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The Control of Cholesterol Metabolism and Plasma Lipid Levels in Infant Rats

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Summary

Infant rats received an i. p. injection of insulin, anti-insulin serum, streptozotocin, antiglucagon serum or dexamethazone. All substances except the antiinsulin serum, raised the plasma triglyceride level. Both antisera decreased plasma cholesterol levels, while streptozotocin, insulin and dexametazone caused an increase. The activity of 3-hydroxy-3-glutaryl CoA reductase in liver and brown adipose tissue changed inversely to the cholesterol level. However, small intestinal enzyme activity was increased by insulin administration inspite of the rise in plasma cholesterol.

Key words
Plasma cholesterol – Plasma lipids – Infant rats – Plasma insulin – Glucagon – 3-hydroxy-3-glutaryl CoA reductase

Introduction

Plasma levels of cholesterol increase considerably after birth in both rats and humans (Hahn 1989). This rise is partly due to milk consumption but seems to have other (probably hormonal) causes as well.

In rat foetuses the plasma insulin levels are very high and fall to low levels after birth (Hahn 1979). At the same time the plasma levels of glucagon increase from very low prenatal values to very high ones soon after birth. These data suggest that perhaps insulin plays a minor role in infant rats. However, overfeeding infant rats by reducing the number of pups in the litter to 3 or 4 causes a rise in the plasma levels of both insulin and cholesterol within 2 days (Hahn 1984) and repeated injections of insulin to newborn rats results in accelerated weight gains and probably elevated cholesterol levels (Hahn 1984).

What then are the roles of insulin and glucagon in the infant rat? Are they different from those assumed in the adult? It is well established, for instance, that glucagon has an hypocholesterolaemic effect in adult rats (Friedman and Byers 1971). Yet in infant animals that have exceptionally high glucagon levels, plasma cholesterol levels are higher than at any other time in life. In view of the above results, it seemed pertinent to examine the effects of eliminating glucagon or insulin transiently from the body. We already attempted this in the case of ketogenesis (Hahn *et al.* 1991) in infant rats. The rate of ketone formation was suppressed by an anti-glucagon serum and enhanced by streptozotocin. In this work we examined the effects of streptozotocin, insulin and anti-insulin and anti-glucagon serum on plasma levels of cholesterol and triglycerides (TG) and on hepatic 3-hydroxy-3-methyl glutarylCoA reductase (HMGR).

Methods

Wistar infant rats were bred in our animal quarters. Litters were reduced to 9 animals one day after birth. They received i.p. injections of streptozotocin (0. 75 mg in 0. 05 M citrate, pH 4.5 per 100 g twice a day i.p.). Other animals received the antibodies to insulin (Antibovine insulin, ICN diluted with saline phosphate buffer 1:100) or to glucagon (Rabbit antiglucagon, ICN, similarly diluted) in a dose of 1 ml/100 g body weight) as described by Hahn *et al.* (1991) or 0.5 U/100 g body weight insulin for 2 days. They were killed on the third day of treatment. Ages on

Table 1
The effect of the injection of hormones and antihormones on plasma cholesterol levels of infant rats aged 11 to 14 days

Substance	Treated (mg/DL)	Control	p<
Stretozotocin	185(9)	123(3)	0.02
Antiglucagon	132(4)	153(5)	0.01
Anti-insulin	150(4)	184(3)	0.001
Insulin	180(5)	146(3)	0.001
Dexamethazone	213(5)	180(4)	0.01

Five to eight rats per group. Standard error in parentheses

Table 2
The effect of treatment on plasma triglyceride levels in infant rats aged 11 to 13 days

Substance	Treated(mg/DL)	Control	p<
Streptozotocin	879(234)	101(10)	0.01
Antiglucagon	158(14)	111(4)	0.01
nti-insulin	68(7)	100(6)	0.001
Insulin	215(11)	100(6)	0 001
Dexamethazone	275(5)	130(4)	0.001

Five to eight rats per group. Standard error in parentheses

Table 3
The effect of hormones and antihormones on HMGR activity in the liver (L), brown fat (BAT) and proximal small intestinal mucosa (P) of infant rats

Substance	Age	Treated	Control	p<
Streptozotocin L	11d	84(4)	131(11)	0.001
	35d	1161(92)	2005(81)	0.001
BAT	11d	18(1.3)	27(3)	0.01
Antiglucagon L	12d	96(3)	80(2)	0.01
Anti-insulin L	12d	22(2)	25(1)	NS
P	13d	28(1.)	20(0.8)	0.01
Dexamethazone L	12d	52(2)	129(4.6)	0.001

Five to eight rats per group. Standard error in parentheses. Values in pmoles/min/mg protein. NS = not significant.

