Effects of the calcium-channel agonist CGP 28392 on insulin secretion from isolated rat islets of Langerhans

Noel G. MORGAN, Catherine D. SHORT, Gwyneth M. RUMFORD and William MONTAGUE

Department of Biochemistry, University of Leicester, Leicester LE1 7RH, U.K.

The rate of insulin secretion from isolated rat islets of Langerhans was affected by a number of dihydropyridine derivatives known to interact with voltage-sensitive Ca^{2+} channels in excitable cells. The channel antagonists nifedipine and nitrendipine were potent inhibitors of glucose-induced insulin secretion in response to both 8 mM- and 20 mM-glucose, although they did not lower the basal secretion rate observed in the presence of 4 mM-glucose. The Ca^{2+} -channel agonist, CGP 28392, also failed to alter the basal rate of insulin secretion. In the presence of 8 mM-glucose, however, 1 μM -CGP 28392 enhanced the insulin-secretion rate to a value approximately double that with 8 mM-glucose alone. This effect was dose-dependent, with half the maximal response elicited by 0.1 μM -CGP 28392, and full enhancement at 10 μM . The response was rapid in onset, with an increase in insulin secretion evident within 2 min of CGP 28392 infusion in perfused islets. Stimulation of insulin secretion by CGP 28392 was correlated with a rapid enhancement of glucose-stimulated $^{45}\text{Ca}^{2+}$ uptake into islets cells, and with a transiently increased rate of $^{45}\text{Ca}^{2+}$ efflux from pre-loaded islets. Stimulation of insulin secretion by CGP 28392 was abolished in the presence of noradrenaline, although under these conditions the rapid stimulation of $^{45}\text{Ca}^{2+}$ influx induced by CGP 28392 was only partially inhibited. In contrast with these results, when islets were incubated in the presence of 20 mM-glucose, CGP 28392 caused a dose-dependent inhibition of insulin secretion. Half-maximal inhibition required approx. 0.2 μM -CGP 28392, with maximal effects observed at 10 μM . Under these conditions, however, the extent of insulin secretion was still only decreased by about 50%, to a value which was similar to that seen in the presence of 8 mM-glucose and CGP 28392. These results suggest that dihydropyridine derivatives can alter the activity of voltage-dependent Ca^{2+} channels in islet cells, and are consistent with the possibility that gating of these channels plays an important role in regulating the rate of insulin secretion after glucose stimulation.

INTRODUCTION

The rate of insulin secretion from the B-cells of the endocrine pancreas is believed to be regulated by changes in the cytosolic free Ca^{2+} concentration ($[\text{Ca}_i^{2+}]$) (see Wollheim & Sharp, 1981, for review), and direct demonstration that glucose induces a rise in islet-cell $[\text{Ca}_i^{2+}]$ has been provided (Rorsman *et al.*, 1984; Deleers *et al.*, 1985). Some of the Ca^{2+} mediating this effect may derive from intracellular pools (Biden *et al.*, 1984), but the well-documented requirement for extracellular Ca^{2+} during maintained stimulation of insulin secretion (Curry *et al.*, 1968; Grodsky & Bennett, 1966; Hales & Milner, 1968; Kikuchi *et al.*, 1978; Morgan & Montague, 1984) suggests that influx of Ca^{2+} from the extracellular medium provides most of the Ca^{2+} responsible for the rise in $[\text{Ca}_i^{2+}]$. Indeed, studies of $^{45}\text{Ca}^{2+}$ fluxes in islet cells have suggested that glucose promotes $^{45}\text{Ca}^{2+}$ influx within 1 min of stimulation (Wollheim *et al.*, 1977). This effect has been correlated with changes in islet-cell electrical activity in response to glucose, which produces a characteristic burst of action potentials after membrane depolarization (Dean & Matthews, 1968; Atwater & Beigelman, 1976; Beigelman & Ribalet, 1980). Removal of extracellular Ca^{2+} leads to inhibition of action-potential propagation (Dean & Matthews, 1970; Matthews & Sakamoto, 1975), and it has been suggested that the action potentials represent an inward flow of Ca^{2+} current (Matthews & Sakamoto, 1975). On the basis of

such observations, the hypothesis has been advanced that the B-cell plasma membrane is equipped with Ca^{2+} -conducting channels whose activity is regulated by the membrane potential. According to this hypothesis, membrane depolarization to a critical threshold potential leads to gating of the Ca^{2+} channels and net Ca^{2+} influx (Matthews & Sakamoto, 1975; Wollheim & Sharp, 1981).

