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Acetyl-salicylic acid impairs insulin-mediated glucose utilization and reduces insulin clearance in healthy and non-insulin-dependent diabetic man

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Summary. The effect of acetyl-salicylic acid (ASA, 3 g per day for 3 days) on glucose utilization and insulin secretion was studied in healthy volunteers and Type 2 diabetic patients using the hyperglycaemic and euglycaemic insulin clamp technique. When in healthy subjects arterial plasma glucose was acutely raised and maintained at +7 mmol/l above fasting level, the plasma insulin response was enhanced by ASA (70 ± 7 vs. 52 ± 7 mU/l), whereas the plasma C-peptide response was identical. Despite higher insulin concentrations, glucose utilization was not significantly altered (control, 61 ± 7; ASA, 65 ± 6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) indicating impairment of tissue sensitivity to insulin by ASA. Inhibition of prostaglandin synthesis was not likely to be involved in the effect of ASA, since insulin response and glucose utilization were unchanged following treatment with indomethacin. In the euglycaemic insulin (1 $mU \cdot kg^{-1} \cdot min^{-1}$) clamp studies, glucose utilization was unaltered by ASA despite higher insulin concentrations achieved during constant insulin infusion (103 ± 4 vs. 89 ± 4 mU/l). In Type 2 diabetic patients, fasting hyperglycaemia (10.6 ± 1.1 mmol/l) and hepatic glucose production (15 ± 2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) fell upon ASA treatment (8.6 ± 0.7 mmol/l; 13 ± 1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). During the hyperglycaemic clamp study, the plasma response of insulin, but not of C-peptide, was enhanced by ASA, whereas tissue sensitivity to insulin was reduced by 30 percent. It is concluded that in healthy and Type 2 diabetic man, ASA impairs tissue sensitivity to the action of insulin. This effect is counterbalanced by an augmented plasma insulin response to glucose, which results from a reduced insulin clearance rate. In Type 2 diabetic patients, the reduction in hepatic glucose production may be responsible for the amelioration of hyperglycaemia following ASA treatment.

Key words: Acetyl-salicylic acid, indomethacin, glucose utilization, insulin sensitivity, insulin secretion, insulin clearance, hepatic glucose production, Type 2 diabetes.

Prostaglandins (PG) may be involved in glucose homeostasis and insulin secretion. It has, however, been reported that PG_E may either inhibit or stimulate insulin secretion depending upon the experimental conditions used [1], and that alterations in glucose homeostasis as noted during PG^E_2 infusion may not be attributed to PG^E_2 itself, but rather to concomitant catecholamine release [2]. Furthermore, different inhibitors of PG synthesis appear to have variable effects on carbohydrate tolerance [1]. Nevertheless, the rise in insulin concentration and the amelioration of hyperglycaemia observed in non-insulin-dependent (Type 2) diabetic patients during treatment with sodium salicylate [3] or acetyl-salicylic acid (ASA) [4] may be attributed to inhibition of PG synthesis by these substances. In healthy subjects a stimulatory action of ASA has been described for insulin secretion in response to intravenous glucose and arginine [5, 6]. In contrast, other inhibitors of PG synthesis, such as indomethacin decrease [7, 8] or fail to affect insulin secretion [9]. As to tissue sensitivity to insulin, it has recently been demonstrated that ASA, apart from enhancing insulin secretion, impairs glucose metabolism, whereas ibuprofen fails to alter glucose disposal [5]. Against this contradictory background the present study was designed (1) to elucidate the various effects of ASA as the most widely used non-steroidal anti-inflammatory drug on insulin secretion, insulin clearance as well as glucose utilization, and (2) to define the mechanism by which ASA reduces hyperglycaemia in Type 2 diabetic man. The euglycaemic insulin and hyperglycaemic clamp techniques [10, 11] were used to quantitate these determinants of glucose homeostasis.

Materials and methods

Healthy subjects

Fourteen healthy volunteers (12 males, 2 females), ranging in age from 20–27 years (mean ± SEM, 23 ± 1 years) participated in the study. Body mass index was 21.5 ± 0.3 kg/m^2. No subject had a family history of diabetes or any previous history of liver, kidney or endocrine disease. All participants were asked to consume a weight-maintaining diet containing at least 250 g carbohydrate per day, and none of them was on any medication during the 8-week period preceding the study.

