The Effect of Chronic Sulfonylurea Therapy on Hepatic Glucose Production in Non-insulin-dependent Diabetes

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SUMMARY

In 20 patients with untreated non-insulin-dependent diabetes mellitus (NIDDM), there was a positive relationship between fasting plasma glucose (FPG) and glucose production rate, calculated by the isotope dilution technique ($r = 0.72$, $P < 0.001$). This suggests that glucose production rate is an important determinant of FPG in untreated NIDDM. Fifteen patients were also studied during therapy with chlorpropamide for 3–6 mo. During therapy, FPG was lower (133 ± 9 vs. 216 ± 20 mg/dl, mean ± SEM; $P < 0.001$), glucose production was lower (59.5 ± 2.0 vs. 77.6 ± 4.9 mg/m^2/min; $P < 0.005$), and there was a significant correlation between the fall in glucose production and the fall in FPG ($r = 0.59$, $P < 0.05$). Fasting IRI levels increased in some, but not all, patients during chlorpropamide (untreated 18 ± 2, treated 21 ± 3 μU/ml; P = NS). However, there was a significant relationship between the percent rise in IRI and the fall in glucose production during treatment ($r = 0.75$, $P < 0.001$). Patients with a rise in fasting insulin during therapy had a greater fall in glucose production than those whose insulin did not rise (25.4 ± 8.1 vs. 7.8 ± 2.4 mg/m^2/min; $P < 0.05$). When a low-dose insulin infusion was given to approximate the increases of portal venous insulin during therapy, similar falls of glucose production occurred. We conclude that inhibition of endogenous glucose production during chronic chlorpropamide therapy is an important mechanism for the lowering of FPG and that enhanced insulin secretion is the reason for the major part of this inhibition. The small fall in glucose production in those patients whose insulin level did not rise during therapy suggests an additional contribution by some other mechanism.

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The hyperglycemia of non-insulin-dependent diabetes mellitus (NIDDM) has been attributed to both glucose overproduction by the liver and glucose underutilization by peripheral tissues.[1,2] The underlying cause of the metabolic abnormalities has in turn been ascribed to both deficient insulin secretion[3] and resistance to insulin action.[4] Thus, it is not surprising that numerous mechanisms have been postulated for the chronic hypoglycemic effects of sulfonylurea therapy in NIDDM. Early reports emphasized the stimulation of insulin secretion[5,6] and the reduction in hepatic glucose production rate during acute intravenous sulfonylurea administration.[7] Some subsequent reports that insulin levels did not appear to increase during chronic therapy[8,9] led to the hypothesis that sulfonylureas may lower fasting glucose levels mainly by a direct extrapancreatic effect on glucose uptake by peripheral tissues.[10] This hypothesis has been supported by findings of enhanced insulin binding to monocytes[11] and increased sensitivity to the effect of insulin on glucose disposal during sulfonylurea therapy.[12,13] Recent reviews have implied that potentiation of insulin action on glucose uptake is principally responsible for the therapeutic effect of sulfonylurea therapy while the beneficial effect of increased insulin secretion is evanescent.[14,15] The possibility of an effect of sulfonylurea therapy on hepatic glucose production is not discussed in these reviews.

Hepatic glucose production is more sensitive than glucose disposal to changes in insulin,[16] and portal insulin levels to which the liver is exposed are considerably greater than peripheral levels.[17] Therefore, small changes in peripheral insulin levels during chronic sulfonylurea therapy are likely to be associated with a larger insulin effect on glucose production than on glucose disposal. For this reason, the present study was designed to determine the effect of chronic sulfonylurea therapy on glucose production rate in

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patients with NIDDM and to examine the role of enhanced insulin secretion in producing this effect.

