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THEOPHYLLINE-INDUCED HYPOCALCEMIA IN SHEEP

By

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LUTHMAN, J., G. JONSON and J. PERSSON: *Theophylline-induced hypocalcemia in sheep.* Acta vet. scand. 1972, *13*, 484—491. — Intravenous infusion of theophylline at rates of 1 and 2 mg/kg/min. caused marked hypocalcemia in intact sheep. The plasma concentration of NEFA increased. The lipolytic but not the hypocalcemic effect of theophylline was completely inhibited by nicotinic acid. This fact suggested that lipid mobilization and hypocalcemia were not related. The effects of parathyroid hormone are mediated by 3′, 5′AMP, and methyl xanthines are known to inhibit the inactivation of 3′, 5′AMP. There is, however, evidence in the literature that excessive amounts of 3′, 5′AMP inhibit rather than increase bone resorption. It was therefore suggested that the used amounts of theophylline caused an excessive accumulation of 3′, 5′AMP in bone tissue, so that bone resorption was inhibited and hypocalcemia developed.

theophylline; phosphodiesterase; cyclic adenosine 3′, 5′ monophosphate; hypocalcemia.

Lipolytic hormones are shown to produce hypocalcemia and it is reported that calcium is taken up in adipose tissue during excessive lipolysis. The literature in this field was recently reviewed by *Luthman et al.* (1972), and it was also reported in this paper that norepinephrine caused hypocalcemia in sheep. The fact that thyroidectomy did not alter the serum calcium response to norepinephrine suggested that calcitonin was not involved. It also appeared as lipid mobilization and hypocalcemia were related, since nicotinic acid reduced the increase in NEFA as well as the fall in serum calcium.

It thus appears as lipolytic hormones are able to induce hypocalcemia and to stimulate the entrance of calcium into adipose

tissue during certain experimental conditions. The question then arises if lipolytic agents other than hormones also cause hypocalcemia. *Alm et al.* (1970) found that theophylline increased the uptake of calcium in rat adipose tissue in vitro and that this effect was inhibited by the antilipolytic agents insulin and nicotinic acid.

The lipolytic effect of methyl xanthines is due to their ability to inhibit the activity of phosphodiesterase (*Butcher & Sutherland* 1962). Phosphodiesterase is the enzyme system which catalyzes the degradation of cyclic adenosine-3′, 5′-monophosphate (3′, 5′AMP). Lipolysis is as most other biochemical processes regulated by the intracellular concentration of 3′, 5′AMP.

Methyl xanthines are lipolytic in sheep (*Luthman & Jonson* 1971) and the primary aim of the present investigation was to study if theophylline like norepinephrine causes hypocalcemia in sheep. Theophylline was chosen because its effect on the central nervous system is much weaker than that of caffeine. Methyl xanthines are also shown to affect carbohydrate metabolism. *Anderson et al.* (1966 a, b) showed that methyl xanthines inhibited the utilization of glucose. This effect should favour the uptake of calcium in adipose tissue as was suggested by *Alm et al.* They found that the calcium uptake was related to the tissue concentration of fatty acids. If carbohydrate metabolism is inhibited, the reesterification of fatty acids decreases and fatty acids accumulate within the tissue.

MATERIAL AND METHODS

The animal material consisted of mature female sheep weighing between 45 and 60 kg. The animals were not fasted when used in the experiments. Three animals were used in the first experiment. An intravenous infusion of physiological saline was given during 40 min. by means of a peristaltic infusion pump. After a few days theophylline in saline was infused at a rate of 1 mg/kg/min. After a recovery period of about two weeks the theophylline infusion was repeated and nicotinic acid at a dose of 5 mg/kg was administered i.v. immediately before and 30, 60 and 90 min. after the start of the infusion.

In a second experiment theophylline was given to two animals at a rate of 2 mg/kg/min.

The drugs used were Teofyllamin (ACO, Stockholm, Sweden) and Nikotinsyra (ACO). Blood was sampled for determination of glucose, NEFA, calcium and inorganic phosphorus. Glucose was determined on whole blood. Commercial reagents were used (Glox, AB Kabi, Stockholm). Plasma NEFA was measured titrimetrically according to *Dole* (1956). The method of *Skerry* (1965) was used for determination of serum calcium. Inorganic phosphorus was analyzed according to *Fiske & Subbarow* (1925). Commercial reagents were used (Sigma Kit No. 670).

RESULTS

The changes in glucose, NEFA, calcium and inorganic phosphorus during and after theophylline infusion at the rate of 1 mg/kg/min. are shown in Fig. 1. NEFA increased from a pre-infusion level of 0.26 ± 0.03 meq./l to a maximum level of 0.95 ± 0.05 meq./l. This value occurred 30 min. after the end of the infusion. Blood glucose did not change during the infusion, but at the end of the observation period the level was 19 mg/100 ml higher than at the start of the experiment. Serum calcium fell from 10.4 ± 0.4 mg/100 ml to 9.0 ± 0.3 mg/100 ml. The lowest value occurred 30 min. after the end of the infusion. Like blood glucose inorganic phosphorus did not change during the infusion, but the level was 1.0 mg/100 ml lower at the end of the experiment. All animals showed slight hyperestesia, but recovered rapidly.

