table 1. Values of physiological parameters during rest and exercise in high ambient temperature

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Ex0</th>
<th>Ex15</th>
<th>Ex30</th>
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</thead>
<tbody>
<tr>
<td>$T_{sk}$, °C</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>trial-C</td>
<td>34.72±0.66</td>
<td>34.98±0.55</td>
<td>36.44±0.33</td>
<td>37.01±0.21</td>
</tr>
<tr>
<td>trial-G</td>
<td>34.69±0.63</td>
<td>34.94±0.53</td>
<td>36.33±0.49</td>
<td>36.85±0.51</td>
</tr>
<tr>
<td>SkBF, mL·min⁻¹·100 g⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trial-C</td>
<td>0.68±1.37</td>
<td>1.06±1.30</td>
<td>8.42±2.16</td>
<td>8.85±2.66</td>
</tr>
<tr>
<td>trial-G</td>
<td>1.24±1.23</td>
<td>0.41±1.06</td>
<td>8.65±2.16</td>
<td>9.48±2.48</td>
</tr>
<tr>
<td>SR, mg·(cm²⁻¹)·min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trial-C</td>
<td>0.01±0.06</td>
<td>0.02±0.06</td>
<td>0.98±0.11</td>
<td>1.26±0.20</td>
</tr>
<tr>
<td>trial-G</td>
<td>0.05±0.04</td>
<td>0.04±0.04</td>
<td>0.89±0.16</td>
<td>1.12±0.21</td>
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<tr>
<td>Adrenaline, ng/mL</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trial-C</td>
<td>0.04±0.01</td>
<td>0.04±0.02</td>
<td>0.16±0.05</td>
<td>0.30±0.15</td>
</tr>
<tr>
<td>trial-G</td>
<td>0.03±0.01</td>
<td>0.04±0.01</td>
<td>0.16±0.04</td>
<td>0.22±0.08</td>
</tr>
<tr>
<td>Noradrenaline, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trial-C</td>
<td>0.19±0.07</td>
<td>0.19±0.06</td>
<td>1.23±0.49</td>
<td>2.04±0.70</td>
</tr>
<tr>
<td>trial-G</td>
<td>0.19±0.05</td>
<td>0.21±0.07</td>
<td>1.13±0.45</td>
<td>1.60±0.62</td>
</tr>
</tbody>
</table>

Values are means±SD for 8 subjects; Rest, the beginning of rest period; Ex0, the beginning of exercise period; Ex15 and Ex30, 15 and 30 min after the beginning of exercise period; $T_{sk}$, mean skin temperature; SkBF, skin blood flow on the forearm; SR, sweat rate on the forearm; Adrenaline, plasma adrenaline concentration; Noradrenaline, plasma noradrenaline concentration; *Significantly different from the value of trial-C at the same time, p<0.05; †/Significantly different from the value of Ex0, p<0.06; and ‡/Significantly different from the value of Ex15, p<0.05.

Figure 3. Threshold $T_{sk}$ for vasodilatation and sweating for trial-C (white) and trial-G (black). Values are represented as mean±SD for 8 subjects. *Significantly different between trial-C and trial-G, p<0.05. †Tendency to differ between trial-C and trial-G, p<0.1.
plasma adrenaline and noradrenaline concentration, and values remained at 0.22±0.08 ng/mL and 1.60±0.62 ng/mL, respectively, which were significantly lower than those in trial-C at 30 min.

Total sweat loss as evaluated by the difference between the body weight before and after the experiment was 100 mg lower in trial-G as compared with that in trial-C (i.e., 690±90 vs 790±90 mg; p<0.01).

The Tes threshold for vasodilatation and sweating are shown in Figure 3. GABA administration decreased the threshold temperature for increased SkBF (vasodilatation) from 36.87±0.28°C to 36.74±0.29°C (p=0.063), and in sweating from 36.93±0.31°C to 36.75±0.27°C (p<0.05).

Discussion

This study presented data related to effects of the oral administration of GABA in humans for temperature regulation during rest and exercise in high ambient temperatures. The principle observation was that the oral administration of GABA induced the inhibition of core temperature increase during rest and exercise in high ambient temperature.

Effect of GABA during rest

GABA activities in the brain relate to temperature regulation during rest and underlying mechanisms of the relationship have been clarified gradually8,10,14,16. Ishiwata et al10 investigated the actions of GABA in the PO/AH area of rats during pharmacological stimulation of GABA in hot ambient temperature. The PO/AH contains both warm- and cold-sensitive neurons that alter their discharge rates corresponding to physiological changes in local temperature or afferent signals from peripheral skin thermoreceptors. Cold-sensitive neurons are important for controlling heat production17,18. Ishiwata et al concluded that GABA largely suppressed cold-sensitive neurons to prevent the activation of heat production, and caused hypothermia in hot ambient temperature. In this study, Tes of trial-C underwent a steady increase of 0.11°C during 20 min rest because of high ambient temperature, while there was no increase in Tes of trial-G (Fig. 2). On that account, there was a difference of 0.07°C in Tes between trial-C and trial-G at 0 min, while there was no such difference at -20 min. These results suggested that the increase in Tes during rest was inhibited by the oral administration of GABA. We considered that the inhibition of Tes during rest as confirmed in our study resulted from the same mechanism as previous studies, that is, the oral administration of GABA suppressed cold-sensitive neurons to prevent the activation of heat production, and induced the inhibition of Tes increase. But there was no difference in V02 representing heat production during rest between trial-C and trial-G. The cause for it was assumed that the method of V02 measurement was not appropriate to detect the subtle distinction. If we designed more precision techniques such as basal metabolic rate measurement, we would have detected a small difference.

