Gymnema sylvestre stimulates insulin release *in vitro* by increased membrane permeability

S J Persaud[1], H Al-Majed[1], A Raman[2] and P M Jones[1]

[1]Physiology Division, School of Biomedical Sciences, King's College, London, UK
[2]Pharmacy Department, School of Health and Life Sciences, King's College London, UK
(Requests for offprints should be addressed to S J Persaud)

Abstract

To determine whether extracts of *Gymnema sylvestre* may have therapeutic potential for the treatment of non-insulin-dependent diabetes mellitus (NIDDM), we examined the effects of an alcoholic extract of *G. sylvestre* (GS4) on insulin secretion from rat islets of Langerhans and several pancreatic β-cell lines. GS4 stimulated insulin release from HIT-T15, MIN6 and RINm5F β-cells and from islets in the absence of any other stimulus, and GS4-stimulated insulin secretion was inhibited in the presence of 1 mM EGTA. Blockade of voltage-operated Ca^{2+} channels with 10 μM isradipine did not significantly affect GS4-induced secretion, and insulin release in response to GS4 was independent of incubation temperature. Examination of islet and β-cell integrity after exposure to GS4, by trypan blue exclusion, indicated that concentrations of GS4 that stimulated insulin secretion also caused increased uptake of dye. Two gymnemic acid-enriched fractions of GS4, obtained by size exclusion and silica gel chromatography, also caused increases in insulin secretion concomitant with increased trypan blue uptake. These results confirm the stimulatory effects of *G. sylvestre* on insulin release, but indicate that GS4 acts by increasing cell permeability, rather than by stimulating exocytosis by regulated pathways. Thus the suitability of GS4 as a potential novel treatment for NIDDM can not be assessed by direct measurements of β-cell function *in vitro*.

Journal of Endocrinology (1999) **163,** 207–212

Introduction

Diabetes mellitus is an endocrine disorder in which glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic β-cells (insulin-dependent diabetes mellitus; IDDM), inadequate release of insulin from the pancreatic β-cells, or insensitivity of target tissues to insulin (non-insulin-dependent diabetes mellitus; NIDDM). NIDDM accounts for up to 90% of the UK diabetic population, and there is an increasing drive to develop novel methods for its treatment, to improve either the insulin output of β-cells or the sensitivity of peripheral tissues to circulating insulin. Of the currently available therapies for NIDDM, only the sulphonylureas are used to stimulate β-cells to secrete more insulin. The others, such as biguanides, α-glucosidase inhibitors and thiazolidinediones, reduce hyperglycaemia independently of increases in circulating insulin.

Sulphonylureas act at a proximal stage in the β-cell stimulus–secretion coupling cascade: they close plasma membrane ATP-sensitive K^+ channels and the consequent decrease in K^+ efflux depolarises the cells, leading to Ca^{2+} influx via voltage-operated Ca^{2+} channels (Henquin 1980, Sturgess *et al.* 1985). Increases in intracellular Ca^{2+} are sufficient to stimulate insulin release from β-cell secretory granules (Jones *et al.* 1985). Novel drugs that act at downstream sites, perhaps directly on the exocytotic release of insulin, would be useful adjuncts to sulphonylureas in the treatment of NIDDM.

The therapeutic potential of *Gymnema sylvestre* R.Br., a woody climber of the Asclepiadaceae family, has been known for many years and it has a key place in Ayurvedic medicine. There are reports that *G. sylvestre* leaf extracts reduce hyperglycaemia in diabetic rabbits (Shanmugasundaram *et al.* 1983), rats (Srivastava *et al.* 1985) and humans (Khare *et al.* 1983, Baskaran *et al.* 1990), and these glucose-decreasing effects may be mediated by increases in insulin secretion (Shanmugasundaram & Panneerselvam 1981). There has been little systematic characterisation of the identities of the insulinotropic agents within the leaf extracts, but aqueous ethanolic extractions of the leaves provide two potentially active fractions, one containing conduritol A, an acid-soluble polyol-polyhydroxy cyclic compound (Miyatake *et al.* 1993), and the other containing a mixture of acid-insoluble triterpenoid saponins (gymnemic acids), designated GS3 and GS4 (Shanmugasundaram *et al.* 1990).

