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Pantethine, a Somatostatin Depleting Agent, Increases Food Intake in Rats[1]

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ABUCHAM, J., J. BOLLINGER-GRUBER AND S. REICHLIN. *Pantethine, a somatostatin depleting agent, increases food intake in rats.* PHARMACOL BIOCHEM BEHAV **33**(3) 585–589, 1989.—During the course of studies of the effects of pantethine, a cysteamine precursor known to deplete tissue concentration of immunoreactive somatostatin, we observed that the subject rats continued to eat despite marked distension of the stomach. To determine whether this effect was caused by drug-altered food intake, we have measured food and water intake in pantethine-injected rats in the fed and fasting state. In three separate experiments, rats allowed free access to food until the morning of study showed significant increased food intake accompanied by an increased stomach content (at 4 hr) of both food and water following the IP injection of pantethine. In one experiment, intake at 3 hours was 0.60 g/100 g b.wt. (pantethine dose 0.74 g/kg b.wt.) and 0.64 g/100 g b.wt. (pantethine dose 1.47 g/kg b.wt.) compared with 0.24 g/100 g b.wt. in saline-treated animals ($p<0.05$). In contrast, pantethine, 1.47 g/kg b.wt., when administered to overnight-fasted rats, significantly inhibited food intake (3-hr intake 1.54 ± 0.16 g/100 g b.wt. in rats injected with pantethine 1.47 g/kg b.wt. as compared with 3.3 ± 0.21 g/100 g b.wt. in saline-injected controls). The intake-stimulating effect of pantethine in ad lib-fed rats was not demonstrable when the drug was administered shortly before the "lights out"-induced feeding at night. These findings indicate that pantethine, a cysteamine precursor, stimulates food intake in satiated rats, depending upon the stage of circadian rhythm, but is inhibitory to intake in fasted animals. We postulate that the effects are mediated directly or indirectly through the disinhibition of central appetite-regulating somatostatinergic pathways but, since cysteamine also inhibits dopamine-beta-hydroxylase, an effect on depletion of appetite-regulating central catecholamines cannot be excluded.

Pantethine Somatostatin Food intake Satiety

DURING the course of studies of the effects of pantethine, a cysteamine precursor, on tissue concentration of somatostatin in the rat (17), we observed that the stomach was markedly distended with both food and liquid when the experiment was terminated four hours after injection of the drug. Although it has been well documented that cysteamine delays gastric emptying (11), the gastric content appeared to be so much greater than that of controls that we considered it likely that the drug may have stimulated food intake as well. Stimulation of food intake in the face of gastric distension would be of particular interest because, under normal circumstances, gastric distension leads to inhibition of eating (7). We postulated that the loss of this response might be indicative of a central action of pantethine, possibly mediated through its known effects in reducing somatostatin immunoreactivity in hypothalamus as well as other parts of the brain (17). Somatostatin is one of several peptides known to modulate eating behaviour following intracerebroventricular injection in the rat (3). We therefore have carried out detailed measurements of food intake in pantethine-treated rats and determined that this agent increases food intake in rats that had been allowed free access to food prior to the experiment. In contrast, pantethine, when administered to starved rats, was found, paradoxically, to inhibit food intake. Further, it was found that the pantethine effect was apparently modified by a circadian rhythm of eating regulation in that it was ineffective when administered in the evening immediately before the onset of the dark.

METHOD

Male Sprague-Dawley adult rats (Taconic Farms) were housed (3/cage) in a room on a 12:12 hr light cycle (lights on at 6:00

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a.m.), at constant temperature (25°C), and were allowed free access to food pellets and water. In Experiment I, animals were individually caged immediately before the drug was injected and food was available as pellets of Purina Rat Chow. In all other experiments, rats were individually caged and given access to glass cups containing ground Purina Rat Chow for 3 days before the experiment. Pantethine (Calbiochem or Sigma) was diluted in saline and administered IP in a dose of 0.74 or 1.47 g/kg b.wt. contained in 0.1 ml/100 g b.wt. Food intake was determined by serial measurements of pellets or cup weight before and 30, 60, 120, and 180 min after pantethine or saline injection.

Effects of Pantethine on Food and Water Intake and Stomach Content in Rats Allowed Free Access to Food (Experiments I, II, III)

In these experiments, rats were tested beginning at 0900 hr, receiving saline or pantethine IP.

Experiment I. Six rats (204 to 282 g b.wt.) were studied in a Latin Square design at two-day intervals. Treatments were either saline, pantethine 0.74 or 1.47 g/kg b.wt. and both water and pellet food were measured.

