HYPOCALCEMIC EFFECT OF ACETYLSALICYLIC ACID IN RATS

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Oral administration of acetylsalicylic acid (ASA) at a dose of 200 mg/kg produced a decrease in both total plasma calcium and plasma ionic calcium levels in rats. The percent changes from controls in both total and ionic calcium were similar, being approximately 13% at 3 hr after the administration. A significant ($p < 0.01$) decrease in plasma calcium level was also observed at 4 hr after oral administration of salicylic acid at a dose level of 177 mg/kg. However, oral administration of other non-steroidal anti-inflammatory agents such as indomethacin failed to decrease plasma calcium levels in rats. The hypocalcemic effect of ASA was recognized in parathyroidectomized rats, but neither in thyroparathyroidectomized nor in thyroidectomized rats. Therefore, the data suggested that the action of ASA might be mediated by stimulation of calcitonin release, but not by inhibition of prostaglandin biosynthesis.

Keywords—salicylates; hypocalcemia; calcitonin; PTH; hypophosphatemia

Recently, it was reported that administration of acetylsalicylic acid (ASA) inhibited hypercalcemic effect of 1α -hydroxyvitamin D_3 ¹⁾ and decreased serum level of total calcium²⁾ in rats. However, the mechanism of hypocalcemia caused by ASA was not clarified. Since ASA is a potent inhibitor of cyclooxygenase in prostaglandins biosynthesis, special interest has focussed on the role of prostaglandins in the process of ASA induced hypocalcemia. The purpose of the present study was to demonstrate the hypocalcemic effect of ASA and to clarify the related mechanism of the action.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 5–7 weeks of age and fed a usual laboratory rat chow, were used. For the 18–20 hr period (overnight) before the experiments the rats were fasted. Each group contained six rats. Thyroparathyroidectomy (TPTx) and parathyroidectomy (PTx) were performed one day before the experiment and thyroidectomy (Tx), explanted parathyroid glands, was

performed 5 days before the experiment using light ether anesthesia. Each experiment was undertaken on a different day. Drugs used were ASA (Iwaki Seiyaku), salicylic acid (Nakarai Chemicals), indomethacin (Sigma), phenylbutazone (Sigma), ketoprofen (Rhodia Yakuhin) and prednisolone (Sigma). Blood was obtained routinely by orbital puncture. Plasma phosphorus level was measured by commercial test set (Iatron). For the analysis of ionic calcium, blood was drawn from the abdominal aorta into heparinized syringes, care being taken not to expose the samples to air. Plasma total calcium and ionic calcium levels were determined by the orthocresol phthalein complexone method³⁾ and with calcium specific electrode (SS-20, Orion Biochemical). Values represent mean $\pm$ standard error. Statistical analyses were performed by using Student's *t* test.

RESULTS AND DISCUSSION

ASA caused marked decrease in both plasma total calcium and ionic calcium in intact rats. The

marked fall in total plasma calcium level from 9.63 ± 0.17 to 8.35 ± 0.22 mg/100 ml at 3 hr after a single oral administration of ASA (200 mg/kg)

TABLE I. *Effect of Anti-inflammatory Drugs on the Plasma Calcium Level in Intact Rats*

Drug	mg/kg <i>p.o.</i>	Changes in plasma calcium level Δ Ca mean $\pm$ SE (mg/100 ml)
Control	—	0.14 ± 0.13
Acetylsalicylic acid	200	$-1.50 \pm 0.13^*$
Salicylic acid	177	$-2.03 \pm 0.13^*$
Indomethacin	10	-0.47 ± 0.21
Ketoprofen	10	-0.19 ± 0.21
Phenylbutazone	50	-0.22 ± 0.10
Prednisolone	1	-0.25 ± 0.26

Forty-two Sprague-Dawley male rats, 5 weeks of age, were divided into groups with 6 rats in each group. The total plasma calcium levels were determined before and at 4 hr after the administration. Significant differences were observed from control, at *: $p < 0.01$.

was associated with a significant ($p < 0.05$) fall in plasma ionic calcium level from 4.56 ± 0.16 to 3.98 ± 0.10 mg/100 ml. The percent changes from controls in both total calcium and ionic calcium levels were similar, being approximately 13% each.

Results presented in Table I show that oral administration of salicylic acid (177 mg/kg), a metabolite of ASA, caused the same hypocalcemic effect, though each dose of anti-inflammatory agents, indomethacin (10 mg/kg), phenylbutazone (50 mg/kg), ketoprofen (10 mg/kg) and prednisolone (1 mg/kg), was without effect on plasma calcium concentration in intact rats. Based on these data, it is tempting to speculate that prostaglandins are not involved in the process of ASA induced hypocalcemia.

The results in Table II show that ASA was still effective in PTx rats but neither in TPTx rats nor in Tx rats. The ASA induced hypocalcemia in PTx rats was dose-dependent and similar to that observed in intact rats. These results indicate that the presence of the thyroid glands was required for ASA to produce hypocalcemia. Since the ASA induced hypocalcemia was accompanied with

TABLE II. *Effect of Acetylsalicylic Acid (ASA) on Plasma Calcium and Phosphorus Levels in Intact, Parathyroidectomized (PTx), Thyroparathyroidectomized (TPTx) and Thyroidectomized (Tx) Rats*

Treatment	mg/kg (<i>p.o.</i>)	Changes in plasma calcium levels (Δ Ca mg/100 ml)			
		Intact	PTx	TPTx	Tx
Control	—	-0.19 ± 0.09	0.39 ± 0.16	0.26 ± 0.07	-0.22 ± 0.38
ASA	200	$-1.37 \pm 0.05^{**}$	$-0.32 \pm 0.07^{**}$	0.02 ± 0.16	-0.21 ± 0.14
	400	$-1.65 \pm 0.16^{**}$	$-0.69 \pm 0.28^{**}$	0.04 ± 0.08	—
		Changes in plasma phosphorus levels (Δ Pi mg/100 ml)			
		Intact	PTx	TPTx	Tx
Control	—	-0.24 ± 0.17	0.22 ± 0.16	-0.50 ± 0.57	-0.95 ± 0.26
ASA	200	$-0.75 \pm 0.24^*$	$-1.73 \pm 0.36^{**}$	-1.19 ± 0.32	-1.32 ± 0.32
	400	$-1.57 \pm 0.24^{**}$	$-1.97 \pm 0.25^{**}$	-1.66 ± 0.20	—

Data represent mean $\pm$ SE of 6 male Sprague-Dawley rats, 7 weeks of age. PTx and TPTx rats in which the total plasma calcium levels were lower than 6 mg/100 ml, and in intact and Tx rats, higher than 9 mg/100 ml after an overnight fast were used. The determinations were carried out before and at 3 hr after the administration. Significant differences were observed from control, at *: $p < 0.05$ and **: $p < 0.01$.

hypophosphatemia, the action of ASA might be mediated by the stimulation of calcitonin release.

However, a significant rise in plasma magnesium level was also observed at 6 hr after ASA administration according to our preliminary data and it was reported that ASA inhibited hypercalcemic effect caused by parathyroid hormone in TPTx rats.⁴⁾ Therefore, it may not be negligible that in intact rats the calcium-rising effect of the parathyroid hormone secreted in response to the fall in ionic calcium after the administration of ASA, might be suppressed by ASA.

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