Acetylsalicylic Acid Restores Acute Insulin Response Reduced by Furosemide in Man

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SUMMARY

Prostaglandin E (PGE) infusion in normal man inhibits the acute insulin response to glucose. In order to determine whether endogenously released PGE might also inhibit insulin secretion, glucose-stimulated insulin responses were investigated in normal volunteers after furosemide (40 mg i.v.), a stimulator of endogenous PGE synthesis. Acute insulin response to glucose (20 g i.v.) was significantly reduced by furosemide (response before furosemide: 36 ± 5 μU/ml; after furosemide: 26 ± 5 μU/ml, m ± SE, mean change 3–10 min, N = 8, $P < 0.01$), whereas glucose disappearance rates were not modified after furosemide. Infusion of lysine acetylsalicylate (LAS), an inhibitor of endogenous PGE synthesis, completely reversed the inhibitory effect of furosemide on insulin secretion and also augmented acute insulin response to glucose (response before furosemide + LAS: 41 ± 6 μU/ml; during furosemide + LAS: 50 ± 7 μU/ml, N = 10, $P < 0.02$). This effect was associated with an increase in glucose disappearance rates ($P < 0.05$).

These findings demonstrate that (1) furosemide inhibits glucose-induced acute insulin responses and (2) LAS completely reverses the inhibitory effect of furosemide and also accelerates glucose disposal. It is suggested that furosemide acts via the release of endogenous PGEs, which are known to inhibit insulin responses in man. DIABETES *28*:841–845, September 1979.

The prostaglandins (PGs), a group of 20-carbon unsaturated fatty acids discovered by von Euler[1] and Goldblatt[2] almost half a century ago, are ubiquitous in mammalian tissues[3] and have potent physiologic activities. The weight of accumulating evidence strongly suggests the existence of a close relationship between these compounds and the endocrine system.[4] Although the influence of PGs on insulin secretion has been extensively investigated, the results of in vitro and in vivo nonhuman studies are conflicting, either inhibition,[5–8] stimulation,[9–11] or no effects[12] of different PGs on basal and stimulated insulin secretion being reported. More recently, Robertson and Chen[13] and Giugliano et al.[14,15] independently reported that intravenous PGE infusion in normal volunteers inhibited acute insulin response to glucose in a dose-dependent fashion and that this inhibition was associated with a decrease in glucose removal rates.

Since the physiologic role of PGs might be to a large extent intracellular,[16] and since peripherally administered PGs may produce a number of side effects, we have investigated the effect of acetylsalicylic acid (ASA), an inhibitor of endogenous PGE synthesis,[17] on insulin secretion. The results of these studies have shown that ASA augmented both basal insulin concentrations and the responses of this hormone to glucose,[18] arginine,[18] and tolbutamide[19] in normal humans. In addition, sodium salicylate partially restored absent acute insulin response to glucose in adult-onset diabetic patients.[20] However, ASA is known to have a variety of effects in humans, so that its effect on insulin cannot be specifically related to inhibition of endogenous PG synthesis.

The present investigation was designed to answer the question whether endogenously released PGE might also reduce insulin secretion and glucose tolerance in humans. For this purpose, we studied the effect of furosemide, a stimulator of PGE synthesis,[21] on acute insulin response and glucose tolerance in normal humans either with or without a concurrent infusion of lysine acetylsalicylate, an inhibitor of PGE synthesis.