Experiment II. Groups of 5 rats (280–360 g b.wt.) were treated with either saline or pantethine 1.47 g/kg and intake of ground food measured at intervals up to 5 hr.

Experiment III. Groups of 4 rats (275–320 g b.wt.) were treated with either saline or pantethine 1.47 g/kg b.wt. At the end of the experiment (4 hr), animals were killed by decapitation, the abdomen was opened, the stomach was isolated with ligatures at the cardia and pylorus and removed. The content of the stomach was emptied into preweighed vials, and both total and dry weight determined by weighing before and after freeze-drying. The experiment was replicated using groups of 4 rats weighing 150–160 g b.wt.

Effect of Pantethine on Food Intake in Overnight Fasted Rats

Experiment IV. Groups of 8 rats (180–210 g b.wt.) were fasted overnight and food intake was measured after saline or pantethine 1.47 g/kg b.wt. IP injection at 0900 hr.

Effects of Pantethine on Food Intake in the Evening

Experiment V. Groups of 5 rats (330–470 g b.wt.) were allowed free access to food during daytime and food intake was measured after saline or pantethine 1.47 g/kg b.wt. IP given at 1800 hr, immediately before "lights off." This experiment was replicated using rats weighing 240–275 g.

Effect of Pantethine on Blood Glucose Concentration

To determine whether the effect of pantethine on food intake was induced by hypoglycemia, blood glucose concentrations were measured before and 30, 60, 90 and 120 min after pantethine administration (1.47 g/kg b.wt. IP) at 0900 hr in 6 free-eating rats. Blood was collected from the tip of the tail and measured using a blood glucose monitor (Accu-chek II, Boehringer Mannheim, Indianapolis, IN).

Statistical Analysis

Food intake and stomach total and dry contents were expressed in g/100 g ±S.E. b.wt.; water intake and stomach water content were expressed in ml/100 g ±S.E. b.wt. Data from replicated experiments were pooled and Student's unpaired *t*-test was used for statistical analysis. Statistical significance was set at $p<0.05$ (two-tailed).

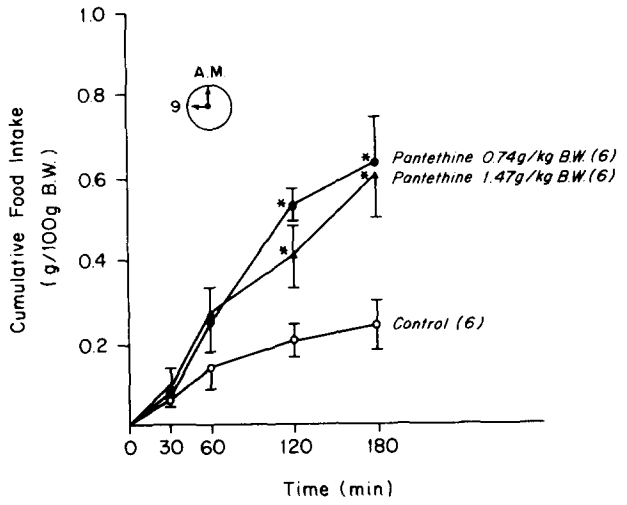

FIG. 1. Effect of morning pantethine administration on pellet food intake in nonfasted rats. Food intake was increased when rats received pantethine at doses of 0.74 or 1.47 g/kg b.wt., IP, as compared to saline treatment. Significant differences from controls are shown by asterisks (*$p<0.05$).

RESULTS

Effects of Pantethine on Food and Water Intake and Stomach Content in Rats Allowed Free Access to Food

Experiment I (Fig. 1). Administration of pantethine in two different doses to free-eating rats in the morning caused a significant increase in pellet food intake which was evident at 60 min and became statistically significant at 120 min. The effects of 0.74 and 1.47 g/kg b.wt. of pantethine on food intake were similar. Cumulative food intake at 180 min was 0.24 ± 0.60 g/100 g b.wt. after saline and significantly increased to 0.60 ± 0.10 g/100 g b.wt. ($p<0.05$) and 0.63 ± 0.14 g/100 g b.wt. ($p<0.05$) after the high and low doses of pantethine, respectively. Water intake, measured at the end of the experiment, was also increased after pantethine, although this difference was not statistically significant due to a wide variation in water intake after pantethine as compared to saline. Water intake varied from 0.15 to 2.43 ml/kg b.wt. (median = 0.67 ml/kg b.wt.) after the low dose and from 0.14 to 2.31 ml/kg b.wt. (median = 0.58 ml/kg b.wt.) after the high dose of pantethine, whereas after saline values ranged from 0 to 0.25 ml/kg b.wt. (median = 0.22 ml/kg b.wt.).