Nicotinic acid completely blocked the lipid mobilizing effect of theophylline, but did not influence the fall in serum calcium.

The higher dose of theophylline, 2 mg/kg/min., caused similar but more marked changes than the lower dose (Table 1). The animals showed severe hyperestesia and one animal died a few hours after the experiment.

Table 1. The effects of infusion of 2 mg/kg/min. of theophylline during 40 min. Mean of two animals.

	Min.									
	0	15	30	40	70	100	130	160	190	220
NEFA meq./l	0.20	0.63	0.93	1.09	1.62	1.68	1.51	1.67	1.40	1.33
Glucose mg/100 ml	36	42	49	62	103	104	116	125	135	140
P mg/100 ml	3.8	4.0	3.7	3.5	2.7	2.2	2.2	2.4	2.8	2.5
Ca mg/100 ml	10.6	10.0	9.7	9.3	8.0	8.1	8.1	8.4	8.2	8.2

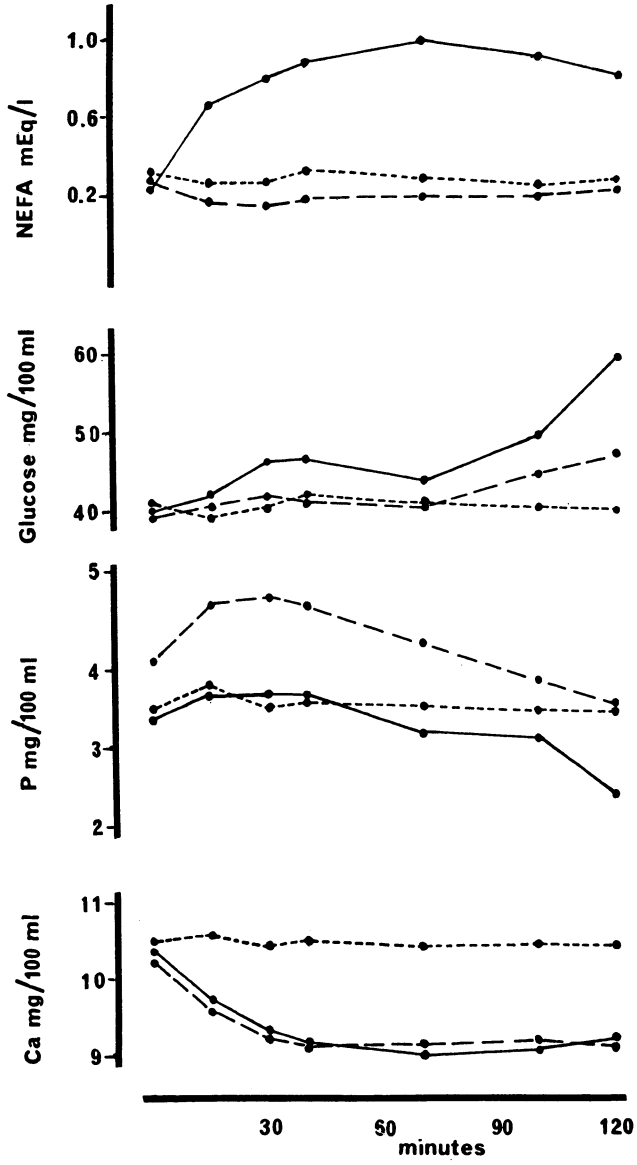

Figure 1. Changes in NEFA, blood glucose, inorganic phosphorus and calcium during and after 40 min. infusion of theophylline at a rate of 1 mg/kg/min. ———. Theophylline and nicotinic acid (5 mg/kg nicotinic acid injected i.v. at 0, 30, 60 and 90 min. — — —. Controls - - - - - -. Mean of three animals.

DISCUSSION

The increase in NEFA after theophylline administration may be caused by two different mechanisms. Theophylline is able to decrease the phosphodiesterase activity in adipose tissue, which leads to accumulation of 3′, 5′AMP and stimulation of lipolysis, and *Strubelt* (1969) presented evidence that the metabolic effects of methyl xanthines in vivo also may be mediated by endogenous catecholamines. As shown in Fig. 1 NEFA increased from 0.26 meq./l to 0.95 meq./l. Serum calcium decreased from 10.4 mg/100 ml to 9.0 mg/100 ml. It seems not likely that hypocalcemia was caused by uptake of calcium in adipose tissue during the increased lipolysis as described by *Akgün & Rudman* (1969) and *Alm et al.* (1970), since the serum calcium response to theophylline was the same also after administration of nicotinic acid, which kept NEFA at normal levels during the whole experimental period. It is not known if there excists any critical NEFA level above which hypocalcemia occurs. In the study of *Akgün & Rudman* NEFA reached levels above 3 meq./l, and similar values were reported by *Luthman et al.* (1972), and it was also suggested that besides increased lipolysis, changes in the calcium transporting mechanisms in the cell were necessary for the accumulation of calcium in adipose tissue.