Direct biochemical evidence for the presence of voltage-dependent Ca^{2+} channels in islet cells is lacking, although studies of the effects of pharmacological agents which affect the functioning of such channels in other tissues have supported the postulate that they are involved in the control of insulin secretion. In particular, the Ca^{2+} -channel antagonists verapamil and D600 have been shown to inhibit glucose-induced insulin release (Devis *et al.*, 1975; Malaisse *et al.*, 1976) and $^{45}\text{Ca}^{2+}$ influx (Malaisse *et al.*, 1976, 1977) in islet cells, and the highly potent dihydropyridine class of Ca^{2+} -channel antagonists have also been shown to inhibit glucose-stimulated insulin secretion (Malaisse & Sener, 1981).

A group of modified dihydropyridine derivatives have been synthesized which have altered properties such that they apparently facilitate Ca^{2+} inflow through voltage-dependent Ca^{2+} channels in a variety of tissues (Schramm *et al.*, 1983; Erne *et al.*, 1984). These compounds may therefore be useful for further investigation of the role of voltage-dependent Ca^{2+} channels in the regulation of insulin secretion. We have studied the effect of one such agent, CGP 28392, on the rate of insulin secretion from

isolated rat islets of Langerhans and demonstrate that this compound enhances insulin secretion in response to sub-maximal glucose concentrations. CGP 28392 also promotes rapid $^{45}\text{Ca}^{2+}$ influx into islet cells and facilitates ^{45}Ca efflux from pre-loaded islets. Our results are therefore consistent with a direct involvement of voltage-sensitive Ca^{2+} channels in regulating insulin secretion. While the present work was in progress, two reports have appeared which demonstrate that a related compound, BAY K 8644, also enhances glucose-induced insulin release in islets of Langerhans (Malaisse-Lagae *et al.*, 1984; Panten *et al.*, 1985).

EXPERIMENTAL

Materials

Nifedipine and nitrendipine were donated by Bayer Ltd., Newbury, Berks, U.K., and CGP 28392 was given by Ciba-Geigy, Basle, Switzerland. Di-n-butyl phthalate was purchased from Hopkin and Williams, Chadwell Heath, Essex, U.K., and dinonyl phthalate from BDH Chemicals, Poole, Dorset, U.K. Collagenase was purchased from Boehringer-Mannheim, Lewes, Sussex, U.K., bovine serum albumin (fraction V) from Armour Pharmaceuticals, Eastbourne, Sussex, U.K., and $^{45}\text{Ca}^{2+}$ and $[^3\text{H}]$ sucrose were obtained from Amersham International, Amersham, Bucks, U.K. All other reagents were of analytical-reagent quality.

Methods

Isolation of islets of Langerhans. All experiments were performed with islets of Langerhans freshly isolated by collagenase digestion of the pancreas from each of two male Wistar rats (250–300 g) as described in detail by Montague & Taylor (1968). Islets were isolated and incubated in a bicarbonate-buffered medium, pH 7.4 (Gey & Gey, 1936), gassed with O_2/CO_2 (19:1) and containing glucose (4 mM), CaCl_2 (2 mM) and bovine serum albumin (1 mg/ml).

Islet incubation conditions. Methods for static incubation and islet perfusion studies of insulin secretion were performed exactly as detailed previously (Morgan & Montague, 1985). Islet $^{45}\text{Ca}^{2+}$ -accumulation experiments were performed as described by Morgan & Montague (1982), and studies of islet-cell ^{45}Ca efflux were undertaken by the methods detailed by Morgan *et al.* (1985). All incubations were carried out at 37 °C, and experiments employing nifedipine, nitrendipine and CGP 28392 were performed in the dark.

Statistical analysis of results. Levels of significance for differences between mean values were estimated by Student's *t* test. Results are presented as mean values $\pm$ S.E.M., with the numbers of observations given in parentheses.