SUBJECTS AND METHODS

Informed consent was obtained from 30 healthy medical students after we fully explained the experimental nature, purpose, and potential hazards of the study. They were free from acute or chronic metabolic or cardiovascular diseases at the time of the study and had no personal or family history

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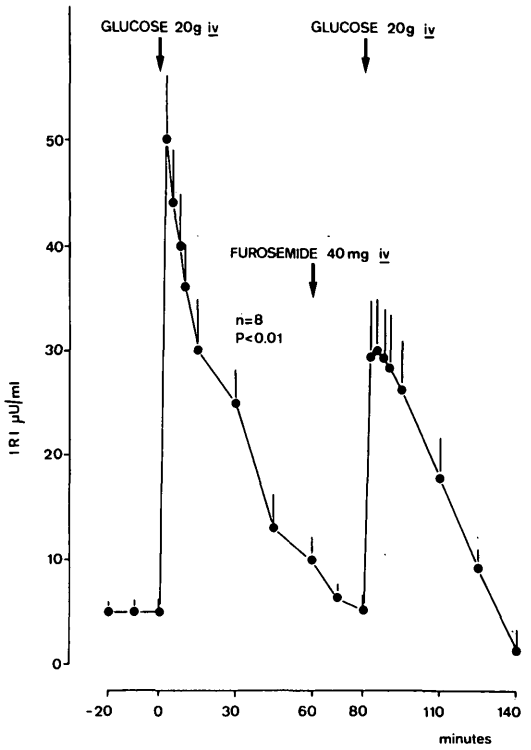


FIGURE 1. Plasma insulin levels in response to glucose pulses given before and after i.v. administration of furosemide in normal humans.

of diabetes. The range of age was 20–27 yr, and the range of relative body weights was −15 to +10% (Geigy, Scientific Tables). The fasting plasma glucose concentration of the various subjects was 65–80 mg/dl, the fasting plasma insulin was 4–12 μU/ml. Each subject was instructed to eat a balanced diet with 250 g of carbohydrates/day for at least 3 days before the experiments. Special care was taken to insure against the recent use of drugs that contain aspirin.

All studies were performed in the morning, after an overnight fast (12 h) and with the subjects supine. Indwelling Teflon catheters were inserted in each antecubital vein, one for infusion, the other for blood sampling. Patency was preserved by a slow saline (0.9%) drip. Glucose stimulation was provided by injecting 20 g of glucose in less than 1 min. Furosemide (40 mg) was administered intravenously 20 min before the second glucose pulse. Lysine acetylsalicylate (LAS, Flectadol, Maggioni, Italy), a hydrosoluble salt of acetylsalicylic acid, was infused intravenously at the constant rate of 72 mg/min. Since 0.9 g of LAS corresponds to 0.5 g of ASA, we really infused 40 mg/min of ASA. Blood samples were drawn in the basal state (−20, −10, 0) and at 3, 5, 8, 10, 15, 30, 45, and 60 min after the pulse. Basal insulin levels were calculated as the mean of the three samples drawn before the first glucose injection. The acute insulin response (AIR) was calculated as the mean of the 3°, 5°, 8°, and 10° min postglucose injection values minus the basal insulin level. Blood specimens were collected in prechilled tubes containing 1.2 mg of EDTA per milliliter of blood as anticoagulant, kept in ice until the end of the study, and then immediately centrifuged at 4 °C. Plasma was separated and stored deep-frozen until assayed.

Plasma glucose was estimated according to Huggett and Nixon,[22] and plasma insulin by using dextran-coated charcoal to separate free from antibody-bound hormone.[23] For insulin values greater than 5 μU/ml, this assay has an intra-assay coefficient of variation of 10% and an interassay coefficient of 20%; the minimal sensitivity of this assay using 0.1 ml of plasma is 2 μU/ml. All samples from one subject were analyzed in the same assay. Glucose disappearance rate (kG) was calculated by the method of the least squares taking the natural logs of the glucose concentrations from 15 to 60 min. Plasma salicylate was measured according to Trinder.[24] Serum sodium and potassium were measured by flame photometry.

Statistical comparison of the results was performed by the Student's *t*-, paired *t*-, test.[25] Results are presented as $\bar{x} \pm SE$.

