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Effect of the new imidazoline derivative S-22068 (PMS 847) on insulin secretion in vitro and glucose turnover in vivo in rats

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Abstract

We have investigated the possible mechanisms underlying the antihyperglycaemic effect of the imidazoline derivative S-22068. In vitro, in the presence of 5 mmol/l glucose, S-22068 (100 μmol/l) induced a significant and sustained increase in insulin secretion from isolated, perifused, rat islets and a marked sensitization to a subsequent glucose challenge (10 mmol/l). S-22068 (100 μmol/l was able to antagonize the stimulatory effect of diazoxide on ^{86}Rb efflux from preloaded islets incubated in the presence of 20 mmol/l glucose. Experiments were also performed to investigate whether S-22068 can alter glucose turnover and peripheral insulin sensitivity in vivo in mildly diabetic rats and obese, insulin resistant, Zucker rats. Neither glucose production nor individual tissue glucose utilization was modified by S-22068 in either group of rats. Similar results were obtained whether the studies were performed under basal conditions or during euglycaemic/hyperinsulinemic clamps. The results suggest that S-22068 exerts part of its antihyperglycaemic effect by promoting insulin secretion without alteration of peripheral insulin sensitivity.

Keywords: Imidazoline; Non-insulin-dependent diabetes mellitus; S-22068; Insulin secretion, in vitro; K^+_{ATP} channels; Glucose metabolism

1. Introduction

There is now increasing evidence that certain imidazoline derivatives can act as potent insulinotropic agents and that they can improve glucose homeostasis under conditions of glucose intolerance in vivo (Wang et al., 1996; Berdeu et al., 1997). The weight of evidence indicates that this capacity is not attributable to α_2-adrenoceptor antagonism, although it was originally proposed that this property might be involved since many of the most active compounds are also α_2-adrenoceptor antagonists. It emerged subsequently, however, that the ability to promote insulin release and to lower plasma glucose is restricted to a sub-family of α_2-adrenoceptor antagonists which also possess an imidazoline ring (Schulz and Hasselblatt, 1988; Östensen et al., 1989; Chan, 1993). Moreover, insulin release and glucose tolerance can be enhanced by several imidazolines which are devoid of α_2-adrenoceptor antagonist activity (Schulz and Hasselblatt, 1989; Bertrand et al., 1992; Berdeu et al., 1997). Thus, the chemical class of imidazoline compounds represents an increasingly attractive source of potential new oral antidiabetic agents.

In this context, Institut de Recherches Internationales Servier (IRIS) has recently synthesized a new imidazoline compound, S-22068, as a prototype antidiabetic agent. In a recent study, we have examined the effects of this agent in

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a rat model of non-insulin-dependent diabetes mellitus, in which diabetes was induced by injection of a low dose of streptozotocin (STZ). These rats exhibited mild fasting hyperglycaemia, glucose intolerance, and impaired insulin secretion, features very similar to those observed in patients with non-insulin-dependent diabetes mellitus, (Thibault et al., 1992; Pelé-Tounian et al., 1998). Acute as well as chronic treatment of diabetic rats with S-22068 greatly improved glucose tolerance and slightly stimulated insulin secretion.

The present work was undertaken to study the mechanism responsible for the antihyperglycaemic effects of S-22068. This has been done, on one hand, by examination of the effects of S-22068 on the process of stimulus–secretion coupling in isolated rat islets and on the other, by monitoring its effects on glucose turnover and utilization, in vivo, under basal or euglycaemic/hyperinsulinemic conditions. The in vivo experiments were performed in normal control rats, in diabetic rats and in obese, insulin resistant, Zucker (*fa/fa*) rats (Terretaz et al., 1986).

2. Materials and methods

2.1. Animals and treatments

All experiments on animals were performed under approval of both institutions.