Effect of GABA during exercise

During exercise, a body temperature increases in proportion to workloads because of heat production in working muscles19. In our results during exercise, Tes in both trials increased continuously until the end of exercise. At the time point when exercise was begun, the difference in Tes values between trial-C and trial-G was 0.07°C although when the exercise time ended, it had increased to 0.23°C. These results indicated that Tes increase during exercise in high
ambient temperature was inhibited by the oral administration of GABA. On the other hand, there was no difference in SkBF between trial-C and trial-G, and SR and total sweat loss were lower in trial-G than in trial-C. Namely, it was conceivable that the inhibition of T_e increase during exercise was induced not by a rise in the heat loss such as vasodilatation or sweating but by other factors. We presumed that there were two factors of the inhibition of T_e increase during exercise. The first factor is the suppression of cold-sensitive neurons in the PO/AH, which also caused the inhibition of T_e increase during rest. The reduction of heat production caused by the suppression of cold-sensitive neurons seemed to induce the inhibition of T_e increase in trial-G. But there was no difference in \( \dot{V}O_2 \) during exercise between trial-C and trial-G like that during rest. We had to design more precision techniques of \( \dot{V}O_2 \) measurement. The second factor is the attenuation of the sympathetic nervous system (SNS). The activation of central GABA function induces the attenuation of SNS activity\(^{22,28}\). In our results, plasma adrenaline and noradrenaline concentrations, which were controlled by the activity of the SNS, during exercise were lower in trial-G than those in trial-C (Table 1). Because the exercise intensity was identical between trial-C and trial-G, plasma catecholamine levels which depended upon exercise intensity\(^{20,21}\) was likely to be the same level. Therefore, our results mean that orally administered GABA induced the attenuation of SNS and lower plasma catecholamine levels. Then, how did the attenuation of SNS induce the inhibition of T_e increase during exercise? Mora-Rodriguez et al\(^{29}\) showed that the reduction in the plasma catecholamine concentrations induced an inhibition of T_e increase during prolonged exercise in man. The observation of relationship between the inhibition of T_e increase and plasma catecholamine levels in our study was consistent with results of the previous study. However, they concluded that the mechanisms responsible for their observations remained unclear. Further studies are necessary to elucidate the relation between plasma catecholamine concentrations and the inhibition of T_e increase. In the present study, we confirmed that the oral administration of GABA inhibited T_e increase and sweating during exercise. It is well established that core temperature increase and dehydration put restrictions on exercise performance in hot environment\(^{30,31}\). Therefore, it can be said that the oral administration of GABA improves exercise performance in hot environment.

**A process of GABA action**

Since GABA could not pass the brain-blood barrier (BBB), it has been long considered that the systemic administration of GABA, as in the present experiment, could not affect the central nervous system (CNS)\(^{32,34}\). From that, the systemic administration of GABA-induced depressor effects on noradrenaline release from sympathetic nerve endings and on blood pressure were thought to be due to the blockade of sympathetic ganglia\(^{35,36}\). Nevertheless, our results lead us to assume that systemically administered GABA might affect the GABA functions of the CNS. The inhibition of T_e increase during rest was consistent with previous investigations concerning centric GABA functions\(^{8,10,58}\). The attenuation of the SNS activity was also observed in previous investigations showing the effects of GABA agonists and antagonists microinjection to hypothalamic area in animals\(^{37,39}\). Besides, the shift of T_e thresholds for vasodilatation and sweating to a lower temperature in trial-G (Fig. 3) gave us the impression that the orally administered GABA might affect the temperature regulation center and shift the set point to a lower temperature. Furthermore, there were some reports suggesting that systemically administered GABA could access certain areas of the brain that lacked or was destroyed the
BBB\(^{34,40,41}\). The hypothalamus is known to partially lack the BBB. Therefore, we thought that GABA in blood reached the hypothalamus through the site which lacked the BBB, participated in the activations of central GABA functions and induced the suppression of cold-sensitive neurons and the attenuation of the SNS.

**Conclusion**

This study demonstrated that the oral administration of GABA inhibited the increases in \(T_{es}\) during rest and exercise in high ambient temperature. \(T_{es}\) inhibition during rest was seemed to be induced by the suppression of cold-sensitive neurons; and during exercise, by the inhibitions of plasma adrenaline and noradrenaline concentrations caused by the GABA-induced attenuation of the SNS. It remained unknown how the inhibitions of plasma adrenaline and noradrenaline concentrations inhibited the increase in body temperature.

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**References**