Glibenclamide Stimulates and Glucose Inhibits Glucagon Release Induced by Calcium Deprivation

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SUMMARY

The effects of low extracellular calcium levels on glibenclamide- and glucose-induced A-, B-, and D-cell secretion were investigated using the perfused pancreas preparation of the rat. At normal (2.6 mM) calcium, glucagon secretion was unaffected by glibenclamide (1 μg/ml) and transiently suppressed by glucose (6.7 and 16.7 mM). Reduction of extracellular calcium to 0.24 mM promptly and persistently enhanced glucagon secretion in the presence of 3.3 mM glucose; this effect of low calcium was, however, diminished ($P < 0.001$) by 54% and 61% when the glucose concentration was increased to 6.7 and 16.7 mM, respectively. In contrast, glibenclamide enhanced the glucagon response to calcium reduction, a twofold stimulation by the drug being observed at all glucose concentrations.

Reduction of calcium to 0.24 mM failed to inhibit first-phase glibenclamide or glucose-induced insulin release; second phase was, however, inhibited whether induced by glibenclamide (in the presence of 6.7 mM glucose) or by glucose 16.7 mM per se. Reduction of calcium uniformly and completely abolished glibenclamide and glucose-induced somatostatin responses. It is concluded that: (1) the glucagon response induced by calcium deprivation is inhibited by glucose but potentiated by glibenclamide, and (2) reversal of a D-cell paracrine effect could underlie the glibenclamide effect. DIABETES *33*:505–509, June 1984.

The effects of sulfonylureas and the interactions of these drugs with glucose on glucagon secretion have not been fully elucidated. We have shown that glibenclamide (a "second-generation" sulfonylurea) and glucose interact on glucagon release, since the drug induces diphasic glucagon secretion (stimulation followed by inhibition) from pancreata perfused with media without glucose, and that these effects are completely abolished by the addition of glucose.[1]

The present study aimed to further investigate interactions between glucose and sulfonylurea on glucagon release by testing the effects of the drug and glucose at normal and reduced calcium concentrations, and by comparing effects on glucagon release with concomitant insulin and somatostatin release. The rationale for this approach was twofold. First, since we have previously shown that moderate calcium deprivation inhibits somatostatin more than insulin secretion,[2] the present experimental design offered a possibility to study glucagon secretion in the absence of a possible paracrine effect by pancreatic D-cells. Second, since reduction of extracellular calcium reportedly desensitizes glucagon release from the influence of glucose,[3,4] our experimental design could, on these premises, be used to test whether the influence of glucose on glibenclamide-induced glucagon secretion is coupled to the efficacy of the hexose in suppressing glucagon secretion.

MATERIALS AND METHODS

Animals and perfusion system. Male Sprague-Dawley rats (Anticimex, Solna, Sweden), weighing 200–250 g, were fed ad libitum with a commercial pelleted diet (Anticimex). The animals were anesthetized by an i.p. injection of 100 mg/kg body wt of pentobarbital. The pancreata were isolated and freed from all adjacent organs by the technique of Loubatières et al.[5] Each organ was perfused by a cannula into the abdominal aorta. The perfusion medium was not allowed to recirculate, and consisted of Krebs-Ringer bicarbonate buffer solution[6] supplemented with 20 g/L of bovine albumin (Sigma, St. Louis, Missouri) and, when not otherwise indicated, 3.3 mM of glucose. The system was operated at a flow rate between 2.7 and 3.0 ml/min. A 20-min preperfusion period with "basal" medium (containing 2.6 mM calcium) was allowed before the start of each experimental protocol. During a 40-min test period, reduction of the calcium concentration of the perfusate was introduced together with (or without) the administration of glibenclamide and elevated concentrations of glucose. Reduction of calcium was