MATERIALS AND METHODS

Subjects. Twenty patients, diagnosed as having NIDDM on the basis of a fasting plasma glucose (FPG) greater than 140 mg/dl,[18] gave informed consent before participation in this study. All had plasma glucose greater than 140 mg/dl at 0730 h after an overnight, 10-h fast. There were 19 males and one female with median percentage ideal body weight 128% (range 95–212%) and median age 57 yr (range 40–68). Thirteen of the patients were previously untreated and were studied in this condition. Eight of these 13 patients were subsequently restudied after 3–6 mo treatment with the sulfonylurea drug chlorpropamide. Doses ranging from 250 to 750 mg daily were determined according to periodic measurements of fasting plasma glucose. The other 7 of the 20 patients were studied first while on chlorpropamide treatment and then 2–3 mo after stopping treatment for the purpose of this study. Thus, a total of 15 patients were studied both during treatment with chlorpropamide and in the untreated state. An additional five patients were studied only while untreated. Except for two patients who were taking 50 mg hydrochlorothiazide daily for mild hypertension during both parts of the study, the subjects were on no medication other than chlorpropamide. Patients were required to refrain from the use of aspirin and aspirin-containing products for at least 1 wk before the studies. Specific instruction was given not to alter dietary habits during the course of the study and in particular not to attempt any weight loss until after completion of the study. Apart from this, no dietary instruction or standardization was attempted. Studies were performed after an overnight fast, and cigarette smoking was not allowed on the day of the study. While on chlorpropamide, patients did not take their medication on the day of the study.

Study protocol. Studies were performed on a metabolic ward, commencing between 0730 and 0800 h. With the patients recumbent, an 8-in, 18.5-gauge catheter (I-Cath, Bard Biomedical, Murray Hill, New Jersey) was introduced via an antecubital vein for the infusion of tritiated glucose and insulin. A 19-gauge butterfly needle was placed in an antecubital vein of the opposite arm for blood sampling. Both lines were kept patent by the slow infusion of 0.9% NaCl. After two blood samples were drawn for measurement of background radioactivity, a primed (25 μCi) constant infusion (0.15 μCi/min) of [3-^{3}H]-glucose (New England Nuclear, Boston, Massachusetts) was begun and continued for 260 min. To determine the effect of small changes of insulin levels on glucose production, regular insulin (purified pork insulin, Eli Lilly and Co., Indianapolis, Indiana) was infused intravenously between 200 and 260 min at the rate of 250 μU/kg/min. Both [3-^{3}H]-glucose and insulin were diluted in 0.9% NaCl for the purpose of infusion.

Venous samples for measurement of glucose, glucose radioactivity, and immunoreactive insulin (IRI) were collected into tubes containing EDTA at 10-min intervals between 160 and 260 min of the tracer infusion. Samples for measurement of immunoreactive glucagon (IRG) were collected into heparinized tubes containing benzamidine at 20-min intervals between 160 and 260 min, while samples for estimation of epinephrine and norepinephrine levels were collected into tubes containing EGTA and glutathione at 190, 200, 250, and 260 min. All samples were stored on ice, and plasma was promptly separated by centrifugation at 4°C, with storage at −20°C until subsequent analysis.

Analytic methods. Plasma IRI levels were assayed by a modification of the double antibody method of Morgan and Lazarow.[19] Plasma glucose was measured by the autoanalyzer glucose-oxidase method (Technicon Instruments). Plasma IRG was measured by radioimmunoassay, employing a C-terminal-directed antiserum.[20] Plasma epinephrine and norepinephrine levels were determined by single isotope enzymatic assay.[21] For IRI and IRG measurements, samples taken from the same subject while on and off chlorpropamide treatment were measured in the same assay. IRI levels basally and during the insulin infusion represent the mean of five samples, while IRG levels are the mean of three samples, and epinephrine and norepinephrine are the mean of two. Fasting plasma glucose level is the mean of five samples drawn between 160 and 200 min of the tracer infusion.

Plasma [3-^{3}H]-glucose specific activity was measured in triplicate using 0.4-ml aliquots of plasma which were deproteinized by the addition of 0.4 ml chilled 0.5 M perchloric acid. Following centrifugation, 0.5-ml aliquots of the supernatant were evaporated to dryness under compressed air to eliminate tritiated water. The residue was reconstituted in 0.5 ml water, added to 4.5 ml of Biofluor (New England Nuclear, Boston, Massachusetts), and counted in a liquid scintillation spectrometer. Counts were corrected for quenching by the method of external standard ratios using a series of quenched standards. Aliquots of the infusate were also counted to determine the concentration of [3-^{3}H]-glucose, and tracer infusion rate was determined by noting the time taken to infuse a certain volume of the infusate (coefficient of variation was less than 2%). Recovery of [3-^{3}H]-glucose from the deproteination procedure was determined by spiking an aliquot from the tracer solution into a plasma sample drawn before the start of the tracer infusion. Comparison with the counts from direct counting of the infusate after correction for dilution and quenching showed a recovery of 97 ± 1% (mean ± SEM). [3-^{3}H]-Glucose counts in deproteinized plasma were corrected for dilution and recovery and then divided by the appropriate plasma glucose level for calculation of specific activity. With [3-^{3}H]-glucose, no correction for recycling of label is necessary.[22] Counts in the plasma samples were all more than 10 times background, and the intra-assay coefficient of variation for measurement of [3-^{3}H]-glucose radioactivity was less than 2%. The interassay coefficient of variation was 6% at a level of radioactivity 80 times background using pooled plasma to which [3-^{3}H]-glucose had been added as a control.