Non-insulin-dependent diabetic patients

Seven Type 2 diabetic patients, whose individual clinical data are given in Table 1, were also enrolled in the study. The known duration of their diabetes was 2 to 18 years. The mean fasting blood glucose

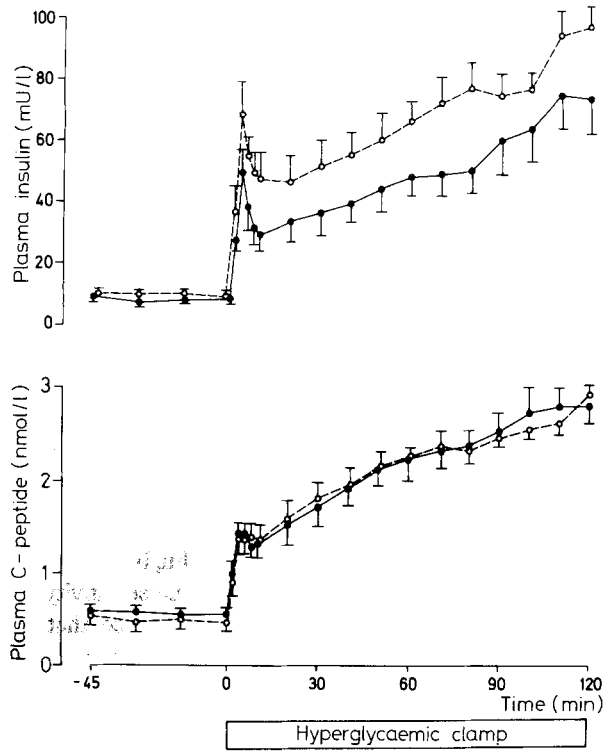

Fig. 1. Plasma concentrations of insulin and C-peptide in response to a square wave hyperglycaemic stimulus (+7 mmol/l hyperglycaemic clamp study) with (O - - - O) and without (●——●) preceding administration of acetyl-salicylic acid in healthy subjects. Mean ± SEM is indicated

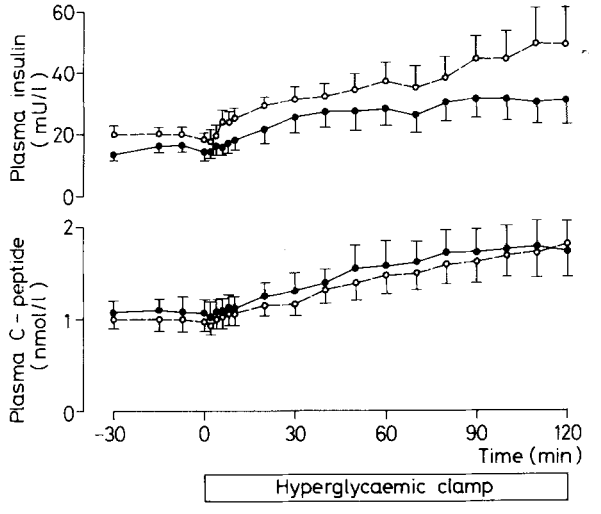

Fig. 2. Plasma concentrations of insulin and C-peptide in response to a square wave hyperglycaemic stimulus (+7 mmol/l) with (O - - - O) and without (●——●) preceding administration of acetyl-salicylic acid in Type 2 diabetic patients. Mean ± SEM is indicated ($n = 7$)

concentration obtained at 2 outpatient visits within the preceding 4 months was 9.4 ± 0.6 mmol/l, HbA_{1c} 6.7 ± 0.5% (normal ≤ 5.8%), urinary glucose loss was 2.9 ± 1.8 g per day without ketonuria. The patients continued their usual diet containing 120-150 g carbohydrate per day. No patient was on any oral hypoglycaemic therapy.

The purpose and potential risks of the study were carefully explained to all subjects before obtaining their written consent to participate. The study protocol was approved by the Ethical Committee of the University Hospital.