Hypocalcemia must thus have been caused by other mechanisms. Methyl xanthines are diuretics and renal loss of calcium can not be excluded. It is also possible that calcitonin was released from the thyroid gland.

Theophylline is, however, known to affect calcium metabolism in other ways. *Wells & Lloyd* (1967) found that theophylline like parathyroid hormone increased serum calcium in parathyroidectomized rats. Theophylline was, however, without effect in intact rats. Later it was shown that theophylline prevented calcitonin-induced hypocalcemia in intact rats (*Wells & Lloyd* 1968). The authors suggested that theophylline like parathyroid hormone increased the concentration of 3′, 5′AMP in bone cells and thus also bone resorption, and that the lack of effect on serum calcium in intact animals was due to a compensatory release of calcitonin. In a later work *Wells & Lloyd* (1969) showed that also dibutyryl-3′, 5′AMP increased serum calcium in parathyroidectomized rats. Dibutyryl-3′, 5′AMP is a derivative of 3′, 5′ AMP which is not so easily inactivated as the natural compound. It was observed that when amounts of dibutyryl-3′, 5′AMP

greater than what was necessary to produce maximum response were administered, the serum calcium response was less. The authors had previously observed (*Wells & Lloyd* 1967) that the combination of high doses of theophylline and parathyroid hormone resulted in a serum calcium increase which was less than that produced by either agent alone. It was also reported that the same phenomenon occurs in vitro in bone tissue cultures (*Wells & Lloyd* 1969). In such systems dibutyryl-3′, 5′AMP increased bone resorption, but when supraoptimal doses were administered the rate of bone resorption decreased. It was therefore concluded that excessive amounts of 3′, 5′AMP may inhibit rather than increase bone resorption. It is possible that the doses of theophylline used in the present study caused an excessive accumulation of 3′, 5′AMP in bone cells, so that bone resorption was rapidly inhibited and hypocalcemia occurred.

It was previously suggested that norepinephrine-induced hypocalcemia probably was mediated by more than one mechanism (*Luthman et al.*). When the lipolytic effect of norepinephrine was reduced by nicotinic acid, the fall in serum calcium was also reduced. This fact favoured the hypothesis that calcium was taken up in adipose tissue. But when the increase in NEFA was completely inhibited, serum calcium was still slightly reduced. It is possible that norepinephrine induces an excessive formation of 3′, 5′AMP in the bone cells and that hypocalcemia in part was caused by decreased bone resorption. This mechanism may also explain the hypocalcemic effect of glucagon. *Tanzer et al.* (1970) found that hypocalcemia occurred both in intact and thyroparathyroidectomized rats after pharmacological doses of glucagon.

Inhibition of bone resorption usually results also in hypophosphatemia. In the present study inorganic phosphorus remained almost unchanged or increased during the infusion of theophylline, but declined later. Inorganic phosphorus is, however, not only regulated by agents affecting calcium metabolism. The intimate relationship between carbohydrate metabolism and phosphate metabolism is known since several years. In intact animals a glucose load is normally followed by hypophosphatemia. This does not occur in insulin deficient animals, and it is generally accepted that insulin increases the transport of phosphate from the extracellular fluid and into the cell. The change in serum inorganic phosphorus following glucose administration has therefore sometimes been used as an indicator

of the peripheral glucose utilization. The literature in this field was summarized by *Burt* (1960). Methyl xanthines are known to inhibit glucose utilization, and it is possible that this effect influenced the level of inorganic phosphorus during the theophylline infusion.

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SAMMANFATTNING

Teofyllin-inducerad hypokalcemi hos får.

Intravenös infusion av teofyllin (1 och 2 mg/kg/min.) förorsakade påtaglig hypokalcemi hos intakta får. Plasmakoncentrationen av NEFA ökade. Den lipolytiska men ej den hypokalcemiska effekten av teofyllin blockerades av nikotinsyra. Det föreföll således som om dessa effekter ej var kopplade. Effekterna av parathormon medieras av 3′, 5′AMP och metylxantiner blockerar effektivt inaktiveringen av 3′, 5′AMP. Det finns emellertid belägg för att excessiva mängder av 3′, 5′AMP hämmar benresorbtionen. Det förefaller därför troligt att teofyllin vid de använda doseringarna gav upphov till en excessiv ackumulering av 3′, 5′AMP i benvävnaden, så att benresorbtionen hämmades och hypokalcemi utvecklades.

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