Effect of Theophylline, Glucagon and Theophylline plus Glucagon on Insulin Secretion in the Premature Infant

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SUMMARY

Serum insulin was measured in thirty premature infants in the first twenty-four hours of life following the administration of theophylline, glucagon, or theophylline plus glucagon. Theophylline (1 mg. per min.) produced a striking rise in serum insulin without change in blood glucose. Neither glucagon (0.4 γ per min.) nor theophylline (0.5 mg. per min.) produced any significant change in the levels of blood glucose and serum insulin. However, the simultaneous administration of these doses of glucagon and theophylline was followed by a progressive rise in blood glucose and serum insulin. The insulin secretion with this combination exceeded that seen with a rapid injection of 300 γ of glucagon. DIABETES *19*:837-41, November, 1970.

Insulin is present in the human fetal pancreas as early as the twelfth week of gestation, and there is a progressive increase in insulin content up to the time of birth.[1] Previous studies[2-10] have shown, however, that full-term and premature infants have either a delayed or absent rise of insulin after glucose administration. These suggest that the newborn pancreas is less sensitive to glucose stimulation than that of the adult.

Recent investigations[11-13] have provided evidence suggesting that insulin release may be dependent on the intracellular accumulation of cyclic 3′,5′ AMP within the beta cell. It has been found, in vitro and in vivo, that adrenocorticotropin and glucagon, which stimulate adenyl cyclase system in the liver, adipose tissue and adrenals,[14-15] also cause an increase of insulin secretion.[12,16-18] A similar effect was also obtained in the isolated perfused pancreas with cyclic AMP,[16] and the effect was mimicked by theophylline[19] which is known in other tissues to inhibit phosphodiesterase thus preventing inactivation of cyclic 3′,5′ AMP.

The following studies were designed to investigate the effect of theophylline with and without glucagon on insulin secretion in the premature infant.

MATERIAL AND METHODS

Thirty premature infants, delivered vaginally, were included in the study. Prematurity was assessed on the basis of weight, maternal dates and clinical appearance.[20-22] The infants were otherwise normal by history and physical examination. The mean gestational age was 33 ± 2 weeks, and birth weights ranged from 1,600 to 2,340 gm.

Under sterile conditions a polyethylene catheter was inserted in the umbilical vein of the fasting infant during the first twenty-four hours of life. All solutions were infused through the catheter and blood samples were drawn after rinsing the catheter with saline. Serial levels of serum insulin and blood glucose were measured after administration of the following substances:

a. A 20 per cent solution of theophylline in water infused at a constantly monitored rate of 1 mg. or 0.5 mg. per min. for periods of one hour.

b. Crystalline glucagon (NOVO) in a total dose of 300 γ intravenously for five minutes or by constant infusion at the rate of 0.4 γ per minute for one hour. In this last experiment theophylline was added at concentration of 0.5 mg. per min. for one hour.

Insulin was determined in triplicate on alcohol extracts of serum[23] by the immunochemical method of Hales and Randle[24] with use of I-125 and binding reagent obtained from the Radiochemical Center, Amersham, England.

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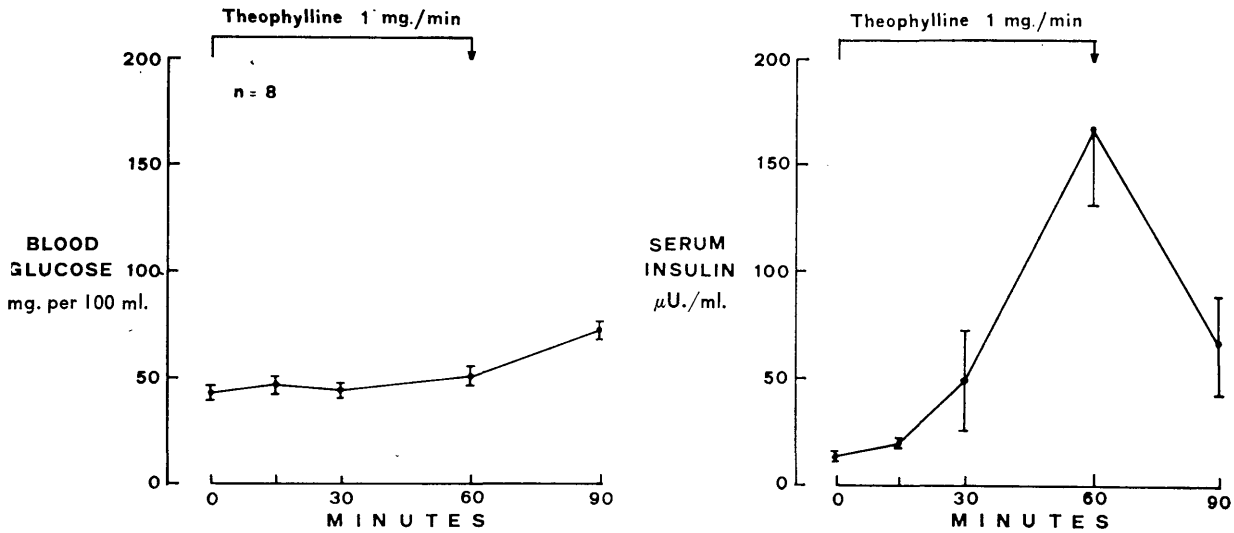

