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Effects of Cyproheptadine on the Feeding and Satiety Centers in the Rat

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OOMURA, Y., T. ONO, M. SUGIMORI AND T. NAKAMURA. *Effects of cyproheptadine on the feeding and satiety centers in the rat.* PHARMAC. BIOCHEM. BEHAV. 1(4) 449–459, 1973.–In order to clarify the effects of cyproheptadine hydrochloride (Cyp) which is known clinically to stimulate the appetite and a subsequent increase in body weight, acute and chronic experiments were carried out. In the acute experiments, changes in single neuronal activity in the lateral area (LH) and ventromedial nucleus (VMH) in the rat hypothalamus by applications of Cyp, Na and Cl were studied by means of multibarreled electrodes. (1) About 60% of the VMH neurons were reduced in firing frequency by Cyp. Most of them were increased in frequency by glucose. (2) About 70% of the LH neurons were increased in frequency by Cyp. However, most of them were inhibited by glucose. (3) The activity of the Cyp sensitive neurons was modulated by stimulations of either the basolateral amygdaloid nucleus or the stria terminalis. (4) In the chronic experiments, food intake and body weight in young rats were significantly increased by Cyp. Thus, it was concluded that Cyp modulates both LH and VMH neurons which might account for its effects on feeding in children.

Single unit discharges Lateral hypothalamic area Effect of cyproheptadine Ventromedial hypothalamic nucleus Glucosensitive neuron

IT HAS been reported clinically that cyproheptadine hydrochloride (periactin, Cyp), which is known to have antiserotonic and antihistaminic actions, increases appetite and food intake in asthmatic and anorexic children. They become more obese and taller than other normal children not given this drug [5, 10, 11, 13, 17, 27, 28]. Although the exact mechanism of this phenomenon is not known, some possible involvement of hypothalamic areas have been suggested [27]. Investigation of the action of Cyp in cats which were implanted with chronic electrodes [2] showed that Cyp increased the amplitude and frequency of the EEG in the lateral hypothalamic area (LH), i.e., feeding center. Food intake and body weight also increased after daily intravenous administration of Cyp. It was suggested that the EEG change corresponded to a decrease of the arteriovenous difference in glucose concentration (Δ-glucose), i.e., glucose utilization.

Previously we demonstrated the presence of glucosensitive neurons in the LH and ventromedial hypothalamic nucleus (VMH) of the rat [21], providing strong evidence for the glucostatic regulation of food intake [14,15]. In the present experiments, we examined Cyp by applying it directly to single hypothalamic neurons in order to determine its effect on LH and VMH neurons, especially glucosensitive neurons, hoping to gain an insight into the drug's mode of action upon the central nervous system. We also studied the influence of the amygdaloid nucleus on Cyp-sensitive neurons, since the lateral part of the basal nucleus of amygdala (AL) and stria terminalis (ST) are known to be involved in feeding behavior [18, 20, 24].

The effects of Cyp upon body weight and various behavioral patterns were also studied.

METHOD

Acute Experiment

Nine Wistar BR 46 rats of both sexes (180–200 g body weight) were used. Animals were placed under light ether anesthesia, the EEG showing slow waves with moderate amplitude. After each animal was fixed in the stereotaxic apparatus, a hole (8 x 5 mm) was drilled in the skull using a dental drill. Dura mater was cautiously removed, and the exposed cortex was covered with 2% agar 2–3 mm thick to prevent drying and suppress pulsations of the brain. Body temperature was kept at 37 ± 1°C.

Microelectrodes. Five-barreled glass microelectrodes (Pyrex tubing, 0.4 mm ID) were made with a vertical microelectrode puller (Narishige) after bundling 5 capillaries together with heat [25]. Distilled water was then placed into the 5 micropipettes according to Tasaki's [29] method, and later replaced with various drugs by means of a hypodermic syringe and a 31 gauge needle. The center electrode was filled with 4 M/l NaCl, and the 4 electrodes surrounding it

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were filled with 14.6 mM/l Cyp (pH 5.5–6.0), 0.2 M/l NaCl, 2 M/l monosodium-l-glutamate (adjusted to pH 8.0 with NaOH) and 0.4 M/l glucose in 0.4 mM/l NaCl solution. Electrodes were prepared 30–100 hr before an experiment and stored at 2–4°C until used. Tips of these 5 barreled pipettes were less than 1 μ in outside dia. The resistance of the center electrode was 20–100 MΩ and that of the surrouding electrodes was greater than 10 MΩ

Recording and stimulation. The coordinates of recording and stimulating sites were taken from the brain atlas of König and Klippel [12]. Unit activity was surveyed at two points: VMH (A. 4.6; L. 0.5; H. −3.2 to −3.8), and LH (A. 4.6; L. 1.5; H. −2.0 to −3.4). Unit discharges of thalamic neurons (thalamus ventralis 2 mm dorsal to LH) were used for control comparison. The center electrode was connected to a preamplifier which is capable of applying current as well as recording[7]. Extracellular unit activity was monitored with an oscilloscope and recorded on magnetic tape simultaneously. The time constant of the amplifier was adjusted to 3 msec. The data were analysed with a pulse counter or from photographic records. Na, Cl, and glutamate ions were released electrophoretically from micropipettes. Cyp and glucose were applied electro-osmotically, the former with negative, the latter with positive current. Current was supplied by a constant current source amplifier [26], which maintains a constant current in spite of occasional changes in electrode resistance during drug injections. We employed previously published [3,25] criteria for detecting the effects of drugs or of current itself. Concentric bipolar stimulating electrodes were placed into AL (A. 4.1; L. 3.8; H. −3.3) and ST (A; 4.4; L. 3.8; H. 0.7), an inhibitory pathway from AL [20]. The outer pole was made of 25 gauge (0.4 mm O.D.) stainless steel tubing insulated with enamel or teflon, while the inner electrode was enamel-coated stainless steel wire. The tip separation was 0.2–0.5 mm and the d.c. resistance was 50–70 kΩ. Rectangular stimulating pulses with 0.1 msec duration were used, the inner electrode negative with respect to the outer.

Quantities of Cyp applied electro-osmotically. Preliminary to experiments, we used C^{14}-Cyp (C^{14}-Cyp, Merk Sharp and Dohme Research Lab., Division of Merk and Co., Inc., Rahway, N.J., U.S.A.) to determine the relationship between the quantity of Cyp released from the electrode and applied current. C^{14}-Cyp (15 mg per 198 μc) was dissolved in dilute HCl solution so that the HCl content of the C^{14}-Cyp solution was the same as that of the Cyp solution actually used in experiments. The C^{14}-Cyp electrode was placed in a planchette filled with 2 ml NaCl (165 mM) and current was applied to eject C^{14}-Cyp from the electrode. After drying the solution in the planchette, the radioactivity of C^{14} was measured with a windowless gas flow counter. Quantities of C^{14}-molecules were calculated from a standard curve previously determined using the original solution.