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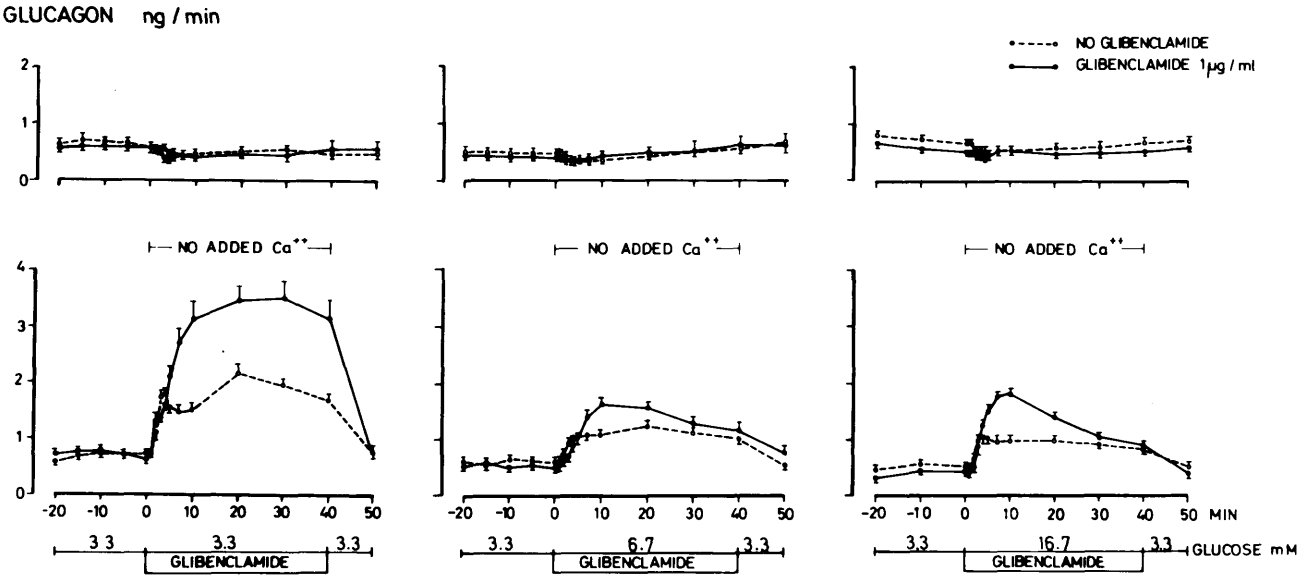

FIGURE 1. Effects of calcium deprivation on glucagon secretion induced by glucose and glibenclamide. Mean ± SEM of the respective number of experiments indicated in Table 1.

achieved by omission of $CaCl_2$ from the incubation medium; this resulted in total calcium concentrations of 0.23 ± 0.05 mM (4 experiments) as measured by atomic absorption. (Corresponding measurements in buffer medium to which calcium had been added were 2.61 ± 0.03, 5 experiments.)

Hormone assays. Insulin was measured radioimmunologically, using charcoal to separate bound and free antibodies.[7] Glucagon was assayed radioimmunologically using a charcoal separation technique and 30K antiserum.[8] Somatostatin was determined by a radioimmunoassay employing our own antibodies (R141E).[1,9]

Presentation of results. Results are expressed as mean ±SEM of incremental or decremental secretory responses, wherein the secretion rates immediately preceding the change in experimental conditions have been subtracted. Levels of significance were calculated using Student's *t* test for paired or unpaired differences as evident or as indicated in text or legend.

RESULTS

Glucagon secretion. At normal calcium, glucose 6.7 or 16.7 mM exerted a small transient inhibition of glucagon secretion (Figure 1). Thus, during 0–5 min secretion rates were decreased by 471 ± 76 and 540 ± 160 pg/5 min by 6.7 and 16.7 mM glucose, respectively, $P < 0.05$. Glibenclamide was, however, without effect (Figure 1, Table 1). Omission of calcium markedly and reversibly enhanced glucagon release in the presence of 3.3 mM glucose. This enhancing effect by low extracellular calcium was, however, clearly diminished by increasing the glucose concentration. The enhancement was significantly ($P < 0.001$) depressed by 54% by the change to 6.7 mM glucose and by 61% by the change to 16.7 mM glucose (Table 1).

The increase by low calcium of glucagon secretion in the presence of 3.3 mM glucose was further enhanced by the addition of glibenclamide (Figure 1, Table 1). This effect of glibenclamide was decreased by higher concentrations of glucose in a fashion parallel to the effects of glucose on the glucagon response to low calcium per se; in other words, a twofold increase due to glibenclamide was constant regardless of the glucose concentration.

Insulin secretion. Omission of calcium from the incubation medium inhibited insulin release induced by 16.7 mM glucose (from 42,741 ± 5284 to 24,769 ± 3895 µU/40 min, $P < 0.02$, Table 1, Figure 2). As shown in Figure 2, this effect was due to inhibition of the second phase (5–40 min) of glucose-induced insulin secretion (from 39,477 ± 5276 to 21,214 ± 3520 µU/35 min, $P < 0.02$), while the first-phase response (0–5 min) was unaffected (3264 ± 193 versus 3555 ± 412 µU/5 min). The effect of glibenclamide together with a nonstimulatory concentration of glucose (3.3 mM) was not inhibited by reduced extracellular calcium. However, low calcium inhibited total (Table 1) as well as second-phase insulin release (5–40 min) when the drug was administered in combination with 6.7 mM glucose, the release deriving from the second phase decreasing from 7943 ± 532 to 4055 ± 746 µU/35 min, $P < 0.01$.