Experiment II (Fig. 2). Pantethine administration to free-eating animals during the morning also increased intake of ground food. This effect was observed as early as 30 min after injection and became significantly different from controls at 60 min and thereafter up to 300 min. As indicated by the slopes of the cumulative food intake curves, pantethine-treated animals ate at a higher rate than controls until 120 min and then decreased eating to a rate similar to control animals.

Experiment III (Fig. 3). In this experiment, food intake was significantly increased as early as 30 min after pantethine injection, remaining significantly elevated in all subsequent measurements. The difference was maximal at 120 min, when pantethine-treated animals had eaten 0.61 ± 0.1 g/100 g b.wt., whereas

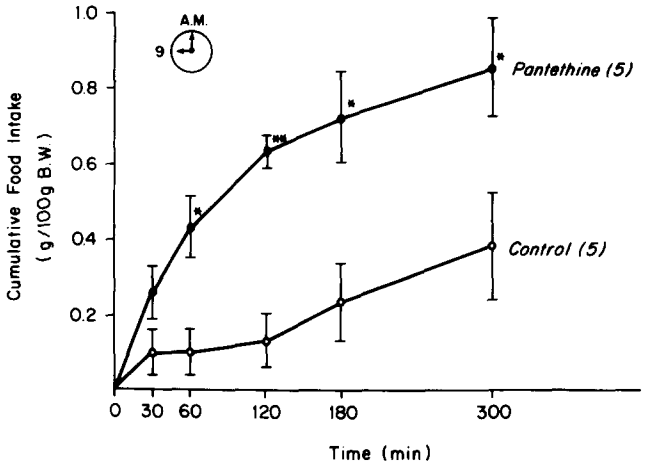

FIG. 2. Effect of morning pantethine administration on ground food intake in nonfasted rats. Pantethine treatment (1.47 g/kg b.wt., IP) led to increased ground food intake as compared to controls, which reached statistical significance at 60 min. Significant differences from controls are shown by asterisks (*$p<0.05$; **$p<0.01$).

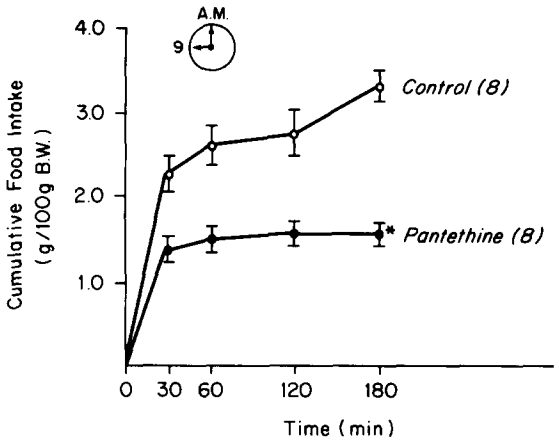

FIG. 4. Effect of morning pantethine administration on ground food intake in overnight-fasted rats. Pantethine administration (1.47 g/kg b.wt., IP) after an overnight fast failed to increase food intake and led to decreased eating as compared to controls. Significant differences from controls are shown by asterisks (*$p<0.05$).

control animals had only eaten 0.05 ± 0.02 g/kg b.wt. ($p<0.0001$). At 180 min, animals were killed and inspection of the stomachs of pantethine-treated animals showed marked distention. The stomach content of pantethine-treated rats was significantly greater than controls, both for dry weight and for water content. Total weight was 3.36 ± 0.27 g/100 g b.wt. vs. 1.12 ± 0.15 g/100 g b.wt. ($p<0.0001$), dry weight was 0.93 ± 0.09 g/100 g b.wt. vs. 0.42 ± 0.07 g/100 g b.wt. ($p<0.001$) and water content was 2.44 ± 0.21 g/100 g b.wt. vs. 0.70 ± 0.09 ml/100 g b.wt. ($p<0.0001$).

Effect of Pantethine on Food Intake in Overnight-Fasted Rats

Experiment IV (Fig. 4). Administration of pantethine in the morning to overnight-fasted rats caused a decrease in food intake as compared to control animals. Although mean food intake in the pantethine-treated group was already less than in the control group by 30 min, this difference increased progressively to reach statistical significance at 180 min, when food intake in controls was 3.31 ± 0.21 g/100 g b.wt. and in pantethine-treated animals was 1.54 ± 0.16 g/100 g b.wt. ($p<0.01$).

