Effects of Arginine and Cold Exposure on Secretory Responses of Insulin, Glucagon and 11-Hydroxy corticosteroids in Piglets

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The effects of the intravenous injection of arginine (1.25 mmoles/kg) on plasma insulin, glucagon, 11-hydroxycorticosteroids (11-OHCS) and glucose were compared in castrated Landrace piglets between the thermoneutral (TN, 22°C) and the cold environment (2°C) groups with constant relative humidity (60%). Plasma insulin rose transiently from 8 μU/ml in initial level to 17 μU/ml 5 minutes after the arginine injection in the TN environment. Likewise, plasma glucagon rose from 122 pg/ml to 253 pg/ml. The cold exposure augmented the insulin and the glucagon responses to arginine. The 11-OHCS level before the arginine injection was 6.5 μg/dl in the TN environment. It increased significantly 4 and 7 days after cold exposure (11.9 μg/dl and 9.4 μg/dl). The arginine injection in the TN environment induced plasma 11-OHCS and glucose. These responses to arginine were augmented by the cold exposure. The potentiation of arginine-induced insulin secretion by the cold exposure in piglets is contrast to the previous results obtained in sheep.—Key words: Arginine, Insulin, Glucagon, Piglet.


When animals are exposed to cold, their neuro-endocrine system changes to a state of the increased sympathetic activity to maintain their nutrient homeostasis. It is known that unstable state of hormonal balance during the course of this adaptation may relate to the occurrence of some infectious diseases via the disfunction of immune system [14] as well as metabolic disorders [4].

Cortisol secretion which has a close relation to the immune response is augmented by several types of stresses such as cold exposure [5], exercise [2], electric shock [16], and confinement [7]. Meanwhile, insulin has also been investigated concerning their effect on the immune system [3, 10, 11]. The secretion of glucagon is reported to enhance by body damages such as burn [33], and severe infections [24].

The previous experiment on sheep revealed that cold exposure reduced insulin secretion [26, 27] and promoted arginine-induced glucagon secretion [28]. These changes have been little studied in pigs. The present study was carried out to know the effect of cold environment on insulin, glucagon, 11-hydroxy corticosteroids (11-OHCS) levels in piglets.

MATERIALS AND METHODS

Animals: Eleven castrated Landrace piglets were used. They were kept in a room controlled 22°C for 20 days. They were fed a concentrate chow (High L, Nippon Haigoshiryo Co., Ltd.) at 11:00 and 17:00 hr and water ad libitum. Each piglet was kept in a free-size cage made of wooden bars. The maximal width and length of each cage were 50 and 120 cm. A steel net coated with polyvinyl resin (Sani Deck, meshes 1/2×3/4 cm) was employed as a floor.

Experimental chamber: Experiments started at 60 days old (mean body weight, 24.1kg) and ended at 70 days old (mean body weight, 30.3 kg). Two of the animals employed were
accommodated in a test chamber (4.23×4.74 ×2.70 m; width, depth, and height). Light-dark cycles (lights on; 6:00-18:00 hr) and relative humidity (60±10%) were maintained constant throughout the experiments. Temperature regulated fresh air was introduced to the test chamber at the rate of 0.06 m³/second with air velocity of approximately 0.15 m/second.

Venous catheterization and blood sampling: Ten days before the onset of the experiment, each animal had a catheter implantation into the external jugular vein. The animals were premedicated with Ketamine-HCL (0.5 ml/kg), followed by halothane anesthesia. The skin of the lower neck above the jugular vein was incised in about 6 cm long, and the vein was partially exposed. Subsequently, an anti-thrombogenic heparinized polyurethane tube (e.d.:1.2 mm, i.d.; 0.8 mm, and 1.:100 mm, Toray Co., Ltd.) was inserted to the vein with an aid of a guide needle. The tube was connected to a silicone rubber tube (e.d.; 2.0 mm, i.d.; 1.0mm, and 1., 400 mm, Fuji Systems Co., Ltd.). A doubled anchor was made with silicone paste near the connection, and was fixed to the connective tissues with silk threads. The free end of the silicone catheter was pulled out at the rear of the neck. Each animal received 200 IU of heparin per 1 ml of sterilized saline through the catheter once a day. A polyethylene tube (5 cm in length) with a sealed end was inserted into the silicone catheter as a stopper. Each animal was given antibiotics (50 mg of Sodium Ampicillin, Toyo Jyozo Co., Ltd., and 40 mg of Doxycycline, Taito Pfizer Co., Ltd.) every day after surgery. Ampicillin was administrated through the venous catheter, and Doxycycline was given in drinking water. Each 7 ml of samples were collected 10 times during an experiment.

