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The hyperinsulinemia produced by concanavalin A in rats is opioid-dependent and hormonally regulated

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Abstract

The present study examines the effect of concanavalin A (Con A) on the blood insulin and glucose levels of rats. Male and female rats treated with Con A (62.5-500 µg/kg) for three days showed a dose- and time-dependent hyperinsulinemia that lasted more than 48 h. Male rats were more sensitive to Con A. Thus, 6 h after treatment with Con A the circulating insulin levels in male rats had increased by 85% (control: 10.2 ± 0.9 mU/l and Con A-treated: 18.8 ± 1 mU/l) compared to only 38% (control: 7.5 ± 0.2 mU/l; Con A-treated: 10.3 ± mU/l) in females. An identical response was seen after 12 h. Con A (250 µg/kg) produced time-dependent hypoglycemia in both sexes but more pronounced in males. There was no correlation between the hypoglycemia and hyperinsulinemia described above. The Con A-induced hyperinsulinemia in rats of both sexes was abolished in gonadectomized animals (intact males: +101 ± 17% vs orchiectomized males: -5 ± 3%; intact females: +86 ± 23% vs ovariectomized females: -18 ± 7.2%). Pretreating intact male and female rats with human chorionic gonadotropin also significantly inhibited the Con A-induced hyperinsulinemia. Estradiol (10 µg/kg, im) significantly blocked the Con A-induced increase in circulating insulin in male rats (101 ± 17% for controls vs 32 ± 5.3% for estradiol-treated animals, $P<0.05$) while testosterone (10 mg/kg, im) had no similar effect on intact female rats. Pretreating Con A-injected rats with opioid antagonists such as naloxone (1 mg/kg, sc) and naltrexone (5 mg/kg, sc) blocked the hyperinsulinemia produced by the lectin in males (control: +101 ± 17% vs naloxone-treated: +5 ± 14%, or naltrexone-treated: -23 ± 4.5%) and females (control: +86 ± 23% vs naloxone-treated: +21 ± 20%, or naltrexone-treated: -18 ± 11%). These results demonstrate that Con A increases the levels of circulating insulin in rats and that this response is opioid-dependent and hormonally regulated.

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- Concanavalin A
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- Blood glucose alterations
- Sex hormones
- Endogenous opioids
- Canatoxin

Introduction

Plant lectins are proteins capable of binding to carbohydrates on mammalian cells and membranes. Such binding can lead to a variety of effects including cell agglutination (1), modification of enzymes or receptors on the cell surface (2-5), stimulation of cell growth (6,7), and various hormone-like responses (8,9).

Concanavalin A (Con A) is a glucose/mannose-binding plant lectin isolated from jack bean (Canavalia ensiformis) seeds that binds extensively to mammalian cell surfaces (10) and exhibits multiplein vitro insulin-like effects (11,12). Thus, Con A stimulates glucose oxidation and hexose transport (13), inhibits epinephrine-stimulated lipolysis in isolated adipocytes (14) and inhibits insulin binding to intact adipocytes and liver cell membranes (12,15).

Lectins and related toxic seed proteins may share common pharmacological and biochemical properties, as has been shown for seeds of the castor bean (Ricinus communis), the jequiriti bean (Abrus precatorius) (16) and kintoki beans (Phaseolus vulgaris) (17).

Although Con A and canatoxin (CNTX), a toxic protein isolated from Canavalia ensiformis seeds, are structurally distinct (for a review, see Ref. 18), both substances induce platelet aggregation (19,20), trigger histamine secretion from peritoneal mast cells (21-23), and induce paw edema (24,25) and neutrophil chemotaxis in the peritoneal cavity of rats (26-28).

The administration of CNTX to rats raises circulating insulin levels (29) and produces hormonally regulated blood glucose alterations (30). Several of the effects described for this toxin (31,32), including hyperinsulinemia (29), are modulated by the endogenous opioid system. A long-lasting hypoglycemia is the main effect produced by CNTX in rats (33,34). The increased insulin levels and consequent hypoglycemia seen in these animals suggested that the toxin may act upon pancreatic islet ß-cells. This hypothesis has been confirmed by the observation that isolated rat pancreatic islets secrete insulin when exposed to CNTX (35).

