Biochemical Pharmacology, Vol. 32, No. 19, pp. 2903–2908, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

THE EFFECTS OF GLIBENCLAMIDE ON RAT ISLET RADIOACTIVE NUCLEOTIDE EFFLUX, ATP CONTENTS AND RESPIRATORY RATES

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(*Received* 8 *November* 1982; *accepted* 2 *May* 1983)

Abstract—In order to assess the effects of sulphonylurea on islet function, changes in radioactive nucleotide efflux, ATP contents and oxygen uptake of low or high glucose cultured rat islets in response to glibenclamide were determined. It was observed that: (1) low glucose cultured islets displayed a prompt increase in radioactive efflux in response to glibenclamide when perfused in the presence of 1.7, 5.6 and 11.1 mM glucose. The response was most prominent in the presence of 5.6 mM glucose. (2) High glucose cultured islet did not respond to glibenclamide with increased radioactive efflux in the presence of 11.1 mM glucose, in contrast to the effects in the presence of 1.7 or 5.6 mM glucose. The sulphonylurea-induced changes could be delayed by adding glibenclamide in parallel with a decrease in glucose from 20 to 1.7 mM. (3) No effects of glibenclamide on radioactive nucleotide efflux from either low or high glucose cultured islets could be observed in the absence of extracellular Ca^{2+}. (4) Glibenclamide decreased the islet ATP content of low glucose cultured islets in the presence of 5.6 mM glucose. On the contrary, the ATP content of high glucose cultured islets was increased by glibenclamide with 1.7 mM glucose present. (5) Islet respiration was increased by adding glucose in both high or low glucose cultured islets, although respiration in the presence of both 5.6 and 11.1 mM glucose was higher in high glucose cultured islets. (6) The addition of glibenclamide decreased oxygen uptake of low glucose cultured islets in the presence of 5.6 and 11.1 mM glucose and that of high glucose cultured islets at all glucose concentrations tested (1.7, 5.6 and 11.1 mM). It is concluded that glibenclamide-induced changes in radioactive nucleotide efflux may reflect metabolic and ionic changes of importance to islet functions.

The mechanism(s) by which hypoglycaemic sulphonylurea stimulates insulin release remains a question of considerable debate. It has been shown that tolbutamide depolarizes the B-cells and increases their electrical activity [1]. Furthermore, the sulphonylurea-induced insulin release is accompanied by an increase in $^{45}Ca^{2+}$ net uptake [2–4]. Tolbutamide may both increase or decrease K^+-permeability [3], depending on the glucose concentration of the perfusion medium. Finally, sulphonylureas have been shown to induce modest decreases in the islet ATP contents without any noticeable effects on islet NADH-generation [5, 6].

In a recent report it was observed that glipizide stimulation of insulin release coincided with a drastic increase in radioactive nucleotide efflux from mouse islets prelabelled with [2-^{3}H]adenosine [7]. Changes in radioactive nucleotide efflux have been assumed to be directly proportional to cytoplasmic AMP and/or ADP concentrations, and an increase in intracellular ATP is therefore accompanied by decreased radioactive nucleotide efflux [7]. The present study was performed in order to elucidate further the mechanism of sulphonylurea stimulation of B-cell function with particular respect to changes in radioactive nucleotide efflux, adenine nucleotide contents and oxygen uptake using glibenclamide.

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MATERIALS AND METHODS

Chemicals. [2-^{3}H]Adenosine was purchased from Amersham (Bucks, U.K.). Firefly extracts (FLE 50), apyrase, EGTA, phosphenolpyruvate and HEPES were from Sigma Chemical Co. (St. Louis, MO). ATP, ADP, AMP, pyruvate kinase and myokinase were obtained from Boehringer (Mannheim, F.R.G.). D-Glucose was bought from Mallinckrodt Chemicals (Paris, KY). Bovine serum albumin (fraction V) was from Miles Laboratories (Slough, U.K.). Collagenase (type CLS) was obtained from Worthington Biochemicals (NJ). Hanks' solution and TC 199 were purchased from Statens Bakteriologiska Laboratorium (Stockholm, Sweden). Streptomycin and penicillin were from Glaxo Laboratories (Greenford, U.K.). Glibenclamide was a gift from Hoechst AG (Frankfurt, F.R.G.) Unisolve 1 was from Koch Light Laboratories (Colnbrook, Berks., U.K.).

