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Feeding Behavior Response of Zucker Rats to Proglumide, a CCK Receptor Antagonist[1]

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McLAUGHLIN, C. L., S. R. PEIKIN AND C. A. BAILE. *Feeding behavior of Zucker rats to proglumide, a CCK receptor antagonist.* PHARMACOL BIOCHEM BEHAV **18**(6) 879–883, 1983.—Proglumide not only antagonizes the gastric secretion induced by histamine, tetragastrin and ligation of the pylorus, but also antagonizes the effects of cholecystokinin (CCK) on contraction of the gallbladder and stimulation of amylase secretion from the pancreas. Since CCK is a putative satiety peptide, it was hypothesized that administration of proglumide would increase food intake by inhibiting the satiety effect of CCK. Zucker obese (638±26 g) and lean (462±24 g) rats, trained to bar press for food pellets, were administered intragastrically 0.25, 0.42 or 0.59 g/kg proglumide 15 min before the end of a 3- or 6-hr fast. Feeding behavior was analyzed during the subsequent 18 hr using an automated data collection system. Food intakes, while not affected by proglumide treatment during the first meal (4.30 vs. 4.30 g) were increased 3 (5.60 vs. 4.92 g, $p<0.02$) and 18 (34.0 vs. 32.5 g. $p<0.008$) hr after treatment. In addition, water intake 18 hr after proglumide treatment was increased (46.8 vs. 41.5 ml, $p<0.001$) and food intake to water intake ratio was decreased (0.74 vs. 0.80 g/ml, $p<0.004$). These responses were not different in obese compared with lean rats or in 3-hrs vs. 6-hr fasted rats. Daily feeding patterns in 6-hr fasted obese rats treated with 0.59 g/kg proglumide were characterized by increased meal frequency (12.4±1.0 vs. 9.6±1.1, $p<0.001$) and decreased average meal size (3.1±0.2 vs. 3.6±0.3 g, $p<0.03$). In 3-hr fasted obese and lean rats serum concentrations of proglumide were 7.1±2.1 mg/dl 20 min and 6.6±1.5 mg/dl 120 min after administration of 0.59 g/kg proglumide, indicating levels remained significantly elevated after 120 min. Since proglumide has been shown previously to be an antagonist to the effects of CCK on gallbladder, gastric and pancreatic function, it is postulated that increased food intake in Zucker obese and lean rats after proglumide administration is a result of antagonism of the receptor for the effect of CCK on satiety.

Cholecystokinin CCK Proglumide Zucker rats Satiety Feeding behavior Food intake Meal patterns

THE structure of proglumide (DL-4-benzamido-N,N-di-n-propyl-glutaramic acid) is similar to that of the C-terminal chain of gastrin. Proglumide is a competitive antagonist of gastrin and in rats inhibited volume and amount of acid secreted in response to administration of histamine and tetragastrin as well as pylorus ligation [9, 12, 16]. Because of these actions proglumide has been administered to patients with gastric and duodenal ulceration [12]. In addition to these effects on gastric secretion proglumide has been shown to antagonize the effects of caerulein, a peptide with CCK-like activity, on enzyme secretion from the pancreas and gallbladder contraction [6]. In dispersed pancreatic acini the dose response curve for CCK-stimulated amylase secretion was shifted to the right by use of proglumide and this effect was correlated with proglumide inhibition of binding of ^{125}I-CCK to receptors and of calcium outflux [5]. The effect of proglumide on the pancreas appeared to be specific for secretagogues that interact with CCK receptors since proglumide did not inhibit amylase secretion stimulated by other secretagogues whose effect is mediated by cyclic AMP or which act through receptors other than those for CCK.

CCK is proposed to be a satiety peptide in the control of food intake [15]. Increased serum concentrations of CCK either by exogenous administration of CCK or by stimulation of endogenous secretion of CCK from the duodenum are associated with decreased food intake [2, 3, 4, 11]. These responses have been accompanied by normal satiety behaviors in rats [1]. Since proglumide antagonizes the effect of CCK on pancreatic secretion and gallbladder contraction, we hypothesized that it may also block the satiety effect of endogenously secreted CCK during a meal and thus elicit increased food intake. In these experiments proglumide was administered to rats intragastrically to determine if food intake could be increased as a result of its CCK-antagonist activity.