Anatomical identification of recording sites. After each experiment, a concentric bipolar stimulating electrode was

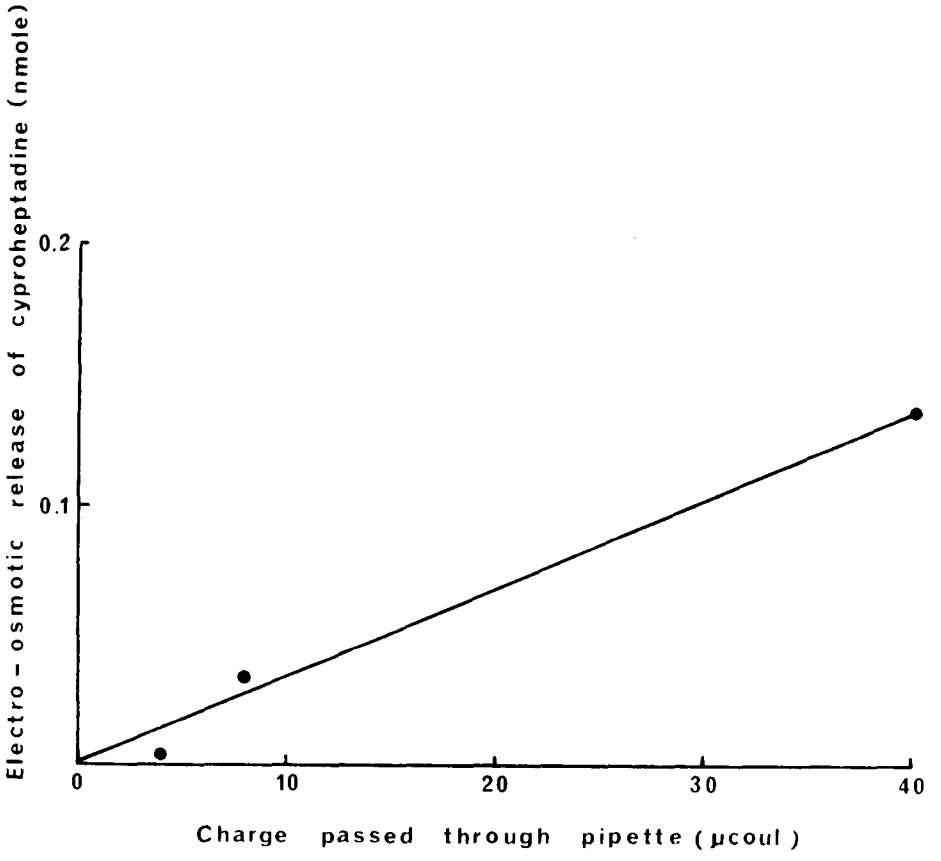

FIG. 1. Electro-osmotic release of Cyp. Lineal relationship between the amount (n mole) or C^{14}-Cyp released from the Cyp pipette at different negative charges (μ coul) applied to the pipette.

placed at the exact coordinates of the microelectrode recording site. An anodal current (5 mA) was applied for 15 sec between the inner and outer pole of the concentric electrode. Then the rat was sacrificed and decapitated and its brain was fixed with neutral 10% Formalin Ringer solution. A Nissl staining procedure was used on the 20–30 μ thick brain sections [25].

Measurement of Food Intake and Weight Gain

Ten young male Wistar rats (35 days old) were used for studying the effects of Cyp on food intake, weight gain and behavior. The humidity and temperature of the animal colony room were maintained at 50 ± 10% and 20 ± 3°C respectively. Rats were housed separately in 20 x 15 x 15 cm cages made of wire mesh. Weight and food intake were measurd twice per day (morning and evening). Cyp (0.5–1.5 mg/kg) syrup was administered orally in the tap water ad lib. Other control rats were administered syrup only or nothing.

RESULTS

Cyp release by electro-osmosis. Quantities of C^{14}-Cyp ejected from electrodes with constant current application correlated well with current strength. However, inward current consistently caused greater amounts of Cyp to be extruded than outward current. Inward current was actually used in experiments with Cyp ejection. Figure 1 shows the results of the calibration procedure. The slope of this curve is 3.5×10^{-12} M/μcoul and from this, the transport number of Cyp was 0.33 M/coul. Glucose, however, is ejcted more effectively with outward current, though both currents are effective for glucose application [21]. The transport number of glucose was 0.53 M/coul. A difference in the transport number of Cyp and glucose can be explained by the difference in molecular size; the molecular weight of the former is twice as heavy as the latter. There were few electrodes from which Cyp could not be released. Responses to the drugs were evaluated by the magnitude of the changes in frequency of unit discharges.

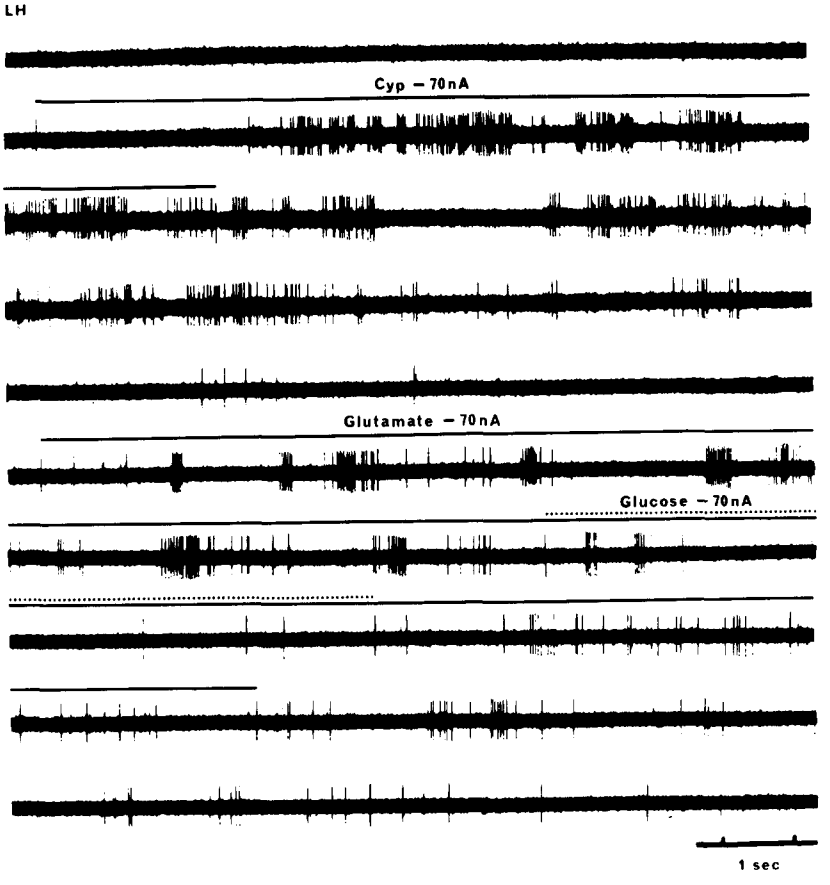

FIG. 2. LH neuron. Effect of Cyp and glucose. Continuous recording from upper to lower. Upper records: Before Cyp application, no spontaneous discharges. Cyp was applied at −70 nA (during the length of upper bar), the unit discharges increased in frequency with a latency of 3 sec. The after effect continued for about 19 sec. Middle records: Spontaneous discharges were so few that glutamate was applied at −70 nA. During the increase in frequency by glutamate glucose was applied at −70 nA (dotted line). Glucose reduced discharge frequency with a latency of about 1.9 sec. Lower records: By the glucose current off the discharge returned to the rate caused by glutamate with an after effect of glucose for about 2 sec. By glutamate current off the spontaneous discharges returned to the original rate.