Somatostatin secretion. Omission of calcium completely inhibited somatostatin release induced by glucose and glibenclamide, whether tested alone or in combination (Figure 3, Table 1).

DISCUSSION

Our results confirm, in a qualitative fashion, an enhancing effect of calcium deprivation on glucagon secretion.[2,4] Furthermore, this study indicates that a glucagon response after reduction of extracellular calcium is regulated by the prevailing glucose concentration, i.e., profoundly and persistently inhibited by transition from 3.3 mM to higher concentrations of glucose. The latter findings are in contrast to those of Leclercq-Meyer et al.,[3,4] who previously reported that omission of calcium not only enhances glucagon secretion from pancreas perfused with glucose-containing media, but also abolishes the inhibitory effect of increasing concentrations

TABLE 1
Effects of extracellular calcium on insulin, glucagon, and somatostatin secretion*

Conditions	No. of experiments	Insulin (μU/40 min)	Glucagon (pg/40 min)	Somatostatin (pg/40 min)
Glucose 3.3 mM				
2.6 mM Ca^{2+}	5	—	−3603 ± 906	−147 ± 182
2.6 mM Ca^{2+} + glibenclamide	5	6181 ± 972‡	−5014 ± 2988	974 ± 333‡
No Ca^{2+}	6	—	41922 ± 3405†	−277 ± 43
No Ca^{2+} + glibenclamide	8	8373 ± 1175‡	95593 ± 9055†‡	−153 ± 76†
Glucose 6.7 mM				
2.6 mM Ca^{2+}	6	3102 ± 686	−1799 ± 1524	161 ± 46
2.6 mM Ca^{2+} + glibenclamide	6	16775 ± 2285‡	2113 ± 2159	1305 ± 148‡
No Ca^{2+}	6	4840 ± 880	19423 ± 2697†	−27 ± 52†
No Ca^{2+} + glibenclamide	6	9239 ± 611†‡	32414 ± 2932†‡	276 ± 128†
Glucose 16.7 mM				
2.6 mM Ca^{2+}	6	42741 ± 5284	−3331 ± 2205	481 ± 65
2.6 mM Ca^{2+} + glibenclamide	6	53558 ± 4964	−1092 ± 2279	1779 ± 279‡
No Ca^{2+}	8	24769 ± 3895†	16468 ± 2025†	−145 ± 66†
No Ca^{2+} + glibenclamide	8	28789 ± 1472†	32722 ± 2615†‡	−36 ± 66†

*Mean ± SEM of respective number of experiments. Results are derived from Figures 1–3 and calculated as the integrated response (basal secretory rates subtracted) for a 40-min test period.
Significance testing (unpaired differences): †$P < 0.05$ or less for effect of calcium deprivation; ‡$P < 0.05$ or less for effect of glibenclamide versus absence of drug.

of glucose on glucagon secretion. In trying to analyze the cause of the divergent results, it should be noted that calcium deprivation was initiated concomitant with a variation of the glucose concentration in our experiments, while, in the previous studies, the change in glucose concentration either preceded that of calcium deprivation or vice versa. Also, calcium deprivation may have been more pronounced in the previous experiments than in the present ones, since measurements of total calcium content in respective calcium-deprived media show moderate differences, namely 0.24 mM in the present experiments versus 0.09–0.21 mM in previous studies.[3,4] Although the exact cause of discrepancies cannot be ascertained, it seems safe to conclude that an enhancing effect of calcium deprivation on glucagon se-