Zucker obese rats have been shown to be less sensitive

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than lean rats to the effect of CCK on pancreatic function and satiety [7,8]. If proglumide antagonized the satiety elicited by CCK released during a meal, which would be demonstrated if food intake were increased, it was hypothesized that the response of obese rats would be less than that of lean rats. However, since obese Zucker rats are more sensitive than lean rats to the effect of CCK on gastric emptying and intestinal transit rate [9], which, however, are likely to be independent of that on food intake, they may be more sensitive to proglumide antagonism of effects of CCK.

METHOD

Five obese and five lean male Zucker rats (initial body weights 638±26 vs. 462±24 g, paired-$t=8.00$, $p<0.001$) were housed individually in Plexiglas cages in a room maintained at constant temperature (20°C) and 12 hr light-dark cycle. They were trained to press a bar to obtain each food pellet (P. J. Noyes Company) using a continuous reinforcement schedule. They were adapted to being fasted for 6 hr starting at "light on" and in the second experiment were adapted to being fasted for 3 hr beginning 3 hr after "lights on." Thus, treatments were administered at the same time of day in both experiments. Each obese rat was paired with a lean rat and each pair of rats was administered control (5.0 ml/kg water), given gastrically using a dosing needle, or one of three doses of proglumide (in 5.0 ml/kg water, pH=8.0). Crossover designs were used for assigning treatments for each dose of proglumide. On the first treatment day 2 pairs were given control and the other 3 pairs were given proglumide treatments; on the subsequent treatment day the control and proglumide treatments were reversed for each pair of rats. The doses of proglumide tested were 0.25, 0.42 and 0.59 g/kg. Fifteen min after treatments were administered, rats were allowed to press the bar to obtain food during the subsequent 18 hr. Each bar press was recorded on a magnetic tape with a time base using a data acquisition system (Massey-Dickinson). The data on the magnetic tape were then stored and analyzed using a Sperry Univac V77 computer. To determine the effects of proglumide on food intake, the parameters measured were size of the first meal after the fast, and food intake 3 and 18 hr later. Feeding behavior was continuously monitored in response to 0.59 g/kg proglumide after both 3 and 6 hr fasts. Water intakes were measured 18 hr after treatment and food intake/water intake ratios were calculated. Analysis of variance was used to determine significance of treatment, length of fast, phenotype and interaction of treatment with length of fast and phenotype for each parameter.

In addition, serum concentrations of proglumide were measured in obese and lean rats 20 min and 2 hr after gastric dosing with 0.59 g/kg proglumide. Rats were fasted for 3 hr, administered proglumide and at the assigned time sacrificed and a portal vein blood sample taken. Serum was frozen and subsequently extracted with dichloromethane. Proglumide concentration was measured using high performance liquid chromatography and gas liquid chromatography and probenecid as an internal standard. (Analysis was performed by Dr. Ray Adams, Scott and White Clinic, Temple, TX) Data were subjected to analysis of variance to determine significance of effects of phenotype and time after administration. Paired-t tests were also used.

RESULTS

Food intake, while not affected during the first meal after a fast, was increased 3 and 18 hr after proglumide administration in both obese and lean rats. Size of the first meal after a fast was larger in obese than lean rats, 5.12 vs. 3.48 g, SE=0.16, $F(1,108)=41.97$, $p<0.001$ and after a 6-hr than 3-hr fast, 4.56 vs. 4.04 g, $F(1,108)=6.58$, $p<0.01$. First meal size was not affected by proglumide compared with control (4.30 vs. 4.30 g) and there was no interaction of treatment with phenotype or length of fast. However, 3 hr after proglumide treatment food intake was increased, 5.60 vs. 4.92 g, SE=0.16, $F(1,108)=5.78$, $p<0.02$, Fig. 1. In addition 3-hr food intake was greater in obese than lean rats, 6.05 vs. 4.47 g, SE=0.16, $F(1,108)=4.73$, $p<0.03$ and in 6-hr than 3-hr fasted rats, 5.62 vs. 4.90 g, $F(1,108)=6.58$, $p<0.01$. Interactions of treatment with phenotype and length of fast were not significant. By orthogonal comparisons 3-hr food intake was increased by 0.42 and 0.42 plus 0.59 g/kg proglumide, $F(1,108)=4.18$, $p<0.04$ and 7.86, $p<0.006$ respectively.