Experimental procedure: The experiment was performed on a thermoneutral(TN) environment (22±1°C) and on days 1, 4, and 7 of cold environment(2±1°C) employing 8 piglets. As a control experiment, other 3 piglets received the same experimental procedure as the cold exposed piglets except for the environmental temperature (22°C). Arginine (L-arginine monohydrochloride) solution was prepared at a concentration of 2.5 M, adjusted to pH 7.4 with 2.5N-NaOH, and given into the jugular vein through the catheter at a dose of 1.25 mmoles/kg in one minute. All experiments were carried out in a period between 9:00 and 11:00 a.m. No food was given during the experiment, but water was freely accessible.

Measurements: A blood sample collected into a heparinized syringe was immediately transferred into a polyethylene tube in ice water and centrifuged at 4°C. A part of a blood sample for glucagon assay was placed in another tube containing 1000 KIU/2 ml blood of Antagasan (trypsin inhibitor, Hoechist) and was centrifuged similarly. Plasma obtained by centrifugation was stocked −20°C until assayed. Plasma insulin was assayed by the radioimmunoassay method by Morgan and Lazarow [19] with a slight modification. Plasma glucagon was determined by the radioimmunoassay [20]. Anti-glucagon serum G42-E, which reacts only with pancreatic glucagon, was employed [22]. Plasma 11-OHCS was fluorospectrometrically determined [17]. Plasma glucose was assayed by the glucose oxidase method by Huggett and Nixon [15]. Rectal temperature and heart rate were measured with a mercury thermometer and a stethoscope, respectively.

Statistical analysis: All the values were expressed as mean ±S.E. Comparison of mean values were made by Student’s test.

RESULTS

In the TN environment, arginine administration to the piglets resulted in a significant rise (P<0.01) in plasma insulin level (Fig. 1). When animals were exposed to cold for 1, 4 or 7 days, the maximum insulin levels observed 5 minutes after the arginine treatment
were higher than that in the TN environment (P<0.01). The plasma glucagon level rose 5 minutes after the arginine injection and was followed by a gradual decline to the basal level for 30 minutes. The cold exposure accelerated the arginine-induced glucagon secretion. The maximum values of glucagon 5 minutes after arginine on days 1, 4, and 7 were significantly higher (P<0.05, P<0.01, and P<0.01) than that in the TN environment.

Plasma 11-OHCS levels in both groups tended to decline soon after the arginine injection, but return to the basal level gradually (Fig. 2). The basal 11-OHCS value before the arginine injection rose by the cold exposure; significant elevations were observed on day 4(P<0.01) and day 7(P<0.05).

Intravenous administration of arginine in the TN group was followed by a slight rise in blood glucose within 5 minutes and then, glucose levels declined under the basal level in 15 minutes (113.3±6.0 mg/dl, Fig. 2). The magnitude of this decrease in plasma glucose levels was intensified by the cold exposure. The hematocrit values virtually unchanged throughout the experiment (Fig. 3). Rectal temperature was not affected by the cold exposure (Fig. 3). Shivering was observed in the piglets during the course of the exposure. Heart rate was not affected by the arginine injection, but enhanced significantly by the cold exposure (Fig. 3).

No change in appetite was noticed between the piglets exposed to cold and the TN condition. The body weight of the control piglets was 22.3±1.2 kg on the first day of experiment and 28.7±1.8 kg on the last day. Those of the piglets for the cold experiment were 24.1±2.1 kg and 30.3±1.7 kg. No significant difference in the rate of the body weight gain was found between both groups(P>0.1).
Fig. 2. Plasma 11-OHCS and glucose responses to intravenous injection of arginine in piglets under the thermoneutral (●) or the cold (○) environment. Symbols are explained in Fig. 1.

Fig. 3. Hematocrit, rectal temperature, and heart rate responses to intravenous injection of arginine in piglets under the thermoneutral (●) or cold (○) environment. Symbols are explained in Fig. 1.

DISCUSSION

Present experiment showed that changes in hormonal levels and heart rate were induced by exposing piglets to cold. It is widely accepted that the sympathetic nervous system is stimulated by cold exposure. The increase in the heart rate by cold exposure will be due to the activation of sympatho-adreno-medullary system which release epinephrine and norepinephrine [25,31]. An elevation of the heart rate in the piglets exposed to the cold was similar to that observed in sheep [25].