RESULTS

The data presented in Fig. 1 demonstrate that nitrendipine and nifedipine both induced a dose-dependent inhibition of insulin secretion from isolated rat islets of Langerhans incubated in the presence of 20 mM-glucose. Half-maximal inhibition was obtained in the presence of approx. 20 nM of either compound (Fig. 1), and complete suppression of the response occurred at 1 μM . Nifedipine

Fig. 1. Effect of nifedipine and nitrendipine on glucose-stimulated insulin secretion

Groups of three isolated islets were incubated with 20 mM-glucose in the presence of increasing concentrations of nitrendipine (●) or nifedipine (○). After incubation for 1 h the medium was sampled, and the extent of insulin secretion determined by radioimmunoassay. Each point represents the mean value $\pm$ S.E.M. for six observations. The basal rate of secretion obtained in the presence of 4 mM-glucose averaged 1.5 ± 0.2 ng/h per islet.

(1 μM) also inhibited insulin secretion in response to 8 mM-glucose, but failed to lower the basal rate of insulin secretion observed in the presence of 4 mM-glucose (Table 1). In contrast with the effect of nifedipine, CGP 28392 (1 μM) enhanced the rate of insulin secretion observed in the presence of 8 mM-glucose (Table 1). This effect was dose-dependent (Fig. 2), with half the maximal response observed at 100 nM-CGP 28392 and full enhancement at 10 μM . Under these conditions the rate of insulin secretion was increased to more than double that observed with 8 mM-glucose alone (Fig. 2). CGP 28392 did not increase the rate of insulin secretion under basal conditions (Table 1). Perfusion of isolated islets in the presence of 8 mM-glucose and CGP 28392 revealed that the agent caused a prompt increase in insulin secretion, which was evident within 2 min and could be maintained for at least 15 min if CGP 28392 infusion was continued (Fig. 3).

In contrast with the enhancement of insulin secretion provoked by CGP 28392 when islets were incubated with 8 mM-glucose, exposure of islets to this agent in the presence of 20 mM-glucose was associated with inhibition

Table 1. Effect of dihydropyridine derivatives on glucose-induced insulin secretion

Groups of three isolated islets were incubated for 1 h under the conditions shown. Portions of the incubation medium were removed after this time and their insulin content was measured by radioimmunoassay. Each value represents the mean $\pm$ S.E.M. of at least eight observations. * $P < 0.001$ relative to 8 mM-glucose alone; ** $P < 0.01$ relative to 20 mM-glucose alone.

Glucose concn. (mM)	Addition to incubation medium...	Insulin secretion (ng/h per islet)		
		None	Nifedipine (10 μ M)	CGP 28392 (1 μ M)
4		1.4 $\pm$ 0.2	1.2 $\pm$ 0.1	1.2 $\pm$ 0.2
8		3.0 $\pm$ 0.3	1.4 $\pm$ 0.1*	6.6 $\pm$ 0.7*
20		10.4 $\pm$ 1.0	1.5 $\pm$ 0.2**	6.2 $\pm$ 0.4**

Fig. 2. Dose-response relationship for stimulation of insulin secretion by CGP 28392

Groups of three islets were incubated in medium containing 8 mM-glucose and increasing concentrations of CGP 28392 for 1 h. After this time, samples of the medium were removed for assay of insulin secretion. Each value represents the mean $\pm$ S.E.M. for 15 observations.

of secretion (Table 1). This effect was also dose-dependent, but required approx. 200 nM-GCP 28392 for half the maximal response (Fig. 4). Furthermore, CGP 28392 decreased the rate of glucose-induced secretion by only 46% at concentrations up to 10 μ M (Fig. 4), whereas nifedipine and nitrendipine abolished glucose stimulation when present at 1 μ M (Fig. 1).

To examine the possibility that the action of CGP 28392 to stimulate insulin secretion in response to

Fig. 3. Dynamics of stimulation of insulin secretion by CGP 28392

Groups of 80 islets were perfused with medium containing 4 mM-glucose for 30 min, when the glucose concentration was increased to 8 mM. Then 15 min later ($t = 0$; arrow) CGP 28392 (1 μ M) was introduced and perfusion was continued for a further 15 min. Samples of perfusion medium were collected at 1 min intervals and their insulin contents measured. Each point is the mean value $\pm$ S.E.M. obtained in three perfusions.