Protocol

All tests were performed with the subjects in the recumbent position after an overnight fast starting between 0700 and 0800 hours. A polyethylene catheter was placed in a retrograde direction in a wrist vein for blood sampling, and that hand was kept in a heated box at 70 °C to ensure arterialization of venous blood. Two types of studies were performed:

Hyperglycaemic (+ 7 mmol/l) clamp. In eight healthy subjects the effect of ASA on insulin secretion and glucose metabolism was examined with the hyperglycaemic clamp technique [10, 11]. The arterial plasma glucose concentration was acutely raised by 7 mmol/l (125 mg/dl) above basal with a priming dose of glucose given in a logarithmically falling fashion over 10 min. Subsequently, plasma glucose was maintained at the desired level by determination of plasma glucose every 5 min and appropriate adjustment of a variable glucose infusion. Blood for insulin and C-peptide measurements was drawn at 2-min intervals for the first 10 min and every 10 min thereafter. Under these steady-state conditions of hyperglycaemic hyperinsulinaemia, the endogenous glucose production is assumed to be completely suppressed in healthy man [12] and the amount of glucose infused (corrected for urinary glucose losses) must therefore equal the rate of glucose taken up by the body. It thus serves as a measure of the body's response to endogenous insulin.

All eight healthy subjects underwent a hyperglycaemic clamp study without any pretreatment (control) and following ASA administration (Colfarit®, Bayer-Pharma, Leverkusen, FRG; 1 g t.i.d. for 3 days, and 1 g one hour prior to the test). Six of these eight subjects also participated in a hyperglycaemic clamp study after pretreatment with another inhibitor of PG synthesis, indomethacin (Indocid, Merck Sharp & Dohme, Haarlem, Netherlands; 50 mg t.i.d. for 3 days, and 50 mg 1 h prior to the test). The same six subjects also volunteered for another hyperglycaemic clamp, in which insulin was infused continously at a rate of 0.25 $mU \cdot kg^{-1} \cdot min^{-1}$ to match the plasma insulin concentrations achieved in the study performed after ASA treatment.

Seven Type 2 diabetic patients also underwent a hyperglycaemic clamp study in combination with tracer glucose kinetic analysis for estimating hepatic glucose production (HGP). To this end, ^{3}H-3-glucose (Amersham International Ltd, UK) was administered in a 55 μCi bolus dose followed by a continuous infusion at a rate of 0.40 μCi/min. The infusion of tritiated glucose was begun 180 min prior to initiation of the clamp. Plasma ^{3}H-glucose specific activity, which reached a plateau in all patients during the 30 min basal period prior to starting the clamp, was determined at 5-10 min intervals throughout the study. All patients were studied before and after ASA administration as described above for healthy subjects.

Euglycaemic insulin clamp: In six healthy subjects, a primed-continuous infusion of crystalline biosynthetic human insulin (Eli Lilly Co., Indianapolis, IN) at rates of 1.0 and 10.0 $mU \cdot kg^{-1} \cdot min^{-1}$ was administered to acutely raise and maintain the arterial plasma insulin concentration at approximately 100 and > 1000 mU/l, respectively, for 120 min. Plasma glucose was maintained at the basal level by a variable glucose infusion as described for the hyperglycaemic clamp study. Under these steady-state conditions of euglycaemic hyperinsulinaemia the amount of glucose infused plus the rate of residual HGP equals the rate of glucose uptake by the body. ^{3}H-glucose was not employed in the studies in healthy subjects to estimate HGP. However, physiologically, HGP is almost completely suppressed by the hyperinsulinaemia achieved and glucose infusion rates therefore largely equal glucose utilization. Only in states of diminished suppression of HGP by insulin will glucose infusion rates underestimate glucose utilization. Low- and high-dose insulin clamp studies, which were performed 4 days apart, were repeated after at least 10 days thereafter following the same ASA regimen as in the hyperglycaemic clamp study.

Analytical procedures

The plasma glucose concentration was determined in duplicate on arterialized venous samples using the glucose oxidase method (Glucostat, Beckman Instruments Corp., Fullerton, Calif., USA). Tritiated

Table 1. Clinical data of Type 2 diabetic patients

Patients	Sex	Age (years)	Body mass index (kg/m^2)	HbA_{1c} (%)	Serum creatinine (μmol/l)	Medication	Duration of diabetes (years)	Blood glucose[a] (mmol/l)
1.	F	63	24.5	9.0	79	-	8	10.7
2.	F	55	25.4	5.8	62	digitoxin	2	7.3
3.	M	49	27.1	6.8	79	-	9	9.3
4.	M	52	25.9	6.2	97	-	5	7.7
5.	M	53	24.8	8.1	88	-	18	11.7
6.	F	40	22.0	5.2	70	clonidine	6	10.5
7.	F	60	25.9	6.0	79	-	5	8.4
Mean		53	25.1	6.7	79		8	9.4
±SEM		3	0.6	0.5	4		2	0.6