Islet preparation. Pancreases were excised from fed Sprague–Dawley rats and minced in a Hanks' solution to pieces of approximately 3–5 mm in diameter under sterile conditions. The minced pancreas was then shaken (approximately 180 per min) in a 37° water bath with collagenase (64 mg/pancreas in

16 ml sterile Hanks' solution) for 17–20 min. The collagenase was washed away and the islets were collected and transferred to culture dishes containing medium TC 199 (5.6 mM glucose) plus 10% calf serum (v/v) with braking pipettes. The islets were cultured free-floating [8]. After 24 hr, some of the islets were transferred to new culture dishes containing TC 199 with 20 mM glucose.

Perfusion experiments. The experimental details have been given elsewhere [7]. Briefly, 60 cultured islets were prelabelled with 0.1 mM [2-^{3}H]adenosine (10 Ci/mmole) for 45 min at 37° in a HEPES-buffered Krebs–Ringer bicarbonate solution (KRBH) with glucose present at the same concentration as during culture. Washed islets were transferred to perfusion chambers and perfused in the KRBH media of composition given in the figures. Effluents were collected as shown. For determining the radioactive nucleotide efflux rates, 800 μl effluent samples were mixed with 4 ml Unisolve 1® for liquid scintillation counting. The filter containing the islets was carefully recovered after each experiment. Fractional outflow rates (F.O.R.) of radioactive efflux, defined as the fraction intracellular radioactive content (in %/min) leaving the islets per minute, were calculated. The basal F.O.R. between minutes 11 and 20 or 11 and 15 was chosen as a reference in each experiment, and all values are expressed in per cent of this for each experiment. Fractions of 100 μl were always assayed for insulin contents [9].

Adenine nucleotide measurements. Islets harvested from culture dishes were incubated for 15 min in the various KRBH media supplemented with glucose, glibenclamide and 0.5 mg/ml albumin as given in Table 1. The incubations were performed at 37° in a 5% CO_2 atmosphere with ten islets in small 'Beckman' tubes, containing 100 μl medium. After 15 min, the medium was rapidly removed and 40 μl 0.02 M NaOH was added. The tubes were then directly frozen in liquid N_2 and stored at −70°. When assaying the tubes were thawed by mechanical vibration [10] after which ATP and total adenine nucleotide contents were determined by bioluminescence [11] as described in detail previously [7].

Respiratory measurements. Respiratory rates were determined in the Cartesian divers [12] in a KRBH buffer with all $NaHCO_3$ replaced by NaCl. The media contained 1.7, 5.6 or 11.1 mM glucose as basal supplements. After 1 hr respiration under such basal conditions, glibenclamide was added by mixing with the side-drop. The experiments were performed at 37° in ambient air.

Statistical calculations. A Student's two-tailed *t*-test was used for all comparisons of chance differences (P) unless otherwise stated.

RESULTS

Effects of glibenclamide on islet radioactive nulceotide and insulin efflux

The effects of glibenclamide on radioactive nucleotide efflux from rat islets cultured at a low glucose (48 hr at 5.6 mM glucose) or a high glucose concentration (24 hr at 5.6 mM glucose + 24 hr at 20 mM glucose), prelabelled with [2-^{3}H]adenosine and finally perfused in the presence of 5.6 mM glucose are shown in Fig. 1. Exposure to glibenclamide caused a prompt 3-fold increase in radioactive nucleotide efflux from the low glucose cultured islets, whereas the high glucose cultured islets only slightly increased their efflux (when comparing the average percentage efflux rate per min during min 18–45 against that during min 14–16, P was < 0.05). When the high glucose cultured islets were perfused at 11.1 mM glucose, no response to glibenclamide was observed, in contrast to the doubling of radioactive nucleotide efflux in response to glibenclamide with the low glucose cultured islets (Fig. 2). As can be seen in Fig. 3, the radioactive nucleotide efflux from high glucose cultured islets slowly increased after an initial drop when the glucose concentration of the medium was decreased from 20 to 1.7 mM. Low glucose cultured islets displayed a similar response, although the onset of the increase was more rapid

Table 1. Effects of glibenclamide on ATP contents of rat islets cultured at low or high glucose