The present study examines the effect of Con A on the blood insulin and glucose levels of rats. The involvement of opioids and a possible sex-related, hormone-dependent susceptibility to the effects of the toxin were also investigated.

Material and Methods

Male and female Wistar rats (200-250 g) were used. All experiments were done between 1:00 p.m. and 4:00 p.m. using rats that had been individually caged with free access to food and water for at least 24 h beforehand.

Con A (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.1 M phosphate buffer, pH 7.0, and administered sc to the animals every 24 h for three days. In most experiments, the dose of Con A was 250 µg/kg. At the desired time, blood samples were obtained from one control and one test group by cardiac puncture under chloral hydrate (350 mg/kg, ip) anesthesia.

Insulin levels were measured by radioimmunoassay (RIA) using a commercial kit (Pharmacia Diagnostics). The assays were performed in duplicate using 100-µl serum aliquots. The intra- and inter-assay coefficients of variation were ≤5.8% and ≤6.5%, respectively. The results are reported as mU of insulin per liter of serum or as a percent of basal levels (taken as 100%). Blood glucose levels were determined using a glucose oxidase method (36) and the results are reported as mmol glucose per liter of blood.

Different groups of rats (six animals per group) were submitted to one of the following protocols: group 1 consisted of intact males and females injected with Con A or vehicle. Group 2 consisted of gonadectomized males and females injected with Con A or vehicle solution three weeks after surgery. Group 3 consisted of intact males and

females pretreated with human chorionic gonadotropin (hCG) in accordance with the schedule used by Reich et al. (37). The animals were pretreated with hCG (40 IU/kg, im) over a three-day period. Treatment with Con A or vehicle was initiated 6 h after the first injection of hCG. Group 4 consisted of intact males and females pretreated every 72 h with three injections of 0.5 ml of depo-estradiol (10 µg/kg in corn oil, im) or depo-testosterone (10 mg/kg in corn oil, im), respectively. In both cases, the schedule described by Pomerantz et al. (38) was used. Treatment with Con A or vehicle solution was started on the eighth day of hormonal pretreatment. Group 5 consisted of intact males and females pretreated with naloxone (1 mg/kg, sc) or naltrexone (5 mg/kg, sc) 20 min before the administration of Con A or vehicle solution.

The results are reported as means ± SEM. Comparison of the means, reported as percent variation, was performed using the Kruskal-Wallis test. Other means were compared by the Student unpaired t-test. In both cases, a P value ≤0.05 was considered to be significant.

Results

Male and female rats treated chronically with Con A (62.5-500 µg/kg) developed hyperinsulinemia. Tables 1 and 2 show that the phenomenon was dose- and time-depend-

Table 1 - Dose dependence of the hyperinsulinemia induced by Con A in male and female rats.

Con A was injected *sc* every 24 h over a three-day period. Blood samples for insulin determinations were obtained 24 h after the last Con A administration. The results are reported as the mean ± SEM for six rats per group. *P≤0.05 compared to the control group (Student unpaired *t*-test).

Con A (µg/kg)	Females			Males		
	Serum insulin (mU/l)			Serum insulin (mU/l)		
	Control	Treated	Δ%	Control	Treated	Δ%
62.5	8.4 ± 0.9	9.5 ± 1.0	+13	10.2 ± 0.5	12.1 ± 2.1*	+19
125	8.0 ± 0.7	10.7 ± 0.6*	+34	9.4 ± 1.0	12.0 ± 0.3*	+27
250	8.2 ± 0.2	15.3 ± 0.3*	+86	8.3 ± 0.2	16.8 ± 0.7*	+101
500	9.0 ± 1.0	12.1 ± 0.5*	+34	11.2 ± 0.6	14.2 ± 0.2*	+27

Table 2 - Time course of the hyperinsulinemia induced by Con A in male and female rats.

Con A (250 µg/kg) was injected *sc* every 24 h over a three-day period. Blood samples for insulin determinations were withdrawn at the times indicated after the last Con A administration. Data are reported as the mean ± SEM for six rats per group. *P≤0.05 compared to the control group (Student unpaired *t*-test).