LH neurons. The effects of Cyp on 15 neurons situtated inside the LH were investigated. Unit discharges increased in 10 neurons, and were unaltered in 5 neurons after Cyp application. Reduction in unit discharge frequency by Cyp has not been observed in LH neurons examined so far (Table 1). It has been found that there are both glucosensitive and osmosensitive neurons in LH. The former are thought to be related to feeding, while the latter, to drinking behavior. Although firing of the glucosensitive neurons was reduced with glucose applications, that of osmosensitive neurons was increased in response to a local increase in osmotic pressure [21]. The effects of Cyp and Na or Cl upon these receptor neurons was studied. As shown in Fig. 2, an LH neuron which fired spontaneously at a low frequency responded to Cyp electro-osmotically applied with −70 nA. It increased firing after a 3 sec latent period and this firing continued for 19 sec after the cessation of the current. Such a long response latency may be due to the glial lamellar structure surrounding hypothalamic neurons [19] as well as the distance from the electrode tip to the neuron. After the firing rate had completely recovered from the Cyp effect, glutamate was ejected with −70 nA from the glutamate pipette. The firing frequency increased presumably because of the general facilitatory action of glutamate. Glucose applied electro-osmotically then reduced this glutamate facilitation with a 1.9 sec latency. The firing level rebounded to that previously produced by glutamate 2 sec after the cessation of glucose current. The firing rate was restored to the initial spontaneous level 20 sec after glutamate application was terminated (Fig. 3 upper). In order to demonstrate that these responses were not due to the effects of inward current or osmotic pressure but to Cyp or glucose, Cyp and chloride ions were applied to the same neuron (illustrated in Fig. 2) each with −70 nA current for comparison. As shown in Fig. 3, Cyp remarkably increased unit discharges of this neuron and this effect continued after cessation of the current, while chloride ions ejected from the NaCl pipette did not alter the spontaneous activity. Thus, these drug effects were not due to a current effect together with the long latency for the appearance of these effects and the long lasting effects after the off current. Moreover, this neuron did not respond to the change of osmotic pressure brought about by chloride application. The discharge rate curve presented in Fig. 4 is another example of these drug effects. As shown to the left of this figure, glutamate applied with −100 nA increased the firing rate. Then glucose was ejected with +70 nA during glutamate administration. The glutamate-induced firing rate was reduced by glucose application. The mean spike frequency was 6.5 ± 6.4 Hz for 18 sec of glutamate application and 3.5 ± 3.2 Hz during the 17 sec of glucose application. The effect of glucose lasted a fairly long time after the interruption of current. After the spike frequency returned to its initial level, Cyp was ejected with −100 nA. The spike frequency increased to 19.2 ± 6.8 Hz for 20 sec with about 1 sec latent period and was followed by about 1 sec aftereffect.

Effects of AL and ST stimulation. It is known that LH neurons receive an inhibitory input through the ST from AL [20]. We investigated whether or not LH neurons whose activity was facilitated by Cyp were inhibited by AL or ST stimulation. As indicated in Fig. 4, after the neuronal activity returned to the initial control firing rate following the first glutamate injection, Cyp application led to a sustained increase in firing rate. Spike frequency was subsequently increased to 33.0 ± 6.7 Hz during the second −100 nA glutamate application. AL was then stimulated repetitively at a frequency 0.8 Hz, and 8 V strength. The spike frequency was reduced to 12.2 ± 4.4 Hz during the 26 sec of AL stimulation. The frequency again increased to 27.8 ± 8.5 Hz for the remaining 10 sec of glutamate application after the AL stimulation was discontinued. Such repetitive stimulation, however, might actually mask the inhibitory effect of AL on LH neurons. An EPSP-IPSP com-

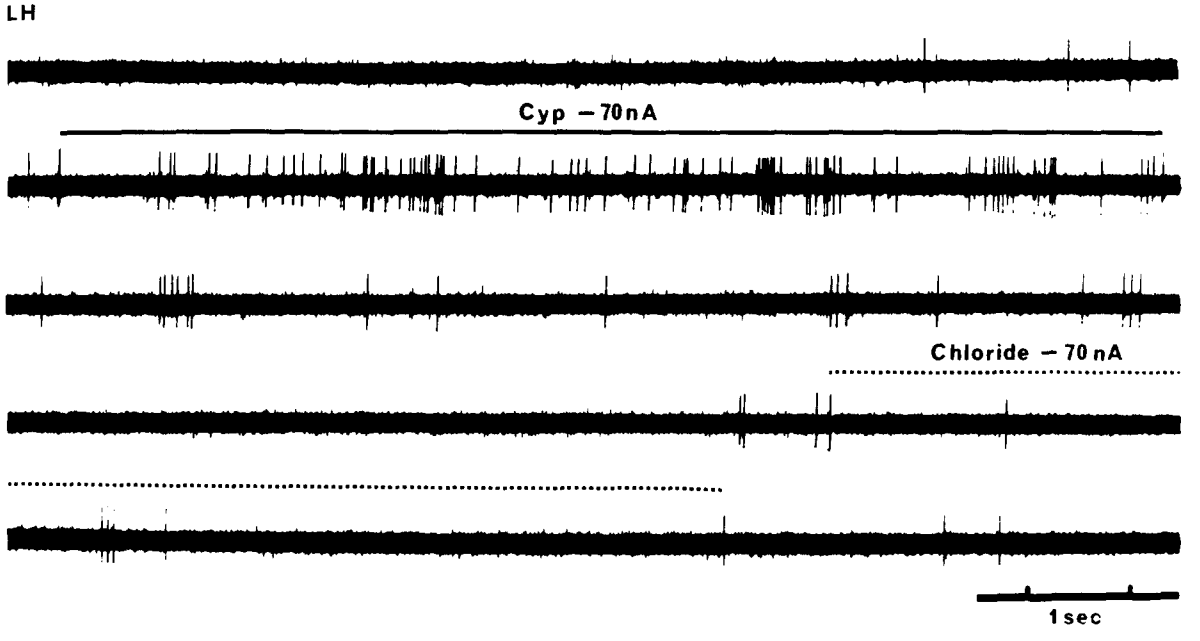

FIG. 3. LH neuron, continuous record from Fig. 2. Cl was applied at −70 nA (dotted line) after the application of Cyp at the same current intensity (continuous line).