2.1.1. Control Wistar rats

Three-month old male Wistar rats (Iffa-Credo, L'Arbresle, France) weighing about 250 g were used in control experiments. They were housed in wire-bottomed cages and maintained at 21 ± 2°C in a room with a 12-h fixed light–dark schedule. Water and standard laboratory chow (UAR, Villemoisson-sur-Orge, France) were freely available.

2.1.2. Streptozotocin-injected rats

To be rendered diabetic, control Wistar rats received an i.v. injection of a low dose (35 mg kg^{-1}) of STZ dissolved in a citrate buffer under ketamine hydrochloride anaesthesia (75 mg kg^{-1}, i.p.) (Mérieux, Lyon, France). This was administered at 6 weeks of age. Glucose homeostasis and insulin secretion were assessed as an index of diabetes by performing a glucose tolerance test 2 weeks after STZ injection.

2.1.3. Male obese Zucker rats

Three-month old genetically obese Zucker (*fa/fa*) rats, weighing around 350–450 g, were used. They were housed in the same conditions as those described above.

2.1.4. Drug administration

In order to study the effects of S-22068 on glucose turnover, animals (either STZ or obese Zucker rats) received a single daily dose of the compound (24 mg kg^{-1}, p.o.). Control rats received saline in place of S-22068.

2.2. Basal glucose turnover rate and clamp studies in obese Zucker and diabetic rats

2.2.1. Measurement of glucose turnover rate

Endogenous glucose production in the basal state, as well as during the glucose clamp studies, was assessed by a primed continuous infusion of 3-[^{3}H] glucose according to the protocol previously described by Burnol et al. (1983). In the basal state, the rate of glucose appearance (Ra) is equal to the rate of glucose disappearance (Rd). These two parameters were calculated from the isotopic dilution equation: $Ra = Rd = F/AS$ where F is the rate of tracer infusion (dpm min^{-1}) and AS is the specific activity of blood glucose at equilibrium (dpm mg^{-1}). Blood glucose and specific radioactivity among these four points varied by less than 5%.

A second aliquot from each blood sample was used to determine the blood glucose concentration on an automated blood glucose meter (Lifescan, Johnson and Johnson, Milpitas, USA). The remaining blood was centrifuged and the plasma kept at -20°C for subsequent determination of insulin.

2.3. Euglycaemic / hyperinsulinemic clamps studies

Clamps studies were performed as described previously (Ferre et al., 1985) in rats fasted for 6 h. Rats were anaesthetized with pentobarbital sodium (75 mg kg^{-1} i.p., Clin-Midy, France). After a 30-min rest period (which allows blood glucose to return to basal values) S-22068 (24 mg kg^{-1}) was injected i.p. The experiment was then started 30 min after this injection. Before all infusions, a 150-ml blood sample was collected for determinations of basal blood glucose and plasma insulin levels. A priming dose of insulin (0.2 pmol/l) was given over a 30-s period through a saphenous vein to rapidly increase the plasma insulin concentration. Following this, insulin was infused at a constant rate of 20 ml min^{-1} (0.6 U kg^{-1} h^{-1}). The infusion of exogenous glucose (10% solution) via a precidor pump (Infors, Basel, Switzerland) was commenced 5 min after beginning the insulin infusion. Blood samples (30 μl) were then collected from the arterial catheter at 5 min intervals for 45 min and the blood glucose concentration was immediately measured using an automated blood glucose meter (Lifescan). The rate of glucose infusion was constantly adjusted throughout the experiment to clamp blood glucose at basal level.

2.4. 2-Deoxyglucose injection and blood and tissue sampling

Either in the basal state or when a steady-state glycaemia was achieved in the glucose clamp experiments,

2-deoxyglucose was injected as a 15-μCi bolus through the arterial catheter. Thereafter, blood glucose samples (20 μl) were collected 1, 3, 5, 10, 20, 40 and 60 min after 2-deoxyglucose injection. Blood samples to be used for determination of glucose concentration or 2-deoxyglucose specific radioactivity were collected throughout the experiment. At the end of the experiment (60 min after the 2-deoxyglucose administration) a blood sample was collected and immediately centrifuged at 4°C and the plasma was frozen at −20°C for subsequent determination of insulin. Then the animal was killed by cervical dislocation. The tissues were removed and their 2-deoxyglucose-6-phosphate tissue content was determined according to the method of Ferre et al. (1985).