FIG. 1. Levels of blood sugar and serum insulin in premature infants following a sixty minute infusion of theophylline (1 mg. per min.).

Concentrations of blood glucose were determined by a specific glucose oxidase enzymic method.[25]

RESULTS

Infusion of theophylline at a rate of 1 mg. per min. for one hour was accompanied by a rapid rise in serum insulin with no change in glucose levels in all subjects. The mean serum insulin level rose from a control value of 13 ± 2 μU. per ml. to a peak of 167 ± 36 μU. per ml. at 60 min. It fell to 65 ± 23 μU. per ml. at thirty minutes after the theophylline was discontinued (figure 1).

Administration of 300 γ glucagon injected in five minutes produced a prompt rise in serum insulin which reached a mean peak of 78 ± 5 μU. per ml. at 5 min. It rapidly declined to 25 ± 6 μU. per ml. and 19 ± 4 μU. per ml. at thirty and sixty minutes respectively. The mean blood glucose level rose gradually from a control value of 39 ± 4 mg. to 79 ± 6 mg. per 100 ml. at sixty minutes (figure 2). The weights and ages of these infants were respectively 2,340, 2,210, 1,980, 1,860 gm.; 20, 18, 18, 20 hours. The hyperglycemic

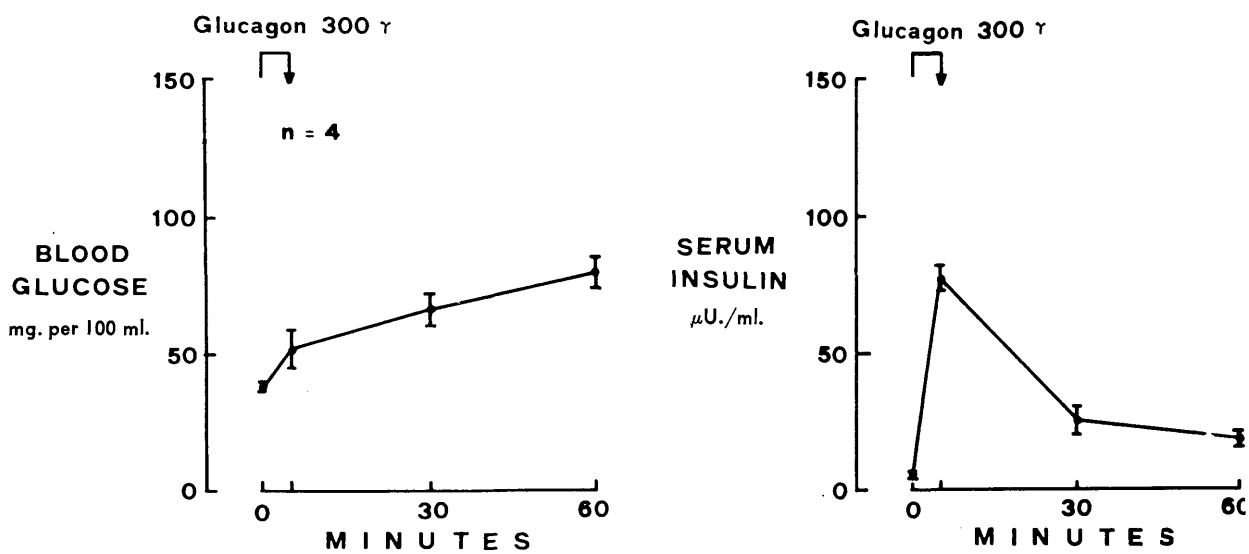

FIG. 2. Effects of a rapid infusion (5 min.) of 300 γ glucagon on blood glucose and serum insulin levels in premature infants.