Time (h)	Females			Males		
	Serum insulin (mU/l)			Serum insulin (mU/l)		
	Control	Treated	Δ%	Control	Treated	Δ%
1	10.2 ± 1.0	11.4 ± 1.0	+12	9.2 ± 0.2	11.4 ± 0.4*	+24
3	12.2 ± 0.1	14.6 ± 0.2*	+20	8.3 ± 0.9	11.3 ± 0.3*	+35
6	7.5 ± 0.2	10.3 ± 0.9*	+38	10.2 ± 0.9	18.8 ± 1.0*	+84
12	9.1 ± 1.0	15.1 ± 1.0*	+66	11.4 ± 0.2	25.6 ± 1.0*	+124
24	8.2 ± 0.2	15.3 ± 0.3*	+86	8.3 ± 0.2	16.8 ± 0.7*	+101
48	10.5 ± 0.9	16.0 ± 0.4*	+52	9.7 ± 0.2	14.6 ± 0.9*	+51

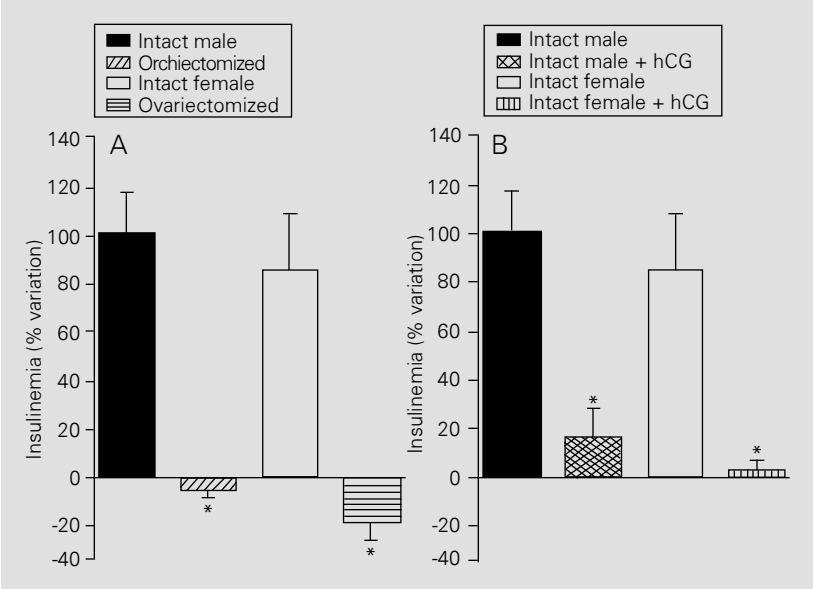

Figure 1 - Castration (A) and pretreatment with human chorionic gonadotropin (hCG) (B) inhibit the hyperinsulinemia produced by Con A in intact male and female rats. Con A (250 µg/kg) was injected *sc* every 24 h over a three-day period. Blood samples for insulin determinations were obtained 24 h after the last administration of Con A or vehicle. Each bar represents the mean ± SEM for six rats per group. The values for the treated animals are reported as the percent variation relative to the vehicle-treated controls taken as 100% (males 8.3 mU/l and females 8.2 mU/l). Gonadectomized males and females were injected with Con A or vehicle three weeks after surgery. Intact males or females received hCG (40 IU/kg) *im* every 24 h over a three-day period. Treatment with Con A or vehicle was started 6 h after the first injection of hCG. *P≤0.05 compared to intact (A) or non-pretreated (B) rats (Kruskal-Wallis test).