Because the main purpose of the present study was to compare glucose utilization between treated and untreated rats (rather than to obtain an absolute estimation of glucose utilization rate in each case) tissue glucose uptake was evaluated by calculating the simplified index, R′g as previously described by James et al. (1985).

2.5. Islet perifusion studies and measurement of islet $^{86}Rb^+$ efflux rates

Pancreatic islets were isolated by collagenase digestion (Montague and Taylor, 1968) from normal Wistar rats (180–230 g) allowed free access to food and water. Estimation of islet cell $^{86}Rb^+$ efflux was performed as described previously (Chan et al., 1991). Samples of the perifusion medium were collected and analysed for $^{86}Rb^+$ after addition of scintillant. $^{86}Rb^+$ efflux rates were determined and expressed relative to the control rate (100%) measured as the efflux over the 10 min preceding introduction of diazoxide. S-22068 was introduced 15 min before diazoxide.

2.6. Chemicals

S-22068 was provided by the IRIS chemistry department. 3-[^{3}H] glucose, and $^{86}Rb^+$ were purchased from Amersham Life Science (Buckinghamshire, UK) and 2-deoxy-1-[^{14}C] glucose was from Dupont NEN (Boston, USA). Collagenase from *Clostridium histolyticum*, specific activity 2.67 U/mg, from Boehringer Mannheim (Germany), Ready-gel scintillant from Beckman (Fullerton, USA), porcine monocomponent insulin Actrapid from Novo (Copenhagen, Denmark).

2.7. Analytical methods

Plasma glucose was determined using a glucose analyzer (Beckman). Insulin concentrations in the plasma and in the perifusate were measured with a radioimmunoassay kit (Sorin, Biomedica, Antony, France). The lower limit of the assay was 19.5 pmol/l with a coefficient of variation within and between assays of 6%.

2.8. Calculations and statistical analysis

Results are expressed as mean ± S.E.M. Differences between means obtained with treated and untreated diabetic or Zucker rats were evaluated by a one-way analysis