[a] Data of bood glucose represent the mean values obtained at the diabetic outpatient service on 2 occasions preceding the study by 4 months

Table 2. Plasma concentrations of glucose and insulin, as well as glucose infusion rate (M), insulin sensitivity (M/I ratio), and metabolic clearance rate of insulin (MCR_I) during the hyperglycaemic ($n=8$) and euglycaemic insulin clamp studies ($n=6$) in healthy subjects (mean ± SEM)

	Glucose (mmol/l)		Insulin (mU/l)			M	M/I	MCR_I
	Basal	Steady state	Basal	Insulin response		20–120 min ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	($\mu mol \cdot kg^{-1} \cdot min^{-1}$ per mU/l × 100)	($ml \cdot kg^{-1} \cdot min^{-1}$)
				Early	Late			
Hyperglycaemic clamp								
A. Control	4.5±0.2	11.4±0.1	8±1	35±5	52±7	61±7	129±20	-
B. +ASA	4.5±0.1	11.4±0.1	10±1[a]	51±8[a]	70±7[b]	65±6	100±15[a]	-
C. +indomethacin	4.7±0.1	11.8±0.1	8±1	31±4	42±5	56±5	137±16	-
D. +i.v. insulin	4.7±0.1	11.5±0.1	11±2	58±8[b]	73±9[a]	90±9[b]	132±19	-
Euglycaemic insulin clamp ($1.0\ mU \cdot kg^{-1} \cdot min^{-1}$)				Steady state				
A. Control	5.2±0.1	5.2±0.2	13±2	89± 4		47±2	55± 4	11.3±0.5
B. +ASA	5.2±0.4	5.2±0.3	14±1	103± 4[b]		50±2	48± 3[a]	9.8±4[b]
Euglycaemic insulin clamp ($10.0\ mU \cdot kg^{-1} \cdot min^{-1}$)								
A. control	4.9±0.2	4.8±0.2	10±1	1362±110		69±3	-	7.5±0.6
B. +ASA	5.1±0.3	4.9±0.2	14±2[a]	1641±165[a]		70±3	-	6.4±0.7[a]

[a, b] Indicates significant difference when compared with the respective control study ([a] $p<0.05$, [b] $p<0.005$)

Table 3. Plasma concentrations, utilization (M) and hepatic production (HGP) of glucose during the hyperglycaemic clamp studies in Type 2 diabetic patients ($n=7$; mean ± SEM)

	Glucose		M	M/I	HGP		
	Basal (mmol/l)	Steady state	20–120 min ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	($\mu mol \cdot kg^{-1} \cdot min^{-1}$ per mU/l × 100)	Basal	20–60 min ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	60–120 min
Hyperglycaemic clamp							
A. Control	10.6±1.1	17.6±1.1	26±3	135±34	15±2	3±2	5±3
B. +ASA	8.6±0.7[a]	15.9±0.7	24±1	84±21[a]	13±1[a]	2±1	4±2

[a] Indicates significant difference when compared with the respective control study without ASA ([a] $p<0.05$)

glucose specific activity was determined by the Somogyi procedure [13] with glucose concentration in the Somogyi filtrates analyzed by the hexokinase reaction (Boehringer, Mannheim, FRG). Determination of plasma insulin and C-peptide were performed by radioimmunoassay (inter- and intraassay coefficient of variation 8% and 6% respectively) as previously described [14]. HbA_{1c} was determined by the microcolumn technique (Biorad, Richmond, CA). Salicylate was measured in plasma colorimetrically on an automatic clinical analyzer (ACA II, Du Pont, Genf, Switzerland) [15].

Calculations

During the clamp studies, the glucose infusion rate required to maintain the desired glucose level was determined by calculating the mean value observed from 20–120 min. The total amount of glucose metabolized (M) was then calculated by correcting for urinary glucose losses and for the, although minor, changes in plasma glucose (space correction) [10]. In the studies employing ^3H-glucose kinetic analysis, HGP in the basal state was determined by dividing the tritiated glucose infusion rate by the steady-state plateau of glucose specific activity achieved during the 30 min preceding the clamp period. As a non-steady-state condition in glucose specific activity exists after glucose infusion, the rate of glucose turnover was calculated by Steele's equations in their derivative form [16] using a total volume of distribution of 280 ml/kg body weight and a pool fraction of 0.65 [17]. The rate of HGP was calculated by subtracting the glucose infusion rate from the rate of glucose appearance as determined by the isotopic tracer technique. During the hyperglycaemic clamp studies, the early and late insulin response were determined by calculating the mean plasma insulin (I) and C-peptide concentrations during the 0- to 10-min and the 10- to 120-min periods. The M/I ratio then provides a measure of the

effectiveness of insulin on tissue glucose uptake. The metabolic clearance rate (MCR) of insulin was calculated by dividing the continuous insulin infusion rate by the mean increment in plasma insulin concentrations.