Incubation conditions	Culture for 48 hr in 5.6 mM glucose		Culture for 24 hr at 5.6 mM glucose + 24 hr at 20 mM glucose	
	%ATP	Total adenine nucleotide content (pmole/islet)	%ATP	Total adenine nucleotide content (pmole/islet)
1.7 mM glucose	38.5 ± 5.7 (12)	8.28 ± 1.40 (12)	33.9 ± 6.7 (11)	8.80 ± 0.68 (12)
1.7 mM glucose + glibenclamide (2.5 μg/ml)	34.0 ± 4.5 (11)	7.68 ± 1.16 (12)	47.8 ± 4.6 (11)*	8.28 ± 1.00 (12)
5.6 mM glucose	40.0 ± 5.0 (11)	8.36 ± 1.24 (11)	38.4 ± 4.7 (11)	9.44 ± 1.08 (12)
5.6 mM glucose + glibenclamide (2.5 μg/ml)	27.2 ± 3.6 (12)*	8.08 ± 1.20 (12)	36.7 ± 5.9 (11)	8.84 ± 0.92 (12)

Ten rat islets cultured at low glucose (48 hr in 5.6 mM glucose) or high glucose (24 hr in 5.6 mM glucose + 24 hr in 20 mM glucose) were incubated for 15 min at 37° before freezing and extracting nucleotides. These were determined by bioluminscence. Means ± S.E.M. are given, with a number of observations in parentheses.

* Denotes $P < 0.05$ when tested with a paired *t*-test against the corresponding per cent ATP in the absence of glibenclamide.

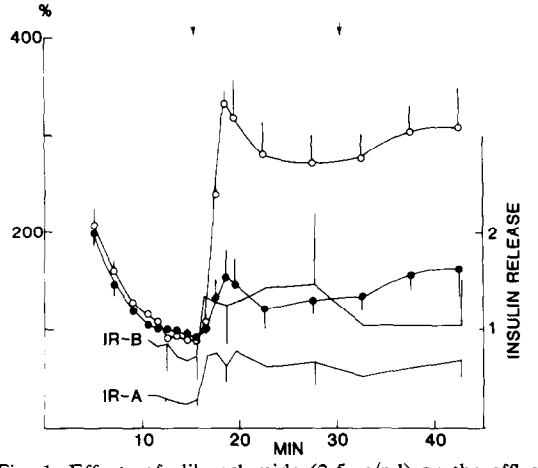

Fig. 1. Effects of glibenclamide (2.5 μg/ml) on the efflux of radioactive nucleotides and insulin from low glucose (open circles) or high glucose (closed circles) cultured rat islets, prelabelled with [2-^{3}H]adenosine. Prelabelled islets were perfused in the presence of 5.6 mM glucose, and glibenclamide was added as indicated by the arrows. Glibenclamide was in these and all other experiments dissolved in alkaline ethanol in a concentrated form so that the final ethanol content in the experiments was 0.3% (v/v). Fractional outflow rates (F.O.R.) for the radioactivity efflux were determined and in each experiment, the average efflux rate between minutes and 11 and 15 was regarded as a 100% control; all other efflux values are expressed in per cent of the latter. The 100% efflux F.O.R. for the low glucose cultured islets was 0.51 ± 0.07 %/min and the corresponding value for the high glucose cultured islets was 0.81 ± 0.15. Effluent samples were collected as shown. Means ± S.E.M. for 4–6 experiments are given. Means and every third S.E.M. for the insulin release (as ng/60 islets and min) from the low glucose (IR-A) or the high glucose (IR-B) islets are also shown.

