2-Deoxy-D-Glucose Stimulates Acid Secretion from Chicken Proventriculus

Haruto NAKAGAWA, Masakazu NISHIMURA, and Norimoto URAKAWA

Department of Veterinary Pharmacology, Faculty of Agriculture, The University of Tokyo, Bunkyo, Tokyo 113

(Received 28 April 1983/Accepted 16 August 1983)

ABSTRACT. Effects of 2-deoxy-D-glucose (2DG) and insulin on gastric acid secretion and blood glucose level were examined to clarify the mechanism of acid secretion in chicken. Proventricular lumen of urethane-anesthetized chicken was continuously perfused with a dilute solution of sodium hydroxide and the change in the pH of effluent was monitored as an indicator of acid secretion. 2DG (50–200 mg/kg, i.v.) continuously lowered the pH level with a latent period of 30–60 min and slightly elevated the blood glucose level. By contrast, insulin (4 I.U./kg, i.v.) decreased the blood glucose to a half of the control level without changing the effluent pH. The duration of pH fall induced by 2DG was dose-dependent. The pH fall induced by 2DG was inhibited by an injection of pyruvic acid (80 mg/kg, i.v.). The pH fall was abolished by bilateral vagotomy at the cervical region and abolished by the administration of atropine (0.5 mg/kg, i.v.), proglumide (200 mg/kg, i.v.) or metiamide (0.5 mg/kg, i.v.). These results suggest that 2DG, which inhibits glucose metabolism, activates the cephalic phase through vagus nerve and thus increase cholinergic, gastrinergic and histaminergic acid secretions.

Secretion of gastric acid is enhanced by hypoglycemia in mammals [4]. 2-deoxy-D-glucose (2DG), which inhibits glucose metabolism, and tolbutamide, which causes hypoglycemia, have been used as useful tools for the study of the cephalic phase of gastric secretion in mammals [3, 5, 10–12, 19, 23, 24]. These agents have been known to stimulate hypothalamus by inhibiting the glucose utilization and thus augment the gastric acid secretion via vagus nerve [13, 23, 25]. In chicken, however, it has been reported that hypoglycemia induced by insulin does not stimulate the acid secretion [2, 17, 18]. It has also been known that shown feeding gives equivocal results and the sight of food fails to change the secretory activity in chicken [8]. From these results it has been suggested that cephalic phase does not play a role in gastric acid secretion in chicken [93]. Recently, however, it has been reported that the electrical stimulation on the vagus nerve enhances the

acid secretion in chicken [14, 20]. Burhol [2] also reported that 2DG stimulates the basal gastric secretion in chicken. These results imply a possibility that cephalic phase regulates gastric secretion also in chicken.

In the present experiments, the effects of 2DG and insulin on gastric acid secretion and blood glucose level were examined to clarify the mechanism regulating the gastric acid secretion through glucose metabolism in chicken.

MATERIALS AND METHODS

Male chicken of Lameat strain (Nikkei Shokusan), weighing 1.5–2.8 kg were used in this experiment. Gastric acid secretion was measured by a continuous recording of pH of proventriculus effluent [20] with some modifications of the original method for rats [6]. Before the experiments all the birds were starved for 24 hr but allowed to drink water. The birds were anesthetized by a single injec-

tion of urethane (1.7 g/kg, i.p.), operated for artificial respiration with air and kept warm by electric heating matt and 60 watt incandescent light. Silicone tube, 6 mm diameter, was inserted into lower esophagus below the crop. Another tube, 6 mm diameter, was inserted into the proventriculus through a cut in the proventriculus-gizzard junction. The operative technique for proventricular perfusion has been described in detail in the previous paper [21].

The proventricular lumen was continuously perfused with a diluted sodium hydroxide solution (1.25×10^{-4} N) at a flow rate of 2 ml/min using a peristaltic pump (Manostat). The pH of the perfusate was monitored by a glass electrode (Toa, pH unit FU20A) and recorded. The right and left basilic veins (*V. basilica*) were cannulated with silicone tubes for the purpose of administration of drugs, collecting blood samples and instillation of physiological saline solution. All the drugs were intravenously injected in a volume of 0.15 to 0.28 ml followed by an injection of 1.0 ml saline solution. The first injection was carried out at more than 1 hr after completing the operation. Blood glucose levels were determined by a glucose oxidative method (Wako Pure Chemicals, Glucose-B test).