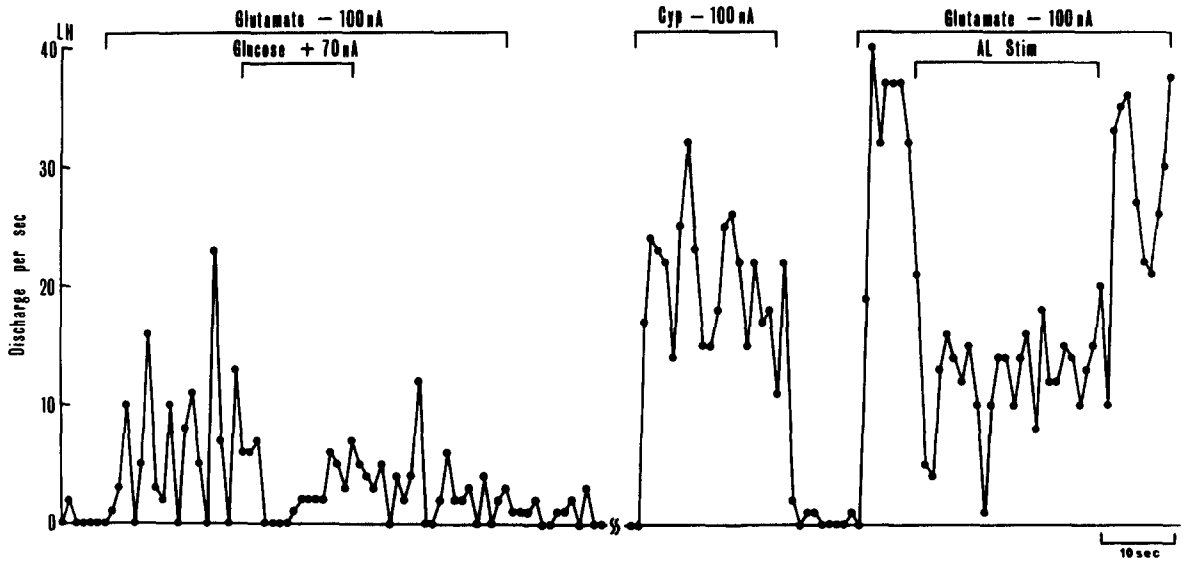

FIG. 4. Effects of Cyp and a repetitive stimulation of AL on a glucose-sensitive LH neuron. Left: Unit discharges increased by glutamate (−100 nA continuous line). This increase was inhibited by glucose (+70 nA). The after effect of glucose continued for a considerable period. Middle: After complete recovery in discharge rate to the original level, Cyp was applied (−100 nA). With 1 sec latency discharge rate increased. After effect lasted for about 2 sec after Cyp off. Right: During facilitation of discharge due to glutamate (−100 nA), AL stimulation reduced the discharge rate.

TABLE 1

NUMBER AND TYPE OF RESPONSES OF VMH, LH AND THALAMUS NEURONS TO CYP GLUCOSE, Na, Cl AND GLUTAMATE AND TO REPETITIVE STIMULATIONS OF BASOLATERAL AMYGDALOID AND STRIA TERMINALIS

Cyproheptadine			Glucose			Sodium			Chloride			Glt			AL			ST		
			↑	↓	no	↑	↓	no	↑	↓	no	↑	↓	no	↑	↓	no	↑	↓	no
LH	↑	10		3	3			3			3	3		1		2			2	
(15)	↓	0																		
	no	5	2																	
VMH	↑	1	1						1			1								
(10)	↓	6	4		2			4			3	3			5			2	1	
	no	3													2			2		
Thalamus	↑	0																		
(12)	↓	0																		
	no	12			6			6			3	3		2						

↑, discharge rate increased ↓, discharge rate decreased no, no effect

plex of 10 msec latency is evoked in LH by AL stimulation and only the EPSP remains during repetitive AL stimulation [20]. Among 4 Cyp-sensitive neurons, two were tested with repetitive AL and the other two, with ST stimulation (0.8 Hz, 5 V). All of these neurons were inhibited by the electrical stimulation (Table 1).

VMH neurons. Ten VMH neurons were sampled. Of these, 6 were inhibited and 1 neuron was facilitated by Cyp application. The other 3 neurons showed no response to Cyp (Table 1). The neuron facilitated by Cyp was also facilitated by Cl application, and, therefore, this neuron might be responsive to the nonspecific actions of other drugs besides Cyp. As shown in Fig. 5, Cyp was applied with +50 nA for 11 sec to a VMH neuron which fired initially at a frequency of 20 Hz. No remarkable change was observed, but Cyp at −50 nA completely inhibited the spontaneous

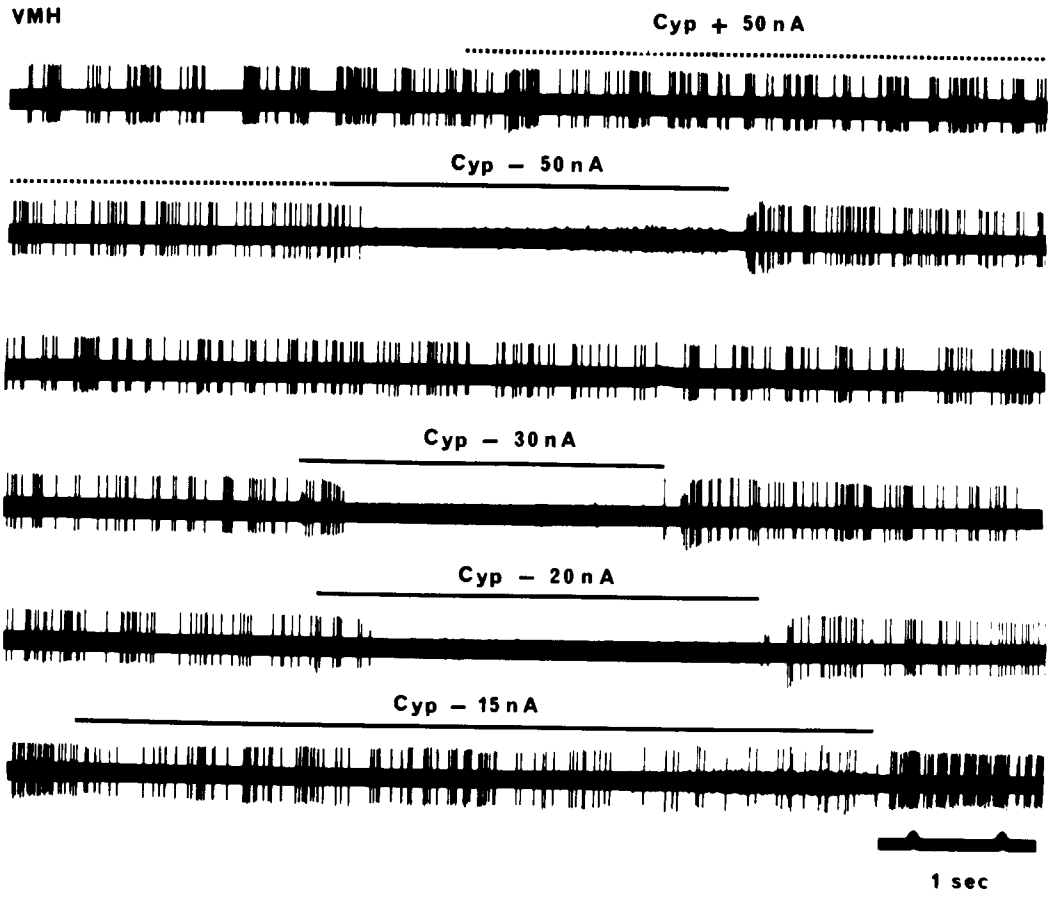

FIG 5. VMH neuron. Continuous record from upper to lower. Upper records: Spontaneous discharges did not change in frequency by Cyp application at +50 nA (dotted line) but disappeared by −50 nA (continuous line). Middle and lower records: Effect of Cyp at −30, −20, −15 nA. −15 nA affected slightly on the spontaneous discharges with a prolonged latency of about 5 sec.