8 mM-glucose resulted from alterations in islet-cell Ca^{2+} fluxes, rates of $^{45}\text{Ca}^{2+}$ influx and efflux from islets treated with the agent were measured. Islets incubated in the presence of 8 mM-glucose rapidly accumulated $^{45}\text{Ca}^{2+}$

Fig. 4. Effect of CGP 28392 on insulin secretion in response to 20 mM-glucose

Groups of three islets were incubated with 20 mM-glucose in the presence of increasing concentrations of CGP 28392 as shown. The medium was sampled after 1 h of incubation and the extent of insulin secretion determined. Each point represents the mean $\pm$ S.E.M. for ten observations. The basal rate of insulin secretion was measured in the presence of 4 mM-glucose (○).

during 10 min of incubation (Table 2), and the extent of this uptake was markedly enhanced by 1 μ M-CGP 28392. In addition, CGP 28392 provoked an efflux of $^{45}\text{Ca}^{2+}$ from pre-loaded islets (Fig. 5). This effect was rapid in onset, but was transient, with the rate of $^{45}\text{Ca}^{2+}$ efflux returning to the control value within 5–6 min. In contrast, when islets were incubated with CGP 28392 in the presence of 20 mM-glucose, the agent markedly inhibited the extent of $^{45}\text{Ca}^{2+}$ influx (Table 2). These results are consistent, therefore, with an effect of CGP 28392 to alter islet-cell Ca^{2+} handling.

Incubation of islets with noradrenaline caused a partial inhibition of CGP 28392-stimulated $^{45}\text{Ca}^{2+}$ influx (Table 3), although, under these conditions, the rate of $^{45}\text{Ca}^{2+}$ influx was still elevated relative to the control value. However, despite this failure of noradrenaline to abolish the stimulation of $^{45}\text{Ca}^{2+}$ influx, it completely prevented any stimulation of insulin secretion by CGP 28392 (Table 3).

Fig. 5. Effect of CGP 28392 on $^{45}\text{Ca}^{2+}$ efflux from pre-loaded islets

Groups of 100 islets were pre-loaded with $^{45}\text{Ca}^{2+}$ for 30 min before transfer to perfusion chambers. They were then perfused for 30 min with medium containing 4 mM-glucose, when the glucose concentration was increased to 8 mM. Then 15 min later ($t = 0$; arrow) 1 μ M-CGP 28392 was also introduced and perfusion continued. Samples of medium were collected at 1 min intervals and their $^{45}\text{Ca}^{2+}$ contents determined. Rates of $^{45}\text{Ca}^{2+}$ efflux are expressed relative to the control rate (100%) averaged over the 5 min immediately before introduction of CGP 28392. Each point represents the mean value $\pm$ S.E.M. for five perfusions.

Table 2. Effect of CGP 28392 on $^{45}\text{Ca}^{2+}$ influx into islet cells

Groups of ten isolated islets were incubated with 8 mM-glucose and CGP 28392 as shown, in the presence of $^{45}\text{Ca}^{2+}$ and [^3H]sucrose. After incubation for 10 min, the islets were separated from the incubation medium and their $^{45}\text{Ca}^{2+}$ contents determined. Results are presented as $^{45}\text{Ca}^{2+}$ accumulation in excess of that in the [^3H]sucrose space. * $P < 0.001$ relative to 8 mM-glucose alone; ** $P < 0.05$ relative to 20 mM-glucose alone.

Incubation conditions	$^{45}\text{Ca}^{2+}$ influx (fmol/islet)
8 mM-Glucose	660 $\pm$ 30 (13)
8 mM-Glucose + 1 μ M-CGP 28392	910 $\pm$ 60* (13)
20 mM-Glucose	1020 $\pm$ 38 (10)
20 mM-Glucose + 1 μ M-CGP 28392	851 $\pm$ 77** (10)

Table 3. Effect of noradrenaline on the stimulation of insulin secretion and $^{45}\text{Ca}^{2+}$ influx by CGP 28392

Groups of isolated islets were incubated with 8 mM-glucose in the presence of CGP 28392 and noradrenaline as shown. The initial rate of $^{45}\text{Ca}^{2+}$ influx and the rate of insulin secretion were determined, and the results are presented as mean values $\pm$ S.E.M. ($n = 10$). * $P < 0.01$ relative to 8 mM-glucose alone; ** $P < 0.001$ relative to absence of noradrenaline.