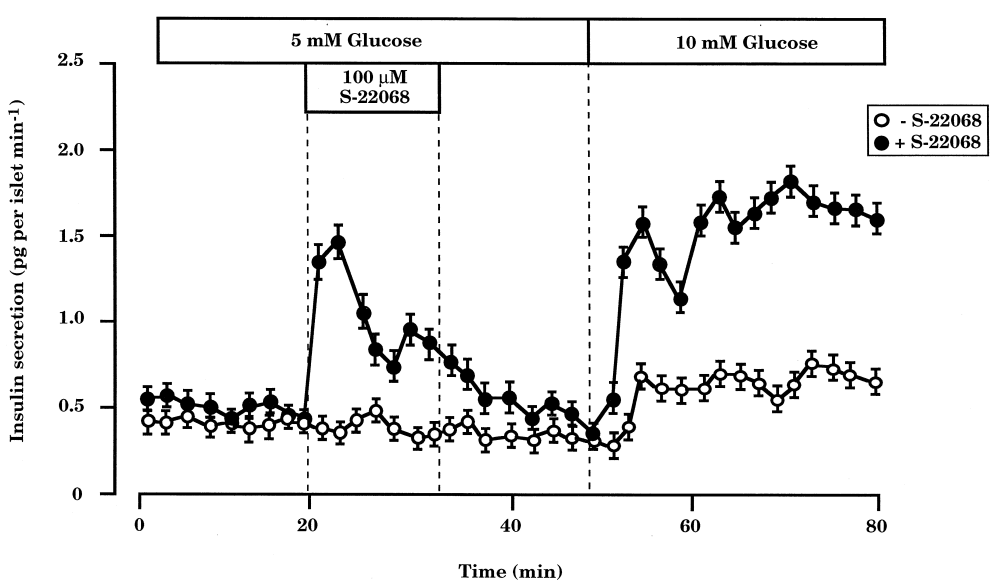

Fig. 1. Effects of S-22068 on insulin secretion. Groups of 100 islets were perifused in a medium containing 4 mmol/l glucose for 30 min. After this time ($t = 0$) 5 mmol/l glucose was introduced. The 100-μmol/l S-22068 was then added during 15 min after a further 20 min. After S-22068 removal, insulin secretion was stimulated with 10 mmol/l glucose through the end of the experiment. Data are presented as mean rates of insulin secretion ± S.E.M. for four different experiments.

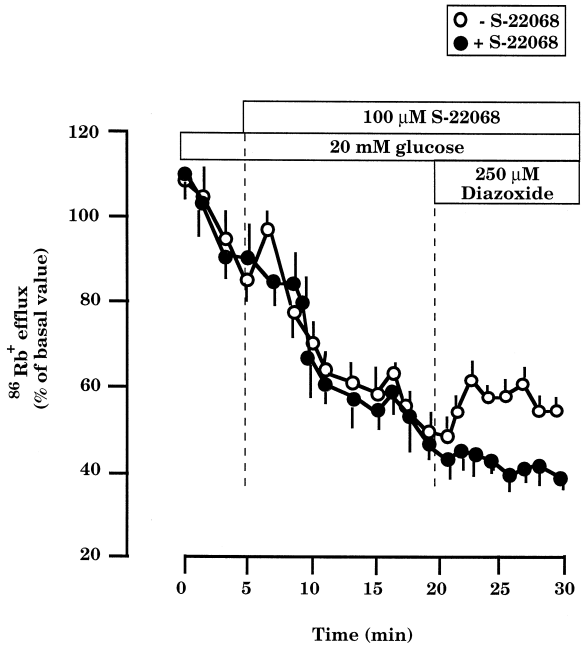

Fig. 2. Effects of S-22068 (100 μmmol/l) on diazoxide (250 μmmol/l)-induced increase in $^{86}Rb^+$ efflux from preloaded islets. Islets of Langerhans were preloaded with $^{86}Rb^+$ and perifused in the presence of 4 mmol/l glucose for 30 min prior to the start of the experiment. At $t = 0$, 20 mmol/l glucose was introduced in test channels, S-22068 was introduced at $t = 5$ min (closed circles). Diazoxide was added at $t = 20$ min to all channels. Results are means of duplicate channels. The experiment was reproduced four times.

of variance and results were considered significant from $P < 0.05$.

3. Results

3.1. Effect of S-22068 on insulin secretion

S-22068 (100 μmol/l) provoked a significant increase in insulin secretion from islets exposed to 5 mM glucose. The increase in secretion followed rapidly after introduction of the imidazoline (Fig. 1) and could be sustained for at least 30 min in the continued presence of S-22068 (not shown). When the compound was removed from the medium, insulin secretion declined to the basal level within 10 min, demonstrating that the secretory response to S-22068 was fully reversible. Treatment of islets with S-22068 did not cause any impairment of their glucose response upon subsequent stimulation (Fig. 1). Indeed, islets which were previously exposed to S-22068 responded to a glucose (10 mmol/l) challenge with an enhanced secretory response compared to islets not treated with the compound (Fig. 1). Thus, perifusion of islets with S-22068 resulted in marked sensitization to glucose such that their secretory response was enhanced significantly when 10 mM glucose was subsequently introduced (Fig. 1).