activity after a latency of 300 msec. The firing frequency regained its spontaneous level following a short aftereffect of 200 msec. Cyp applied with −30 nA also inhibited the spontaneous firing, but the latency to the appearance of the drug effect was elongated. In general, the less current was applied, the longer was the latency and the weaker was the response observed. For example, when Cyp was applied with −15 nA, the latent period was elongated to 5 sec, and the spontaneous firing was not inhibited completely. The period of after effect was also shortened as the applied current was reduced. These reductions in spontaneous firing were not due to current or osmotic effects, but to a specific action of Cyp. As shown in Fig. 6, the activity of another neuron which fired spontaneously at 2 Hz was inhibited by Cyp application with −15 nA. In this case, the precise latency is unknown, but firing was completely inhibited after 5 sec. Cyp with +15 nA was not effective. Subsequent Na and Cl application by positive or negative current did not alter the firing rate. Figure 7 illustrates the inhibitory effects of different doses of Cyp. Current doses were decreased in the following order; +50, −50, +30, −30, +20, −20, +15, −15 nA. Only inward currents were effective. From this, the threshold dose and time from the current application to the onset of the effect, namely the latent period, were assumed. As the applied current was decreased from −50 nA to −20 nA, the latent period was gradually elongated, and the aftereffect was shortened. Activity was not completely inhibited with −15 nA and the latency was elongated to 5 sec. The mean spike frequency was 20.2 ± 3.5 Hz during the application of Cyp with +50 nA. When the polarity was changed to negative, the impulses were completely inhibited. The spike frequency recovered to 20.6 ± 3.7 Hz, virtually the same as the initial level in 21 sec after the cessation of current. The spike frequency of 16.4 ± 2.4 Hz before and after the +30 nA current was reduced to 0 Hz with −30 nA, and after the current was ceased it recovered the spontaneous firing rate (15.4 ± 5.1 Hz). Comparable results were observed with a Cyp application at −20 nA. However, when Cyp was applied with −15 nA, activity decreased from 12.6 ± 2.4 Hz to 8.9 ± 5.9 Hz. The control spike frequencies before and after Cyp applications were gradually reduced, which is perhaps attributable to the effect of Cyp accumulation. The threshold current for a Cyp effect on VMH neurons seemed to be −15 nA.

The amplitude of recorded spikes was always more than 1 mV, so the distance between a neuron and electrode tip was assumed to be about 10 μm at most. The diffusion constant of Cyp at 38°C was calculated to be 0.64 x 10^{-5} cm^2/sec, based upon the molecular weight according

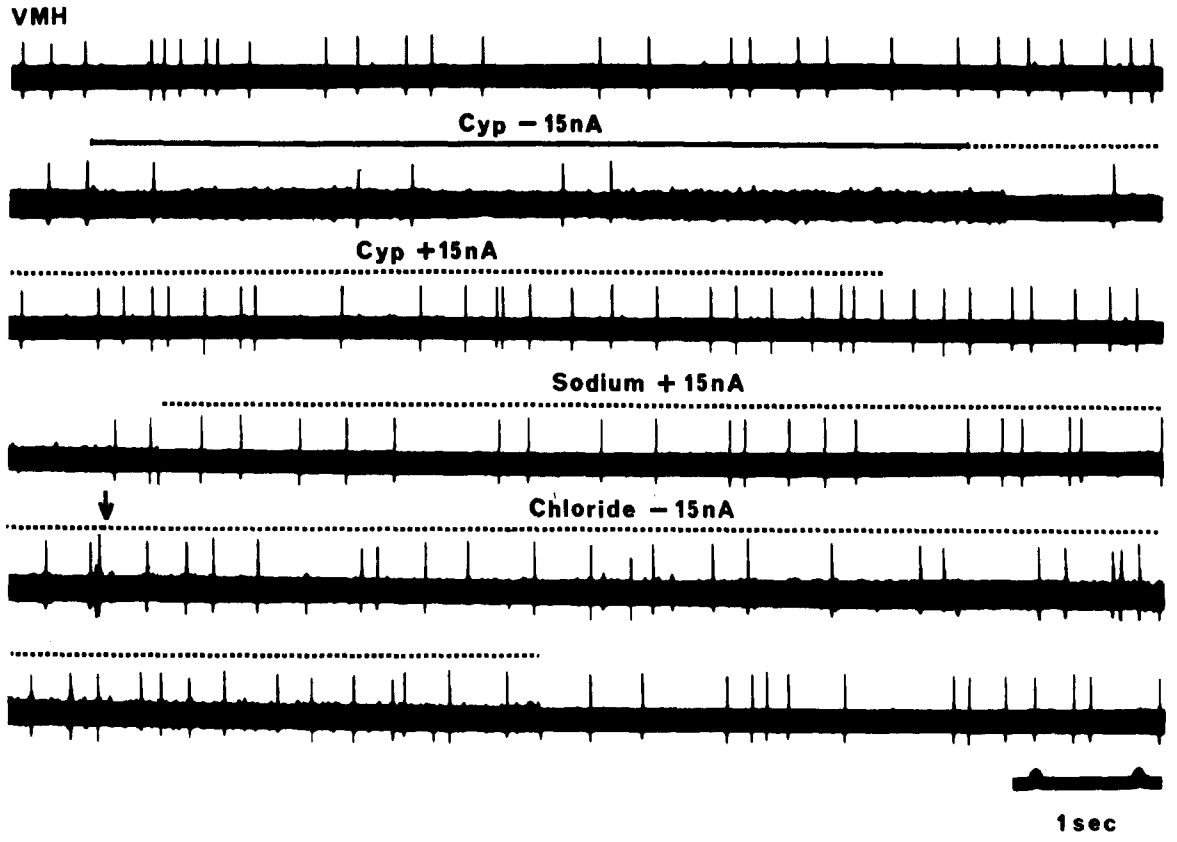

FIG. 6. VMH neuron. Cyp reduced spontaneous discharges at −15 nA. Na and Cl have no effect.