Administration of proglumide compared with control increased 18 hr food intake, 34.0 vs. 32.5 g, SE=0.4, $F(1,98)=8.85$, $p<0.004$, Fig. 1. Food intake 18 hr after treatment was greater in obese than lean rats, 35.5 vs. 31.0 g, $F(1,98)=71.72$,

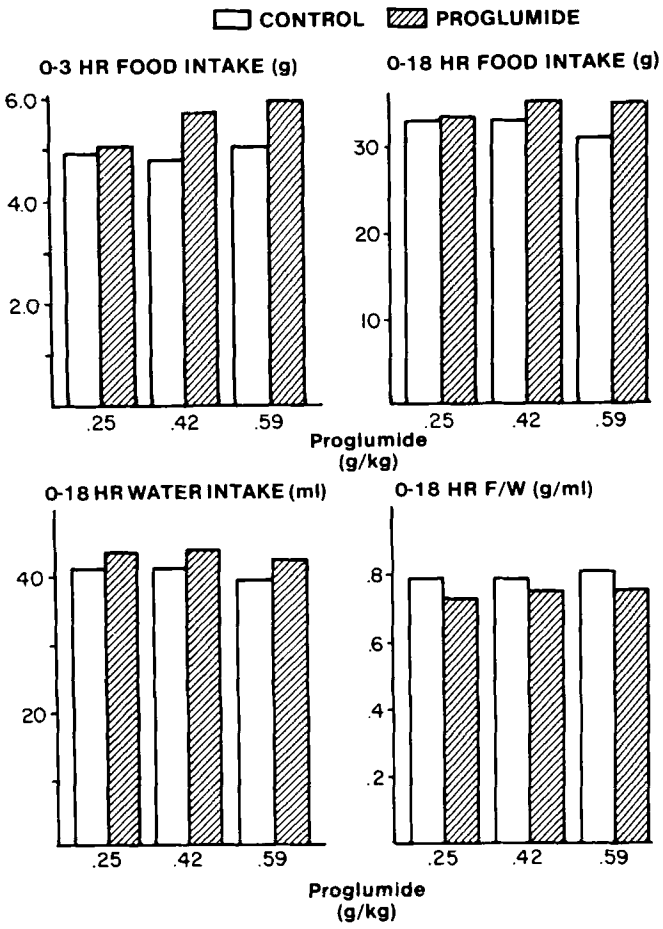

FIG. 1. Food intake, water intake and food intake/water intake ratio (F/W) of Zucker obese and lean rats in response to intragastric administration of proglumide. Standard errors for 0–3 hr food intake, 0–18 hr food intake, 0–18 hr water intake and 0–18 hr F/W ratio are 0.26 g, 0.41 g, 0.6 ml and 0.01 g/ml respectively.

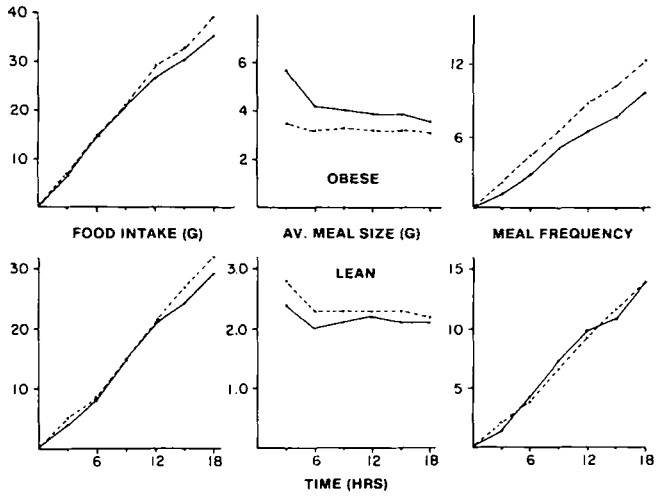

FIG. 2. Cumulative food intake, average meal size and cumulative meal frequency of 6-hr fasted Zucker obese and lean rats in response to intragastric administration of water or 0.59 g/kg proglumide. See text for statistical analysis.

$p<0.001$ and was greater after a 6-hr than 3-hr fast, 34.5 vs. 32.0 g, $F(1,98)=23.28$, $p<0.001$. Orthogonal comparisons of response to dose of proglumide demonstrated that both 0.42 and 0.59 g/kg increased 18-hr food intake, $F(1,98)=5.03$ and 5.01 respectively $p<0.03$. There was no interaction of treatment with length of fast, phenotype or dose; however interaction of treatment, length of fast and phenotype was significant, $F(1,98)=3.89$, $p<0.05$ and orthogonal comparisons demonstrated that proglumide treatment increased food intake in 3-hr but not 6-hr fasted obese rats, $F(1,98)=6.06$, $p<0.02$ and in 6-hr but not 3-hr fasted lean rats, $F(1,98)=6.18$, $p<0.01$.