3.2. Effect of S22068 on diazoxide-induced changes in islet $^{86}Rb^+$ efflux

One of the principal steps in the stimulus–secretion coupling process for insulin secretion is the closure the ATP-sensitive K^+ channels (K^+_{ATP} channel). This, in turn, leads to plasma membrane depolarization and the entry of calcium ions (Cook and Hales, 1984). In vitro, the permeability of the membrane to potassium can be conveniently evaluated by using $^{86}Rb^+$ as tracer. When islets were preloaded with $^{86}Rb^+$, and then perifused with 20 mmol/l glucose, a progressive decrease in $^{86}Rb^+$ efflux was observed (Fig. 2). In these experiments a 20-mmol/l glucose concentration was used for complete K^+_{ATP} channel closure, then allowing maximal diazoxide effect. Therefore, the ability of the compound to reverse the effect of diazoxide could be investigated under the best conditions. Addition of S-22068 (100 μmol/l) to the perifusion medium did not cause any measurable change in the rate of $^{86}Rb^+$ efflux. Treatment of islets with diazoxide (250 μmol/l) triggered an increase in $^{86}Rb^+$ efflux in control channels, but this was prevented by the presence of S-22068 (Fig. 2).

3.3. Effect of chronic treatment with S-22068 (24 mg kg^{-1}) on glucose turnover in diabetic rats

As shown in Table 1, plasma glucose was significantly higher in diabetic rats than in the other groups. Plasma insulin levels were significantly decreased in diabetic rats, whereas obese Zucker were highly hyperinsulinemic (Table 1).

After chronic administration of S-22068 (over 15 days) basal glucose turnover was not different between control and treated diabetic rats (6.2 ± 0.4 vs. 6.6 ± 0.4 mg min^{-1} kg^{-1}). Basal glucose uptake in tissues was also unaffected by chronic S-22068 treatment (Table 2).

Table 1
Plasma glucose and insulin concentrations in the different groups of rats in the basal state (values are means ± S.E.M. Number of cases in parenthesis)

	Wistar rats	Diabetic rats	Lean Zucker rats	Obese Zucker rats
Glucose (mmol/l)	5.2 ± 0.2 (18)	8.9 ± 0.3* (14)	4.9 ± 0.2 (18)	5.4 ± 0.2 (15)
Insulin (pmol/l)	210 ± 20 (18)	145 ± 21 (14)	180 ± 15 (18)	1190 ± 90* (15)

*$P < 0.05$. Significantly different from control Wistar or lean Zucker rats.

3.4. Effect of S-22068 on glucose turnover and utilization by individual tissues during an euglycaemic / hyperinsulinemic clamp

During euglycaemic/hyperinsulinemic clamp experiments insulin was infused at a rate of 0.6 U kg^{-1} h^{-1} in the first set of experiments and glucose turnover rates were increased to similar values in normal, untreated diabetic and S-22068-treated diabetic rats (Table 2). Indeed, no differences were found in either of the parameters studied between S-22068-treated and -untreated diabetic rats. In all groups of rats, hepatic glucose production was abolished by the insulin infusion and individual tissue glucose utilization was not significantly affected by the administration of S22068 (Table 1).

In a second set of experiments, the rate of insulin infusion was then reduced to 0.2 U kg^{-1} h^{-1}, to reveal a possible effect of the compound that could have been masked by a maximal insulin concentration. However, again, no differences were observed between the experimental groups under these conditions (Table 2).