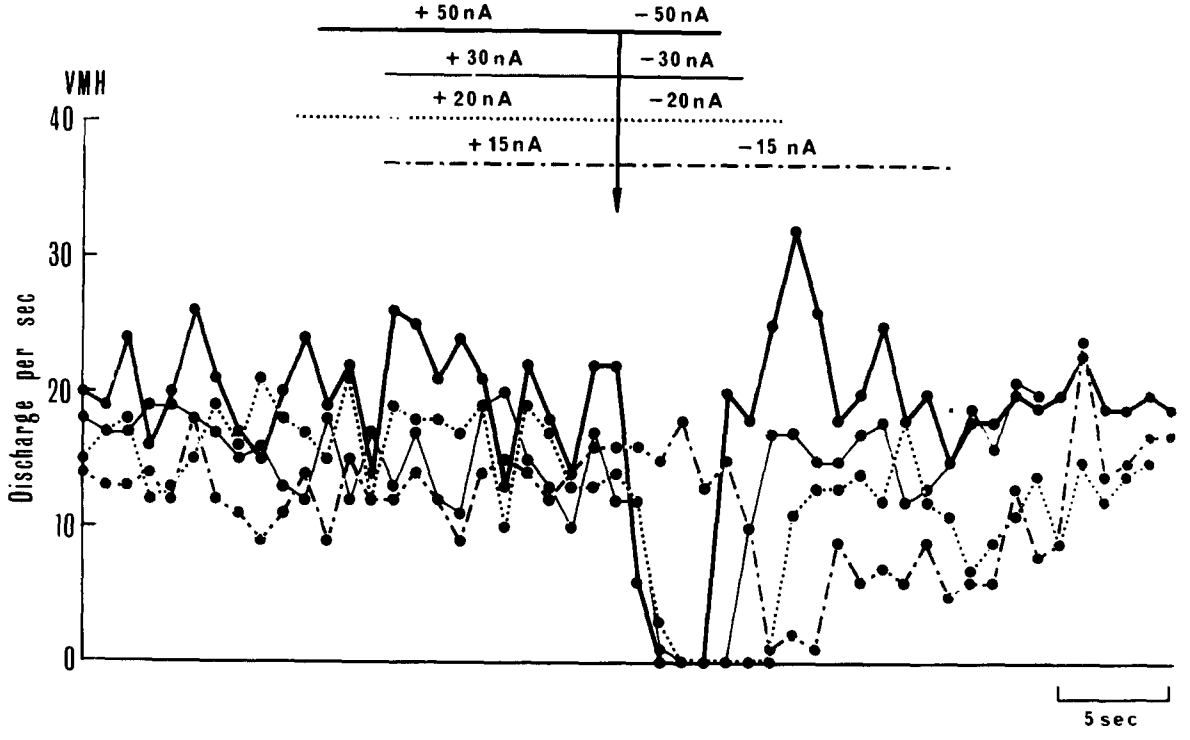

FIG. 7. VMH neuron. Cyp was effective on negative currents. +50 and −50 nA, thick continuous line; +30 and −30 nA, thin continuous line; +20 and −20 nA, dotted line; +15 and −15 nA, broken line. At the arrow applied current was switched from positive to negative. The length of the lines indicate the duration of the applied currents.

TABLE 2

THRESHOLD CONCENTRATION OF CYPROHEPTADINE ON THE VMH NEURON

$$C = \frac{8.25 \times 10^{-6} ni}{ZDr} \operatorname{erfc} \frac{r \times 10^{-4}}{2\sqrt{Dt}}$$

C:	Concentration	M/L
i:	Current intensity	-15×10^{-9} A
t:	Duration	5 sec
r:	Distance between pipette tip and cell membrane	10μ
n:	Transport number	0.33 M/coul X 96500
d:	Diffusion coefficient	0.64×10^{-5} cm^2/sec
Z:	Valency	1
C:	6.4×10^{-6} M/L	

to Curtis' hypothesis [4]. The transport number was 0.33 M/coul as illustrated in Fig. 1. Threshold concentration near the surface of the neuronal membrane was estimated to be 6.4×10^{-3} M/l, as indicated in Table 2.

Responses of a single VMH neuron to Cyp, glucose, Cl and AL stimulation are shown in Fig. 8. The control spontaneous firing (5.6 ± 0.8 Hz for 11 sec) was significantly reduced by 17 sec of Cyp application at −70 nA to 2.8 ± 1.6 Hz. A slight aftereffect seems to have persisted for over 7 sec following the Cyp injection. Repetitive AL stimulation (8 V, 0.8 Hz) resulted in a slight facilitation of the low firing rate which followed Cyp application. The spike frequency increased to 4.9 ± 1.9 Hz by this stimulation. This might be due to an increase of background excitability of VMH neurons. Then glucose was applied with +70 nA during AL stimulation, and the firing rate increased markedly after a 2 sec latency (Fig.8, middle, left). Mean spike frequency during the glucose application was 28.5 ± 22.3 Hz and it returned to 5.6 ± 1.8 Hz after a subsequent glucose aftereffect. After cessation of AL stimulation, the frequency further decreased to 3.2 ± 1.5 Hz in 9 sec. Thereafter glucose was again applied with +70 nA. Spike frequency increased to 15.2 ± 11.4 Hz for 5 sec from 5.1 ± 1.9 Hz before the application and returned to the initial level shortly after cessation of the glucose current. Some differences in spike frequency are obvious between glucose application only and AL stimulation paired with glucose application. This might be due to a general facilitatory effect of AL stimulation. Chloride alone has no effect upon this neuron.