The drugs used were as follows: 2-deoxy-D-glucose (2DG), pyruvic acid and atropine sulfate (Wako Pure Chemicals), insulin (Novo Yakuhin), metiamide (donated by Smith, Klein and Fujisawa) and proglumide (donated by Kaken Kagaku). Pyruvic acid solution was adjusted to pH 7.2 with sodium hydroxide (0.1 N) prior to use.

RESULTS

Effects of 2DG and insulin on blood glucose level

Blood glucose levels of chicken were

maintained between 200 and 250 mg/100 ml for at least 6 hr after completing the operation for acute proventricular fistel. The control blood glucose level of 219 ± 11 mg/100 ml (Mean $\pm$ S.E., $n=4$) did not change up to 2 hr of the 2DG injection (100 mg/kg, i.v.) and slightly increased thereafter (Fig. 1). In contrast, insulin (4

Fig. 1. Effects of 2DG 100 mg/kg i.v. (○) and insulin 4 I.U./kg i.v. (□) on blood glucose. * and **: Significantly different from control (the value at 0 min), $P<0.05$ and $P<0.01$, respectively.

Fig. 2. Effects of 2DG and insulin on the pH of proventricular perfusate.

- (A) 2DG 50 mg/kg i.v.
- (B) 2DG 100 mg/kg i.v.
- (C) Insulin 4 I.U./kg i.v.

I.U./kg, i.v.) significantly reduced the blood glucose level from 224 ± 15 mg/100 ml ($n=4$) to 130 ± 26 mg/100 ml ($n=4$, $P<0.05$) after 1 hr of dosing (Fig. 1).

Effect of 2DG and insulin on gastric acid secretion

The acidity of the proventricular effluent from untreated chicken was at a constant level between pH 5 to 7 during the 12 hr observation periods. 2DG (50 to 200 mg/kg, i.v.) lowered the pH with a latent period of 30–60 min; lower dose of 2DG (50 mg/kg, i.v.) produced an un-

stable pH decrease, while higher dose (100 to 200 mg/kg, i.v.) induced a constant decrease (Fig. 2-A and B). The pH fall lasted for 1 to 6 hr depending on the dose of 2DG (data not shown). Insulin (0.5 to 8.0 I.U./kg, i.v.), on the other hand, did not change the pH of the proventricular effluent in most of the chicken as shown in Fig. 2-C, although a slight decrease in pH was observed in 1 out of 8 chicken injected with 4 I.U./kg insulin.

The properties of the pH fall induced by 2DG
The pH fall induced by 2DG (100 mg/

Fig. 3. Effect of pyruvic acid (80 mg/kg i.v.) on the pH fall induced by 2DG (100 mg/kg i.v.).

Fig. 4. Effect of bilateral vagotomy on the pH fall induced by 2DG.

- (A) Vagotomy before the administration of 2DG (100 mg/kg i.v.).
(B) Vagotomy after the administration of 2DG (100 mg/kg i.v.).

Fig. 5. Inhibitory effects of ATR, PGM or MTM on the pH fall induced by 2DG (100 mg/kg i.v.).

- (A) ATR; Atropine sulfate (0.5 mg/kg i.v.).
(B) PGM; Proglumide (200 mg/kg i.v.).
(C) MTM; Metiamide (0.5 mg/kg i.v.).

kg, i.v.) lasted for approximately 4 hr (Fig. 2-B). Pyruvic acid (80 mg/kg, i.v.), administered 2 hr after the 2DG injection, increased the mean pH level (Fig. 3).

In bilaterally vagotomized birds, the effluent pH was not affected by a 2DG injection (Fig. 4-A). However, the decreased effluent pH in the 2DG-treated chicken was returned to the control level by the bilateral vagotomy (Fig. 4-B).

Atropine (0.5 mg/kg, i.v.) injected after the onset of the pH fall induced by 2DG (100 mg/kg, i.v.) transiently antagonized the effect of 2DG (Fig. 5-A). The duration of the effect of atropine was dose-dependent. Proglumide (200 mg/kg, i.v.), an antigastric agent [22], and metiamide (0.5 mg/kg, i.v.), a histamine H_2 -receptor blocking agent [1], also transiently antagonized the effect of 2DG (Fig. 5-B and C). The basal level of the proventricular pH was not altered by the bilateral vagotomy (Fig. 4-A) or by administration of atropine, proglumide or metiamide (data not shown, $n=3-6$ each).