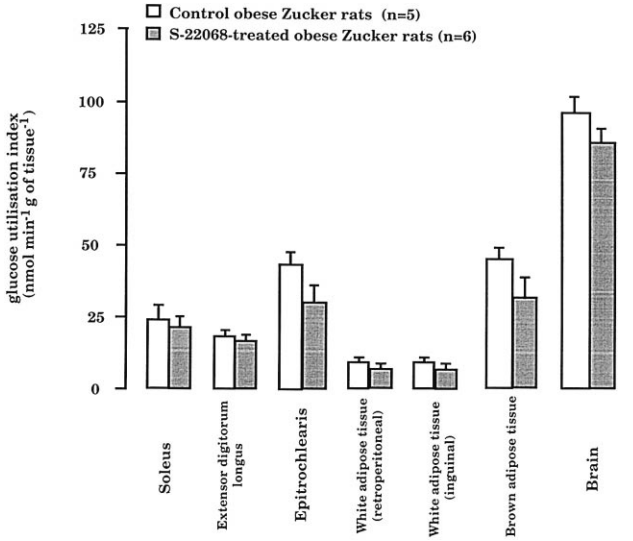

Fig. 3. Glucose utilization during an euglycaemic/hyperinsulinemic clamp in various tissues of obese Zucker (*fa/fa*) rats chronically treated with S-22068 (24 mg kg^{-1} day^{-1}) or saline. Values are means ± S.E.M. All the differences were not significant.

Table 2
Effect of S-22068 on whole body glucose utilization and hepatic glucose production under basal conditions and during euglycaemic/hyperinsulinemic clamp in diabetic and obese Zucker rats
Rats received daily a single oral administration of S-22068 (24 mg kg^{-1}) or saline during 15 days before the day of experiment. Experiments were performed in rats fasted from 6 h. Glucose turnover rate was assessed by a primed followed by a continuous infusion of 3-[^{3}H] glucose. Each value represents the mean ± S.E.M. of four to six different experiments. All the differences between treated and untreated rats were not significant.

	Glucose utilization (mg min^{-1} kg^{-1})	Hepatic glucose production (mg min^{-1} kg^{-1})
Basal conditions		
Untreated normal rats	6.5 ± 0.6	6.5 ± 0.6
Untreated STZ rats	6.6 ± 0.4	6.6 ± 0.4
STZ rats treated with S-22068	6.2 ± 0.4	6.2 ± 0.4
Untreated obese Zucker rats	4.6 ± 0.5	4.6 ± 0.5
Obese Zucker rats treated with S-22068	5.1 ± 0.5	5.1 ± 0.5
Under insulin infusion		
Flow rate: 0.6 U kg^{-1} h^{-1}		
Untreated normal rats	11.6 ± 0.3	None
Untreated STZ rats	13.1 ± 1.1	None
STZ rats treated with S-22068	13.5 ± 1.9	None
Untreated obese Zucker rats	7.9 ± 0.8	0.7 ± 0.1
Obese Zucker rats treated with S-22068	7.9 ± 1.2	1.0 ± 0.1
Flow rate: 0.2 U kg^{-1} h^{-1}		
Untreated STZ rats	7.8 ± 0.3	2.3 ± 0.3
STZ rats treated with S-22068	8.9 ± 0.6	1.7 ± 0.8

Similar experiments were next performed in obese insulin-resistant Zucker *fa/fa* rats. In these rats, infusion of insulin at 0.6 U kg^{-1} h^{-1} did not completely abolish hepatic glucose output (due to insulin resistance) but glucose turnover rate was unaffected by S-22068 (Table 2). Glucose uptake into individual tissues was slightly decreased by S22068 but the difference did not reach statistical significance (Fig. 3).

4. Discussion

Recently, we have presented evidence that the new imidazoline compound, S-22068, is very effective at lowering basal hyperglycaemia and promoting improved glucose tolerance in diabetic rats (Pelé-Tounian et al., 1998). The basis of these effects were not fully defined but it was suggested that they resulted, in part, from an insulinotropic action on the pancreatic B-cell, since insulin levels under basal conditions and after a glucose load were slightly higher in treated than in untreated diabetic rats. To investigate these issues further, we have now studied both insulin secretion and glucose metabolism in response to S-22068, in a variety of experimental models.

4.1. Effects of S-22068 on pancreatic B-cell function

In confirmation of our previous in vivo experiments, it was observed that S-22068 caused a direct stimulation of insulin secretion from isolated, perifused, rat islets. The response was rapid and readily reversible on removal of the drug (Fig. 1) but could be sustained if the infusion was maintained.

