Pharmacology Biochemistry and Behavior, Vol. 53, No. 4, pp. 839–842, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02090-X

Inhibition of Food Intake in Rats by the K^+ Channel Opener Cromakalim

E. DEL PRETE,[1] T. A. LUTZ AND E. SCHARRER

Institut für Veterinär-Physiologie, Universität Zürich, Winterthurerstr. 260, CH-8057 Zürich, Switzerland

Received 7 February 1995; Revised 20 August 1995; Accepted 28 August 1995

DEL PRETE, E., T. A. LUTZ AND E. SCHARRER. *Inhibition of food intake in rats by the K^+ channel opener cromakalim.* PHARMACOL BIOCHEM BEHAV 53(4) 839–842, 1996.—We studied the effect of the K^+ channel opener cromakalim, which exhibits antihypertensive properties, on food intake in rats. Intraperitoneally injected cromakalim induced a dose-dependent (0.1, 0.5, and 1.0 mg/kg body wt.) reduction in food intake, which was associated with an inhibition of gastric emptying. The anorectic effect was not influenced by subdiaphragmatic vagotomy. Cromakalim's anorectic effect did not appear to be due to a learned taste aversion. Therefore, an intact abdominal vagus is not a prerequisite for cromakalim's anorectic effect.

Cromakalim K^+ channel opener Anorexia Food intake Subdiaphragmatic vagotomy Taste aversion Gastric emptying

IN THE LAST FEW YEARS, much interest has been focused on a group of compounds known as K^+ channel openers. These compounds open adenosine triphosphate (ATP)-sensitive K^+ channels in a variety of tissues including smooth, cardiac, and skeletal muscle, pancreatic β-cells, and neurones. They are therapeutically interesting because of their vasorelaxant and antihypertensive effect (5). K^+ channel openers (e.g., cromakalim) induce an outward K^+ current and hyperpolarize the cell membrane (17). In pancreatic β-cells, the ability to open K^+ channels is reflected by the inhibition of insulin release (5). However, in vivo, cromakalim was found to have minimal effects on plasma glucose and insulin levels at concentrations producing large falls in blood pressure (18). Some effects of cromakalim on K^+ channels in cardiac muscle have also been detected. The changes (negative inotropic effect) are observed at concentrations higher than those required to produce K^+ channel opening in vascular smooth muscle (8). Cromakalim has also been shown to decrease neuronal excitability and reduce epileptiform discharges in hippocampal slices (1). Because of its hyperpolarizing effect on smooth muscle cells of the gastrointestinal tract, cromakalim reduces intestinal motility (2); in gastrointestinal preparations, cromakalim was capable of inhibiting spontaneous tone and also reduced agonist-induced contractions (8).

Because gastrointestinal motility—in particular, the rate of gastric emptying—seems to be related to the control of food intake (12,13,20), the effect of cromakalim on gastric emptying and voluntary food intake was tested in rats. As both gastric emptying and food intake were inhibited by cromakalim and gastric distension seems to produce a satiety stimulus (6,15,16) signalled to the brain through vagal afferents (7), we also investigated cromakalim's anorectic effect in rats after subdiaphragmatic vagotomy. Furthermore, we studied whether cromakalim's anorectic effect is associated with taste aversion, because a severe aversive effect of a drug may suppress food intake (9).

METHODS

Animals, Housing Conditions, and General Procedures

The experiments were performed with male rats (ZUR:SIV rats; Institut für Labortierkunde, Universität Zürich) housed individually in a temperature-controlled (21 ± 1°C) colony room and kept on an artificial 12 L : 12 D cycle. The rats had a body weight of 400–530 g (Experiments 1–3). Rats used in Experiment 4 had a mean body weight of 207 g. The rats had access to the food in the home cages. They were adapted to the housing conditions for at least 2 weeks. They also were adapted to the test procedure [weighing of food cups to measure cumulative food intake, and intraperitoneal (IP) injections] and to the diets. For the taste aversion study, however, rats were naive to the saccharin diet. The vagotomized (VAG)

[1] To whom requests for reprints should be addressed.

and sham-vagotomized (SVAG) rats were fed a high-carbohydrate, low-fat diet (HC diet) (Table 1), because such a diet is well tolerated following subdiaphragmatic vagotomy (11). The rats used in the others experiments (Experiments 1, 2, and 4) were fed a powdered medium-fat diet (MF diet) (Table 1). The diets were isoenergetic (16.5 kJ/g). For the taste aversion study, saccharin (1%) was added to the MF diet [saccharin diet (SD)].