Calculations. Because glucose concentration was falling slightly during the 40 min of baseline glucose turnover measurements (160–200 min of tracer infusion), non-steady-state equations[23] were used to calculate the rates of glucose appearance and disappearance both before and during the insulin infusion. Before this calculation, variation in levels of plasma glucose and [3-^{3}H]-glucose specific activity were smoothed using the sliding fit method.[24] A value of 200 ml/kg was used for glucose distribution volume,[25] and 0.65 was used as the pool fraction.[26] The coefficient of variation for the [3-^{3}H]-glucose counts during the baseline period

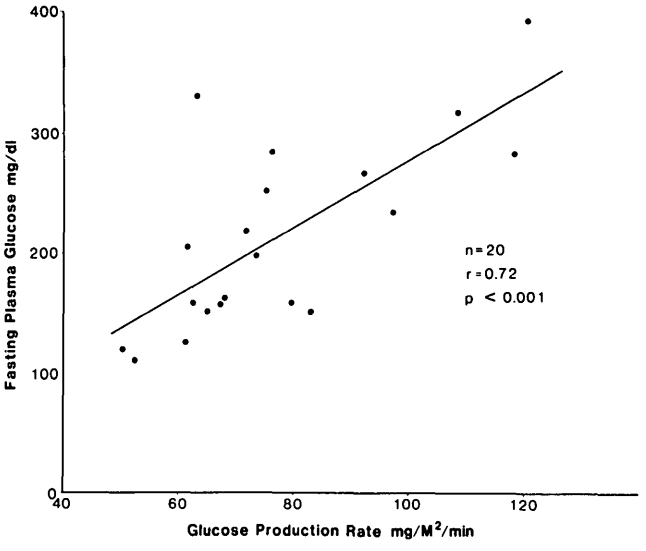


FIGURE 1. Correlation between fasting plasma glucose during the tracer infusion and glucose production rate in 20 patients with untreated NIDDM. Despite the suppressive effect of hyperglycemia *per se* on glucose production, those patients with the highest glucose levels had the highest production rates.

averaged less than 3%. Both on and off treatment, glucose production rate reached a plateau after 25 min of the insulin infusion. Thus, for comparison of the insulin effect in the same patients while on and off chlorpropamide, the average glucose production rate between 25 and 55 min of the insulin infusion (225 and 255 min of the tracer infusion) was utilized.

Statistical analyses included Student's *t* test using paired and unpaired samples, as appropriate. Tests for correlation were done by linear regression analysis except when the distribution was not normal, in which case Spearman's rank correlation was used.[27] All values for plasma glucose levels, glucose production rates, and hormone levels are expressed as mean ± SEM.

RESULTS

Untreated patients. For the 20 untreated patients, the plasma glucose level from 160 to 200 min of the tracer infusion was 214 ± 18 mg/dl and glucose production rate was 77.4 ± 4.5 mg/m²/min. As shown in Figure 1, there was a significant correlation between these two sets of measurements ($r = 0.72$, $P < 0.001$). Thus, glucose production rate was highest in those subjects with the highest FPG level.

Effect of therapy. Individual data for the 15 subjects studied both while untreated and on chlorpropamide therapy are shown in Table 1. Glucose production rate during treatment was significantly lower (59.5 ± 2.0 vs. 77.6 ± 4.9 mg/m²/min; $P < 0.005$) than in the untreated state. FPG was also lower (133 ± 9 vs. 216 ± 20 mg/dl; $P < 0.001$), and there was a significant correlation between the fall in glucose production during treatment and the fall in FPG ($r = 0.59$, $P < 0.05$, Spearman's rank correlation). The effect of chlorpropamide on FPG and glucose production is illustrated in Figure 2. There was no significant change in body weight while on or off treatment (untreated 90 ± 3 kg, treated 92 ± 3 kg). As summarized in Table 2, basal levels of IRG, epinephrine, and norepinephrine did not change with treatment.