All data are presented as the mean ± SEM. Statistical significance between means was calculated by the Student's paired two-tailed t-test.

Results

Healthy subjects

Fasting state. In the basal state, plasma concentrations were 4.8 ± 0.2 mmol/l for glucose, 0.53 ± 0.07 nmol/l for C-peptide, and 9.9 ± 0.9 mU/l for insulin. After ASA treatment, which raised plasma salicylate concentration into the therapeutic range, 906 ± 94 μmol/l, plasma glucose (4.8 ± 0.2 mmol/l), and C-peptide (0.50 ± 0.10 nmol/l) were unchanged, whereas insulin was increased slightly to 12.5 ± 0.6 mU/l ($p < 0.02$).

Hyperglycaemic clamp (+ 7 mmol/l; Table 2). When plasma glucose was acutely raised and maintained at 11.4 ± 0.1 mmol/l (coefficient of variation 4 ± 1%), a biphasic insulin response was observed. The early and late plasma insulin responses were 57 ± 21% ($p < 0.05$) and 45 ± 13% ($p < 0.005$), respectively, higher after ASA as compared to the intraindividual control study. However, plasma C-peptide responses in the control and ASA studies were identical (Fig. 1). Plasma insulin concentrations observed in the hyperglycaemic clamp studies with ASA were closely mimicked by the additional infusion of insulin (70 ± 7 vs. 73 ± 9 mU/l). Pretreatment with indomethacin did not alter the plasma insulin response to the square wave stimulus of hyperglycaemia.

The amount of glucose metabolized by the entire body (M) was 61 ± 7 μmol · kg^{-1} · min^{-1} in the control study. This was not significantly altered by pretreatment with ASA, 65 ± 6, despite higher insulin concentrations. Tissue sensitivity to insulin, as calculated by the M/I ratio was therefore diminished by 21 ± 4% by ASA ($p < 0.005$; Table 2). In contrast, glucose utilization and insulin sensitivity were unaltered by the administration of indomethacin. In the hyperglycaemic clamp study, in which insulin concentrations observed following ASA administration were mimicked by i.v. insulin, glucose utilization was markedly increased (90 ± 9 μmol · kg^{-1} · min^{-1}; $p < 0.005$), whereas insulin sensitivity remained unchanged as compared to the control study.

Euglycaemic insulin clamp (1.0 mU · kg^{-1} · min^{-1}; Table 2). As in the hyperglycaemic clamp studies, plasma glucose was kept close to the desired level, as demonstrated by a small coefficient of variation (4 ± 1%). Insulin infusion caused the hormone's plasma concentration to increase to 89 ± 4 mU/l in the control study, and to 103 ± 4 mU/l following ASA treatment ($p < 0.005$). Thus, calculated MCR of insulin was reduced by 13 ± 2% by ASA ($p < 0.005$). Glucose infusion rate (M) necessary to maintain euglycaemia was 47 ± 2 μmol · kg^{-1} · min^{-1} in the control, which was not different from the 50 ± 2 seen after ASA administration. Correcting for the higher insulin concentrations achieved following ASA by calculating an M/I ratio, insulin sensitivity was reduced by ASA (48 ± 3 vs. 55 ± 4 μmol · kg^{-1} · min^{-1} per mU/l × 100; $p < 0.05$).

Euglycaemic insulin clamp (10.0 mU · kg^{-1} · min^{-1}; Table 2). Steady-state insulin concentrations obtained during the high-dose insulin infusion were higher after ASA, 1641 ± 165 vs. 1362 ± 110 mU/l in the control study ($p < 0.05$), corresponding to a 19 ± 4% decrease in MCR of insulin by ASA. Insulin-mediated glucose uptake without ASA, 69 ± 3 μmol · kg^{-1} · min^{-1}, was identical after ASA treatment, 70 ± 3. Since glucose metabolism plateaus at insulin concentrations above 500 mU/l [18], the M/I ratio, which assumes a linear relationship of these parameters, was not calculated.