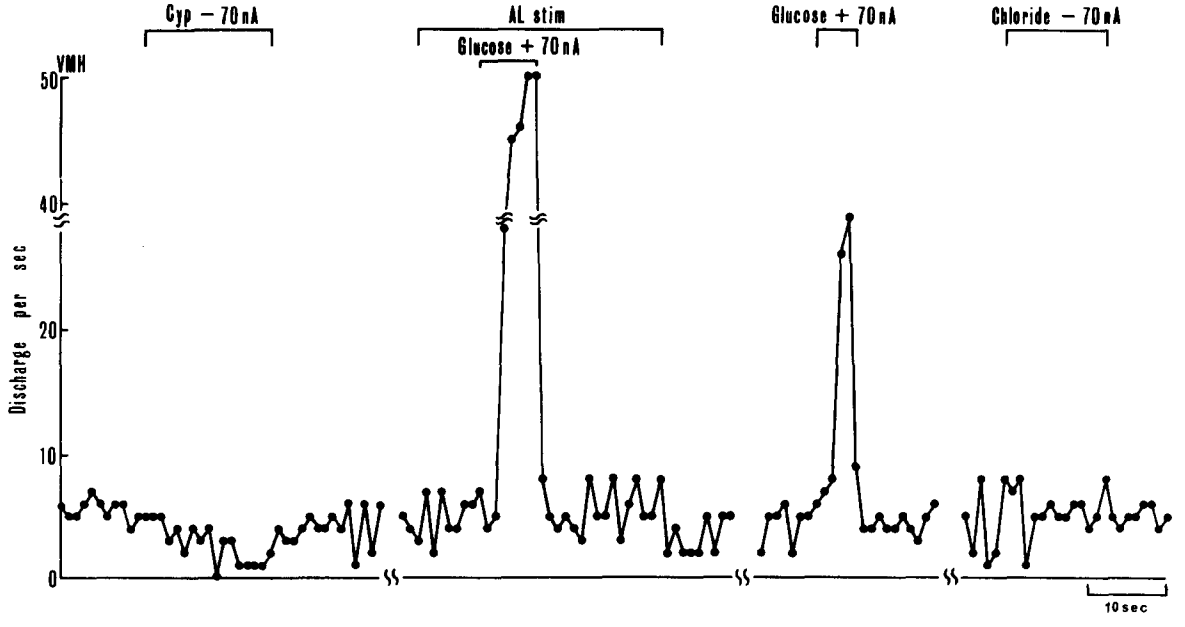

FIG. 8. Effect of Cyp and a repetitive stimulation of the Al on a glucosensitive VMH neuron. Most left: Cyp reduced the rate of spontaneous discharges with a latency of about 3 sec. Middle left: Repetitive stimulation of the AL brought a slight facilitation. During the stimulation a glucose application at +70 nA increased the discharge rate with a latency of about 2 sec. Middle right: Glucose alone at +70 nA. Right: No effect of a Cl application (−70 nA).

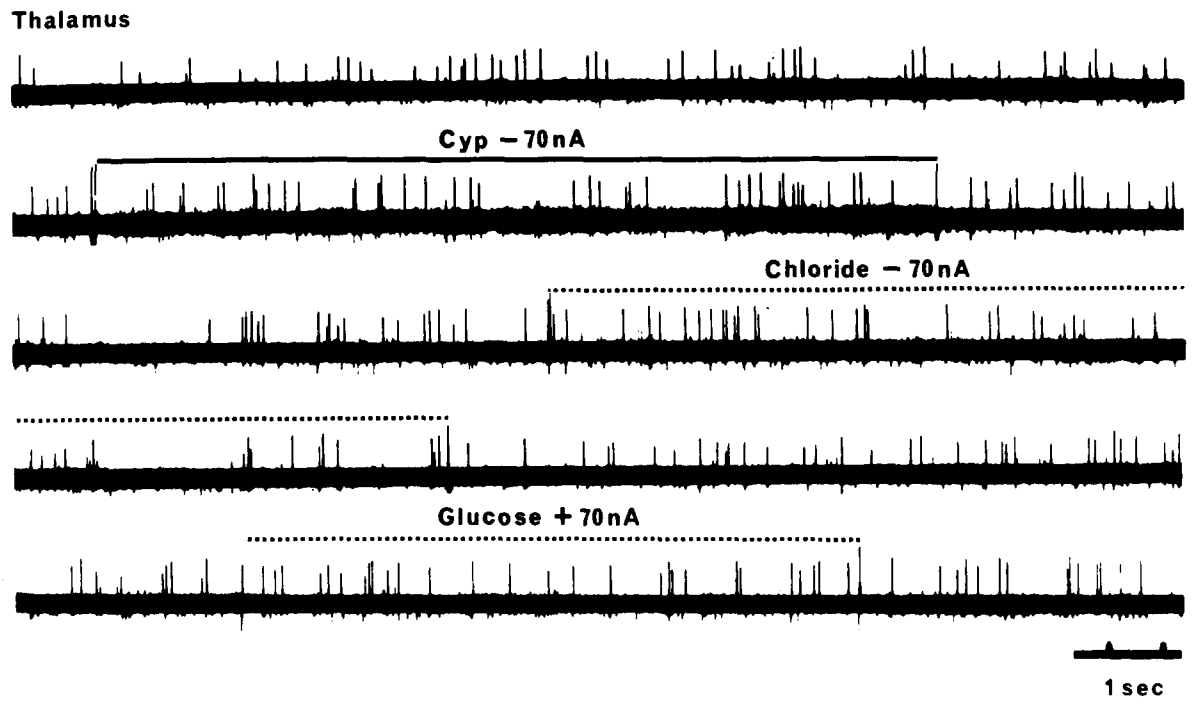

FIG. 9. No effect of Cyp, glucose and Cl on the thalamic neuron. Continuous recording from upper to lower. Cyp at −70 nA (continuous line), Cl at −70 nA (dotted line) and glucose at +70 nA (dotted line) are applied successively.

Effect of AL and ST stimulation. It is known that VMH neurons are facilitated by AL stimulation mainly via the monosynaptic pathway of the ventral amygdalohypothalamic tract [6, 24, 25, 30]. It has been suggested [16] that an EPSP followed by an IPSP would be recorded in VMH following AL stimulation. A polysynaptic facilitatory pathway through the ST has also been found (Oomura and Ono, unpublished observation). As shown in Fig. 8, neurons which responded to Cyp and glucose were also facilitated by AL stimulation. As also illustrated in Table 1, six VMH neurons inhibited by Cyp were studied with repetitive stimulation. Among them five neurons were facilitated. The effect of ST stimulation was also tested on three out of these neurons, two of which were facilitated. These results suggest that a facilitatory input from AL terminates to Cyp sensitive VMH neurons.

Thalamic neurons. Cyp was applied to 12 thalamic neurons as well as both LH and VMH neurons. None of them responded to Cyp applied even with a current intensity of more than −100 nA. The neuronal activity shown in Fig. 9 was recorded 2 mm dorsal to the center of LH. Spikes appeared spontaneously at about 2 Hz. Cyp was applied through the same electrode which had been effectively used with LH and VMH neurons, but without effect. Then, Cl was applied with −20 nA through the NaCl electrode, and glucose was tested with both positive and negative currents. None of these chemicals altered the discharge rate of these neurons.

Summary of the Cyp effect. The effects of several drugs on LH neurons are listed in Table 1. Unit discharges of 15 neurons within the LH were recorded extracellularly. Unit discharges of 10 neurons were facilitated with Cyp application and glucose was applied to 6 of these. Three out of these 6 neurons were inhibited, while 3 were not affected by glucose applications. Two out of 5 neurons which were not responsive to Cyp were faciliated by glucose, but since the effect of sodium on these neurons was not tested, it is difficult to state whether they were glucosensitive or osmosensitive. It is obvious that the effect of Cyp was not due to nonspecific osmotic pressure changes but to some specific action of the drug, because 3 neurons which were responsive to Cyp were not affected by Na and Cl adminstration. Moreover, activity of all Cyp-sensitive neurons was inhibited by both AL and ST stimulation. These facts suggest that Cyp-sensitive neurons might be principal neurons concerned with the feeding mechanism. In regard to the neurons within the VMH, unit discharges of six out of 10 neurons were inhibited with Cyp and glucose was applied to these all six. Four of them were glucosensitive neurons. Neither Na nor Cl had any effect but glutamate had the nonspecific facilitatory effect. Moreover, activity of almost all Cyp-sensitive neurons were facilitated by both AL and ST stimulation. These facts suggest that Cyp-sensitive neurons are the principal ones concerned with a mechanism of satiety. Only one neuron responded both to Cl and glucose which might be excluded from the specific Cyp-sensitive neuron. As shown in Table 1, Cyp insensitive thalamic neurons were not affected by glucose, Na or Cl. Three out of 5 thalamic neurons were facilitated by glutamate which has a nonspecific facilitatory effect. These control experiments suggest that Na, Cl, glucose and Cyp do not affect any but VMH and LH neurons.