Water intake after 18 hr was also increased by proglumide treatment, 46.8 vs. 41.5 ml, SE=7, $F(1,105)=32.93$, $p<0.001$. Water intake was greater in obese than lean rats, 46.0 vs. 42.2 ml, $F(1,105)=16.39$, $p<0.001$ and there was an interaction of treatment with phenotype, with water intake response to proglumide greater in obese than lean rats, 49.8 vs. 42.2 ml and 43.8 vs. 40.7 ml in obese and lean rats, respectively, $F(1,105)=5.90$, $p<0.02$. Use of orthogonal comparisons demonstrated that water intake was increased by each dose of proglumide, $F(1,105)=7.28$, $p<0.008$, 10.80, $p<0.001$ and 15.27, $p<0.001$ respectively.

Food intake to water intake ratio was decreased by proglumide, 0.74 vs. 0.80 g/ml, SE=0.01, $F(1,107)=9.17$, $p<0.003$. Food intake to water intake ratio was greater after a 6-hr fast than after a 3-hr fast, 0.80 vs. 0.74 g/ml, $F(1,107)=11.55$, $p<0.001$ but there was no difference in the ratio for obese compared with lean rats and there was no interaction of treatment and length of fast or phenotype. Orthogonal comparisons of response to each dose showed that food intake to water intake ratio was decreased after 0.25 and 0.59 g/kg proglumide, $F(1,107)=4.22$, and 4.16 respectively, $p<0.04$.

Meal patterns were analyzed after administration of 0.59 g/kg proglumide in rats fasted 3 and 6 hr. Average food intake during the 6 3-hr periods was increased in obese, 20.8 vs. 19.1 g, SE=0.8, $F(1,102)=7.17$, $p<0.009$ but not lean rats. Interval to the second meal was decreased more in obese compared with lean rats, 120 vs. 199 min, SE=15, $F(1,12)=5.86$, $p<0.03$; continued decreases in intervals between meals would have resulted in increased meal frequency. However, in obese rats trends for increased meal frequency and average meal size during the first 3 hr were not significant.

Proglumide affected feeding patterns more in 6-hr than 3-hr fasted rats, Fig. 2. As after the 3-hr fast, after the 6-hr fast proglumide treatment decreased the interval to the second meal in obese and lean rats, 125 vs. 192 min, SE=16, $F(1,13)=6.99$, $p<0.02$. Continued decreased interval between meals resulted in increased average meal frequency cumulated during the 3-hr intervals in obese, 7.4 vs. 5.5, SE=0.4, $F(1,102)=22.72$, $p<0.001$, which was different from the response of lean rats, $F(1,102)=12.67$, $p<0.001$. Average meal size was decreased in obese rats, 3.2 vs. 4.3 g, SE=0.1, $F(1,106)=32.55$, $p<0.001$ but not lean rats. Cumulative food intake was increased by proglumide treatment in obese and lean rats, average of 6 3-hr periods 2.12 vs. 19.7 g, SE=0.1, $F(1,103)=7.56$, $p<0.007$. Thus, in obese rats increased food intake occurred because of increased meal frequency in spite of decreased meal size, and in lean rats increased food intake occurred because of trends for increased meal size during the last 9 hr (2.3 vs. 2.1 g, NS) with no effect on meal frequency.

Analysis of serum concentrations of proglumide showed that 20 min after administration of 0.59 g/kg proglumide, concentrations were 7.07 ± 3.7 and 7.12 ± 2.5 mg/dl for obese and lean rats respectively. Two hours later serum concentration of proglumide in lean rats was decreased 40% to 4.33 ± 2.1 mg/dl while in obese rats was increased slightly to 8.89 ± 1.7 mg/dl. Serum concentrations of proglumide 2 hr after administration were lower in lean rats than obese rats (paired-$t=2.07$, $p<0.05$).

DISCUSSION

Proglumide, a gastrin and CCK receptor antagonist, increased food intake in Zucker lean and obese rats. The response to proglumide was not observed during the first meal after a fast, but became significant by 3 hr. Even though proglumide was administered 15 min before rats were allowed to bar press for food, it apparently was not absorbed rapidly enough to compete effectively for the CCK receptor for satiety during the first meal.