The decline of the plasma glucose level by the arginine injection was preceded by a slight rise. The degree of the hypoglycemia tended to be promoted by the prolongation of exposure to cold (Fig. 2). This promotion of hypoglycemia may be caused by the enhancement of arginine-induced insulin secretion since insulin peaks observed in this study are sufficient to counteract the elevation of plasma glucose in swine [30].
The present data indicated that arginine-induced glucagon secretion was promoted in the piglets exposed to cold. Glucagon which is one of the most important hormones in regulating the glucose metabolism is considered to be a kind of stress-related hormone. Some observations are available concerning glucagon increase following burns [33], external wounds [18], and severe infections [24]. Few reports have been investigating the level of glucagon following cold stress. Our previous report [28] showed that arginine-induced glucagon secretion in sheep was accelerated remarkably by reducing the room temperature from 20°C to 0°C.

Plasma corticosteroids levels affect the state of the immune response [1, 14]. Shimizu et al. [29] observed that cold exposure delayed the initiation of the immune response in piglets inoculated with transmissible gastroenteritis viruses. In the present study, piglets exposed to cold had a gradual rise in plasma 11-OHCS levels, and reached the maximum level on day 4. The result was in agreement with the report of Rafai et al. [23] who exposed pigs (body weight, 36 kg) to cold (from 22°C to 5°C) and found that the plasma cortisol level increased gradually, and reached its maximum between 48 and 72 hrs.

The present study showed that the basal values of plasma insulin tended to increase and arginine-induced insulin secretion was augmented by exposing piglets to cold. The result was not consistent with our previous reports in which cold exposure to sheep abolished insulin secretion induced by arginine or other insulino-secretagogues [26, 27]. We found that the suppression of insulin was attributable to stimulation of adrenergic-α-receptor of B cells in pancreatic islets in sheep [26]. It is well known that cold exposure enhances urinary excretion of catecholamines and the heart rate in many kinds of animals [31]. Moreover, inhibition of insulin release by epinephrine has been shown in number of different species [6, 26] including pigs [12]. Therefore, it is very enigmatic that insulin secretion was enhanced in a cold environment in piglets.

There have been observations that arginine-induced insulin secretion is not affected by epinephrine which is released by cold stimulation [9], and that arginine-induced insulin release is not mediated by cyclic-AMP [8] which is thought to play as a second messenger in releasing insulin in normal condition [32]. We have observed that among insulino-secretagogues such as glucose and tolbutamide, the inhibition of insulin secretion by cold exposure was least manifested by arginine in sheep [27]. By testing these drugs also in piglets in future, some clues to make clear the phenomenon that arginine-induced insulin secretion is promoted by cold exposure in piglets will be provided.

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REFERENCES


要約

仔豚における寒冷曝露時のアルギニン静脈内投与にともなう血中インスリン、グルカゴンおよび11-ハイドロキシコルチコステロイドの変化：高橋秀之（農林水産省家畜衛生試験場研究第4部）——去勢雄仔豚を常温環境（22℃、相対湿度60％）あるいは寒冷環境（2℃、相対湿度60％）において第1, 4および7日にアルギニンを静脈内投与し、血漿インスリン、グルカゴン、11-ハイドロキシコルチコステロイド（11-OHCS）およびグルコースの濃度を観察した。常温環境では投与5分後に血漿インスリンが一過性に上昇して17 μU/ml（基礎レベル8 μU/ml）となり、血漿グルカゴンも上昇して253 pg/ml（基礎レベル122 pg/ml）となった。寒冷環境では、アルギニンに対するインスリン、グルカゴンの分泌反応は常温環境におけるよりも増強された。血漿11-OHCSの常温環境下でのアルギニン投与前後の値は6.5 μg/mlであった。寒冷曝露により、11-OHCS値は上昇し、曝露第4日（11.9 μg/dl）および7日（9.4 μg/dl）の値は常温環境のそれにくらべて有意に高かった。アルギニン投与により、常温環境では血漿11-OHCS値は投与直後に一時減少し、血漿グルコース濃度も投与15分後には低下したが、寒冷環境下ではこれよりさらに低い値を示した。このような仔豚の寒冷環境におけるアルギニン投与に対するインスリン上昇反応はヒマンのそれとは異なる所見であった。