Table 4
Changes as a percentage of control values in HMGR activity and plasma levels of cholesterol(CHOL) and triglycerides (TG) due to the indicated treatments

Treatment	Parameter		
	HMGR	CHOL	TG
Streptozotocin	−36	+50	+870
Antiglucagon	+20	−14	+42
Anti-insulin	+101	−19	−32
Insulin	−12	+23	+115
Dexamethazone	−60	+18	+111

that day are shown in the tables. Blood was collected from the neck region after decapitation. The small intestine was removed, rinsed thoroughly with cold saline and then the mucosa from the first third was expressed using a spatula and placed on ice. A piece of liver and brown adipose tissue from between the shoulder blades were also isolated and kept on ice. The tissues were homogenized in 2.25 mM sucrose, 25 mM TRIS pH 7.8 and centrifuged at 8000 rpm for 20 min. The supernatant was respun at 105 000 rpm for 60 min at 4 °C. The pellet was rehomogenized in about 1 ml of 100 mM sucrose, 50 mM KCl, 40 mM KH_2PO_4 and 30 mM EDTA, pH 7.2. Fresh dithiotreitol was added to give a final 20 mM solution. The "t" test was used to examine statistical significance. Cholesterol and triglycerides were determined using commercial kits (Biopacific Diagnostic Inc., W. Vancouver, B.C., Canada). The term "antihormone" is here taken to mean the antibody to a homone.

Results

In infant rats insulin raises the plasma cholesterol level, as reported previously (Hahn *et al.* 1977). The insulin antiserum, as expected, has the opposite effect. However, streptozotocin administration was found to act on the cholesterol level in the same way as insulin, suggesting that this drug has other effects in addition to its insulin suppressing action. The antiglucagon serum also lowers cholesterol levels (Tab. 1). It is apparent from Table 2 that plasma triglyceride levels are elevated after insulin, antiglucagon serum and streptozotocin administrations and decreased only by the anti-insulin serum. Dexamethazone raises both cholesterol and triglyceride levels.

Table 3 shows how HMGR activity was affected by hormones and antihormones in infant rats. It can be seen from the table that whenever plasma cholesterol levels are raised HMGR activity is decreased significantly except for the effect of anti-insulin serum, where the difference is not significant. Table 4 summarizes the data. It is obvious that the anti-insulin serum and streptozotocin have opposite effects on all the indicators studied again strongly suggesting that streptozotocin acts at a different level than the antiserum. This is also indicated by the very large spread of TG values after streptozotocin.

Discussion

It is apparent from Table 4 that the effects of the different substances were not the same on the levels of cholesterol and triglycerides. Thus streptozotocin and the anti-insulin serum acted in a similar fashion on TG levels but in opposite ways on those of cholesterol. Similarly, insulin and the antiglucagon serum both raised TG levels but had opposite effects on plasma cholesterol levels and liver HMGR activity. Obviously the *in vivo* effects of hormones and antihormones are more complex than might be expected, probably because compensatory mechanisms are initiated by the administration of the former. These data indicate that in the infant rat at least, streptozotocin may have effects additional to those due to the suppression of insulin action. Insulin raises both cholesterol and TG plasma levels in infant rats and its antiserum has an opposite effect, presumably because now the action of glucagon preponderates.

It is tempting to speculate that the cholesterol elevating effect of insulin in infant rats is due to the hormone suppressing cholesterol-7-alpha-hydroxylase (Subbiah and Yunker 1984) the rate limiting enzyme of bile acid formation, thus leading to a greater retention of cholesterol in the plasma. The same effect would not occur in adult rats in whom both levels of insulin and hydroxylase activity are much higher. However it will require much more work to unravel the developmental relationships between hormones and cholesterol metabolism.

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