EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF ALIPHATIC DIAMINES ON INGESTIVE BEHAVIOR IN THE RAT

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Abstract—We examined the pharmacological effects of intracerebroventricularly administered aliphatic diamines on ingestive behavior in male rats adapted to a 4 hr per day feeding and drinking schedule. 1,2-Ethanediamine (ETD), 1,3-propanediamine (PRD), 1,4-butanediamine (putrescine, PUT), 1,5-pentanediamine (cadaverine, CAD) and 1,6-hexanediamine (HED) suppressed feeding and drinking behavior in a dose-dependent manner, but not unless a relatively high dose (over 80 μg) was given. The approximate anorectic potency was HED>CAD>PUT>ETD>PRD. A sedation was also produced in fairly good parallel to these alterations in feeding and drinking behavior. Thus, there appears to be a relationship between the length of the carbon chain and the potency of the pharmacological action, and these inhibitory effects on feeding and drinking behavior are probably not due to a specific action on the regulatory system for ingestive behavior, but rather to a nonspecific action.

The aliphatic diamines, PUT and CAD, occur in the mammalian brain (1-4), and PUT is known to be the direct precursor of spermidine (5), and associated with cell growth or proliferation (6). The existence of a pathway from PUT to γ-aminobutyric acid (GABA) in the mammalian brain has been reported (7-10). Moreover, part of the endogenous PUT is localized within nerve endings (11), and the active uptake system for ornithine, a direct precursor of PUT, into nerve endings has also been reported (12). A significant increase in CAD content in both the brain and the blood of dormant mice has been reported (2), and a high concentration of monoacyl-CAD was found in the blood of schizophrenic patients (13, 14).

Despite this evidence which suggests physiological roles of these diamines, few pharmacological studies have been reported (15-17), and a relatively high concentration of PUT is reportedly localized in the hypothalamus (4), a regulatory center for ingestive behavior (18). We examined the pharmacological effects of these diamines, as well as a few structurally related substances, on feeding and drinking behavior in rats, and our findings are reported herein.

MATERIALS AND METHODS

Animals and surgery: Male Wistar rats weighing 325-447 g at the time of drug administration were used. The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a stainless steel cannula modified hypodermic needle (0.6 mm dia.)
with an inner obdulator (0.3 mm dia.) was implanted stereotaxically into the left lateral ventricle, according to the atlas of Pellegrino and Cushman (19). After at least one week recovery, the rats were housed individually in wire mesh cages (26 x 36 x 19 cm) in a room maintained at 20-24°C with a 12 hr light-dark cycle (9:00-21:00).

Procedures: Each rat was trained to a 4 hr (16:00-20:00) per day feeding and drinking schedule for at least one week until the body weight stabilized or increased, and was conditioned at least three times to the type of handling to which it would be exposed on the day of injection. At 16:00, each rat was removed from its cage and an intracerebroventricular (icv) administration of each drug dissolved in a 20 μl volume of Ringer’s solution was given through the injection cannula (0.3 mm dia.) using a Hamilton microsyringe. The injection cannula was kept in the guide cannula for 30 sec in order to diffuse the drug solution sufficiently. Then powdered food (CE-2, Clea Japan, Inc.) and water were fed to the rats using a glass cup and a graduated glass tube, respectively. The amount of food and water consumed was measured every hour for 4 hr after the injection, and the body weight was also measured at the end of this feeding and drinking period. At the end of the experiment, pentobarbital anesthesia was administered and the cannula placement was confirmed by infusion of 0.2% solution of Evans blue dye in a volume of 20 μl and the overflow from the outlet which was opened above the cisterna magna.

Drugs: Drugs used are as follows: 1,2-

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**Fig. 1.** Effects of 1,2-ethanediamine (ETD) on food intake, water intake and body weight. ○ O Ringer control (N=7). ● ● 80 μg (N=4). ▲ ▲ 160 μg (N=5). ■ ■ 240 μg (N=5). Each value represents mean and SEM. *p<0.05, **p<0.01, compared to Ringer control.
ethanediamine dihydrochloride (ETD, 80–240 μg, pH 4.4–4.5, Nakarai Chemicals, LTD.), 1,3-propanediamine dihydrochloride (PRD, 80–240 μg, pH 3.6–3.7, Tokyo Kasei Kogyo Co., LTD.), putrescine dihydrochloride (PUT, 80–240 μg, pH 5.9–6.0, Nakarai), cadaverine dihydrochloride (CAD, 80–240 μg, pH 4.3–4.6, Tokyo Kasei) and 1,6-hexanediamine dihydrochloride (HED, 80–120 μg, pH 4.5–4.6, Nakarai). All these chemicals were freshly dissolved in sterile Ringer’s solution. Dosage of each drug is expressed in terms of its salt.