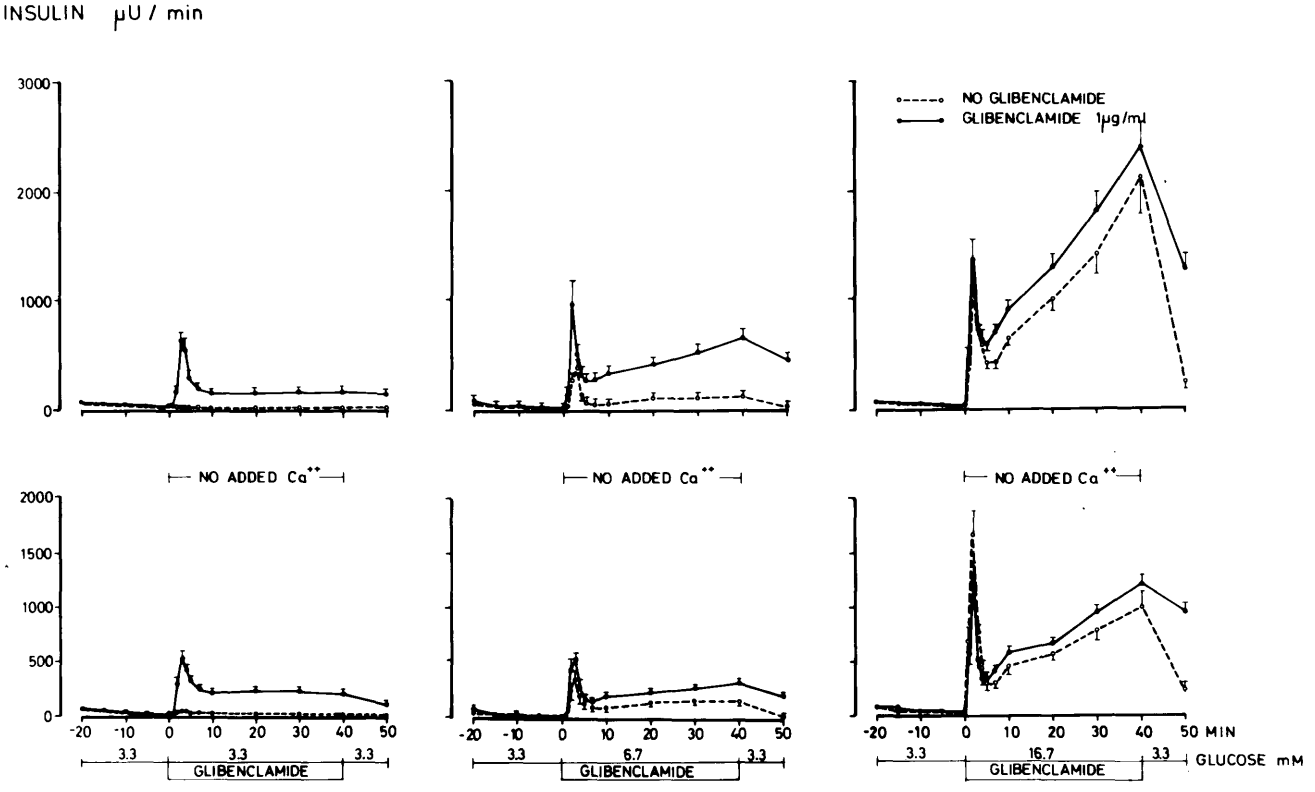

FIGURE 2. Effects of calcium deprivation on insulin secretion induced by glucose and glibenclamide. Mean ± SEM of the respective number of experiments indicated in Table 1.