RESULTS

The effect of furosemide on insulin secretion and glucose tolerance in normal subjects. All normal subjects had fasting plasma glucose below 100 mg/dl. In all subjects, there was an immediate insulin response after the first glucose pulse (20 g i.v.); insulin returned to basal values by 80 min (Figure 1). Furosemide, which was given intravenously at 60 min, did not produce any significant change in the mean prestimulatory insulin level. After the second glucose pulse, the acute insulin response was significantly less than that observed after the first glucose pulse (before furosemide: 36 ± 6 μU/ml; after furosemide: 26 ± 5 μU/ml, N = 8, P < 0.01). In control experiments, in which saline rather than furosemide was administered (Figure 2), the acute insulin responses to the first and second glucose pulses were almost identical (first response: 34 ± 5 μU/ml; second response: 36 ± 6 μU/ml, N = 10, P = NS), nor was there a difference between the first insulin responses in furosemide experiments and controls (Table 1).

FIGURE 2. Plasma insulin levels in response to glucose pulses in normal humans.

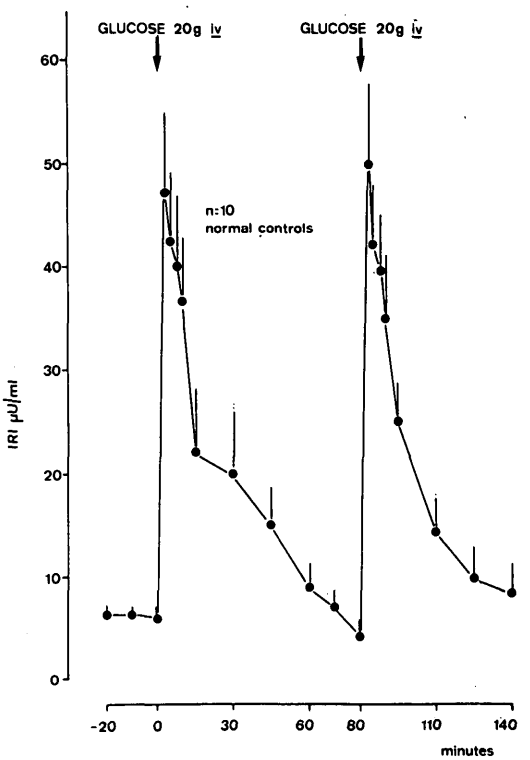


TABLE 1
Plasma insulin levels before (basal) each glucose pulse and acute insulin responses (AIR) in the various experiments performed

		Pulse I (μU/ml)			Pulse II (μU/ml)	
		Basal	AIR	P*	Basal	AIR
Saline (N = 10)	Mean ± SE	6 ± 1	34 ± 5		5 ± 1	36 ± 5
Furosemide (N = 8)	Mean ± SE	5 ± 1	36 ± 5	<0.01	5 ± 1	26 ± 5
Furosemide + LAS (N = 10)	Mean ± SE	8 ± 1	41 ± 6	<0.02	25 ± 5	50 ± 7
LAS (N = 8)	Mean ± SE	6 ± 1	36 ± 5	<0.02	17 ± 3	45 ± 7

* Values of P indicate significant differences between the first and the second insulin responses.

The glucose disappearance rate was similar after both the first and second glucose pulses in the group receiving furosemide (first pulse: kG = 2.20 ± 0.14; second pulse: kG = 2.20 ± 0.17, P = NS). Similarly, there was no difference in the glucose removal rates in the control group (first pulse: kG = 2.42 ± 0.19; second pulse: kG = 2.35 ± 0.18, P = NS) (Table 2).

The effect of LAS on insulin secretion reduced by furosemide in normal subjects. In this section, the same experiments with furosemide were repeated in 10 normal subjects as above, with the sole adjunct of a LAS infusion (60–160 min). LAS completely reversed the inhibitory effect of furosemide on insulin secretion and also augmented acute insulin response to the second glucose pulse (first response: 41 ± 6 μU/ml; second response: 50 ± 7 μU/ml, N = 10, P < 0.02). LAS itself caused an increase in circulating insulin levels before the second glucose pulse (preinfusion level, 60 min value: 15 ± 4 μU/ml; 40 min after onset of LAS infusion: 25 ± 5 μU/ml, P < 0.05 (Figure 3). The glucose disappearance rate was increased after the second glucose pulse as compared with that observed after the first (first pulse: kG = 2.11 ± 0.15; second pulse: kG = 2.53 ± 0.15, P < 0.05) (Table 2).