Initially, it was suggested that the stimulation of insulin secretion by imidazoline compounds may reflect the blockade of α_2-adrenoceptors since the majority of active compounds are also α_2-adrenoceptors antagonists. However, this view has now been superseded by a model in which imidazolines are proposed to interact with a binding site which controls the open state of K^+_{ATP} channels in the B-cell membrane. Numerous experiments performed either in isolated islets (Chan and Morgan, 1990; Chan et al., 1991; Brown et al., 1993), pancreatic B-cells (Plant and Henquin, 1990; Plant et al., 1991; Jonas et al., 1992; Ishida-Takahashi et al., 1996) and RINm5F or HIT-T15 insulin-secreting cells (Chan et al., 1991; Dunne, 1991; Rustenbeck et al., 1995; Zaitsev et al., 1996) have demonstrated that imidazolines (including phentolamine, clonidine, alinidine, antazoline, tolazoline, cibenzoline) promote insulin secretion by a mechanism involving closure of the ATP-sensitive K^+ channel.

The present studies suggest that S-22068 may have a similar action in the B-cell since, in common with other imidazoline secretagogues, it was able to antagonize the effects of diazoxide (an opener of K^+_{ATP} channels) on both $^{86}Rb^+$ efflux (Fig. 2) and insulin secretion from rat islets. Moreover, we observed that S-22068 was able to sensitize the cells to a subsequent glucose challenge (Fig. 1). This effect suggests that exposure of the cells to S-22068 induced a state of heightened responsiveness which resulted in amplification of the secretory response when the glucose concentration was subsequently raised. We have not investigated the detailed time course of this phenomenon but the present results show that removal of S-22068, as much as 20 min prior to the increase in the glucose concentration, was associated with potentiation of the response to the nutrient. This was not due to any prolonged elevation of insulin secretion at the time of glucose addition since removal of S-22068 was followed by a rapid decline in the secretion rate to the basal level. In this respect, S-22068 differs from certain other imidazolines (such as phentolamine or antazoline) or related compounds (Dickinson et al., 1997) whose stimulatory actions in vitro are only slowly reversible.

4.2. Effects of S-22068 on glucose turnover

Previously, we reported that administration of S-22068 to diabetic rats resulted in normalization of hyperglycaemia (Pelé-Tounian et al., 1998). A key difference between diabetic and obese Zucker rats is the development of marked insulin resistance in the latter, as evidenced by the failure of insulin infusion to completely block hepatic glucose output in the obese animals. This contrasted markedly with the situation in diabetic rats, where infusion of insulin resulted in total blockade of hepatic glucose output.

Therefore, the present results emphasize that the ability of S-22068 to restore euglycaemia in vivo was independent of the presence or absence of hepatic insulin resistance.

The euglycaemic/hyperinsulinemic clamp experiments confirmed that the diabetic rats were not insulin resistant at either the hepatic or the peripheral (muscle, adipose tissue) level. These observations are in keeping with those reported by other workers who have demonstrated that insulin resistance only develops in diabetic rats given higher doses of the diabetogenic drug or when STZ is administered later in life (Blondel et al., 1989; Portha et al., 1989). After chronic treatment with S-22068, neither whole body glucose turnover nor glucose utilization in a selection of insulin-responsive tissues and in the brain were modified at either of the insulin infusion rates used. Exactly equivalent results were obtained in obese Zucker (*fa/fa*) rats. Thus, we can conclude from these results that chronic oral treatment with S-22068 is not effective in modifying peripheral tissue sensitivity to insulin. However, it cannot be excluded that a more prolonged treatment could result in significant changes in insulin sensitivity.

Taken as a whole, the present results reveal that S-22068 may exert an influence on whole body glucose homeostasis at least in part via its insulinotropic effect rather than by increasing glucose metabolism.

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