Intravenous administration of proglumide has decreased within 1 min pancreatic secretion and gall bladder contraction stimulated by caerulein [6]. However, in studies of stimulated gastric acid secretion proglumide has been administered intraperitoneally or subcutaneously and responses were not measured until 30 min later [6, 9, 16]. Thus comparisons of these results with the ones of the present paper are difficult because (1) proglumide was administered orally and (2) responses could be measured within 15 min of administration. However, volume of gastric acid secretion was decreased in 20 min in the perfused stomach by proglumide administered subcutaneously.

In the present experiment the meal of the rats ended in approximately 20 min or 35 min after proglumide was administered orally. To be effective in increasing food intake the proglumide should have been absorbed by the 15 min before feeding was allowed. By 20 min concentrations of approximately 7.0 mg/dl were achieved; however, this is only $^1/_5$ that effective in inhibiting amylase secretion stimulated by CCK in pancreatic acini and binding of CCK to its receptor (10^{-3} M or 33 mg/dl) [5]. Although in rats serum

proglumide concentrations did not increase 2 hr after administration, the sustained levels may have resulted in sustained binding to the CCK receptor and may have antagonized the CCK released during the meal. The interval to the second meal was shorter when the high dose of proglumide was administered and after 3 hr food intake of rats treated with proglumide was higher than that of rats treated with control.

Although serum concentrations of proglumide were not measured in rats 18 hr after treatment, in humans the ED_{50} dose 12 hr after treatment with proglumide was 0.85 g/kg, $2^1/_2$ times higher than that $2^1/_2$ hrs after treatment. Thus 12 hr after treatment in rats concentrations of proglumide would be expected to be sufficiently high to increased food intake [12].

The doses of proglumide which increased food intake (0.25 to 0.59 g/kg) were in the range of those administered to rats in previous studies to decrease gastric acid secretion stimulated by histamine, tetragastrin and pyloric ligation [9, 12, 16]. In rats administration of 0.25 g/kg/hr proglumide decreased gastric acid secretion stimulated by 2.5 mg/kg histamine [12]. Gastric acid secretion stimulated by intravenous infusion of 0.06 mg/kg/hr of tetragastrin or 6 mg/kg/hr histamine was decreased by ED_{50} doses of approximately 0.25 and 0.50 g/kg proglumide, respectively. Since the stimulated increase in acid output was similar, this result suggests that tetragastrin-stimulated gastric acid secretion was inhibited more readily by proglumide [9]. In rats with gastric fistulas proglumide (1 g/kg) completely inhibited the gastric acid stimulatory effects of tetragastrin (0.25 g/kg IM), histamine (5 mg/kg SC), and bethanechol (1 mg/kg SC) administered 30 min later [16]. In rats in which the pylorus was ligated, increases in gastric acid volume and total acid secreted occurring after 4–5 hrs and gastric ulceration present after 17–18 hrs were antagonized by administration of 0.5 to 1.0 g/kg proglumide at the time of ligation [9,16]. Thus, the doses we used are within the range used previously to inhibit stimulated gastric acid secretion and ulcer formation. Even lower doses of proglumide (0.005–0.050 g/kg) antagonized contraction of the gall bladder in guinea pigs and contraction of the pylorus in rats, stimulated by caerulein, a peptide with CCK-like activity [6].

Zucker obese rats are less sensitive than lean rats to the effects of CCK on food intake and, since proglumide is a CCK antagonist [7,8], it was hypothesized that obese rats would also be less sensitive to the effects of proglumide on food intake. While food intake responses 3 and 18 hr after administration of these doses of proglumide overall were not different in obese and lean rats, feeding behavior was affected more in obese than lean rats by administration of the highest dose of proglumide (0.59 g/kg).. Proglumide in 3-hr fasted rats decreased first intermeal interval more and increased 18-hr food intake more in obese than lean rats and in 6-hr fasted rats increased meal frequency and decreased meal size in obese but not lean rats, although 18-hr cumulative food intake was increased in both lean and obese rats. Thus, if anything, proglumide was more effective in obese than lean rats. One possible reason for increased responsivity of obese rats is that more proglumide was administered per animal, since dose was calculated on a per body weight basis and the obese rats weighed 38% more than the lean rats. Although obese rats are less sensitive than lean rats to the effects of CCK on satiety, they are more sensitive to the effects of CCK on gastric emptying and transit rate; thus the increased response of obese rats to proglumide, the CCK antagonist, suggests that gastric emptying and transit rate, as affected by CCK do not influence food intake.

