Effects of Serotonin Antagonist, Cyproheptadine, and Serotonin Depletor, *p*-Chlorophenylalanine, on Feeding in Rats Kept at High and Temperate-Ambient Temperatures

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Abstract The present study was conducted to examine the participation of serotonin (5 HT) in the regulation of appetite in rats acclimated to hot (33°C) and temperate (26°C) environments. The central administration of noradrenaline exerted a stimulative effect on food intake at 26 and 33°C. However, the increment of food intake after noradrenaline administration at 33°C was less than that at 26°C (0.4 vs. 1.0 g). Cyproheptadine, a 5HT antagonist, did not stimulate feeding in rats given noradrenaline at the two temperatures. Food intake was increased by cyproheptadine alone at 26°C, but not at 33°C. Thus, serotonergic activity might not be correlated with the suppression in eating elicited by noradrenaline in the hot environment. In a daytime, there was not a significant difference in food intake between the two temperatures before *p*-chlorophenylalanine (PCPA), a 5HT depletor, was given. Food intake was significantly ($p < 0.01$) increased by PCPA in daytime and nighttime at the two temperatures. However, the food intake at 33°C was significantly ($p < 0.01$) less than that at 26°C. The results of the present study suggest that the activation of serotonergic neurons may not be the main factor controlling food intake inhibitory in a hot environment.

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Some neurotransmitters are believed to have an inhibitory influence over eating behavior. A monoamine, serotonin (5 - hydroxytryptamine, 5 HT), is a convincing candidate for these neurotransmitters. Central and peripheral injections of 5 HT, its precursors, agonist and presynaptic releasers have consistently produced a suppression of feeding²⁾.

Conversely, blockade of 5 HT receptors and selective depletion of 5 HT are correlated with overeating and increased body weight. BAXTER *et al.*¹⁾ reported that subcutaneous injection of the 5 HT receptor blocker, cyproheptadine, 4-(5 H-dibenzo [a, d] cycloheptan-5-

ylidene)-1-methylpiperidine, increased food consumption in fasted rats. BREISCH *et al.*³⁾ reported that rats given *p*-chlorophenylalanine (PCPA), a serotonin depletor, intraventricularly began overeating 3 days after the injection and continued to display marked hyperphagia.

Animals acclimated to a high ambient temperature have lower food intake than those acclimated to a moderate ambient temperature. HAMILTON⁴⁾ showed that during chronic, mild (32°C) and severe (35°C) heat stress over a 21 days period, rats were observed to regulate food intake at a lower than normal levels. As for pig, feed consumption decreased as the air

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temperature increased from 40 to 100°F, and it appeared that the decrease was more rapid at higher temperatures⁵⁾. WINCHESTER and MORRIS⁹⁾ reported that the decline in feed intake of cows appeared to begin only after the temperature reached 90°F while intake of lactating cows began to decline at about 70°F. In our previous study, feeding elicited by noradrenaline was suppressed in the hot environment⁷⁾. In addition, it has been demonstrated that 5HT is particularly effective in suppressing feeding elicited by the injection of noradrenaline into the para-ventricular nucleus (PVN)⁸⁾. The present series of experiment were conducted to examine the participation of 5HT in the regulation of appetite in rats acclimated to hot and temperate environments.

Materials and Methods

Male rats of Wistar strain weighing 350 to 400 g were used. They were kept under standard lighting conditions of 12 h/day artificial light, from 6 : 00 to 18 : 00 h. For intraventricular injection, a guide cannula of stainless steel was first fixed to the skull under sodium pentobarbital anesthesia. The cannula was 0.7 mm in outer diameter and 14 mm in length with a guard on the outside at 2 mm from the tip bottom. Employing a stereotaxic instrument the tip of the cannula was placed 6.5 mm anterior to the lambda and 1.3 mm left of the sagittal suture. The injection cannula, stainless steel, with an outer diameter of 0.3 mm, was inserted into the brain through the guide cannula to a distance of 4.5 mm under the guard; that is 17.4 mm from the top of the guide cannula. After a recovery period of 7 days, the animals were used for two kinds of experiments.

In the 1st experiment, the effect of a serotonin-receptor blocker, cyproheptadine, on short time eating was investigated in freely-fed rats at temperate (26°C) and high (33°C) ambient temperatures. The animals were divided into

two groups, and acclimated to the two temperatures for 7 days. After the acclimation period, the experiment was started at 11 : 00 a. m. Cyproheptadine hydrochloride (Sigma, St. Louis, USA) dissolved in saline (0.85%) was administered intraperitoneally at a dose of 15 mg/kg body weight, and soon later 10 µg of L-noradrenaline bitartrate (Wako, Osaka, Japan) dissolved in 10 µl of saline was injected into the lateral ventricle. Soon after the injection, the rats (n=8 per each group) were individually separated into one cage. They were given pre-weighed chow pellets (CE-2, Clea Japan, Inc.), and food intake during 2 hours was calculated as the difference between the original weight of pellets and those left in the cage plus the amount of crumbs found under the cage. The rats were allowed free access to chow pellets before the drugs were given.