Cromakalim (BRL-34915; Sigma Chemical Co., St. Louis, MO) was dissolved in DMSO and saline was added (10 μl DMSO/ml saline). The injection of vehicle (10 μl DMSO/ml saline) served as control. Rats were injected IP with 2 ml/kg body wt. (Experiment 4: 6 ml/kg body wt.). Rats were distributed into groups according to food intake measured during the preceding light phase, because the experiments were performed during the light phase. In Experiments 1-3 two groups, and in Experiment 4 three groups of rats with similar baseline food intake (light phase) were formed.

Experiment 1: Effect of Different Doses of Cromakalim on Food Intake of Intact Rats

A total of 32 intact rats were injected with 0.1, 0.5, and 1.0 mg cromakalim/kg body wt. or vehicle at the beginning of the light phase, after 12 h of food deprivation. At these doses, cromakalim has been shown to reduce blood pressure in rats and other animal models (3,18). Cumulative food intake was measured by weighing the food cups ($\pm$0.1 g) just before injection and 0.5, 1, 2, 4, 6, and 12 h afterward. Spillage of food was accounted for. Rats were food-deprived for 12 h to increase basal intake (19).

Experiment 2: Effect of Cromakalim on Gastric Emptying

After 12 h of food deprivation, 15 rats had access to feeding cups for 1 h before light onset. At this time, the feeding cups were removed and cromakalim (0.5 mg/kg body wt.) or vehicle was injected IP. Then, 2 h later, rats were anesthetized, the stomach was removed, and the stomach content was transferred into small plastic cups and dessicated in a vacuum oven (70°C, 30 mbar, 24 h). The dry matter of gastric content was related to the dry matter of food ingested. This value, expressed as a percentage (percent residual gastric content), was then used as a measure for gastric emptying.

TABLE 1

COMPOSITION OF DIETS (g)

	MF Diet	HC Diet
Casein*	13.00	13.00
Corn starch	46.00	76.67
Soybean oil	3.41	3.33
Beef tallow	9.42	0
Lard	5.17	0
Mineral mixture†	4.00	4.00
Vitamin mixture‡	3.00	3.00
Diluent§	16.00	40.00
Total	100.00	100.00

MF diet: medium-fat, medium-carbohydrate diet; HC diet: high-carbohydrate, low-fat diet.

*Säurekasein (UFAG, Sursee), crude protein content 89.0%, supplemented with 1% D,L-methionine.

†A 1-kg mineral mixture contained 162.14 g Ca, 80.75 g P, 66.31 g Na, 90.88 g K, 38.99 g Mg, 102.00 g Cl, 2.92 g Fe, 665 mg Mn, 174 mg Cu, 411 mg Zn, 27 mg J, 63 mg F, 13 mg Co, and 9 mg Se.

‡A 1-kg vitamine mixture contained 700,000 IU D_3, 4.91 g E, 1.80 g C, 1.00 g B_1, 0.60 g B_2, 0.45 g B_6, 1.20 mg B_{12}, 1.80 g nicotinic acid, 1.50 g pantothenate, 100 mg folic acid, 3 mg biotin, 18.75 g choline.

§Polyethylene powder (Lupolen®, BASF, Ludwigshafen, FRG).

Experiment 3: Effect of Cromakalim on Food Intake of SVAG and VAG Rats

For total subdiaphragmatic vagotomy, rats (approximately 140 g) were anesthetized and laparatomized, and the anterior abdominal vagal trunc was ligated and cut between two ligatures immediately subdiaphragmatically. Similarly, the posterior vagal trunc was visualized by manipulating the esophagus, ligated with two ligatures and lesioned in between. SVAG rats underwent the same procedure except for ligation and transsection of the vagal truncs. For this experiment, 16 VAG and 14 SVAG rats were injected with 0.5 mg cromakalim/kg body wt. or vehicle at light onset after 12 h of food deprivation. Cumulative food intake was measured as described in Experiment 1. In a second trial, after 1 week, treatments were switched to have each rat act as its own control.