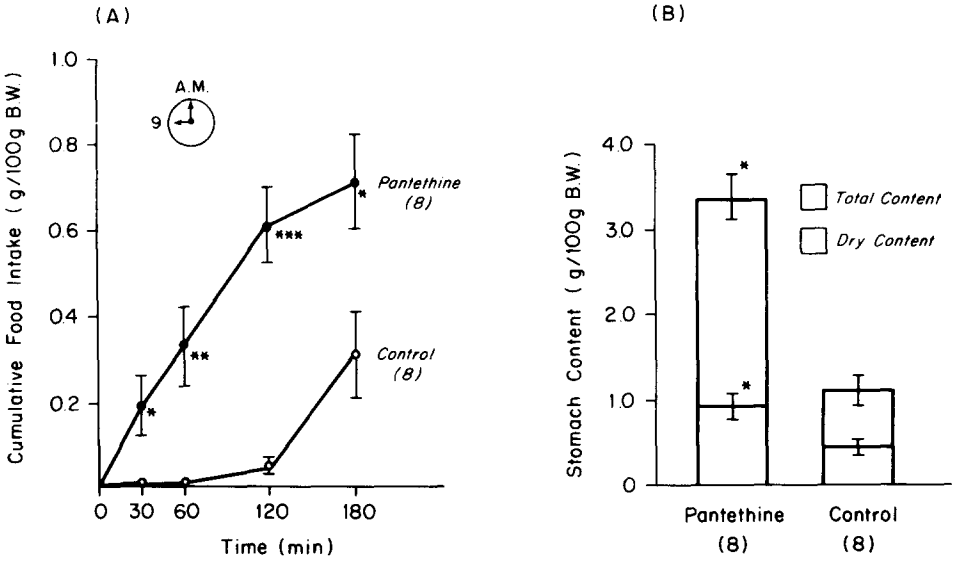

FIG. 3. Effect of morning pantethine administration on ground food intake and stomach content in nonfasted rats. Rats received pantethine (1.47 mg/kg b.wt., IP) or saline. Food intake was measured during 180 min and animals were then killed by decapitation and stomach content was measured. (A) Pantethine eating-stimulatory effect was already significant at 30 min after injection. (B) Total and dry stomach content were significantly higher in pantethine-treated rats as compared to controls. Significant differences from controls are shown by asterisks (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

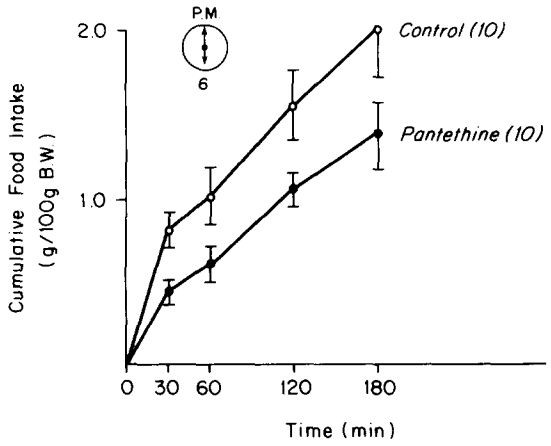

FIG. 5. Effect of pantethine administration on ground food intake in the evening. There was no significant difference on food intake between pantethine-treated rats and controls when pantethine (1.47 g/kg b.wt., IP) was administered in the evening, when lights went off.

Effect of Pantethine on Food Intake in the Evening

Experiment V (Fig. 5). To determine whether the effect of pantethine was time of day dependent, this experiment was started in the evening, immediately before lights were turned off, when rats become more active and there is a burst of eating. In contrast to the increased food intake observed in rats given pantethine in the morning, rats tested in the evening tended to eat less than controls, although the differences were not statistically significant.

Effect of Pantethine on Blood Glucose Concentration

Blood glucose levels were not significantly decreased after pantethine administration. Mean basal blood glucose levels were 69.2 ± 3.3 mg/100 ml and remained virtually unchanged after 30 and 60 min of pantethine administration (69.3 ± 7.1 mg/100 ml and 62.7 ± 6.2 mg/100 ml, respectively), increasing slightly but nonsignificantly at 90 and 120 min to 74.5 ± 5.4 mg/100 ml and 85.3 ± 7.6 mg/100 ml, respectively.