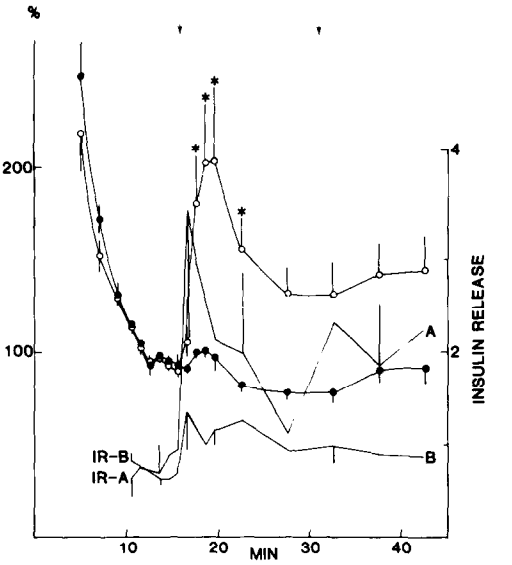

Fig. 2. Effects of glibenclamide (2.5 μg/ml) on the efflux of radioactive nucleotides and insulin in the presence of 11.1 mM glucose from cultured rat islets prelabelled with [2-^{3}H]adenosine. Fractional outflow rates of the radioactive efflux were expressed in per cent of 100% control (0.44 ± 0.09 and 0.76 ± 0.24 %/min for the low and high glucose cultured islets, respectively) as described in the legend to Fig. 1. The efflux from low glucose (open circles) or high glucose (closed circles) cultured islets is shown. Glibenclamide was added as indicated by the arrows. Means ± S.E.M. for 4–6 experiments are given. *denotes $P < 0.05$ when tested with a paired t-test against 100% control. Means and every third S.E.M. for the insulin release values (given as ng insulin released per 60 islets and min) from the low glucose (IR-A) or high glucose (IR-B) cultured islets are also shown.

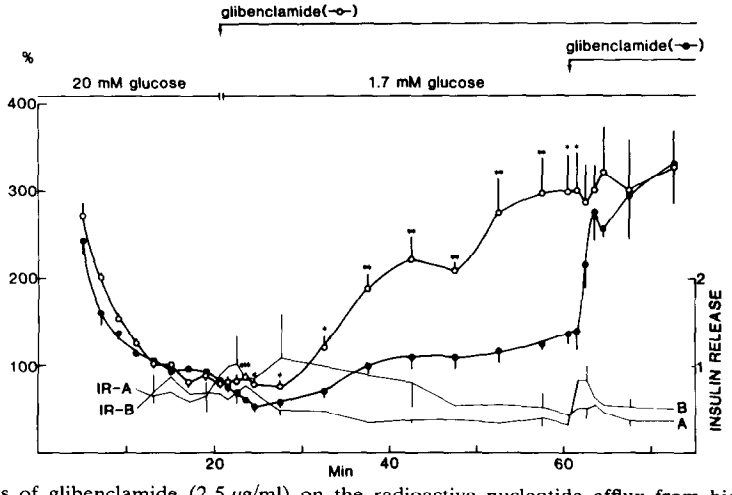

Fig. 3. Effects of glibenclamide (2.5 μg/ml) on the radioactive nucleotide efflux from high glucose cultured rat islets prelabelled with [2-^{3}H]adenosine. The glucose concentration of the perfusion medium was changed as shown in the figure for both experimental groups. Open circles shows the effects of glibenclamide on the radioactive efflux when added parallel to the decrease in glucose, whereas the closed circles show the efflux changes when glibenclamide was added later as shown. The efflux values are expressed as in Fig. 1, although the 100% control values are based on the average F.O.R. rates between minutes 11 and 20. These were 0.39 ± 0.09 and 0.35 ± 0.06 %/min for the closed and open circle experiments, respectively. Means ± S.E.M. for four experiments are given. The corresponding insulin release values (as ng insulin per 60 islets and min) with every third S.E.M. for the open circle (IR-A) or closed circle (IR-B) radioactive efflux experiments are also shown. *, ** and *** denote $P < 0.05$, 0.01 and 0.001, respectively, when tested against efflux in the absence of glibenclamide.

(results not shown). When adding glibenclamide 40 min after decreasing the glucose concentration in the perfusion medium, the high glucose cultured islets responded with a rapid increase in radioactive nucleotide efflux (Fig. 3). When adding glibenclamide simultaneously with the decrease in the glucose concentration, no immediate effects were observed. Subsequently, however, the radioactive nucleotide efflux gradually increased in parallel with, though to a much greater extent than, the increase in efflux from controls. The radioactive nucleotide efflux approached an apparent plateau 30 min after glibenclamide addition. No effects of glibenclamide on radioactive nucleotide efflux in the presence of 5.6 mM glucose from low or high glucose cultured islets could be observed when the perfusion was performed in the absence of extracellular Ca^{2+} (Fig. 4).