Experiment 4: Investigations of the Aversive Properties of Cromakalim

This study was performed with 32 intact male, drug-naive rats. The rats received the MF diet during 2 weeks of adaptation. After 12 h of food deprivation, at light onset, the saccharin containing diet [1% saccharin in MF diet (SD)] as conditioning stimulus (CS) was presented for 2 h (1st day). Immediately after the removal of the SD, the rats were injected with 0.5 mg/kg body wt. cromakalim [unconditioned stimulus (US), n = 11], vehicle (n = 11), or lithium chloride (LiCl, 76.2 mg/kg body wt., n = 10). The rats then fasted for 10 h until dark onset, when MF was offered again. On the 3rd day at light onset, all rats were offered both the MF diet and the SD, and the intake of both diets was measured after 6 and 12 h. Relative intake of the SD (percent of total intake) was determined and compared in rats previously injected with cromakalim, vehicle, or LiCl. Rats were excluded from the evaluation of the relative intake of the SD if they had not ingested food by 6 and 12 h after presentation of the MF diet and the SD. Lithium chloride was used as a positive control, because its aversive properties are well established (14) and the reduction of food intake induced by LiCl reflects a learned taste aversion (5). The concentration of the LiCl and NaCl in the injected solutions was 0.3 mol/l, and cromakalim was dissolved in 0.3 mol/l NaCl. The volume injected was 6 ml/kg body wt.

Statistics

Results are presented as mean $\pm$ SEM. The treatment groups were compared using unpaired Student's t-test (Experiment 2). Analysis of variance (ANOVA) with the Bonferroni posthoc test (Experiments 1 and 4) or a paired Student's t-test as posthoc test (Experiment 3) was used when more than two groups were compared. $p < 0.05$ was considered significant.

RESULTS

Effect of Different Doses of Cromakalim on Food Intake in Intact Rats

Cromakalim induced a dose-dependent reduction in food intake (Fig. 1). The reduction in food intake induced by cro-

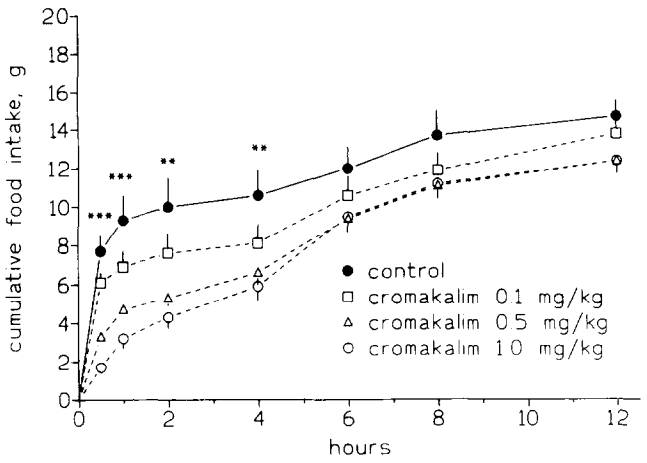

FIG. 1. Influence of cromakalim (0.1, 0.5, and 1.0 mg/kg, IP) on cumulative food intake of intact rats. Injection at light onset, after 12 h food deprivation. Values are means ± SEM, $n = 8$ each. ***$p < 0.001$; **$p < 0.01$ (ANOVA). Posthoc Bonferroni: 0.5 h PI: $p < 0.01$, 0.1 vs.0.5 mg/kg; $p < 0.001$, 0.1 vs. 1.0; 0.5 and 1.0 mg/kg vs. control. At 1 h PI: $p < 0.05$, 0.1 vs. 1.0 mg/kg; $p < 0.01$, 0.5 mg/kg vs. control; $p < 0.001$, 1.0 mg/kg vs. control. At 2 h PI: $p < 0.05$, 0.5 mg/kg vs. control; $p < 0.01$, 1.0 mg/kg vs. control. At 4 h PI: $p < 0.05$, 0.5 and 1.0 mg/kg vs. control.