In the 2nd experiment, the effect of PCPA on diurnal pattern of food intake was investigated at the two environmental temperatures. Every rats was stereotaxically implanted with a guide cannula of stainless steel directed into the left ventricle. The method of the operation was the same as the 1st experiment. After a recovery period of 7 days, the animals (n=11) were divided into two groups, and acclimated to temperate (26°C) and hot (33°C) ambient temperatures. Five days after the acclimation, each rat was given pre-weighed powdered chow (CE-2, Clea Japan Inc.) at 18 : 00 h. Food intake in a nighttime was measured from 18 : 00 to 6 : 00 h, and that in a daytime was from 6 : 00 to 18 : 00 h. The food intake was measured daily. Seven days after the beginning of measurement of food intake, the rats were anesthetized with ether for easier injection, and received 3 mg of PCPA in 20 µl of saline into the ventricle at a rate of 10 µl/min. The dose of PCPA was determined according to BREISCH *et al.*³⁾. Over 7 days after the injection, the food intake was measured daily.

After the experiments, the animals were killed by ether and injected 10 µl of 1% Evans

blue solution. The brains were removed and sectioned for verifying adequacy of placement of the cannula. The dye was distributed not only in the lateral ventricle but also in the third ventricle in every case. Data were analyzed by two-way analysis of variance according to the 2×2 fractional scheme. And the statistical significance between two values was determined by *t*-test.

Results

The central administration of noradrenaline exerted a stimulative effect on food intake at both temperatures (Table 1). However, the increment of food intake after noradrenaline administration at 26°C was greater than that at 33°C (1.0 vs. 0.4 g). An additive increment of food intake was not observed after cyproheptadine administration in rats given noradrenaline simultaneously. Food intake was increased by cyproheptadine only when the drug was administered at 26°C. Food intake was gradually increased after PCPA administra-

tion, and reached the peak three to four days after the administration. The means of food intake for five days, from five days to one day before and from three days to seven days after PCPA administration, were shown in Table 2. In a daytime, there was not a significant in food intake between 26 and 33°C before PCPA was given. In a nighttime, however, food intake at 26°C was significantly ($p < 0.01$) greater than that at 33°C. Food intake was significantly ($p < 0.01$) increased after PCPA administration at the both temperatures. In addition, food intake at 26°C was significantly ($p < 0.01$) greater than that at 33°C in daytime and nighttime.

Discussion

In the present study, noradrenaline increased food intake in rats in hot and temperate environments. However, the increment of food intake by noradrenaline in the hot environment was less than that in the temperate environment. Serotonin (5 HT) is one of neurotransmitters causing satiety²⁾. We expected

Table 1. The effects of intraventricular injection of noradrenaline (NA, 10 µg) and intraperitoneal administration of cyproheptadine (CYP, 15 mg/kg BW) on food intake in rats kept at temperate (26°C) and hot (33°C) ambient temperatures¹⁾

	SAL		NA		Analysis of variance ²⁾		
	SAL	CYP	SAL	CYP	NA	CYP	NA×CYP
26°C	0.9±0.2 ^a	1.6±0.1 ^b	1.9±0.2	1.5±0.1	**	NS	**
33°C	0.1±0.1	0.3±0.1	0.5±0.1	0.7±0.1	**	NS	NS

¹⁾ Values represent means for eight rats with SE.

²⁾ **: $p < 0.01$, NS: not significant.

^{a, b}: Significant difference between saline (SAL)- and CYP-treatment in each treatment ($p < 0.01$).

Table 2. The effects of intraventricular injection of *p*-chlorophenylalanine (PCPA, 3 mg) and ambient temperatures on food intake of rats in daytime and nighttime¹⁾

	pre-PCPA		post-PCPA		Analysis of variance ²⁾		
	26°C	33°C	26°C	33°C	Temp.	PCPA	Temp.×PCPA
Daytime	4.8±0.3	3.9±0.3	9.6±0.7 ^a	6.8±0.4 ^b	**	**	*
Nighttime	12.4±0.1 ^a	8.2±0.2 ^b	17.1±0.3 ^a	13.1±0.7 ^b	**	**	NS

¹⁾ Values represent means of food intake for five days with SE. The numbers of rats used were seven and four at 26 and 33°C, respectively.