At present the location of the receptors for the effect of CCK on satiety is unknown. There are CCK-responsive cells in the gallbladder (except in rats) [6], pancreas, stomach [12,13], small intestine [14] and parts of the brain [2]. Proglumide has been shown in the gallbladder to inhibit caerulein-stimulated contraction [6], in the pancreas to inhibit CCK-stimulated amylase secretion, calcium flux and binding [5], and in the stomach to inhibit caerulein-stimulated gastric secretion [6]. However, while CCK administered in the lateral cerebroventricles of sheep decreased food intake, proglumide similarly administered did not increase food intake (M. A. Della-Fera, personal communication). These results may be contrasted with those rats in which similar injections of CCK in the lateral ventricle did not decrease food intake, and peripheral administration of proglumide increased food intake. Differences in these responses may be due to species and/or route of administration. It has been proposed that since gastric, but not splenic or hepatic, vagotomy abolishes the feeding response to CCK, the receptors for the satiety effect of CCK may be in the stomach [14]. Others have shown that contraction of gastric smooth muscle, gastric acid secretion and pyloric contraction are stimulated by CCK and that the latter two actions are antagonized by proglumide. Thus, it seems likely that if the receptors for the satiety effect of CCK are on the stomach, they too would be inhibited by proglumide. Indeed, we have shown that administration of proglumide increased food intake in rats, presumably by inhibiting the satiety effect of CCK.

REFERENCES

1. Antin, J., J. Gibbs, J. Holt, R. C. Young and G. P. Smith. Cholecystokinin elicits the complete behavioral sequence of satiety in rats. *J Comp Physiol Psychol* **89**: 784–490, 1975.
2. Della-Fera, M. A. and C. A. Baile. Cholecystokinin octapeptide: continuous picomole injections into the cerebral ventricles suppress feeding. *Science* **206**: 471–473, 1979.
3. Gibbs, J., J. D. Falasco and P. R. McHugh. Cholecystokinin-decreased food intake in rhesus monkeys. *Am J Physiol* **230**: 15–18, 1976.
4. Gibbs, J., R. C. Young and G. P. Smith. Cholecystokinin-decreased food intake in rats. *J Comp Physiol Psychol* **84**: 488–495, 1973.
5. Hahne, W. F., R. T. Jensen, G. F. Lemp and J. D. Gardner. Proglumide and benzotript: members of a different class of cholecystokinin receptor antagonists. *Proc Natl Acad Sci USA* **78**: 6304–6308, 1981.
6. Mantovani, P., G. L. Puccinin and M. C. U. Adorni. Effecto della Xylamide su alcune azioni della caeruleina. *Arch Int Pharmacodyn Ther* **189**: 319–327, 1971.
7. McLaughlin, C. L. and C. A. Baile. Decreased sensitivity of Zucker obese rats to the putative satiety agent cholecystokinin. *Physiol Behav* **25**: 543–548, 1980.
8. McLaughlin, C. L., S. R. Peikin and C. A. Baile. Decreased pancreatic exocrine response to cholecystokinin in Zucker obese rats. *Am J Physiol* **242**: G612–G619, 1982.

9. Moos, A. B., C. L. McLaughlin and C. A. Baile. Effects of CCK on gastrointestinal function in lean and obese Zucker rats. *Peptides* **3**: 619–622, 1982.
10. Narumi, S., T. Hirata, K. Gomaibashi and M. Kanno. Inhibitory effects on gastrin secretion and ulcer formation of proglumide in rats. *J Takeda Res Lab* **29**: 85–91, 1970.
11. Peikin, S. R., C. L. McLaughlin and C. A. Baile. Trypsin inhibitors and food intake in Zucker obese rats. *Fed Proc* **41**: 386–1982.
12. Rovati, A. L. Inhibition of gastric secretion by anti-gastrinic and H_2-blocking agents. *Scand J Gastroenterol* **11**: 113–118, 1976.
13. Saladino, C. F., M. Walden, J. M. Berkowitz and M. Praissman. Characterization of specific receptors on isolated gastric glands for peptides of the gastrin-cholecystokinin family. *Gastroenterology* **82**: 1168–1171, 1982.
14. Scott, L. P. and R. W. Summers. Correlation of contraction and transit in rat small intestine. *Am J Physiol* **230**: 132–137, 1976.
15. Smith, G. P., C. Jerome, B. J. Cushin, R. Eterno and K. J. Simansky. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science* **213**: 1036–1037, 1981.
16. Watanabe, K., F. Kudo and H. Fukuda. Effects of DL-4-benzamido-N, N-di-n-propyl-glutaramic acid (Proglumide) on gastric secretion and ulceration in rats. *Pharmacometrics* **4**: 285–291, 1970.