Chronic experiments. Ten male young rats (100 ± 3 g body weight) were tested. Cyp (0.5−1.5 mg/day) in tap water or syrup was administered orally every day for 10 days. As illustrated in Fig. 10, food and water intake of the

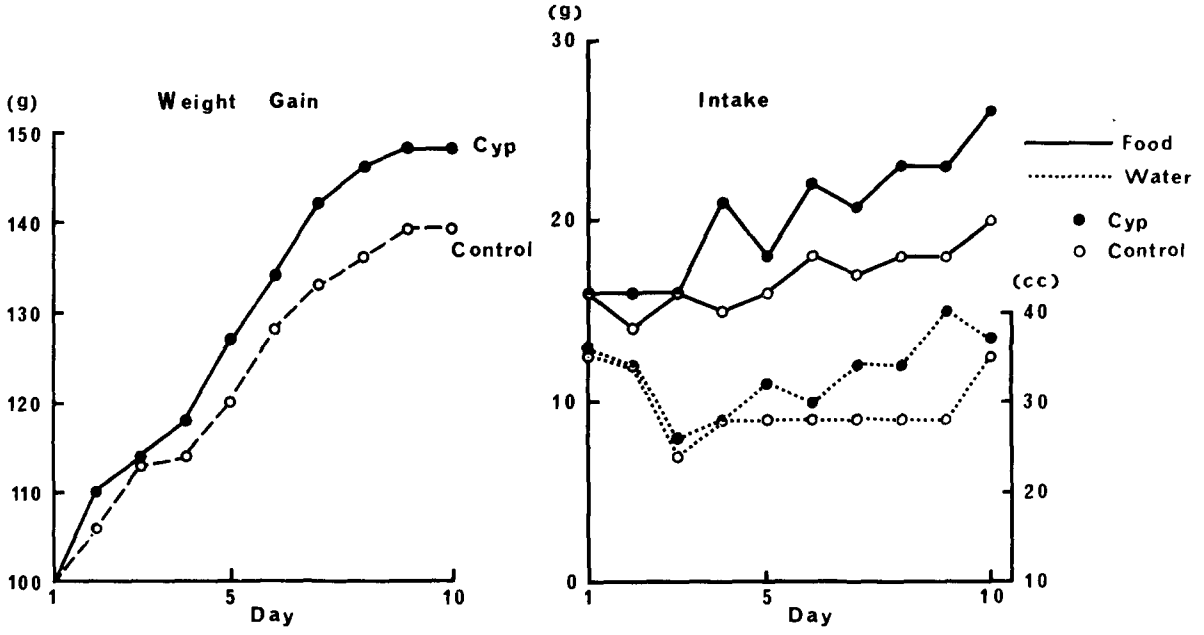

FIG. 10. Increases in food and water intake (right) and body weight (left) of a rat (100 g, age 35 days) by Cyp-syrup (mixed in water) (0.5 mg/kg body weight/day) (filled circles). Control rat (open circles).

Cyp group was increased significantly above that of the control group even only 1 day after the start of Cyp administration. Body weight was obviously increased in the Cyp group after the first week of Cyp administration.

DISCUSSION

From the acute experiments reported in this paper, it is obvious that Cyp directly activated LH neurons and inhibits VMH neurons in the hypothalamus which is known to be involved in the regulation of food intake. It is also established that there is a reciprocal relation between LH and VMH neurons [22,23], that is, if one's activity is facilitated, the activity of the other is inhibited. The glucostatic theory [14,15] was proposed in order to explain the regulation of food intake. According to this theory, glucosensitive VMH neurons are activated by the increase in the blood glucose arterio-venous difference, Δ-glucose, occurrring in the post-ingestional phase, and they in turn inhibit the activity of LH neurons. On the other hand, a decrease in Δ-glucose lowers the activity of VMH neurons, and thereby LH neurons are activated to evoke feeding behavior. There are many reports which support the glucostatic theory. The existence of the glucosensitive neuron has actually been demonstrated [21] by electro-osmotic application of glucose to single neurons in both LH and VMH [21]. These glucosensitive neurons respond to glucose in a stepwise manner, as the quantity of glucose molecules ejected from a micropipette near them is increased by corresponding increases in the electro-osmotic currents. VMH neurons are facilitated and LH neurons are inhibited by glucose application. From these facts, and taking into account the glucostatic theory, there might be a neuronal circuit betweeen VMH and LH consisting of these glucosensitive neurons. Feeding and satiety may be controlled by activity of this circuit. It is important that the action of Cyp should be further investigated in relation to this aspect of the neuronal system.

EEG changes in LH, but not VMH, have been found after intravenous application of Cyp [2]. Moreover, a reduction of Δ-glucose was noticed at the same time. It was suggested that the reduction of available blood glucose concentration affected LH neurons. In that experiment, it was not obvious whether Cyp had a direct or an indirect effect upon hypothalamic neurons, possibly acting through a decrease of blood glucose. However, it has also been suggested [1] that Cyp is not related to blood glucose concentration. The threshold concentration of Cyp applied to VMH neurons was 6.4×10^{-3} M/l. Similarly that of glucose was 2×10^{-3} M/l [21], and for ACh 4.3×10^{-4} [25]. Since blood glucose concentration is 3.6×10^{-3} M/l on the average, approximately the same amount of blood glucose is effective on LH and VMH neurons when applied locally. This concentration is comparable to Cyp and ACh at threshold. Cyp-sensitive VMH and LH neurons were affected by repetitive AL and ST stimulation. The former were facilitated and the latter were inhibited. This, meaningfully, may suggest that Cyp sensitive neurons in VMH and LH function as principal neurons in the regulation of feeding behavior. In the chronic experiments, young rats took more food and gained more weight than control rats after only a short period of Cyp administration. This result is well in accord with that of the acute experiments.

In a preliminary experiment, we compared the difference in food intake and weight gain between rats given Cyp and those with bilateral VMH lesions using 9 male rats (170 g body weight). Four bilateral-VMH lesioned rats took more food and water than two control rats. Obesity was

conspicuous in the VMH lesiond group. Moreover, we observed behavioral patterns of these rats in open field tests. VMH lesioned rats seemed to be hypoactive and hyperreactive as already mentioned [8]. However, rats which were given Cyp orally were hyperactive and noticeably different from VMH lesioned rats. Several rats were tested in an open field maze after administration of Cyp at a dosage which was enough to evoke an increase of food intake. These rats showed hyperactive and anxielytic state. Hyperactivity with the emotional stability might be an indication of LH hyperactivity [9]. It is known that activity of LH neurons is directly related to level of consciousness [22]. Cyp might enhance hyperactivity and the level of consciousness by activating LH neurons. There has been no answer regarding the mechanism of Cyp action which would satisfactorily explain the clinical reports that this drug evokes appetite and subsequently facilitates the development of weight and height in asthmatic and anorexic children. The results of the present experiments explain this mechanism.

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