Type 2 diabetic patients

Fasting state. Basal plasma concentrations were 10.6 ± 1.1 mmol/l for glucose, 1.07 ± 0.15 nmol/l for C-peptide, and 15 ± 2 mU/l for insulin. Following ASA treatment, which raised plasma salicylate concentration to 728 ± 100 μmol/l, plasma glucose fell to 8.6 ± 0.7 mmol/l ($p < 0.05$), insulin was increased to 20 ± 2 mU/l ($p < 0.05$), whereas plasma C-peptide remained essentially unchanged, 0.98 ± 0.12 nmol/l. Thus, the molar insulin/C-peptide ratio was augmented by ASA from 0.10 ± 0.01 to 0.14 ± 0.01 ($p < 0.005$). Basal HGP, which at steady state conditions of fasting and negligible urinary glucose loss (mean, 0.1 μmol · kg^{-1} · min^{-1}) equals glucose utilization, fell from 15 ± 2 to 13 ± 1 μmol · kg^{-1} · min^{-1} upon ASA ($p < 0.05$; Table 2).

Hyperglycaemic clamp (+ 7 mmol/l; Table 3). When plasma glucose was acutely raised and maintained close to the desired level of + 7 mmol/l above fasting concentration (coefficient of variation 3 ± 0.3%), the response in plasma insulin was characterized by the absence of an early concentration peak within the first 10 min and the failure to significantly increase thereafter. In contrast to plasma insulin, which was augmented by about 40% by ASA administration, C-peptide concentrations were unchanged by ASA. Accordingly, the insulin/C-peptide ratio was increased by ASA (0.12 ± 0.01 vs. 0.18 ± 0.01, $p < 0.01$). Despite higher insulin concentrations, glucose utilization was unaltered by ASA (26 ± 3 vs 24 ± 1 μmol · kg^{-1} · min^{-1}). Therefore, tissue sensitivity to the action of insulin was reduced by 30 ± 9% ($p < 0.05$). Suppression of HGP by increasing hyperglycaemia was incomplete before (4 ± 2 μmol · kg^{-1} · min^{-1}) and after ASA treatment (3 ± 1 μmol · kg^{-1} · min^{-1}; $t = 20$–120 min).

Discussion

Several studies have demonstrated that salicylate and ASA increase basal [19] and stimulated insulin concentrations in normal subjects [5, 19] and in non-insulin-de-

pendent diabetic patients [3, 4, 20]. In particular, the defective acute insulin response to intravenous glucose characteristic for Type 2 diabetic patients can partially be restored by the infusion of ASA [21]. Since in normal man treatment with other inhibitors of PG synthesis, such as ibuprofen [5] or indomethacin [7, 8], is associated with unaltered or decreased insulin responsiveness to glucose, respectively, it may be assumed that the different effects of these drugs may be due to actions apart from inhibiting PG synthesis. As to experiments in vitro, sodium salicylate has been shown to augment glucose-induced insulin release by the isolated perfused rat pancreas [22], and by monolayer cultures of rat pancreatic cells [23], but not by hamster islets [24].