²⁾ *: $p < 0.05$, **: $p < 0.01$, NS: not significant.

^{a, b}: Significant difference between 26 and 33°C in each treatment ($p < 0.01$).

that serotonergic activity might suppress feeding in the hot environment. Cyproheptadine blocks 5HT receptors, thus suppressing the activity of 5HT neurons. Nevertheless, food intake was not additionally increased by cyproheptadine in rats given noradrenaline in the hot environment. Consequently, serotonergic activity might not be correlated with the suppression in eating elicited by noradrenaline in the hot environment. On the other hand, cyproheptadine alone increased food intake only in the temperate environment. LEIBOWITZ *et al.*⁸⁾ have suggested that 5HT may suppress feeding by directly acting to increase the firing of PVN satiety neurons, and that α 2-adrenergic receptors on 5HT presynaptic terminals reduce 5HT release and consequently disinhibit eating. The failure to increase food intake by the 5HT antagonist suggests that there may be satiety neurons besides serotonergic neurons, directly activating satiety neurons within or without the PVN in the hot environment. The reduced increment of food intake after noradrenaline in the hot environment may be due to the same mechanism in the case of cyproheptadine alone, because both noradrenaline and cyproheptadine suppress serotonergic neurons.

Rats are a nocturnal animal, and eat more food in nighttime than in daytime. Interestingly, there was not significant difference in food intake in the daytime between hot and temperate environments before PCPA was given. Following PCPA administration, however, feeding was activated in both daytime and nighttime, and food intake at the low temperature was greater than that at the high temperature. The activation of feeding neurons may be suppressed by satiety neurons in the hot environment. PCPA is a drug which disturbs 5HT synthesis and then depletes 5HT. BREISCH *et al.*³⁾ showed that hyperphagia was related to drug dose and to the degree and duration of 5HT depletion. If hyperphagia after PCPA is caused by inactivation of

serotonergic neurons, the reduced increment of food intake in the hot environment suggests that satiety neurons besides serotonergic neurons may be activated in the hot environment. HOLMES *et al.*⁶⁾ have concluded that elevated activity of noradrenergic neurons, rather than decreased serotonergic activity, in the medial basal hypothalamus may cause hyperphagia following PCPA administration. If noradrenaline stimulates feeding through inhibiting 5HT release as suggested by LEIBOWITZ *et al.*⁸⁾, the decrease in food intake in the hot environment can be explained by activation of satiety neurons besides serotonergic neurons. The results of the present study suggest that the activation of serotonergic neurons may not be the main factor controlling food intake inhibitory in a hot environment.

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暑熱ならびに快適環境に馴化したラットの摂食に対するセロトニン 阻害薬シプロヘプタジンおよびセロトニン合成阻害薬 *p*-クロロフェニルアラニンの影響

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本研究の目的は、暑熱環境における食欲減退にセロトニンが関与する可能性をラット（雄、体重 350～400 g）を用いて検討した。実験 1 では、試薬投与前 7 日間、暑熱（33℃）および快適環境（26℃）下でラットを飼育した。試薬投与直前まで自由採食とした。ノルアドレナリン（10 μg）を側脳室投与すると摂食が刺激され、2 時間当たりの採食量は増加した。しかしながら、33℃ 下の採食量の増加は 26℃ 下の採食量の増加よりも少なかった（0.4 vs. 1.0 g）。セロトニン拮抗薬シプロヘプタジン（15 mg/kg 体重）をノルアドレナリンと同時に投与しても、採食量がノルアドレナリン単独投与の場合より多くなることはなかった。したがってセロトニン神経の活性化は、ノルアドレナリンによる摂食が暑熱環境下において抑制される要因ではない。シプロヘプタジン単独投与後 2 時間の採食量を測定すると、26℃ 下では採食量が増加したが、33℃ 下では増加しなかった。実験 2 において、7 日間の温度馴化後、1 日当たりの採食量を明期および暗期に分けて 5 日間測定したところ、明期の採食量は両環境間で差がなかった。セロトニン合成阻害薬 *p*-クロロフェニルアラニン（PCPA, 3 mg）を側脳室投与すると、両環境下で、明期および暗期の採食量が増加した。PCPA 投与 3 日後から 7 日後までの 5 日間の採食量は、明期および暗期ともに 33℃ 下の方が 26℃ 下よりも少なかった。以上の結果は、セロトニン神経の活性化が暑熱環境下における食欲の減退に関与しないことを示唆している。

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