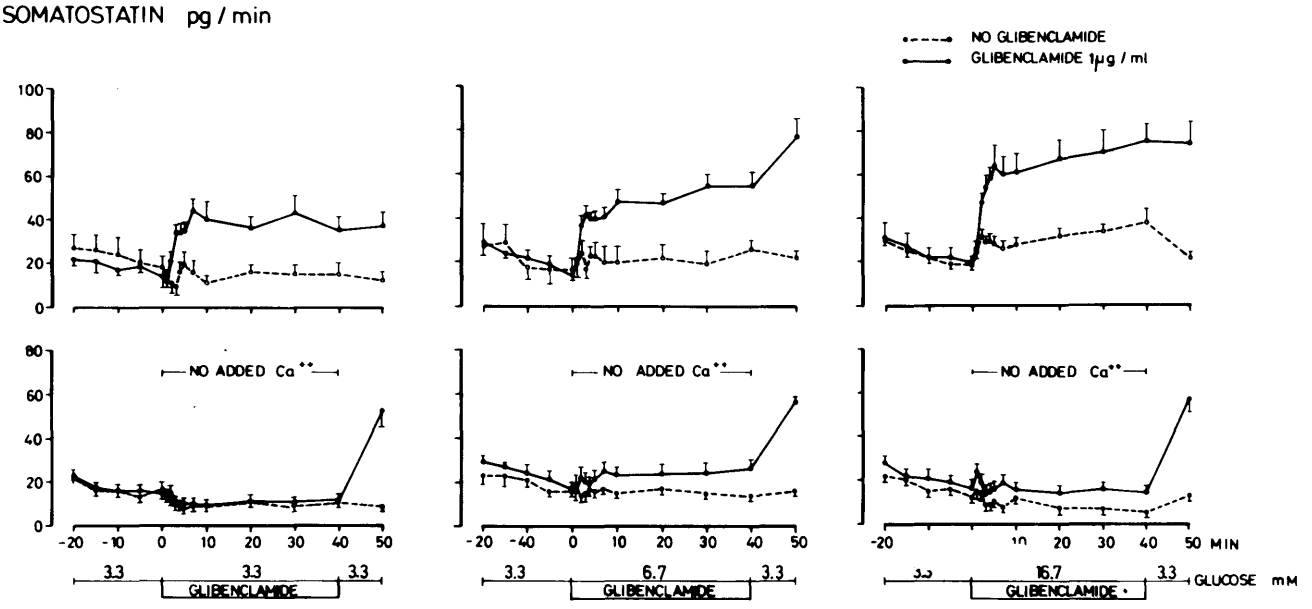

FIGURE 3. Effects of calcium deprivation on somatostatin secretion induced by glucose and glibenclamide. Mean ± SEM of the respective number of experiments indicated in Table 1.

cretion is not, under all conditions, coupled to complete insensitivity to glucose.

The mechanisms behind the effects of low calcium levels on glucagon secretion are unclear as are, in general, the role(s) of calcium in the secretion of glucagon. Difficulties in defining roles of calcium for glucagon release stem from the complex nature of effects of this ion on glucagon secretion. The action of calcium on glucagon secretion has thus been demonstrated to depend on the substrate present in incubation media, the type of secretagogue, and the previous calcium environment.[3,4,10-13] An absolute requirement for minimal amounts of calcium seems to be established, since glucagon secretion is abolished by the calcium-chelating agent EGTA.[14] A subphysiologic level of calcium may, however, be either inhibitory or stimulatory in relation to normal calcium, depending on the substrate and/or secretagogue present. Thus, the stimulatory effects of arginine[10] as well as of nutrients such as fumarate, glutamate, and pyruvate[11] are inhibited by calcium reduction to the subphysiologic concentrations presently and previously employed. An enhancing effect due to calcium deprivation has, so far, been found only when glucose[3,4] or α-ketoisocaproic acid[15] were present in the incubation medium. From these findings, the conclusion was drawn that inhibition by normal calcium of glucagon secretion may be coupled to the metabolism of certain nutrients.[15]

In light of the above discussion, how should one interpret the stimulating effects of glibenclamide on glucagon in a low-calcium medium? Considering different alternatives, it may be relevant that sulfonylureas lower levels of glycogen and ATP in B-cells.[16] If such effects are also operative in the A-cell, then glibenclamide could counteract metabolic effects due to glucose per se. Since glucose may modulate sensitivity to calcium by virtue of metabolic effects, it follows that the drug could oppose such regulation. Paracrine effects could also be important for the glibenclamide effect on glucagon release. It is possible that stimulation of glucagon release by glibenclamide is inhibited by a concomitant somatostatin response at normal calcium, and is demasked only at low calcium, which abolishes glibenclamide-induced somatostatin secretion. Such a notion is supported, first, by findings that glibenclamide induced a diphasic effect (a small stimulation followed by pronounced inhibition) when tested at normal calcium but in the total or almost total (1.1 mM) absence of glucose.[1] The inhibition phase coincided with marked stimulation of somatostatin release. Second, glibenclamide suppressed the effect of arginine on glucagon release and enhanced its stimulatory action on somatostatin secretion.[17] According to this reasoning, a somatostatin response could, in some situations, cause frank inhibition and in others inhibit stimulation of glucagon secretion. Needless to say, these and other alternatives for mechanisms of action remain speculative.

Regarding insulin secretion, it is interesting to note that a clear inhibitory effect of calcium deprivation on insulin release was observed in relation to prominent second-phase secretion, whether induced by 16.7 mM glucose or by the combination of 6.7 mM glucose and glibenclamide. These results seem compatible with previous observations that indicate that the second, but not the first, phase of (at least) glucose-induced insulin release is dependent on flow of calcium into the B-cell.[18] Regardless of whether or not the influence of extracellular calcium in our experiments can be explained along these lines, our results suggest that the interaction between glucose and sulfonylurea is de facto modulated by extracellular calcium levels, since the stimulatory effect of glibenclamide on insulin secretion induced by 6.7 mM glucose seemed less marked at low, rather than at normal, extracellular calcium levels.

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