Additions to the incubation medium		Insulin secretion (ng/h per islet)	$^{45}\text{Ca}^{2+}$ influx (fmol/10 min per islet)
CGP 28392 (1 μM)	Noradrenaline (2 μM)		
—	—	3.5 ± 0.3	318 ± 22
+	—	$5.9 \pm 0.5^*$	$531 \pm 52^*$
—	+	$0.4 \pm 0.1^{**}$	298 ± 25
+	+	$0.5 \pm 0.1^{**}$	$417 \pm 29^*$

DISCUSSION

The data obtained in the present study demonstrate that a variety of structurally related dihydropyridine derivatives can influence the rate of glucose-induced insulin secretion from isolated rat islets of Langerhans. These agents are believed to exert their influence by specific interaction with voltage-dependent Ca^{2+} channels located in cell plasma membranes (Braunwald, 1982; Schwartz & Triggle, 1984). The observation that nifedipine and nitrendipine, inhibitors of voltage-sensitive Ca^{2+} -channel activity, both acted as potent inhibitors of glucose-stimulated insulin secretion (Fig. 1; Malaisse & Boschero, 1977) supports the belief that gating of such channels represents an important component of the stimulus-secretion-coupling mechanism of the pancreatic B-cell (Dean & Matthews, 1970; Wollheim *et al.*, 1978; Wollheim & Sharp, 1981).

In contrast with the inhibitory effects of nifedipine and nitrendipine, the modified analogue CGP 28392 actually enhanced insulin secretion in response to 8 mM-glucose (Table 1; Fig. 2). This response was rapid in onset (Fig. 3) and correlated with a rapid stimulation of $^{45}\text{Ca}^{2+}$ influx into islet cells incubated with CGP 28392 and 8 mM-glucose (Table 2). The functional interaction of CGP 28392 with cells has been attributed to binding at a site which is close to the voltage-dependent Ca^{2+} channel (Erne *et al.*, 1984; Loutzenhiser *et al.*, 1984), and it has been shown that this interaction induces changes in the properties of the channel. In particular, CGP 28392 increases both the probability of channel opening (Brown *et al.*, 1984) and also the mean open time of the channel (Kokubun & Reuter, 1984). Our results, demonstrating that CGP 28392 promotes $^{45}\text{Ca}^{2+}$ influx into islet cells (Table 2), therefore suggest that voltage-sensitive Ca^{2+} channels are responsible for mediating the enhanced Ca^{2+} influx seen at sub-maximal glucose concentrations. This conclusion is at variance with that of Lebrun *et al.* (1982), who demonstrated that changes in islet-cell Ca^{2+} fluxes in response to 8.3 mM-glucose are relatively resistant to the Ca^{2+} -channel antagonist verapamil, and suggested therefore that, at low concentrations, glucose facilitates Ca^{2+} inflow by a voltage-insensitive mechanism. It is unlikely, however, that CGP 28392 facilitated Ca^{2+} influx by a voltage-insensitive mechanism in our study, since it failed to stimulate insulin secretion at 4 mM-glucose (Table 1), conditions under which the B-cell membrane is not depolarized, but enhanced secretion at

8 mM-glucose, when membrane depolarization occurs (Atwater & Beigelman, 1976; Ribalet & Beigelman, 1979; see Wollheim & Sharp, 1981, for review). Furthermore, nifedipine abolished glucose stimulation at 8 mM-glucose, providing further evidence for the operation of voltage-sensitive Ca^{2+} channels under these conditions. In the study by Lebrun *et al.* (1982), islet-cell $^{45}\text{Ca}^{2+}$ -efflux rates were used to estimate indirectly changes in Ca^{2+} influx, and in the present investigation we have also demonstrated that CGP 28392 transiently facilitates $^{45}\text{Ca}^{2+}$ efflux from islets perfused in medium containing 8 mM-glucose (Fig. 5), consistent with the enhancement of Ca^{2+} influx seen under these conditions (Table 2).

