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EFFECT OF CALCIUM CHLORIDE ON ASPIRIN-INDUCED HYPOINSULINEMIA IN RATS

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Salicylates are widely used as analgesics, antipyretics and anti-inflammatory agents. Administration of salicylates has been found to change blood sugar levels; however, these observations have been inconsistent. Some investigators observed a hyperglycemic effect in rats (1), dogs (2), and humans (3,4), while others reported a hypoglycemic effect in normal (5) and adrenalectomized (6) rats, and in normal and mildly diabetic patients (7,8). The mechanism of action of these salicylate effects, especially on insulin secretion, also remains controversial. Some investigators found that salicylates had no effect on insulin secretion in isolated rat (9) and hamster (10) islets, in dogs (11,12), and in humans (4), while other groups reported that salicylates reduced insulin secretion in fragments of rabbit pancreas (13), in rats (5), and in dogs (14), and others reported that salicylates stimulate insulin secretion in normal and diabetic humans (15-17).

We recently observed that, in thyroparathyroidectomized rats, acetylsalicylate (aspirin) inhibited bone resorption (i.e. hypercalcemic effect) caused either by parathyroid hormone (PTH) or by the active form of vitamin D_3, and caused a hypocalcemic state in normal rats. Administration of aspirin, however, did not affect the hypocalcemic effect of calcitonin (CT) (18). From these findings we considered the possible interrelationships in the effect of salicylates *in vivo* on glucose and calcium homeostasis. To examine this, we have studied the effect of administration of aspirin and/or calcium chloride on serum insulin, glucose and calcium in rats.

MATERIALS AND METHODS

Male Wistar rats, weighing 200 g and fed a usual laboratory rat chow, were used. To examine a time course of the effect of aspirin, rats were divided into six groups (four rats per group). The first three groups received 200 mg/kg, i.p., of aspirin (Sigma Chemical Co., St. Louis, MO) in saline with phosphate buffer (pH 7.4). The second three groups (control) received vehicle only. Serum samples were collected by bleeding via the abdominal aorta at 30, 90 and 150 min after the administration of aspirin, and the levels of insulin, calcium and glucose were measured. The dose dependency of the effect of aspirin was also examined at dosage levels of 200 and 50 mg/kg, i.p., at 60 min after drug administration; each group contained four rats.

Next, the effect of administration of calcium chloride was examined with or without aspirin treatment. Group 1 received aspirin and calcium chloride; group 2, aspirin and vehicle; group 3, vehicle and calcium; and group 4, vehicle only. Calcium chloride (in a phosphate buffer, pH 7.4) was administered 15 min after aspirin and serum samples were collected 60 min after aspirin. Dosage levels of aspirin and calcium chloride were 200 mg/kg, i.p.,

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and 2 mg Ca/kg, i.p., respectively. To examine if there is a time dependency in the effect of calcium chloride in aspirin-treated rats, calcium chloride (2 mg Ca/kg, i.p.) was given to the rats 30 and 45 min after aspirin, and serum samples were collected 30 and 45 min after calcium chloride, respectively.

Serum insulin was determined by radioimmunoassay (19) using purified rat insulin (kindly supplied by Drs. R. E. Chance and M. A. Root of Eli Lilly Laboratories, Indianapolis, IN) as standards. Serum calcium and glucose were measured by the orthophthalein complexone method (20) and the glucose oxidase method (21).

RESULTS AND DISCUSSION

Administration of aspirin caused marked decreases in both serum insulin and calcium, although glucose levels remained comparable to those of the control group (Fig. 1).

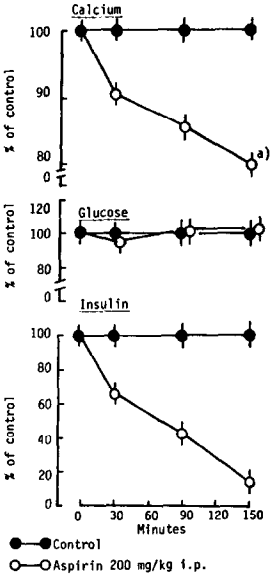

Fig. 1. Time course of effects of aspirin on serum insulin, calcium and glucose in rats. Data are shown as percentage of control. Each point and vertical bar represent a mean of four rats and a standard error of the mean. "a)" indicates a significant difference from control ($P < 0.001$, Student's t-test).

A similar reduction in insulin level without affecting serum glucose level has been observed in dogs (14). These effects increased with time, and changes in insulin levels paralleled those in calcium levels (Fig. 1). The effect of aspirin was also dependent at dosage levels of 50 and 200 mg/kg, i.p., when the effect of aspirin was measured 60 min after its administration: aspirin, at 50 mg/kg, reduced serum insulin and calcium to 70 and 95 percent of control levels, respectively, while at 200 mg/kg, it reduced both variables to 50 and 92 percent (Fig. 2). The lower dosage level of aspirin is compatible to that used in humans.