makalim was significant (ANOVA) until the 4th h postinjection (PI) (30 min PI: $F = 27.79$, $p < 0.001$; 1 h PI: $F = 10.72$, $p < 0.001$; 2 h PI: $F = 6.83$, $p < 0.01$; 4 h PI: $F = 4.74$, $p < 0.01$). The injection of 1.0 mg cromakalim/kg body wt. induced a significant reduction in food intake compared to control rats 0.5 h PI ($p < 0.001$, Bonferroni posthoc test), 1 h PI ($p < 0.001$), 2 h PI ($p < 0.01$), and 4 h PI ($p < 0.05$). It also induced a significant reduction in food intake compared to the injection of 0.1 mg cromakalim/kg body wt. (0.5 h PI: $p < 0.001$; 1 h PI: $p < 0.05$). The anorectic effect of cromakalim was also present after the injection of 0.5 mg/kg body wt. compared to control rats (0.5 h PI: $p < 0.001$; 1 h PI: $p < 0.01$; 2 and 4 h PI: $p < 0.05$). Half an hour after injection, the rats injected with 0.5 mg cromakalim/kg body wt. also ate significantly less than those injected with 0.1 mg cromakalim/kg body wt. ($p < 0.01$). The lowest dose of cromakalim (0.1 mg/kg body wt.) only induced a nonsignificant reduction in food intake compared to control rats.

Effect of Cromakalim on Gastric Emptying

As a measure of gastric emptying, we used the stomach content as a percentage of the individual size of the prefed meal (dry weight). Two hours after the end of the meal, cromakalim-injected rats (0.5 mg/kg) had a significantly greater stomach content than control rats (61.7 ± 4.3% vs. 35.6 ± 6.3%, $p < 0.01$).

Effect of Cromakalim on Food Intake of SVAG and VAG Rats

The anorectic effect of cromakalim was similar in both SVAG rats and VAG rats (Fig. 2). However, the cumulative food intake of the control SVAG rats 1 h after injection was significantly higher than that of VAG control rats (6.1 ± 0.5 g vs. 4.3 ± 0.3 g) (ANOVA: $F = 21.79$, $p < 0.001$; posthoc Bonferroni: $p < 0.01$). At 12 h after cromakalim injection, hypophagia was still present in both VAG and SVAG rats, and the control VAG rats ate significantly more than the control SVAG rats (14.5 ± 0.5 g vs. 12.6 ± 0.8 g, ANOVA: $F = 13.30$, $p < 0.001$; posthoc Bonferroni: $p < 0.05$).

Investigation of the Aversive Properties of Cromakalim

On the aversion test day (3rd day after presenting the SD as the CS), when both the SD and the familiar MF diet were offered, the rats injected with LiCl on the US-CS pairing day avoided the SD (percent intake of the SD after 6 h ($n = 7$) and 12 h ($n = 9$): 0% of the total intake). The SD intake of the rats previously injected with cromakalim was 89.6 ± 6.5% and 77.4 ± 10.9% of total intake 6 h ($n = 5$) and 12 h