DISCUSSION

It is known in mammals that the hypoglycemia induced by insulin [4, 23, 25] or tolubutamide [7] stimulates the hypothalamus to increase the acid secretion through vagus nerve. However, Long [18] and Lipunova [17] have reported that insulin did not stimulate the acid secretion in chicken, though it decreased the blood glucose level. Further, Burhol [2] reported that insulin rather decreased the gastric secretion. It is known that the blood glucose level in chicken (200–230 mg/100 ml) is twice as high as that in dogs or rats (approximately 100 mg/100 ml) [9, 16, 23]. In the present experiments, insulinization lowered the blood glucose level in chicken from $224 \pm$

15 mg/100 ml to 130 ± 26 mg/100 ml, which is still comparable to the normal blood glucose level in mammals. In contrast, insulin decreased the blood glucose level from 102.5 ± 11.8 mg/100 ml to 20.0 ± 5.1 mg/100 ml in rats [23] and from 100 mg/100 ml to 50 mg/100 ml in dogs [9]. Such a difference in the absolute blood glucose levels in insulin-treated mammals and chicken might explain the inability of insulin to increase acid secretion in chicken.

2DG is phosphorylated to 2DG-6-phosphate (2DG-6-P) at a rate similar to that of the phosphorylation of glucose. This product is not further metabolized, and inhibits glucose metabolism [10]. It was reported that the derivatives of glucose such as 2DG and 3-methylglucose, that inhibit metabolic pathways of glucose, stimulate hypothalamus and augment acid secretory activity through vagus nerve in mammals [13]. However, 2DG has shown to have hyperglycemic effect in dogs [10] and ducks [15]. In the present data, 2DG increased gastric acid secretion, supporting the previous report Burhol [2]. It was also found that 2DG slightly increased the blood glucose level. Further, it was found that the stimulatory effect on acid secretion by 2DG was prevented by injection of pyruvic acid, a metabolic intermediate of glucose. These findings support the hypothesis that hypothalamus is susceptible to the lack of metabolic intermediate of glucose, but not glucose itself, and stimulates the gastric secretion. It was also observed that the enhancement of acid secretion by 2DG was abolished by bilateral vagotomy, atropine, proglumide or metiamide. Accordingly, it may be suggested that the gastric acid secretion induced by 2DG in chicken is a cephalic phase-like response which is mediated via vagus nerve and partly due to gastri-

nergic and histaminergic transmissions.

ACKNOWLEDGEMENT. We are very grateful to Dr. Hideaki Karaki of our Department for his helpful advice on this manuscript.

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要 約

ニワトリの胃酸分泌におよぼす 2-deoxy-D-glucose の影響: 中川治人・西村昌数・浦川紀元 (東京大学農学部家畜薬理学教室) ——ニワトリの胃酸分泌と血糖値に対する 2-deoxy-D-glucose (2DG) の影響を検討した。ニワトリ (ラミート種, 雄, 1.5~2.8 kg) にウレタン麻酔, 人工呼吸, 生理食塩水の補液および保温を施し, その腺胃に急性フィステルを装着し, 胃内灌流液の pH を連続記録した。2DG (50~200 mg/kg *i.v.*) は30~60分間の潜伏期の後, 持続的な pH 低下を示し, 血糖値はわずかに上昇した。一方, insulin (4 I.U./kg *i.v.*) は血糖値を約 1/2 に減少させたにもかかわらず灌流液 pH を変化させなかった。2DG による pH 低下反応時間は濃度依存的であった。2DG 反応は pyruvic acid (80 mg/kg *i.v.*) により一部抑制され, 頸部迷走神経切断により完全に消失した。また 2DG 反応は, atropine (0.5 mg/kg *i.v.*), proglumide (200 mg/kg *i.v.*) および metiamide (0.5 mg/kg *i.v.*) により抑制された。以上の成績から 2DG は, 迷走神経を介する脳相を刺激し, コリン, ガストリンおよびヒスタミン作動性胃酸分泌を促進することが示唆された。