Examination of the dose-response characteristics for enhancement of insulin secretion by CGP 28392 (Fig. 2) revealed that approx. 0.1 μM was required for half the maximal response. This is in good agreement with the dose-response curves for stimulation of $^{45}\text{Ca}^{2+}$ uptake into neuroblastoma-glioma cells (Freedman & Miller, 1984) and of Ca^{2+} -dependent quin-2 fluorescence in human platelets (Erne *et al.*, 1984). These observations are consistent, therefore, with the possibility that opening of voltage-sensitive Ca^{2+} channels plays a primary role in mediating the rise in intracellular free Ca^{2+} which leads to enhanced insulin secretion at 8 mM-glucose.

The observation that stimulation of insulin secretion by CGP 28392 was sensitive to inhibition by noradrenaline (Table 3) suggests that the release of insulin occurred by the normal exocytotic mechanism and was not the result of cell damage caused by CGP 28392. The inhibition of secretion induced by noradrenaline occurred despite an enhanced rate of $^{45}\text{Ca}^{2+}$ influx in the presence of CGP 28392 (Table 3), which supports our previous suggestion that noradrenaline does not act primarily to inhibit Ca^{2+} influx into islet cells, but induces a state of desensitization in the B-cell, whereby a rise in Ca^{2+} influx is not able to initiate a secretory response (Morgan & Montague, 1985).

It has been suggested that gating of voltage-sensitive Ca^{2+} channels does not contribute to the basal rate of Ca^{2+} inflow into islet cells, which maintains insulin secretion under resting conditions (Wollheim & Sharp, 1981). Our results support this idea, since nifedipine blockade failed to lower the basal rate of insulin secretion in the presence of 4 mM-glucose (Table 1) and CGP 28392 did not stimulate secretion under these non-depolarizing conditions (Table 1).

An interesting observation arising out of the present

study concerns the failure of CGP 28392 to enhance insulin secretion further when islets were incubated with 20 mM-glucose. Indeed, under these conditions the agent was inhibitory (Table 1; Fig. 4). This suggests that when islet-cell Ca^{2+} channels are predominantly in the open state, the interaction of CGP 28392 at its receptor site may be inhibitory to Ca^{2+} influx. The agent is therefore only a partial agonist with respect to channel activation. Similar blockade of Ca^{2+} channels by CGP 28392 under fully stimulating conditions has also been reported in NG108-15 cells (Freedman & Miller, 1984). Studies of the dose-response relationship for inhibition of insulin secretion in response to 20 mM-glucose revealed that this response was slightly less sensitive to CGP 28392 than was stimulation at 8 mM-glucose (Figs. 2 and 3) and that the agent was an order of magnitude less potent as an antagonist than was nitrendipine (Fig. 1). Furthermore, whereas nitrendipine (1 μM) abolished glucose-induced insulin secretion, CGP 28392 was still only partially antagonistic at 10 μM .

In conclusion, therefore, the results of the present study provide further evidence that voltage-sensitive Ca^{2+} channels play a crucial role in regulating Ca^{2+} influx and insulin secretion in the pancreatic B-cell, under stimulating conditions. The results also confirm that noradrenaline does not exert its inhibitory effect in the pancreatic B-cell by directly preventing the gating of voltage-sensitive Ca^{2+} channels.

We are grateful to Professor H. Brunner and Dr. M. Meier of Ciba-Geigy Ltd. for supplying a sample of CGP 28392. These studies were supported by a grant from the British Diabetic Association.