In an additional eight subjects, the effects of LAS alone on insulin secretion and glucose tolerance were investigated (Figure 4). As seen in the above experiments, LAS significantly increased both the circulating insulin levels before the second glucose pulse (preinfusion value, 60 min value: 7.5 ± 2.5 μU/ml; 40 min after onset of infusion: 17.5 ± 3.5 μU/ml, P < 0.02) and the acute insulin response to the second glucose injection (first response: 36 ± 6 μU/ml; second response: 45 ± 7 μU/ml, P < 0.02). Even here, there was a statistically significant increase in the mean glucose disappearance rate after the second glucose pulse (first pulse: kG = 2.40 ± 0.20; second pulse: kG = 2.95 ± 0.25, P < 0.05) (Table 2).

No significant change in the serum sodium and potassium concentrations was observed after furosemide (before furosemide: serum sodium 140 ± 3 meq/l, serum potassium 4.2 ± 0.1 meq/l; after furosemide: serum sodium 141 ± 4 meq/l, serum potassium 4.2 ± 0.2 meq/l). Urinary sodium excretion increased after furosemide with a maximum between 30 and 60 min. The plasma salicylate levels averaged 21 ± 4 mg/dl at 60 min and 26 ± 7 mg/dl at 100 min after onset of infusion. All subjects were perfectly comfortable during the test.

DISCUSSION

The results of this study show that intravenous furosemide administration significantly inhibits the acute insulin response to an i.v. glucose load in normal subjects. Infusion of LAS, an inhibitor of endogenous PG synthesis, completely reversed the inhibitory effect of furosemide and also augmented glucose-stimulated acute insulin response in normal subjects. LAS itself caused a rise in circulating insulin levels before the glucose injection. Interestingly, there was no evident deterioration of glucose tolerance in the furosemide experiments; conversely, LAS increased glucose disappearance rates. None of these effects were observed in the control studies.

Since there are no reported studies concerning the effect of furosemide on acute insulin response in humans, the mechanism of this inhibition is speculative. One possible candidate might be prostaglandin E, which inhibits glucose-induced insulin secretion in humans.[13–15] However, we have not measured plasma or urinary PGs in this study; on the other hand, Weber et al.[26] recently reported that within 10 min after furosemide (40 mg i.v.) there was an immediate increase in peripheral plasma of the free PG precursor fatty acid, arachidonic acid. Furthermore, there is considerable evidence that following the administration of furosemide, bumetanide, and ethacrynic acid, the immediate increase of renal blood flow, which parallels the initial renin release, is caused by increased PGE formation.[21] Thus, it seems likely that the mechanism of furosemide action upon insulin secretion may be through the synthesis of endogenous PGE.

TABLE 2
Plasma glucose levels before (basal) and at various times during the experiments performed

		Pulse I (mg/dl)				Pulse II (mg/dl)						
Time		Basal	5′	15′	60′	Basal	5′	15′	60′	1st kG*	P	2nd kG
Saline	x̄ ± SE	70 ± 3	250 ± 12	190 ± 10	65 ± 6	55 ± 2	225 ± 12	180 ± 9	62 ± 4	2.42 ± 0.19		2.35 ± 0.18
Furosemide	x̄ ± SE	75 ± 4	240 ± 10	170 ± 8	61 ± 4	60 ± 3	215 ± 10	177 ± 7	65 ± 5	2.20 ± 0.14		2.20 ± 0.17
Furosemide + LAS	x̄ ± SE	80 ± 4	235 ± 12	180 ± 10	70 ± 9	60 ± 2	205 ± 10	165 ± 8	53 ± 2	2.11 ± 0.15	<0.05	2.53 ± 0.15
LAS	x̄ ± SE	70 ± 4	250 ± 13	185 ± 10	64 ± 5	50 ± 3	200 ± 10	185 ± 10	50 ± 3	2.40 ± 0.20	<0.05	2.95 ± 0.25

* Glucose disappearance rates after the first and the second glucose pulses are designated 1st kG and 2nd kG, respectively.