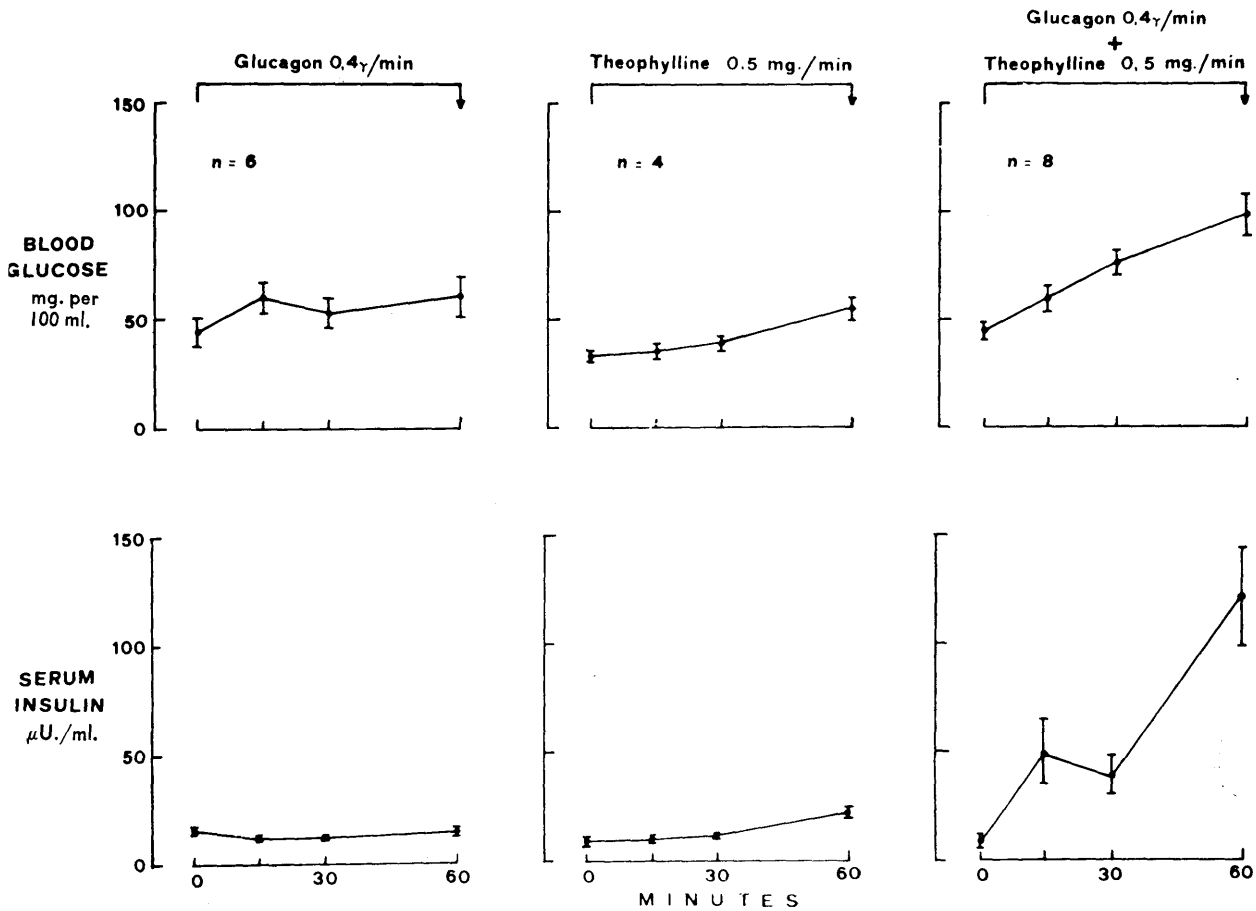

FIG. 3. Blood glucose and serum insulin levels in premature infants following a 60 min. infusion of glucagon (0.4 γ per min.) and theophylline (0.5 mg. per min.) singly or combined.

response was comparable to that reported by other workers[26,27] in premature infants of the same age receiving a similar dose of glucagon.