A consistent but small and non-significant increase in insulin output was observed 1–3 min after glibenclamide addition under all conditions (Figs. 1–3). Although the onset of these changes in insulin release was parallel to that of radioactive efflux, the peak insulin values were reached 2 min before the peak radioactive efflux values. A 10-fold increase in the glibenclamide concentration resulted in a strongly enhanced insulin response without any further changes in nucleotide efflux (not shown).

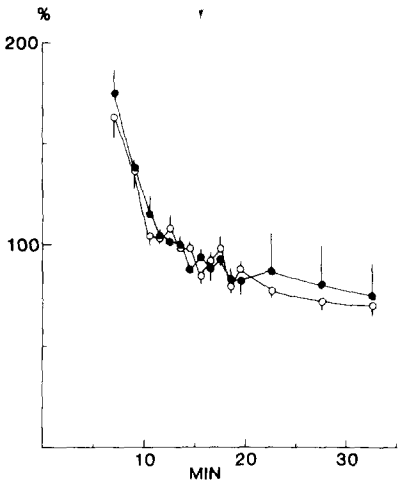

Fig. 4. Effects of glibenclamide (2.5 μg/ml) on the radioactive nucleotide efflux from low glucose (open circles) or high glucose (closed circles) cultured islets. The perfusion medium lacked Ca^{2+} and was supplemented with 5.6 mM glucose and 0.5 mM EGTA throughout. Glibenclamide was added as indicated by the arrow. Means ± S.E.M. for 3–4 experiments are shown, expressed in per cent of the average F.O.R. between min was 11 and 15 for each experiment. The 100% level was 0.26 ± 0.05 and 0.27 ± 0.06 %/min for the low and high glucose cultured islets, respectively.

Effects of glibenclamide on islet adenine nucleotide contents

The total intracellular adenine nucleotide content was not affected by glibenclamide addition at either 1.7 or 5.6 mM glucose with either low or high glucose cultured islets (Table 1). The percentage ATP decreased significantly when adding glibenclamide in the presence of 5.6 mM glucose to low glucose cultured islets. On the other hand, glibenclamide increased intracellular ATP when added in the presence of 1.7 mM glucose to high glucose cultured islets. Otherwise, no significant changes induced by glibenclamide were observed on the percentage contribution of ATP to the total adenine nuleotide content.

Effects of glibenclamide on islet oxygen uptake

The addition of glibenclamide decreased islet respiration when added to low glucose cultured islets respiring in the presence of 5.6 and 11.1 mM glucose

Table 2. Effects of glibenclamide on respiratory rates of low or high glucose cultured rat islets

	Culture at 5.6 mM glucose 48 hr (low glucose culture)		Culture at 5.6 mM glucose 24 hr + 24 hr at 20 mM glucose (high glucose culture)	
Additions to the media	Respiratory rates (nl O_2/islet and hr)	Changes in % of initial respiratory rates	Respiratory rates (nl O_2/islet and hr)	Changes in % of initial respiratory rates
1.7 mM glucose	3.25 ± 0.62 (7)		3.77 ± 0.69 (8)	
		−2.6 ± 1.2		−19.8 ± 5.7*
1.7 mM glucose + glibenclamide (2.5 μg/ml)	3.18 ± 0.67 (7)		3.20 ± 0.67 (8)	
5.6 mM glucose	4.09 ± 0.79 (7)		5.01 ± 0.80 (8)	
		−8.1 ± 3.2*		−24.8 ± 3.6***
5.6 mM glucose + glibenclamide (2.5 μg/ml)	3.82 ± 0.82 (7)		3.94 ± 0.74 (8)	
11.1 mM glucose	4.66 ± 0.81 (7)		6.75 ± 1.17 (8)	
		−16.4 ± 3.1**		−20.7 ± 3.4**
11.1 mM glucose + glibenclamide (2.5 μg/ml)	4.03 ± 0.82 (7)		5.99 ± 1.22 (8)	

Low or high glucose cultured islets were initially allowed to respire in 1.7, 5.6 or 11.1 mM glucose before adding glibenclamide at the same glucose concentration by mixing with the side-drop. The changes in oxygen uptake were expressed in per cent of the initial respiratory rates when glibenclamide was added with *, ** and *** denoting $P < 0.05$, 0.01 and 0.001, respectively, with a paired t-test. Means ± S.E.M. are given, with the number of observations in parentheses. All six experimental groups were run in parallel.

and high glucose cultured islets in the presence of 1.7, 5.6 and 11.1 mM glucose (Table 2).