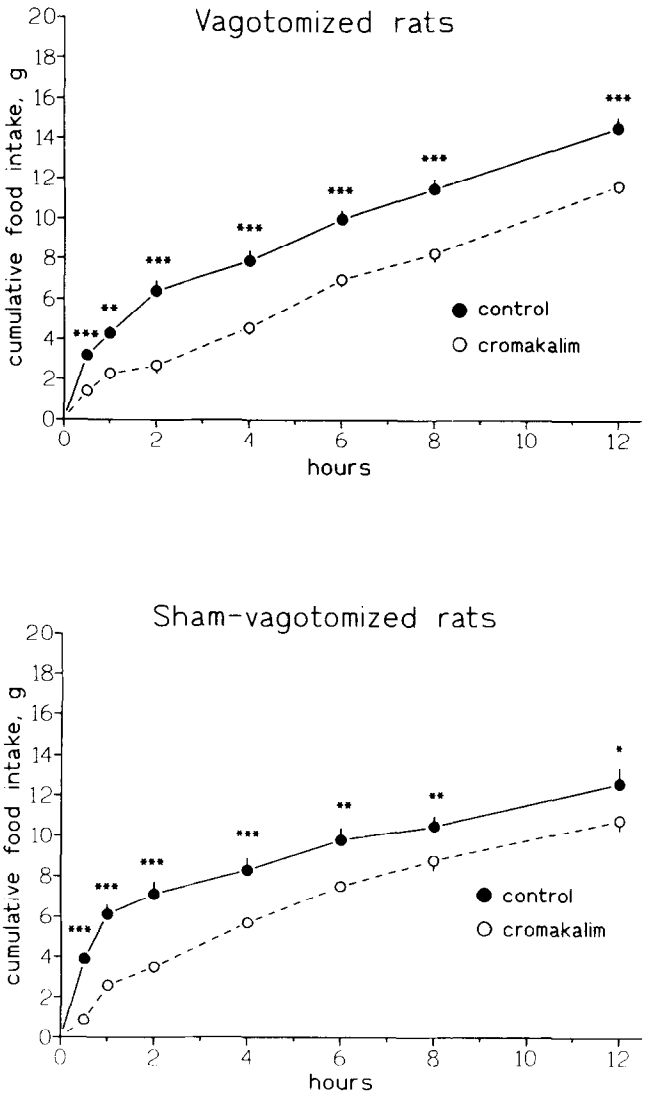

FIG. 2. Influence of cromakalim (0.5 mg/kg, IP) on cumulative food intake of subdiaphragmatically vagotomized ($n = 16$ each) or sham-vagotomized ($n = 14$ each) rats. Injection at light onset, after 12 h food deprivation. Values are means ± SEM. Significant differences between cromakalim-treated rats and respective control rats (posthoc paired Student's t-test: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

(n = 10) after presenting both the MF diet and the SD. The rats injected on the 1st day with vehicle showed a preference for the SD [100.0 ± 0% of total intake after 6 h (n = 6) and 81.5 ± 12.4% of total intake after 12 h (n = 10) (ANOVA after 6 h: F = 346.0; $p < 0.001$, and posthoc Bonferroni: $p < 0.001$ vehicle or cromakalim vs. LiCl; ANOVA after 12 h: F = 20.94; $p < 0.001$, and posthoc Bonferroni: $p < 0.001$ vehicle or cromakalim vs. LiCl]. Total food intake (SD + familiar MF diet) did not differ significantly among the three groups of rats (cromakalim, vehicle, and LiCl) after 6 and 12 h.

DISCUSSION

The present study shows for the first time a dose-dependent anorectic effect of the K^+ channel opener cromakalim. This is an interesting result considering the antihypertensive effect of K^+ channel openers, because obesity is a risk factor for the development of hypertension. Cromakalim also inhibited gastric emptying. This finding is not surprising as it has been documented that potassium channel openers have the ability to relax gastrointestinal smooth muscle (2,8). A role of the delayed gastric emptying in the inhibition of feeding induced by cromakalim, however, is not supported by the failure of total subdiaphragmatic vagotomy to counteract the anorectic effect of cromakalim, because stomach distension elicits a vagally mediated satiety signal under certain conditions (7). The results show that the anorectic effect of cromakalim is probably not mediated by afferent vagal fibres originating from the abdomen. Cromakalim similarly reduced food intake in subdiaphragmatically vagotomized and in SVAG rats. However, It cannot be excluded from the present study that cromakalim may partly exert its anorectic effect via transmission by afferents of the splanchnic nerves (4), or via the secondary release of a peripheral humoral factor which then could act independently of the vagus. It remains to be investigated whether injection of cromakalim elicits the release of peripheral satiety agents whose actions do not depend on the intact abdominal vagus. This applies to bombesin, for instance (10).

The suppression of food intake induced by cromakalim does not seem to be due to the induction of a conditioned taste aversion. In the food selection test (SD vs. the familiar MF diet), the LiCl-injected rats totally avoided the SD, whereas the SD intake of the cromakalim-injected rats did not differ significantly from the SD intake of the vehicle-injected rats. The anorectic effect of cromakalim therefore probably cannot be explained by a learned taste aversion.

