Effects of Cyproheptadine and $p$-chlorophenylalanine on Muscimol-Induced Eating in Rats Kept at High and Temperate-Ambient Temperatures

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Abstract  Food intake in animals kept in a hot environment is generally less than that in a temperate environment. Serotonin has been reported to inhibit appetite in the central nervous system. The central mechanisms, through which food intake is inhibited in a hot environment, have been often examined. In this study, the effects of serotonin antagonist, cyproheptadine, and $p$-chlorophenylalanine (PCPA), which depletes the brain of serotonin, on eating elicited by i.c.v. injection of muscimol were investigated in rats kept in hot (33°C) and temperate (26°C) environments. Muscimol-induced eating was inhibited by cyproheptadine in a dose-dependent manner at both temperatures. Food intake per day was gradually increased after PCPA administration at both temperatures. The pretreatment with PCPA had little effect on food intake for 2 hours after muscimol injection at 26°C. On the other hand, food intake for the short period was suppressed even after muscimol injection in rats pretreated with PCPA at 33°C. It was therefore possible that the stimulatory effect of muscimol on dopamine neurons might have been suppressed in the hot environment.


Key words: cyproheptadine, $p$-chlorophenylalanine, muscimol, food intake, hot environment

Animals kept in a hot environment have a lower food intake than those kept in a moderate environment8). It has been reported that eating behavior is induced by intraventricular injection of the gamma-aminobutyric acid (GABA) agonist, muscimol, in nondeprived rats in a dose-dependent manner10). Food intake increased by muscimol in rats kept in the hot environment was less than that in rats kept in the temperate environment8). Hence it is suspected that there may be central mechanisms regulating food intake in response to the change of ambient temperature. Serotonin has been considered to be one of neurotransmitters correlated with the satiation process in the brain2). The serotonin antagonist, cyproheptadine, has been reported to stimulate appetite13). In addition, an intraventricular injection of $p$-chlorophenylalanine which depletes the brain of serotonin is

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Fig. 1. Effect of cyproheptadine (CYP, 5 and 15 mg/kg i. p.) on food intake increased by muscimol (MUS, 250 ng i. c. v.) in freely-fed rats at 26 and 33°C. The results are the least square means ± S. E. for 9 to 15 rats. 

Followed by hyperphagia⁴,⁷). On the other hand, it has been shown that hot environment increases brain serotonin metabolism and stimulates the firing of midbrain serotonin-containing neurons in the rats¹³). The objective of this study is to investigate the effects of modulation of serotonergic systems on muscimol-induced eating, thereby elucidating central mechanisms controlling appetite in response to the change of ambient temperature.

Materials and Methods

Male rats of the Wistar strain weighing 300 to 400 g (n = 104) were used. They were kept under standard lighting conditions of 12 h/day of artificial light, from 6:00 to 18:00 h. The animals were stereotaxically implanted with a stainless steel guide cannula directed into the left lateral ventricle. After a recovery period of 5 days, the animals were divided into two groups and kept at temperate (26°C) and hot (33°C) ambient temperatures for 7 days.

Two separate experiments were done. In the 1st experiment, after the acclimation period, 5 and 15 mg of cyproheptadine (Sigma) dissolved in 2 ml of saline and saline alone were administered i. p. at a level of 2 ml/kg. Soon after the drug administration, 250 ng of muscimol (Sigma) dissolved in 10 μl of saline and saline alone were injected i. c. v. The dose of cyproheptadine was determined according to GHOSH and PARVATHY⁵), and that of muscimol was determined according to MORLEY et al.¹⁰). The 2nd experiment, after the acclimation period, 3 mg of p-chlorophenylalanine (PCPA, Sigma) dissolved in 20 μl of saline was injected i. c. v. Food intake per day was weighed every day from 4 days before to 5 days after PCPA injection. Four days after PCPA administration, 250 mg of muscimol dissolved in 10 μl of saline was injected. The dosage of PCPA was determined according to BREISCH et al.⁴). Saline was given i. c. v. for the control
Fig. 2. Food intake per day before and after the administration of \(p\)-chlorophenylalanine (PCPA, 3 mg \text{i.c.v.}) in rats at 26 and 33\(^\circ\)C. Muscimol (MUS, 250 ng \text{i.c.v.}) was administered 4 days after PCPA administration. The results are means\(\pm\)S.D. for 3 to 9 rats.

**: p<0.01. SAL: Saline.

Fig. 3. Effect of pretreatment with \(p\)-chlorophenylalanine (PCPA, 3 mg \text{i.c.v.}) on food intake increased by muscimol (MUS, 250 ng \text{i.c.v.}) in freely-fed rats at 26 and 33\(^\circ\)C. Food intake was not increased in rats treated with PCPA at 33\(^\circ\)C. The results are the least square means\(\pm\)S.E. for 3 to 6 rats.

N.S.: non-significant. SAL: Saline.

Fig. 4. Effect of pretreatment with \(p\)-chlorophenylalanine (PCPA, 3 mg \text{i.c.v.}) on food intake increased by muscimol (MUS, 250 ng \text{i.c.v.}) in freely-fed rats at 26 and 33\(^\circ\)C. Food intake was not increased in rats treated with PCPA at 33\(^\circ\)C. The results are the least square means\(\pm\)S.E. for 3 to 6 rats.