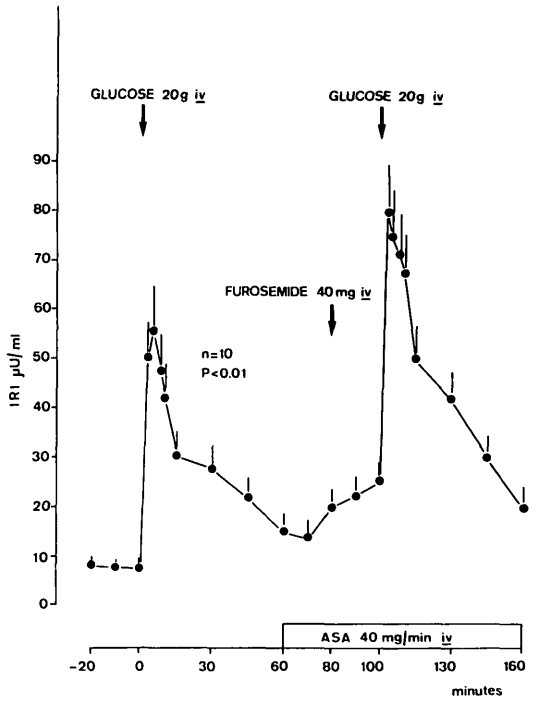


FIGURE 3. The effect of an infusion of acetylsalicylic acid upon plasma insulin response to glucose pulses given before and after i.v. administration of furosemide in normal humans.

The reversal of furosemide-induced inhibition of insulin secretion by LAS, which is an effective inhibitor of endogenous PGE synthesis, seems to support this hypothesis.

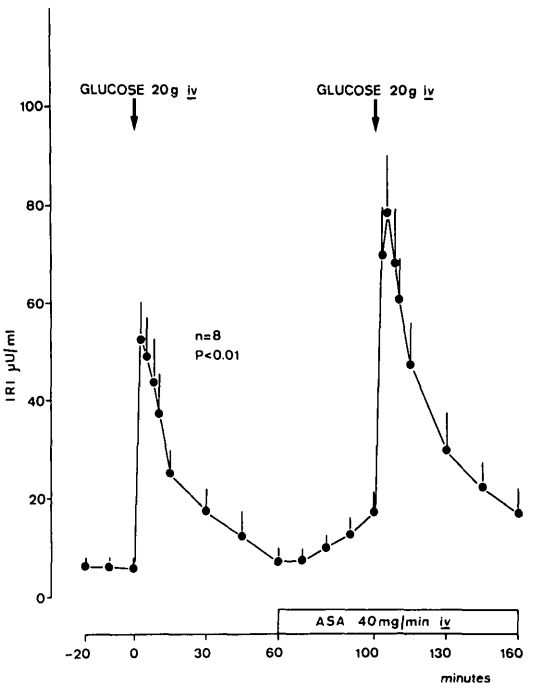
In the perfused rat pancreas, furosemide has been shown to stimulate rather than reduce insulin secretion to glucose.[27] In the same experiment, however, ASA, which is generally reported as a stimulator of insulin secretion in humans, blocked the second phase of glucose-induced insulin release. It seems likely that this discrepancy between in vivo and in vitro results could be explained by the species specificity of the phenomenon.