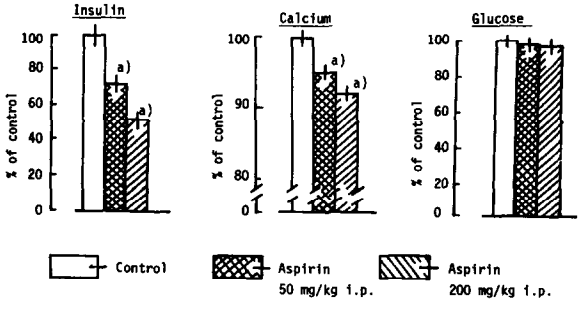

Fig. 2. Aspirin dose response effect on serum insulin, calcium and glucose in rats. Data are shown as percentage of control. Each column and vertical bar represent a mean of four rats and standard error of the mean. "a)" indicates a significant difference from control ($P < 0.001$, Student's t-test).

The reduced insulin level caused by aspirin was restored nearly to the control level in rats given calcium chloride 15 min after aspirin (Table 1). Similar results were obtained when rats received aspirin orally and calcium chloride intraperitoneally (data not shown). This restoration of insulin was not observed in the rats given calcium chloride 30 min after aspirin, although there was some increase in the insulin level (Table 1). When calcium chloride was given as late as 45 min after aspirin, no increase in the reduced insulin level was observed. Thus, the time of calcium chloride administration appears to be important in restoring the reduced insulin level.

Table 1. Effects of calcium on the levels of serum insulin, calcium and glucose with or without aspirin treatment*

Expt.	Aspirin (mg/kg, i.p.)	$CaCl_2$ injection		Assay time after aspirin (min)	Serum level		
		mg Ca/kg i.p.	Time after aspirin (min)		Insulin (μU/ml) Mean ± S.E.	Calcium (mg/100 ml) Mean ± S.E.	Glucose (mg/100ml) Mean ± S.E.
I	0	0		60	65.0 ± 5.7	10.0 ± 0.1	151.8 ± 1.7
	0	2	15	60	78.1 ± 3.9†	10.1 ± 0.2	155.3 ± 4.1
	200	0		60	31.4 ± 2.8‡	9.2 ± 0.2‡	154.2 ± 6.7
	200	2	15	60	59.8 ± 5.4§	9.4 ± 0.3‡	153.1 ± 9.0
	200	2	30	60	41.0 ± 3.2‡ §	9.9 ± 0.3§	143.9 ± 5.0
II	0	0		90	66.0 ± 6.2	10.4 ± 0.1	150.0 ± 7.9
	200	0		90	28.3 ± 6.1‡	9.1 ± 0.1‡	155.0 ± 7.2
	200	2	45	90	22.8 ± 7.9‡	9.6 ± 0.4	144.1 ± 9.2

* Each group includes four rats.

† Indicates significant difference from control ($P < 0.01$, Student's t-test).

‡ Indicates significant difference from control ($P < 0.001$, Student's t-test).

§ Indicates significant difference from group treated with aspirin only ($P < 0.001$, Student's t-test).

In contrast to serum insulin, serum calcium was not changed when calcium chloride was injected 45 min before blood sampling (Table 1). However, the effect of calcium chloride on serum calcium has been reported to be temporary (22), which could explain our results. In fact, a significant increase in calcium was observed when calcium chloride was given 30 min before blood sampling (Table 1).

It should be noted that calcium chloride alone increased the serum insulin level by 20 percent when it was given to normal rats (Table 1), indicating a direct relation between changes in calcium level and insulin secretion. This effect, however, was not as striking (i.e. 44 percent recovery) as that observed in the aspirin-treated group.

These findings suggest that the effect of aspirin on glucose homeostasis, especially on the regulation of insulin secretion, may not be a direct action, but a secondary one caused by its action on calcium homeostasis. Our results not only confirm the observation by Arnold and Fernstrom (5), but also offer some clues to their results. They reported

that intraperitoneal administration of sodium salicylate to rats caused reduction in serum insulin. However, a marked rise in serum glucose levels following an oral glucose load failed to raise the reduced insulin levels in salicylate-treated rats. This is not surprising, since our results suggest that hypocalcemia might have prevented glucose-stimulated insulin secretion. Evidence for inhibition of glucose- and arginine-stimulated insulin secretion in vivo by hypocalcemia was also noted by others in post-paturient cows (23) and a patient with pseudo-hypoparathyroidism (24), respectively.

We cannot explain, at present, why serum glucose levels did not change significantly. Other hormones such as glucagon, epinephrine and cortisol might counteract the effect of aspirin on serum glucose. Effects of aspirin on serum insulin levels may differ among experimental animals and human (5, 13-17). A difference in the sensitivity to salicylates of the regulatory system for calcium homeostasis may explain the discrepancy.

In summary, the present data demonstrated that aspirin decreased serum levels of insulin and calcium and suggest that the reduction in serum levels of insulin by aspirin may be secondary to a fall in serum calcium.

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