N.S.: non-significant. SAL: Saline.
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tures (Fig. 2). The food intake per day at 26°C was greater than that at 33°C before and after the drug administration. Food intake was increased after muscimol injection in rats pretreated with saline and PCPA at 26°C (Fig. 3). On the contrary, food intake was suppressed for at least 2 hours after muscimol injection in rats pretreated with PCPA at 33°C. However, food intake per day in rats pretreated with PCPA was greater than that in the control rats on the 5th day when food intake for the short period after muscimol injection was suppressed.

Discussion

Serotonin has been reported to inhibit eating, when injected into the brain\(^2\). It was reported that food intake was increased by serotonin antagonist, cyproheptadine, in rats that were made to fast\(^1\). On the contrary, muscimol-induced eating was inhibited by cyproheptadine in a dose-dependent manner. The drug has been reported to have the property of blocking dopamine receptors\(^12\). Dopamine has a dual role in the control of eating. It has been reported that dopamine in the perifornical hypothalamus is associated with inhibition of eating behavior\(^9\). On the other hand, MORLEY et al.\(^10\) reported that muscimol-induced eating was suppressed by the dopaminergic antagonist, haloperidol. The contradictory results observed following cyproheptadine administration might be due to the suppression of dopamine neurons rather than to that of serotonin neurons. In addition, the suppressed effects of cyproheptadine on muscimol-induced eating was stronger in the temperate environment than in the hot environment, suggesting that dopaminergic systems might be suppressed in the hot environment.

It has been shown that serotonin concentration in the forebrain gradually decreased after PCPA administration, reaching the minimum level 2 days after the administration\(^7\) and maintaining the level for several days. HOLMES et al.\(^7\) have suggested that the hyperphagia elicited by PCPA may be due to an activation of noradrenaline neurons, although the mechanism lying between serotonin depletion and hyperphagia is still a matter of controversy. The gradual increase in food intake, keeping less in the hot environment than in the temperate environment, was observed after PCPA administration in both environments, suggesting that noradrenaline turnover may not be affected by the change of environmental temperature, and that there may be other eating-stimulatory systems which are suppressed in the hot environment. MORLEY et al.\(^10\) reported that muscimol-induced eating was not blocked by the α-adrenoceptor antagonist, phentolamine. Therefore the suppression of eating-stimulatory systems other than α-adrenergic systems might cause the suppression of muscimol-induced eating in rats pretreated with PCPA in the hot environment.

It was reported that serotonin neurons in the forebrain were activated in a hot environment\(^3\). STEIGRAD et al.\(^11\) have suggested that the serotonin binding capacity in the rat forebrain may be increased after PCPA administration. Under the condition such as a hot environment, therefore, the prolonged depletion of serotonin after PCPA administration may cause an up-regulation of serotonin receptors in the forebrain. BORSINI et al.\(^3\) have shown that eating elicited by muscimol depends on a dopaminergic mechanism, and that dopamine-dependent eating is suppressed by drugs which stimulate the brain serotonin function. The failure of muscimol in stimulating eating in rats pretreated with PCPA might be correlated to the up-regulation of serotonin receptors in some areas of the forebrain, in which the serotonergic systems are exclusively activated in response to the hot environment. Further research, e.g. examining the possibility that the stimulatory effect of muscimol on dopamine neurons may be suppressed by activated serotonin neurons in a hot environment, will be...
References


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高温ならびに快適環境で飼育したラットのムシモール
によって刺激された採食に及ぼすシプロヘプタジン
およびp-クロロフェニルアラニンの影響

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高温環境で飼育された動物の採食量は、一般に快適環境のものよりも少ない。セロトニンは中枢にお
いて食欲抑制に働く神経伝達物質と考えられている。本研究では、高温環境（33℃）および快適環境
（26℃）で飼育したラットを用い、セロトニン神経系を抑制的に修飾する2種類の薬剤が、γアミノ酪
酸アゴニストのムシモール（muscimol）を中枢投与した後の採食量に与える影響について調べ、高温
環境における食欲抑制の機構を明らかにしようとした。ムシモールを側脳室内に投与すると由温環境
下で採食行動が誘発された。ムシモール投与後2時間の採食量は、33℃下の方が26℃下よりも少なかった。
ムシモール投与後の採食量は同時に投与されたシプロヘプタジンによって用量依存的に減少した
が、26℃下の方が大きく減少した。セロトニン合成阻害剤のpクロロフェニルアラニン（PCPA）を
側脳室内に投与すると、両環境温度下で1日当りの採食量が増加した。また、1日当りの採食量は、
PCPA投与後も33℃下の方が26℃下よりも少なかった。PCPAまたは生理食塩水を投与して4日後に
ムシモールを側脳室内投与し、2時間当りの採食量を測定した。26℃下では、PCPA投与区（n=6）
では5.2gとなり、生理食塩水投与区（n=5）の5.8gとはほとんど差はなかった。一方、33℃下では、
生理食塩水投与区（n=4）は3.8gであるが、PCPA投与区（n=3）は少なくともムシモール投与後
2時間は採食行動が抑制された。これらの結果から、高温環境下では、ドーパミン神経系の活性化が抑
制され、採食が抑制される機構が示唆された。

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