The fact that furosemide did not produce any evident deterioration of glucose disposal despite the reduced acute insulin response to glucose, which is thought to be a major determinant of intravenous glucose tolerance,[28] may be explained in part by our previous results. In fact, we have previously reported that PGE_1, infused in normal humans at the constant rate of 0.2 μg/kg/min, resulted in a 24% inhibition of the acute insulin response to glucose and in no deterioration of glucose disposal;[14] with the infusion of a higher dose of PGE_1 (0.5 μg/kg/min), the inhibition was 40% and this was accompanied by a decrease in glucose removal rates.[15] In addition, Robertson and Chen[13] found impaired glucose tolerance after PGE_2 (10 μg/min) in normals. Since the inhibition of the acute insulin response by furosemide in this study was about 30%, it is possible that a dose-related phenomenon exists between the reduction of acute insulin response and glucose tolerance. Alternatively, furosemide may enhance peripheral glucose uptake independent of insulin. However, Jackson and Nellen[29] found no evidence of deterioration of test results of either the glucose tolerance test or tolbutamide test in any of their 19 patients investigated before and after a 3-wk treatment with furosemide, 80 mg daily.

In general, previous reports dealing with the effects of benzothiadiazines or related diuretics upon glucose tolerance indicated that these drugs may have an injurious effect on carbohydrate metabolism. Wilkins,[30] Finnerty,[31] and Freis[32] were the first to report that treatment with thiazide preparations may cause glucose intolerance and perhaps also provoke diabetes mellitus in subjects who had not previously suffered from this disorder. Goldner et al.[33] studied 20 nondiabetic and 20 diabetic patients who were given various benzothiadiazines orally. The nondiabetic group showed no rise in blood glucose or change in their glucose tolerance test while receiving the diuretics, whereas 6 of the diabetic patients had increased hyperglycemia and glycosuria. Shapiro et al.[34] investigated 15 subjects with chemical diabetes and 15 normal controls before and after treatment with chlorthalidone. They found a reduction in carbohydrate tolerance in the disposed patients, but none in the control group. By contrast, Wolff et al.[35] reported that the diabetogenic action of benzothiadiazine derivatives was observed to occur in nonobese patients without a family history of diabetes as well as in obese hypertensive patients with such a family history. In one study only was serum insulin-like activity (ILA) assayed in patients with diabetes mellitus precipitated or worsened by benzothiadiazine administration.[36] This revealed low levels of both typical and atypical types of serum ILA, which rose, but only partially, toward normal after withdrawal of the drug. More recently, Lewis et al.[37] reported deterioration of glucose tolerance in hypertensive patients on prolonged diuretic treatment. Glucose tolerance did not show any significant change after 1 or 3 yr of therapy, whereas it was significantly reduced after 6 yr.

The cause of deterioration of carbohydrate metabolism by thiazide diuretics seems to be linked to endogenous potassium depletion,[38] which in turn causes impairment of

FIGURE 4. Plasma insulin levels in response to glucose pulses given before and during an infusion of acetylsalicylic acid in normal humans.



both insulin response to the glycemic stimuli[39] and glucose utilization in peripheral tissues.[40] The observation of Rapoport and Hurd,[41] who were able to normalize glucose tolerance by replenishing potassium stores depleted by chlorothiazide, is interesting in this context. On the other hand, an endogenous potassium depletion cannot explain the diabetogenic action of diazoxide, a sodium-retaining benzothiadiazine, which causes a reversible form of diabetes in humans[38] without causing any change in body potassium balance.[42] The potent diabetogenic action of diazoxide seems to be due to blockade of insulin secretion, either by a direct effect or by the interaction with α-adrenergic pathways.[38]

Thus, the mechanism(s) of the diabetogenic actions of ordinary thiazide diuretics and also diazoxide seem different from that of furosemide. It is interesting to note that Dargie et al.[43] found no evidence of depletion of total body potassium in subjects receiving continuous treatment with furosemide (40–120 mg daily) for 1 yr.

In conclusion, the action of furosemide on insulin secretion should be taken into account when it is used for long-term treatment of normals or maturity-onset diabetic patients. When diuretic therapy is mandatory, salicylate adjunct may be taken into consideration.

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