Fasting IRI levels were not significantly higher during treatment for the group as a whole (untreated 18 ± 2, treated 21 ± 3 μU/ml). However, an increase of fasting IRI was observed in subjects 1–8 (see Table 1) during treatment, and, as illustrated by Figure 3, there was a significant correlation between the percent rise in fasting insulin with treatment and the fall in glucose production ($r = 0.75$, $P < 0.001$). Furthermore, the eight patients whose fasting insulin level was higher during therapy (ΔIRI = 9 ± 2 μU/ml) had a

TABLE 1
Individual data in NIDDM patients studied while untreated (U) and on chlorpropamide therapy (Rx)

Subject number	Age	Sex	Wt (kg)	IBW (%)	Fasting insulin (μU/ml)		FPG* (mg/dl)		Glucose production (mg/m²/min)	
					U	Rx	U	Rx	U	Rx
1	56	F	104	197	22	36	332	187	63.3	53.8
2	60	M	102	135	15	28	393	138	120.5	57.0
3	57	M	119	175	29	42	218	160	71.6	69.8
4	60	M	94	142	10	21	267	123	92.1	53.1
5	66	M	83	116	10	19	284	227	118.3	67.0
6	48	M	92	135	21	27	199	114	73.5	52.1
7	66	M	91	122	7	9	158	91	79.6	57.6
8	63	M	78	111	11	12	205	121	61.9	53.3
9	50	M	94	127	18	17	158	128	67.2	65.9
10	57	M	80	112	12	11	158	118	62.5	50.9
11	62	M	84	118	8	7	126	110	61.3	59.5
12	64	M	87	136	24	22	151	149	83.0	78.5
13	68	M	70	106	12	10	285	116	76.5	57.1
14	53	M	86	114	28	23	163	124	67.7	58.7
15	49	M	92	130	38	23	152	91	65.1	57.8
$\bar{x}$	59		90	132	18	21	216	133†	77.6	59.5‡
SEM	2		3	6	2	3	20	9	4.9	2.0

* FPG = fasting plasma glucose during the tracer infusion.
† $P < 0.001$ U vs. Rx.
‡ $P < 0.005$ U vs. Rx.

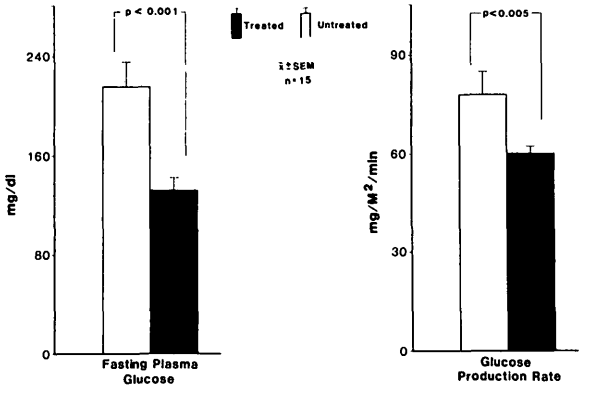


FIGURE 2. The effect of 3–6 mo chlorpropamide therapy on fasting plasma glucose and glucose production rate in 15 patients with NIDDM. The fall in plasma glucose correlated with the fall in glucose production rate (r = 0.59, P < 0.05).

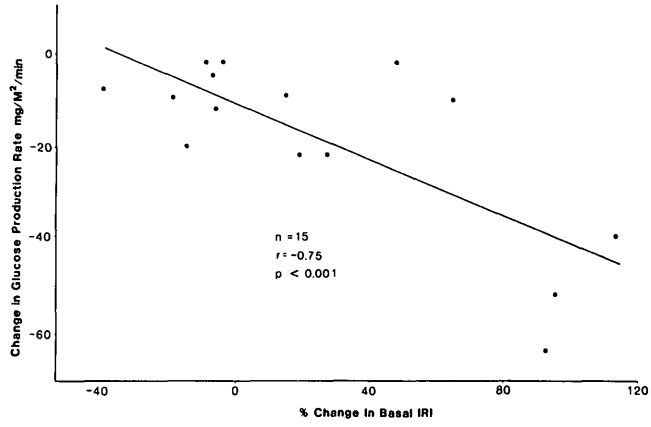


FIGURE 3. Correlation between the percent change in basal IRI during chlorpropamide therapy and the fall in glucose production rate. Patients who had the largest increase of basal IRI had the biggest fall in glucose production rate during treatment.

significantly greater fall in glucose production than those whose insulin level did not rise (25.4 ± 8.1 vs. 7.8 ± 2.4 mg/m²/min; P < 0.05). However, as illustrated in Figure 4, the latter group still had a significant fall in glucose production rate during chlorpropamide therapy. As we have shown previously,[28] those patients who have a rise in insulin during therapy are generally those who had a higher pretreatment level of fasting hyperglycemia. This is demonstrated by the correlation in these 15 patients between pretreatment FPG and the percent rise in insulin with treatment (r = 0.75, P < 0.005).