Statistics: Since the preinjected baseline of food and water intake differed among the groups, the volume of intake was transformed into percentage of the preinjected intake, and then the difference between the two groups was evaluated by Student’s t-test (two-tailed).

RESULTS

ETD suppressed feeding and drinking behavior in doses over 80 μg without showing a clear dose-response relationship (Fig. 1). This inhibitory effect was no longer apparent at 4 hr after injection. There was a slight but significant decrease in body weight.

PRD did not inhibit feeding and drinking behavior when doses of 80 and 160 μg were given, but 240 μg of PRD exerted a slight inhibitory effect on feeding behavior during the first hour following injection. There was no change in body weight (Fig. 2).

PUT (160 and 240 μg) suppressed feeding and drinking behavior, in a dose-dependent manner. This suppression of ingestive be-

![Fig. 2. Effects of 1,3-propanediamine (PRD) on food intake, water intake and body weight. ○ Ringer control (N=7), ● 80 μg (N=5), ▲ 160 μg (N=5), ■ 240 μg (N=5). Each value represents mean and SEM. *p<0.05, **p<0.01, compared to Ringer control.](image-url)
behavior was not observed on the day after the dosing. Body weight was also reduced (Fig. 3).

A similar effect was also observed after injection of CAD. In doses of 160 and 240 μg, CAD suppressed feeding and drinking behavior during the 4 hr following injection, in a dose-dependent manner. By the next day, however, this inhibitory effect had disappeared. Body weight loss was also produced (Fig. 4).

An 80 μg of HED did not suppress feeding behavior, whereas it did exert a slight suppression on drinking behavior during the first 2 hours after injection. With a dose of 120 μg, HED suppressed feeding behavior for 4 hr, and drinking behavior for 2 hr. This suppressive effect was long lasting. On and after day 2, none of the animals ate or drank, and all had died by day 7 (Fig. 5).

The percent decreases in food intake induced by diamines at the various periods after icv injection are summarized in Fig. 6. During the first hour after injection, all diamines except PRD produced marked anorexia. The values during this period for each drug were as follows: PUT 91.1%, CAD 81.4%, ETD 46.3%, HED 44.8%, PRD 7.5% and Ringer’s solution (RING) 2.1%. The inhibitory effects of these diamines decreased and all but disappeared one day after the injection. HED, however, markedly inhibited feeding behavior during the 4 hr-feeding period between 24 and 28 hr after injection. The percent decrease induced by each drug during this period was as follows: HED 87.4%, PUT 18.5%, CAD 11.1%, ETD 8.9%, PRD 4.7% and RING 11.5%.

![Fig. 3. Effects of putrescine (PUT) on food intake, water intake and body weight.](image-url)
The results of water intake are summarized in Fig. 7. The time course of suppression elicited by each diamine was similar to the occurrence of anorexia. During the first hour, the percent decrease in water intake induced by each drug was as follows: PUT 100%, CAD 98.9%, ETD 54.9%, HED 47.2%, PRD 2.7% and RING 13.4%. During the period of 24–28 hr, each value was as follows: HED 89.9%, CAD 27.1%, PUT 11.5%, PRD 7.1%, ETD 5.7% and RING 2.6%.

Although a state of sedation was apparent in the course of all the diamines, the animals did readily respond to external stimuli. No other behavioral changes were observed.

DISCUSSION

The anorectic effect of diamines characteristically showed short latency and duration, and there was a close relationship between the anorectic potency and the length of carbon chain, except for PRD. The nanomolar dose of each diamine represented in Fig. 6 is as follows: ETD 1203, PRD 1088, PUT 993, CAD 914 and HED 635. Therefore, the anorectic potency was approximately HED>CAD=PUT>ETD>PRD. This result is in good agreement with several other reports which described the insulin-like effect on lipid and glucose metabolism in satiated rat adipocytes (20), and the inhibitory effect on ornithine decarboxylase (ODC) in rat ovarian (21), and Ehrlich ascites-carcinoma cells from mice (22).