In summary, the association of the anorectic effect of the K^+ channel opener cromakalim with an inhibition of gastric emptying does not depend on an intact abdominal vagus and does not seem to be due to aversive properties of the drug. Further investigations are necessary to elucidate the mechanism of cromakalim-induced anorexia.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the help of W. Langhans (ETH Zürich, Switzerland) for showing them the technique of subdiaphragmatic vagotomy.

REFERENCES

1. Alzheimer, C.; ten Bruggengate, G. Action of BRL 34915 (cromakalim) upon convulsive discharges in guinea pig hippocampal slices. Naunyn-Schmied. Arch. Pharmacol. 337:429–434; 1988.
2. Buchheit, K.-H.; Bertholet, A. Inhibition of small intestine motility by cromakalim (BRL-34915). Eur. J. Pharmacol. 154:335–337; 1988.
3. Clapham, J. C.; Hamilton, T. C.; Longman, S. D.; Buckingham, R. E.; Campbell, C. A.; Ilsley, G. L.; Gout, B. Antihypertensive and haemodynamic properties of the potassium channel activating (−)enantiomer of cromakalim in animal models. Drug Res. 41:385–391; 1991.
4. Deutsch, J. A.; Jang Ahn, S. The splanchnic nerve and food intake regulation. Behav. Neural Biol. 45:43–47; 1986.
5. Edwards, G.; Weston, A. H. The pharmacology of ATP-sensitive potassium channels. Annu. Rev. Pharmacol. Toxicol. 33:597–637; 1993.
6. Geliebter, A.; Westreich, S.; Gage, D.; Hashim, S. A. Intragastric balloon reduces food intake and body weight in rats. Am. J. Physiol. 251:R794–R797; 1986.
7. Gonzales, M. F.; Deutsch, J. A. Vagotomy abolishes cues of satiety produced by gastric distension. Science 212:1283–1284; 1981.
8. Hamilton, T. C.; Weston, A. H. Cromakalim, Nicorandil and Pinacidil: Novel drugs which open potassium channels in smooth muscle. Gen. Pharmacol. 20:1–9; 1989.
9. Kulkosky, P. J. Conditioned food aversions and satiety signals. Ann. NY Acad. Sci. 443:330–347; 1985.
10. Lee, M. C.; Schiffman, S. S.; Pappas, T. N. Role of neuropeptides in the regulation of feeding behavior: A review of cholecystokinin, bombesin, neuropeptide Y and galanin. Neurosci. Biobehav. Rev. 18:313–323; 1994.
11. Lutz, T. A.; Del Prete, E.; Scharrer, E. Subdiaphragmatic vagotomy does not influence the satiating effect of amylin. Peptides 16:457–462; 1995.
12. Mc Hugh, P. R.; Moran, T. H. The stomach: A conception of its dynamic role in satiety. Prog. Psychobiol. Physiol. Psychol. 11: 197–232; 1985.
13. Mc Hugh, P. R.; Moran, T. H. The stomach and satiety. In: Kare, M. R.; Brand, J. G., eds. Interaction on the chemical senses with nutrition. New York: Academic Press; 1986:167–180.
14. Nachmann, M.; Ashe, J. H. Learned taste aversion in rats as a function of dosage, concentration, and route of administration of LiCl. Physiol. Behav. 10:73–78; 1973.
15. Paintal, A. S. A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst. J. Physiol. 126:255–270; 1954.
16. Pappas, T. N.; Melendez, R. L.; Debas, H. T. Gastric destension is a physiological satiety signal in the dog. Dig. Dis. Sci. 34:1489–1493; 1989.
17. Quast, U. Do the K^+ channels openers relax smooth muscle by opening K^+-channels? Trends Pharmacol. Sci. 14:332–337; 1993.
18. Quast, U.; Cook, N. S. In vitro and in vivo comparison of two K^+ channel openers, diazoxide and cromakalim, and their inhibition by glibenclamide. J. Pharmacol. Exp. Ther. 250:261–277; 1989.
19. Scharrer, E.; Del Prete, E.; Giger, R. Hepatic branch vagotomy enhances glucoprivic feeding in food-deprived old rats. Physiol. Behav. 54:259–264; 1993.
20. Scharrer, E.; Geary, N. Regulation der Futteraufnahme bei Monogastriden. Übers. Tierernährg. 5:103–122; 1977.