Infusion of glucagon or theophylline for one hour at the rate of 0.4 γ and 0.5 mg. per min. respectively produced no significant elevation of blood glucose or serum insulin. However, the simultaneous administration of these doses of glucagon and theophylline was followed by a marked rise in serum insulin and blood glucose. The mean serum insulin level rose from a control value of 8 ± 3 μU. per ml. to a peak of 120 ± 23 μU. per ml. at sixty minutes. The mean blood glucose rose from a control level of 38 ± 6 mg. to 93 ± 10 mg. per 100 ml. at sixty minutes (figure 3).

DISCUSSION

Theophylline and glucagon, which are both thought to act through the adenyl cyclase system in other tissues, stimulated insulin secretion in infants in the present study. The administration of these substances was associated with a rise of the serum insulin which occurred either in the absence of or prior to any significant changes in blood glucose, indicating that they directly stimulated insulin release from the pancreatic beta cell. Theophylline and glucagon in low doses had a synergic effect and together produced a much greater effect on the serum insulin and blood glucose than each one separately. These results could be interpreted as suggesting that theophylline, which blocks phosphodiesterase, causes cyclic 3′,5′ AMP to accumulate to a concentration that activates insulin secretion and that glucagon enhances this process.

Turtle et al.[28,29] have reported that the administration of theophylline to the rat produces a linear increase in insulin secretion in the absence of hyperglycemia and the concomitant administration of glucagon causes even greater insulin release. The potentiation by theophylline of the action of glucagon in our infants

is consistent with the observation of potentiation of cyclic nucleotide action in various tissue preparations and with the demonstrated ability of the methylxanthines to inhibit the phosphodieterase that inactivates 3′,5′ AMP.[30]

Our data are consistent with the view that the beta cell may be equipped with an adenyl cyclase system and that the rate of insulin release may be partially controlled by the antagonistic activities of adenyl cyclase and phosphodiesterase. Furthermore, our studies seem to indicate that the insensitivity to hyperglycemia of the newborn's beta cell resides with the mechanism which controls insulin release and not with the ability to produce this hormone. In vitro studies have revealed a difference between rat fetal pancreas and adult pancreas in respect of the ability of glucose and glucagon to stimulate insulin release. Lambert et al.[31] observed that explants of fetal rat pancreas required caffeine in the media to enable glucose or glucagon to stimulate insulin release; caffeine alone was also effective in causing the release of insulin.

The possibility that the beta cell of the newborn is not able to accumulate stimulatory levels of 3′,5′ AMP necessary for insulin release might perhaps be shown to provide the explanation for our results.

ACKNOWLEDGMENT

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Stevioside Toxicity

A recent review (*Nutrition Reviews* 28:95, 1970) briefly mentioned stevioside as a possible new sweetening agent. This is a glycoside which can be extracted in 6 to 7 per cent yield from the leaves of *Stevia rebaudiana,* a wild shrub of Paraguay (M. Bridel and R. Lavielle, *Compt. Rend.* 193:636, 1932), and is said to be 300 times as sweet as sucrose. The leaves have long been used as a sweetening agent in Paraguay (R. T. Bottle, Manufact. Chem., January 1964, p. 64; F. Bell, Chem. Ind., July 17, 1954, p. 897). Bell refers to a report of the Instituto Agronomico Nacional of Paraguay, made in 1945, in which it was suggested that a stevioside industry be established in Paraguay with the conviction that this would be commercially profitable.

However, as is now the common experience with food additives, attractive features must be weighed against unattractive features. R. I. Dorfman and W. R. Nes (Endocrinology 67:282, 1960) studied the antiandrogenic activity of steviol and dihydrosteviol, which are the diterpinoid acids derived from the sweet glycoside. In the chick comb test, three trials with 2 to 3 mg. of steviol demonstrated a tendency toward antiandrogenic activity; two trials at the 0.5 mg. level were negative. Dihydrosteviol failed to produce a significant inhibition at the 0.5 and 1.5 mg. levels, but significant interference with the action of testosterone was demonstrated at the 3.0 mg. level. With this dose the inhibition was between 51 and 63 per cent. Dihydrosteviol neither stimulated the seminal vesicles, prostate, or levator ani in young male rats, nor was this compound effective in inhibiting the action of testosterone in this test at dose levels of 5 or 20 mg.

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