Regarding the onset of behavioral change, the time was the shortest with ETD. It has been reported that ETD releases catecholamines from the isolated bovine adrenal
gland (23), and also inhibits the storage of tritiated norepinephrine in the cerebral synaptic vesicles from rat brain (24). It is therefore possible that the effect of ETD is due to catecholamine-release in the brain. In addition, the physicochemical characters, i.e., small size and low dissociation constant (25), may be involved in the rapid action.

PRD had no apparent effect on feeding and drinking behavior. Recently, Pösö et al. have found that PRD inhibits ODC activity from regenerating rat liver (26–28), but since there are no findings regarding the effect of PRD in the brain, a discussion of the present results awaits further support from experimental data.

PUT and CAD exerted nearly equal effects on ingestive behavior. The duration of feeding and drinking deficits induced by PUT was short, probably because PUT is rapidly metabolized after icv administration (29, 30). In recent years, it has been reported that PUT is metabolized not only to polyamines but also to GABA (7–9), and to γ-glutamyl-PUT (10, 31) in the brain. However, the physiological function of these pathways remains unknown. In any case, considering the rapid onset and the short duration, it is reasonable to assume that metabolites of PUT are not involved in the behavioral action. The latest findings are that PUT injected into the cerebroventricle of the chick does induce behavioral and biochemical changes, increases in locomotor activity, vocalization and head jerks, decreases in glutamate-decarboxylase activity and GABA content in the brain (17). As such findings were nil in our experiments, the

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Fig. 5. Effects of 1,6-hexanediamine (HED) on food intake, water intake and body weight. ○ ○ Ringer control (N=7), ● ● 80 μg (N=4), ▲ ▲ 120 μg (N=6). Each value represents mean and SEM. *p<0.05, **p<0.01, compared to Ringer control.
differences are probably due to the species used. A specific uptake system for PUT and CAD which is quite different from that of amino acids has been found in the brain (32), and it was suggested that PUT and CAD may play an important role in brain functions.

HED had a characteristic mode of action compared with other diamines. Anorexia and

![Fig. 6. Anorectic intensity of all the chemicals at each period after injection. Numbers on each column represent dose of each amine. RING: Ringer control, ETD: 1,2-ethanediamine, PRD: 1,3-propanediamine, PUT: putrescine, CAD: cadaverine, HED: 1,6-hexanediamine.](image)

![Fig. 7. Adipsogenic intensity of all the chemicals at each period after injection. Numbers on each column represent dose of each amine. RING: Ringer control, ETD: 1,2-ethanediamine, PRD: 1,3-propanediamine, PUT: putrescine, CAD: cadaverine, HED: 1,6-hexanediamine.](image)
adipsia induced by HED were rapid in onset, and long lasting. HED reportedly exerts other potent actions, e.g., potent cytotoxicity on the rat embryo and human amnion cell cultures (33), causes a decrease in the threshold of neuromuscular excitability, increases blood leukocyte and liver glycogen levels, the appearance of renal disorders and alteration in the phagocytic activity of neutrophils, when rats were exposed to an atmospheric contiguity of 1.25 mg/m³ of HED for 4 hr per day and 8 days (34). These findings are in accord with our results concerning the potent effect of HED, though a comparison between the peripheral actions and the effect on ingestive behavior may not be valid. It is of interest that HED which has the longest carbon chain (C=6) among the chemicals used exerted a long lasting and potent action, as compared with the other diamines given.

Recently, a number of investigators have suggested that not only the hypothalamus (18) but also other regions, globus pallidus (35), amygdala (36), nigrostriatal system (37) and ascending noradrenergic bundle (38) may be involved in ingestive behavior. Thus, it is reasonable to assume that these aliphatic diamines exert anorectic effects on these regions in close proximity to the cerebroventricles. The adipsia produced all but paralleled the onset of anorexia. The regulatory system of drinking behavior is apparently different from that of feeding behavior (39), and the regulatory center has been demonstrated in the dorsolateral perifornical incertal region of the hypothalamus (40, 41).

In conclusion, considering that the dosage of each diamine was considerably large compared with other transmitter candidates such as noradrenaline or acetylcholine which are involved in ingestive behavior (42), and that sedation was also simultaneously produced, it is reasonable to assume that these endogenous diamines PUT and CAD, not to mention other unphysiological diamines, are not specifically involved in ingestive behavior, and that their inhibitory effects are due to nonspecific actions. However, another possibility is that these substances have functional roles in the brain.

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