In the present study, plasma insulin concentrations in fasting healthy subjects were higher after a 3-day treatment course with ASA. Also the increase in the hormone's plasma concentration in response to a standardized square wave hyperglycaemic stimulus was about 50 percent higher after ASA administration. This applied to both, the early and late phases of insulin secretion. In contrast to plasma concentrations of insulin, those of C-peptide were almost identical in the fasting state as well as during the hyperglycaemic stimulus (Fig. 1). Even though the early and late responses in insulin secretion to hyperglycaemia were characteristically reduced in Type 2 diabetic patients [25], the hormonal pattern of augmented insulin but unchanged C-peptide concentrations was comparable to that found in normal man. Thus, the behaviour of plasma C-peptide as a reflexion of insulin secretion [26] strongly suggests that the higher insulin concentrations seen after ASA treatment are not brought about by a change in basal and stimulated insulin secretion rate as commonly implied in the literature, but rather by a diminution of insulin clearance. To validate this assumption, euglycaemic insulin clamp studies were performed in healthy subjects with two different insulin doses (1.0 and 10.0 $mU \cdot kg^{-1} \cdot min^{-1}$). Despite identical insulin infusion rates before and after ASA treatment, the plasma insulin concentrations achieved were consistently higher during the latter condition. Thus, the higher plasma insulin levels observed after ASA in the basal state and in response to glucose result from an ASA-induced reduction in unsulin removal. However, due to possible limitations in the interpretation of C-peptide as a measure of insulin secretion [27], an increase of the latter upon ASA cannot be completely ruled out. An effect on plasma insulin is not seen, when indomethacin or ibuprofen [5] is employed instead of ASA. Although a great deal of in-vitro data directly demonstrate that ASA along with other inhibitors of PG synthesis stimulate insulin secretion [22, 23], species differences may exist [24] and in-vitro experiments may not accurately reflect hormonal events in intact man. Several studies have demonstrated that salicylate or ASA lower plasma glucose in normal subjects [19] and Type 2 diabetic patients [3, 4]. Importantly, the improvement of glucose tolerance seems to depend upon continuing B cell function, and no improvement in metabolic control becomes therefore apparent in insulin requiring diabetic patients [6]. In the present study glucose utilization in response to a square wave plateau of hyperglycaemia was unchanged by ASA in healthy as well as diabetic man. When, however, glucose utilization was corrected for the higher plasma insulin concentrations achieved, tissue sensitivity to the action of endogenously secreted insulin as calculated by the M/I ratio was reduced by about 20–30 percent. To validate this estimate of insulin sensitivity, further hyperglycaemic clamp studies were performed in healthy subjects with insulin concentrations strictly comparable to those seen in the ASA treated group by the additional infusion of exogenous insulin. Estimation of the necessary insulin infusion rate was made possible by our previous observation that exogenous insulin does not modify glucose-induced insulin secretory response, but simply adds to the plasma insulin response [28]. Using this approach, glucose utilization was markedly enhanced and thus calculated insulin sensitivity was normal. This line of reasoning not only confirms the inhibitory action of ASA on tissue sensitivity to insulin, but gives evidence for the validity of calculating an M/I ratio for estimating tissue sensitivity to insulin. No such an effect on insulin sensitivity was found for other inhibitors of PG synthesis, such as for indomethacin given in a dose equipotent to ASA in regard to PG inhibition [29] or for ibuprofen in the study by Newman et al. [5]. Whether the effects of ASA treatment on insulin action are due to interference at binding or postreceptor sites remains to be established.

When in the euglycaemic insulin clamp studies plasma insulin was raised to concentrations within the still physiological range of 100 mU/l, glucose utilization was not altered by ASA treatment. When, however, correction was applied for the higher insulin concentrations achieved after ASA by calculating the respective M/I ratio, impaired effectiveness of insulin on tissue glucose uptake was unveiled. Since HGP, which also contributes to glucose input, was not determined in healthy subjects, glucose utilization may have been underestimated. However, HGP is known to almost completely suppress (95–100 percent) at these insulin concentrations [18]. Thus, only if ASA had reduced suppression of HGP, had glucose utilization and thus insulin sensitivity during ASA treatment been underestimated. This explanation, however, appears to be highly unlikely on the basis of the results from the hyperglycaemic clamp study and from the fact that, if at all, PG synthesis inhibitors attenuate HGP [30].

Our data also reconcile how salicylate possibly improves glucose tolerance in Type 2 diabetic patients [3, 4, 21]. It appears that impairment of tissue sensitivity to the action of insulin, which is characteristic for these patients [31], may even deteriorate further upon ASA treatment. This event, however, is counterbalanced by augmented circulating insulin concentrations due to a reduction in insulin clearance. The latter mechanism may help to explain the restraining effect of ASA on

HGP. Reports of enhanced plasma insulin response to glucose stimulation by ASA in non-insulin-dependent diabetic patients [3, 4, 21] are in support of this concept.

In conclusion, treatment with ASA impairs insulin action in healthy and in Type 2 diabetic man. Impaired glucose utilization is, however, counterbalanced by an augmented plasma insulin response to glucose, which results from a reduced clearance rate of insulin. In Type 2 diabetic patients a reduction in HGP due to greater insulin availability appears to be responsible for the blood glucose lowering effect of ASA treatment. Since no effects on glucose utilization and plasma insulin response to glucose are seen after treatment with other PG inhibitors, such as indomethacin or ibuprofen, the ASA induced metabolic and hormonal events may be regulated by PG independent mechanisms.

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