Response to Insulin infusion. There was no difference between the rise in IRI levels produced by the insulin infusion in untreated and treated patients (ΔIRI = 15 ± 1 μU/ml for both groups). IRG, epinephrine, and norepinephrine levels were unchanged by the insulin infusion, as shown in Table 1. In untreated patients, the insulin infusion resulted in a fall in glucose production rate of 13.5 ± 2.5 mg/m²/min. During the 60 min of the infusion, glucose concentration fell from 214 ± 20 to 191 ± 20 mg/dl. Based on the rate of fall of glucose level during the 40 min preceding the insulin infusion, a fall to 204 ± 20 mg/dl would have been predicted during this period in the absence of infused insulin. In treated patients the insulin infusion produced a fall in glucose production rate of 12.3 ± 2.0 mg/m²/min, and it was not significantly different from the fall in the same patients while untreated. During the insulin infusion, glucose concentration fell from 131 ± 9 to 112 ± 8 mg/dl. A fall to 124 ± 9 mg/dl would have been expected during this period in the absence of the insulin infusion. In the group of patients whose fasting insulin levels rose during treatment, insulin infusion lowered glucose production rate by 15.1 ± 3.8 mg/m²/min before and by 11.4 ± 1.9 mg/m²/min during chlorpropamide therapy. This difference was not significant.

TABLE 2
Hormone levels basally and during insulin infusion (250 μU/kg/min)

	Untreated (N = 15)	Treated (N = 15)
IRI μU/ml		
Basal	18 ± 2	21 ± 3
Insulin infusion	33 ± 2*	36 ± 4*
IRG pg/ml		
Basal	76 ± 6	72 ± 6
Insulin infusion	76 ± 6	69 ± 6
Epi pg/ml		
Basal	40 ± 3	41 ± 6
Insulin infusion	45 ± 4	47 ± 4
Norepi pg/ml		
Basal	249 ± 19	259 ± 29
Insulin infusion	251 ± 16	271 ± 27

Insulin (IRI), glucagon (IRG), epinephrine (Epi), and norepinephrine (Norepi) are compared in NIDDM patients both while untreated and on chlorpropamide therapy.
* P < 0.001 compared with basal.

DISCUSSION

This study of non-insulin-dependent diabetic patients has demonstrated that chronic treatment with the sulfonylurea drug chlorpropamide results in a fall in endogenous glucose production rate. The importance of endogenous glucose production to the maintenance of fasting hyperglycemia in these patients is suggested by the positive correlation between glucose production rate and glucose concentration in the untreated state (Figure 1). This relationship is particularly impressive since hyperglycemia, independently of changes in insulin, inhibits glucose production.[29] The finding that the fall in FPG during chlorpropamide treatment was correlated with the fall in glucose production rate suggests that the glucose lowering effect of the drug may be mediated, in part, by inhibition of glucose production. Again, the fall in glucose production during treatment may be even more significant since the restraining influence of hyperglycemia on glucose production is partially removed. There is no direct comparison in this study between the contribution of reduced glucose production versus enhanced glucose disposal to the lowering of glucose concentration. However, it is likely that both mechanisms are important since enhancement of glucose disposal by sulfonylurea drugs has been demonstrated by other studies.[12,13]

The mechanism by which chlorpropamide therapy lowers glucose production rate was also addressed by this study. The importance of enhanced insulin secretion is suggested by the relationship between the percent rise in insulin level and the fall in glucose production rate (Figure 3). Thus, although a small drop in glucose production was observed in

patients whose basal insulin levels did not increase during therapy, the fall in glucose production was significantly greater in those whose insulin levels rose during treatment (Figure 4).