DISCUSSION

The present study was an attempt to characterize and elucidate a possible mechanism by which sulphonylureas stimulate nucleotide efflux from prelabelled islets. Rat islets were chosen since sulphonylurea-induced changes in islet ionic and metabolic fluxes have previously been determined in this species [3, 6]. The specific use of glibenclamide with islets cultured under the present conditions was motivated by a recent study in which the insulin response under these conditions was determined [13].

The glibenclamide concentration used in the present study (2.5 μg/ml) has been shown to stimulate insulin release in batch incubations of identically treated rat islets [13]. However, in the conditions of the present study, this particular glibenclamide concentrations appeared insufficient to induce a significantly increased insulin release to the effluents. Thus, the immediate relation between these two phenomena is presently unclear.

An increase in radioactive nucleotide efflux can sometimes simply be due to decreased intracellular ATP, since the most permeable nucleotides are AMP and ADP, and the concentrations of these increase when ATP decreased [7]. Furthermore, a Ca^{2+}-dependent component of nucleotide permeability may exist, as suggested by previous data on nucleotide efflux [7, 14]. Although the present data on the effects of glibenclamide on radioactive nucleotide efflux cannot be interpreted in detail, it seems that in certain circumstances the increased radioactive efflux may be due to decreased intracellular ATP, as for instance when glibenclamide was added to low glucose cultured islets. It is also evident that there was a dissociation between the stimulated radioactive efflux and high ATP contents when glibenclamide was added to high glucose cultured islets in the presence of 1.7 mM glucose. In this case, the alternative explanation, that radioactive nucleotide efflux depends on cytoplasmic Ca^{2+}, could be valid since it is known that sulphonylurea increases $^{45}Ca^{2+}$ net uptake [3, 4, 6]. In concordance with this, the absence of extracellular Ca^{2+} abolished the nucleotide efflux response to glibenclamide.

A striking feature of the present findings is that the nucleotide efflux in the presence of glibenclamide seems dependent on the metabolic state of the islets, i.e. the rapidity of NADH generation. Thus, the high glucose cultured islets, which displayed increased rates of O_2 consumption at 5.6 and 11.1 mM glucose, released much less radioactivity when glibenclamide was added. Furthermore, when descreasing the glucose concentration from 20 to 1.7 mM during perfusion of high glucose cultured islets, there was a decrease in the nucleotide efflux rate, which could be prevented by simultaneous addition of glibenclamide. Therefore, it seems plausible that glibenclamide and intracellular NADH-generation act synergically towards an increase in radioactive nucleotide efflux not associated with a fall in ATP. This interpretation is supported by the observation that islet respiration drops very rapidly after withdrawal of a substrate [15] compared to the decrease in ATP, as measured by nucleotide efflux in this and a previous study [7].

The previous finding in obese hyperglycaemic mice of enhanced islet oxygen uptake after glibenclamide addition [16] could not be reproduced in this study. At variance with these observations there was a consistent inhibition of islet respiration, which tended to be more marked in conditions with higher respiratory rates. No simple explanations of this discrepancy are immediately apparent, although it may reflect species differences. Nevertheless, the present findings of inhibited respiration after glibenclamide addition may be due to either decreased ATP (as a consequence of changes in membrane cation fluxes), which has been suggested as a stimulator of islet respiration [14], or changes in the intracellular ionic composition. That all secretory, metabolic and ionic changes are secondary to inhibited respiration seems, however, unlikely considering the lack of correlation between changes in intracellular ATP and respiration in the present study and the previously shown correlation between stimulation of respiration and insulin release [15, 17].

In conclusion, the present data suggest that glibenclamide-induced changes in radioactive nucleotide efflux are related to islet functions. No satisfactory explanation can yet be presented of this phenomenon, although under certain conditions it may reflect changes in islet ATP contents and under other conditions changes in transmembrane Ca^{2+} fluxes.

Acknowledgements—I am grateful for technical assistance from Ing-Britt Hallgren. Discussions with Drs. Claes Hellerström and Arne Andersson were very much appreciated, as well as the provision of a computer programme by Dr. Ulf Eriksson for calculating F.O.R. This work was supported by funds from the Swedish Medical Research Council (12X-109), the Swedish Diabetes Association, and the Clas Groschinsky Memorial Foundation.

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