DISCUSSION

In this study we have shown that systemic administration of pantethine has significant effects on food intake in rats depending upon the prior feeding status and time of day. When administered in the morning to rats having had free access to food, pantethine increases food intake. In contrast, when administered in the morning to rats fasted overnight, pantethine significantly decreases food intake. When administered in the evening, immediately before "lights off," pantethine had no effect on the burst of eating that normally occurs at the beginning of the dark cycle.

There are several possible explanations of the increase in eating caused by pantethine. Through its conversion to cysteamine, this agent could act to deplete central somatostatinergic pathways which, directly or indirectly, influence feeding behavior. Other possible explanations are considered below. Pantethine has previously been shown to reduce whole brain and hypothalamic immunoreactive somatostatin concentration (17); it is our presumption that it acts through the known conversion to cysteamine (14), which is an equally potent reducer of immunoreactive somatostatin (21). Further evidence that pantethine exerts effects through its cysteamine breakdown product is our finding of marked distension of the stomach in pantethine treated animals, a change also observed in cysteamine-treated animals and attributed to gastric atony secondary to reduced myoelectric activity in the stomach (15). That reduction of brain immunoreactive somatostatin is accompanied by reduced bioactive somatostatin release is indicated by the finding of Srikant and Patel (20) that somatostatin receptors are up-regulated in cysteamine-treated rats.

Centrally administered somatostatin has previously been shown to modify food intake. When given to fed animals, it reportedly inhibits eating, while, in fasted animals, food intake is enhanced (2). Our findings in this experiment would correspond to these induced effects, since disinhibition of central somatostatin in fed animals would be expected to increase food intake, and in fasted animals to decrease intake. That somatostatin effects could be time-related has been previously suggested by Nicholson *et al.* (13), who showed that potassium-induced release of somatostatin from the hypothalamus, in vitro, is greater in animals killed during the day than early in the night. Somatostatin may work within the brain directly on neurons influencing food drive or indirectly by modifying secretion of other appetite-regulating peptides such as growth hormone releasing hormone (GHRH), thyrotropin releasing hormone (TRH), opioids and neuropeptide Y. GHRH is found in the hypothalamus (18,19), stimulates eating when injected intracerebroventricularly (23), and its release is inhibited by somatostatin (16). The central release of TRH, an anorexogenic peptide (24), is suppressed by somatostatin (6). Opioid peptides and neuropeptide Y have been shown to increase food intake when centrally administered to rats and a role for these substances as endogenous regulators of appetite has been suggested (3, 4, 18, 20). The effects of somatostatin on central opioid and neuropeptide Y release have not been reported, but it is likely that somatostatin exerts an inhibitory effect in view of the almost universal inhibiting effect of somatostatin on neuropeptide secretions.

Pantethine effects may be mediated by changes in central catecholamines. Cysteamine, and presumably pantethine as well, is a potent inhibitor of dopamine-beta-hydroxylase, and hence a depletor of central norepinephrine and epinephrine (22), although it is not known whether changes in catecholamine secretion could be induced as early as 30 minutes, the time shown in this study to be associated with behavioural changes. Depletion of norepinephrine and of epinephrine, and increase in dopamine were observed as early as 2 hours, the first interval studies (22). Catecholamines exert important effects on food drive. As outlined by Leibowitz (9), alpha-2 agonist stimulation of the paraventricular nucleus is a potent inducer of eating in the rat, while beta agonists and dopamine, acting in the lateral hypothalamus, exert inhibitory effects on food intake. Since the effects of cysteamine are probably not region- or catecholamine-specific, mixed effects on food intake could likely occur and it would be difficult to predict, a priori, what the overall effect on catecholamine depletion would be. Further, the effects of the appetite stimulatory catecholamine system apparently depend upon the physiological state of the animal (8).

Prolactin deficiency, a known effect of pantethine administration (17), is unlikely to be involved in the eating stimulatory effect of pantethine, since food intake has been shown to increase (10) or remain unchanged after prolactin administration (5).

Pantethine-induced eating was accompanied by marked gastric distension and by retention of food and water. The analogous cysteamine effect is due to inhibition of gastric tone (15), and increased gastric acid secretion due to somatostatin disinhibition of oxyntic and gastrin-producing cells (12). Somatostatin disinhibition may also lead to increased secretion of gut cholecystokinin (1). The fact that gastric distension, elevated gastrin, and elevated cholecystokinin, all known inhibitors of food intake, fail to suppress eating in the food satiated rat supports the view that pantethine probably acts centrally to overcome peripheral satiety signals.

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