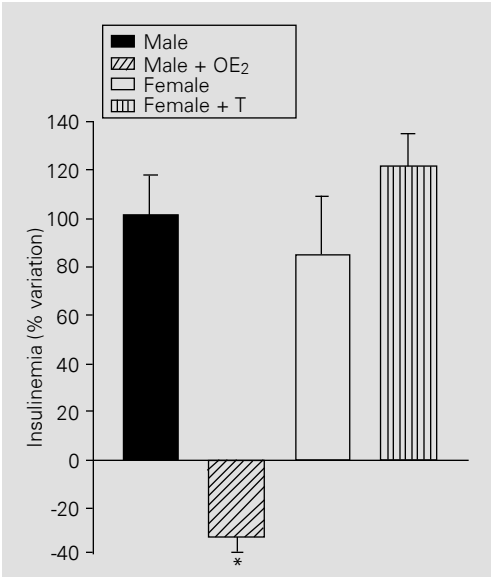

Figure 2 - Effect of pretreatment with estradiol and testosterone on the hyperinsulinemia produced by Con A in intact male and female rats. Male rats were pretreated with estradiol (OE_2, 10 µg/kg) and female rats with testosterone (T, 10 mg/kg). In both cases, the hormone was injected *im* every 72 h. Treatment with Con A (250 µg/kg) or vehicle was started on the eighth day of hormonal pretreatment. The values are reported as the percent change relative to the vehicle-treated controls taken as 100% (males: 8.3 mU/l and females: 8.3 mU/l). *P≤0.05 compared to non-pretreated rats (Kruskal-Wallis test).

ent, respectively, and that it lasted for more than 48 h.

Male rats were more sensitive to the hyperinsulinemic effect of Con A (Table 2). Thus, 6 h after the treatment with Con A (250 µg/kg), the circulating insulin levels in males had increased by 85% compared to only 38% in females. An identical situation was observed at 12 h (males, +124%; females, +66%).

The increase in circulating insulin levels produced by Con A in intact male and female rats was blocked when the animals were orchiectomized and ovariectomized, respectively. Similarly, pretreating intact male and female rats with hCG significantly inhibited the Con A-induced hyperinsulinemia (Figure 1).

Figure 2 shows that pretreating intact male rats with estradiol significantly inhibited the Con A-induced hyperinsulinemia while there was no significant change in intact female rats pretreated with testosterone.

Pretreating rats of both sexes with the opioid antagonists naloxone and naltrexone inhibited the hyperinsulinemic effect of Con A (Figure 3).

Male and female rats chronically treated with Con A (250 µg/kg) showed a biphasic and time-dependent change in blood glucose levels (Table 3). The early phase lasted for approximately 6 h after the last Con A injection while the late phase continued for up to 24 h and ended by 48 h. In the early phase of the response male rats exhibited hypoglycemia while female rats were hyperglycemic. However, 12 h after the last treatment with Con A (late phase), a persistent hyperglycemia was detected in males, while in females there was a significant but transient decrease in the circulating glucose levels (18%). Thus, rats of both sexes exhibited hypoglycemia but the response was more marked in males.

Discussion

The present results demonstrate that Con

A significantly increased the levels of circulating insulin in rats via a mechanism that was opioid-dependent and hormonally regulated. Changes in blood glucose levels were also detected in the lectin-treated rats.

Males were more sensitive than females to the hyperinsulinemic effect of Con A (Table 2). In addition, the increase in plasma insulin levels produced by Con A in rats of either sex completely disappeared in gonadectomized animals (Figure 1A), indicating that sex hormones play an important role in this phenomenon. The hyperinsulinemia produced by Con A was inhibited when rats of either sex were pretreated with hCG (Figure 1B). Since castration is known to elevate the circulating gonadotropin concentrations in male and female animals (39), the increase in insulin produced by Con A also appears to be modulated by these hormones.

Morphine and ß-endorphin are powerful stimuli for insulin secretion (40,41). Several investigators (42-44) have demonstrated that the effects of opioid-like peptides, in addition to being sex related, are also hormonally regulated. Our findings that the pretreatment of Con A-injected rats with opioid antagonists such as naloxone and naltrexone (45) completely blocked the hyperinsulinemia induced by this lectin (Figure 3) are consistent with the foregoing reports.

As described for CNTX (18,29,35), the Con A-induced hyperinsulinemia may also have resulted from a secretory action of this lectin on pancreatic ß-cells. However, this hypothesis was not supported by our data showing that male rats are more sensitive than females to Con A-induced hyperinsulinemia (Table 2) since it is well known that estrogens (46) and progesterone (47) intensify the secretory response of ß-cells. Estradiol significantly blocked Con A-induced increase in plasma insulin levels in male rats while no significant change was seen in intact females treated with testosterone. This is an unusual finding since testosterone generally reduces the secretory response of pancreatic ß-cells in response to glucose (48).