REFERENCES

- Atwater, I. & Beigelman, P. M. (1976) *J. Physiol. (Paris)* **72**, 769-786
- Beigelman, P. M. & Ribalet, B. (1980) *Diabetes* **29**, 263-265
- Biden, T. J., Prentki, M., Irvine, R. F., Berridge, M. J. & Wollheim, C. B. (1984) *Biochem. J.* **223**, 467-473
- Braunwald, E. (1982) *N. Engl. J. Med.* **307**, 1618-1627
- Brown, A. M., Kunze, D. L. & Yatani, A. (1984) *Nature (London)* **311**, 570-572
- Curry, D. L., Bennett, L. L. & Grodsky, G. M. (1968) *Am. J. Physiol.* **214**, 174-178
- Dean, P. M. & Matthews, E. K. (1968) *Nature (London)* **219**, 389-390
- Dean, P. M. & Matthews, E. K. (1970) *J. Physiol. (London)* **210**, 265-275
- Deleers, M., Mahy, M. & Malaisse, W. J. (1985) *Biochem. Int.* **10**, 97-103
- Devis, G., Somers, M. D. G., van Obberghen, E. & Malaisse, W. J. (1975) *Diabetes* **24**, 547-551
- Erne, P., Burgisser, E., Buhler, F. R., Dubach, B., Kuhn, H., Meier, M. & Rogg, H. (1984) *Biochem. Biophys. Res. Commun.* **118**, 842-847
- Freedman, S. B. & Miller, R. J. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5580-5583
- Gey, G. O. & Gey, M. K. (1936) *Am. J. Cancer* **27**, 45-76
- Grodsky, G. M. & Bennett, L. L. (1966) *Diabetes* **15**, 910-913
- Hales, C. N. & Milner, R. D. G. (1968) *J. Physiol. (London)* **199**, 177-187
- Kikuchi, M., Wollheim, C. B., Cuendet, G. S., Renold, A. E. & Sharp, G. W. G. (1978) *Endocrinology (Baltimore)* **102**, 1339-1349
- Kokubun, S. & Reuter, H. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4824-4827
- Lebrun, P., Malaisse, W. J. & Herchuelz, A. (1982) *Am. J. Physiol.* **242**, E59-E66
- Loutzenhiser, R., Ruegg, U. T., Hof, A. & Hof, R. P. (1984) *Eur. J. Pharmacol.* **105**, 229-237
- Malaisse, W. J. & Boscher, A. C. (1977) *Horm. Res.* **8**, 203-209
- Malaisse, W. J. & Sener, A. (1981) *Biochem. Pharmacol.* **30**, 1039-1041
- Malaisse, W. J., Devis, G., Pipeleers, D. G. & Somers, G. (1976) *Diabetologia* **12**, 77-81
- Malaisse, W. J., Herchuelz, A., Levy, J. & Sener, A. (1977) *Biochem. Pharmacol.* **26**, 735-740
- Malaisse-Lagae, F., Matthias, P. C. F. & Malaisse, W. J. (1984) *Biochem. Biophys. Res. Commun.* **123**, 1062-1068
- Matthews, E. K. & Sakamoto, Y. (1975) *J. Physiol. (London)* **246**, 421-437
- Montague, W. & Taylor, K. W. (1968) *Biochem. J.* **109**, 333-339
- Morgan, N. G. & Montague, W. (1982) *Biochem. J.* **204**, 111-125
- Morgan, N. G. & Montague, W. (1984) *Biosci. Rep.* **4**, 665-671
- Morgan, N. G. & Montague, W. (1985) *Biochem. J.* **226**, 571-576
- Morgan, N. G., Rumford, G. M. & Montague, W. (1985) *Biochem. J.* **228**, 713-718
- Panten, U., Zielmann, S., Schrader, M.-T. & Lenzen, S. (1985) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **328**, 351-353
- Ribalet, B. & Beigelman, P. M. (1979) *Am. J. Physiol.* **237**, C137-C146
- Rorsman, P., Abrahamsson, H., Gylfe, E. & Hellman, B. (1984) *FEBS Lett.* **170**, 196-200
- Schramm, M., Thomas, G., Towart, R. & Franckowiak, G. (1983) *Nature (London)* **303**, 535-537
- Schwartz, A. & Triggle, D. J. (1984) *Annu. Rev. Med.* **35**, 325-329
- Wollheim, C. B. & Sharp, G. W. G. (1981) *Physiol. Rev.* **61**, 914-973
- Wollheim, C. B., Kikuchi, M., Renold, A. E. & Sharp, G. W. G. (1977) *J. Clin. Invest.* **60**, 1165-1173
- Wollheim, C. B., Kikuchi, M., Renold, A. E. & Sharp, G. W. G. (1978) *J. Clin. Invest.* **62**, 451-458