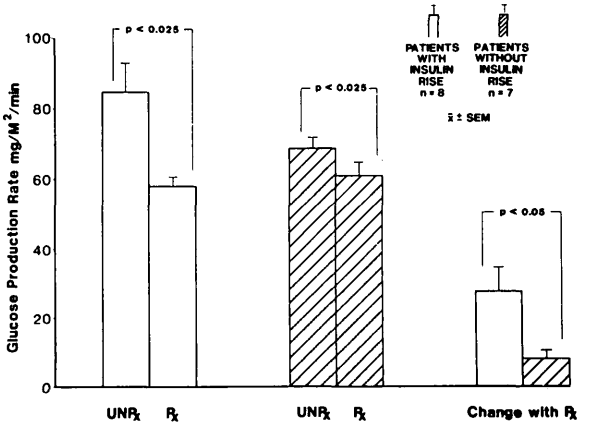
In this latter group, the ability of increased basal insulin to account for most of the fall in glucose production rate is supported by the findings during the insulin infusion study. If endogenous insulin secretion did not change during the low-dose insulin infusion, the average rise in portal insulin level would be equal to the observed increase of peripheral insulin of 15 μU/ml. This increase of insulin caused an average fall in glucose production rate of 15.1 mg/m²/min. Assuming 60% hepatic extraction of insulin,[30] an increase of 15 μU/ml in portal insulin level due to endogenous secretion would produce a peripheral insulin rise of approximately 6 μU/ml. By comparison, chlorpropamide therapy produced a peripheral rise in basal insulin of 9 μU/ml and an average fall in glucose production rate of 25.4 mg/m²/min in those patients whose basal insulin increased. Thus, small increases of peripheral insulin levels could reflect increases of portal insulin of sufficient magnitude to cause similar falls of glucose production to those observed during chlorpropamide. We cannot exclude the possibility that small increases of portal insulin levels also occurred in subjects whose peripheral insulin levels did not appear to change during chlorpropamide.

In a more hyperglycemic group of patients, we have found a consistent rise in fasting insulin levels during chlorpropamide treatment.[28] As in the present study, there was a correlation between the pretreatment FPG and the percent rise in insulin during treatment. Those patients with marked hyperglycemia had the greatest increases in insulin while those who had only modest hyperglycemia showed little or no rise in insulin. In the present study we wanted to assess the relationship between glucose production and FPG over a wide range of hyperglycemia. Therefore, we included a number of patients with relatively mild fasting hyperglycemia. Even in such patients with mild hyperglycemia, a period of increased insulin secretion occurs during the initiation of chlorpropamide treatment.[8,31] The lack of a sustained elevation of insulin levels in such patients may be related to the fall of glucose levels during therapy. In acute studies in normal and NIDDM subjects we have found that the fall of plasma glucose during intravenous tolbutamide masks the β-cell stimulatory effects of the drug.[32,33] Our current findings during chronic sulfonylurea therapy suggest that the stimulatory effect of sulfonylureas is not lost, but may be offset by the loss of the potentiating effect of hyperglycemia on β-cell function.[3] Thus, the observed insulin level during therapy may underestimate the contribution of enhanced insulin secretion to the control of hyperglycemia.

There was a significant fall in glucose production rate during chlorpropamide therapy in the group whose basal insulin levels did not rise. This finding could be due to several factors. First, an initial rise in insulin may have a sustained effect on hepatic metabolism.[34] Second, small rises in portal insulin may not have been detected peripherally. Third, some previous studies have shown a fall in glucagon secretion as the result of chronic sulfonylurea therapy,[35,36] although such an effect was not demonstrated in the present study. Fourth, a direct effect of the drug on the liver must be considered, as chlorpropamide has been shown to directly inhibit glucagon-stimulated gluconeogenesis in perfused rat liver.[37] Finally, previous studies in mice have found increased hepatic insulin receptors as a result of sulfonylurea treatment.[38] An increase of hepatic receptors could result in greater suppression of hepatic glucose production by a given level of insulin.

In summary, this study suggests that increased hepatic glucose production is an important contributor to hyperglycemia in NIDDM and that a fall in glucose production rate is an important mechanism for the hypoglycemic effect of chronic chlorpropamide therapy. Enhanced insulin secretion appears to be a significant cause of this fall in many patients. Some reduction in hepatic glucose production without a sustained increase in insulin secretion also occurs, but the mechanism for this reduction remains to be determined.

FIGURE 4. The effect of chlorpropamide therapy (Rx) on glucose production rate in two groups of patients, separated on the basis of the effect of therapy on their basal IRI levels. The group whose basal IRI increased during treatment had a greater fall in glucose production than those without a rise in basal IRI. However, the latter group still had a significant fall in glucose production during chlorpropamide therapy.



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