The regulatory mechanism that maintains the systemic glucose balance involves hormonal, neural, and autoregulatory factors. Hypoglycemia is only an indication that the rate of glucose efflux from the circulation exceeds that of glucose influx. The time course of the hyperinsulinemia (Table 2) and blood glucose alterations (Table 3) in-

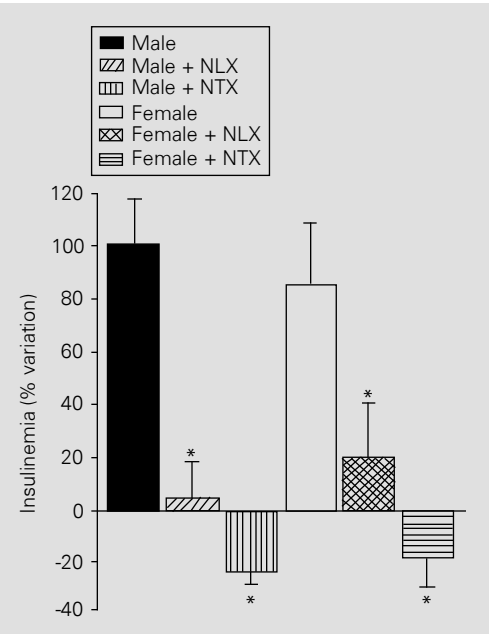

Figure 3 - Naloxone and naltrexone inhibit the hyperinsulinemia induced by Con A in rats. Intact male and female rats were pretreated *im* with naloxone (NLX, 1 mg/kg) or naltrexone (NTX, 5 mg/kg) 20 min before the injection of Con A (250 µg/kg) or vehicle. The animals were bled for insulin determination 24 h after the last treatment with Con A or vehicle. The values are reported as the percent change relative to vehicle-treated controls taken as 100% (males, 8.3 mU/l and females 8.2 mU/l). *P≤0.05 compared to non-pretreated rats (Kruskal-Wallis test).

Table 3 - Time course of the blood glucose alterations induced by Con A in male and female rats.

Con A (250 µg/kg) was injected *sc* every 24 h over a three-day period. Blood samples for glucose determinations were obtained at the times indicated after the last Con A administration. The results are reported as the mean ± SEM for six rats per group. *P≤0.05 compared to the control group (Student unpaired *t*-test).

Time (h)	Females			Males		
	Blood glucose (mmol/l)			Blood glucose (mmol/l)		
	Control	Treated	Δ%	Control	Treated	Δ%
1	9.0 ± 0.2	10.6 ± 0.2*	+18	10.2 ± 0.3	8.5 ± 0.2*	-17
3	8.8 ± 0.4	10.8 ± 0.3*	+23	9.0 ± 0.1	6.0 ± 0.3*	-33
6	9.1 ± 0.1	10.8 ± 0.1*	+19	8.9 ± 0.2	7.4 ± 0.2*	-17
12	8.8 ± 0.2	7.2 ± 0.1*	-18	8.1 ± 0.1	9.4 ± 0.1*	+16
24	8.4 ± 0.2	8.6 ± 0.1	-2	10.5 ± 0.1	13.6 ± 0.2*	+29
48	9.0 ± 0.3	8.1 ± 0.5	-10	11.0 ± 0.3	10.6 ± 0.1	-4

duced by Con A in rats of both sexes was not positively correlated with each other, indicating that these two responses were not necessarily related. However, it is interesting to note that the blood glucose alterations induced by the lectin in rats, like hyperinsulinemia, are sex-related, hormonally regulated and opioid-dependent (49), suggesting that a common pathophysiological mechanism is involved in these events.

The present data demonstrate that Con A significantly increased blood insulin levels in rats via an opioid-dependent, hormonally regulated pathway. Exactly how sex hormones and opioids are involved in this hyperinsulinemia is currently under investigation in our laboratory.

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