Online version via http://www.endocrinology.org
0022–0795/99/0163–207

Conduritol A has been reported to have small stimulatory effects on basal insulin secretion through an undefined mechanism (Billington *et al.* 1994). GS3 and GS4, which would be devoid of conduritol, have been reported to reduce hyperglycaemia in diabetic rats, increase insulin release *in vivo* and *in vitro*, and increase β-cell number after streptozotocin-induced diabetes (Shanmugasundaram *et al.* 1990). GS3 is a fairly crude fraction prepared by acid precipitation from a hydroalcoholic extract of *G. sylvestre* leaves. Most studies have been performed using GS4, which is purified from GS3 by reprecipitation with acid of GS3 solubilised in alkali (Shanmugasundaram *et al.* 1990). GS4 has been used clinically to treat NIDDM, and it was shown to increase serum insulin concentrations, normalise blood glucose concentrations and reduce the requirement for sulphonylurea (Baskaran *et al.* 1990). The potential use of GS4 in the treatment of diabetes is intriguing, and in the current study we have examined whether GS4, prepared according to a previously described method (Shanmugasundaram *et al.* 1990), exerts insulinotropic effects on β-cell lines and on isolated islets of Langerhans. We have also investigated its mode of action *in vitro*, to assess its potential for the treatment of NIDDM.

Materials and Methods

Dried leaves of *G. sylvestre* were a gift from Cipla Ltd (Mumbai, India). They were authenticated by the Herbarium at the Royal Botanic Gardens, Kew. A voucher specimen (reference Gy11 12) is deposited in the Pharmacognosy Museum, Department of Pharmacy, King's College London. Isradipine was purchased from Research Biochemicals International (Herts, UK). Tissue culture media, antibiotics and foetal calf serum were obtained from Life Technologies (Paisley, UK). All other reagents were of analytical grade from BDH (Poole, Dorset, UK) or Sigma Chemical Co. (Poole, Dorset, UK). Rats were supplied by King's College London Animal Unit. HIT-T15 β-cells were purchased from the American Type Culture Collection (Rockville, MD, USA), MIN6 β-cells were kindly provided by Professor J-I Miyazaki (University of Tokyo, Japan) and RINm5F β-cells were from Professor A J Bone (University of Brighton, UK).

Tissue preparation

Islets of Langerhans were isolated from the pancreata of male Sprague–Dawley rats (200–250 g) by collagenase digestion as previously described (Jones *et al.* 1993). Approximately 400–500 islets were obtained from each pancreas and islets were used immediately after isolation for all experiments. Pancreatic β-cell lines were maintained in culture in DMEM (MIN6) or RPMI (HIT-T15, RINm5F) supplemented with 5–15% foetal calf serum, 100 μg/ml streptomycin and 100 U/ml penicillin, in a humidified atmosphere of 5% CO_2.

Preparation of Gymnema *fractions*

GS4 was prepared as follows: dried *Gymnema* leaves (1 kg) were ground to a coarse powder in a coffee grinder. The powder was placed in a large beaker and aqueous ethanol (50%; 6 l) was added to cover the grounds. Steam, generated separately, was bubbled through the mixture for 3–4 h. After cooling, the material was filtered and ethanol removed from the filtrate by heating over a boiling water bath to leave a thick brown viscous fluid. Sulphuric acid (98%) was then added until the mixture reached pH 3. The precipitate that formed after the mixture was left at 4 °C overnight, corresponding to the GS3 fraction, was collected by filtration and then redissolved in aqueous potassium hydroxide (0·1 M, 400 ml). GS4 was precipitated from this solution by the addition of sulphuric acid to pH 3 and overnight cooling as before. The precipitate was collected by filtration and freeze-dried. Chlorophyll was removed from the methanol-soluble components of GS4 by size exclusion chromatography on Sephadex LH-20 gel using methanol as eluent, yielding a major fraction, termed F2. A major component of F2, termed F43, was isolated in semi-pure form by silica gel column chromatography and preparative thin layer chromatography (TLC) using chloroform–methanol–water mixtures. TLC analysis (data not shown) indicated that GS4 and F2 contained a complex mixture of compounds with chromatographic characteristics typical of triterpenoid saponins. The major component of GS4 and F2 was identified as gymnemic acid VIII by comparison of spectral data with values given in the literature (Yoshikawa *et al.* 1992). This compound was not available in sufficient quantities for testing in the present studies. However, F43 contains a mixture of gymnemic acid VIII and at least one other compound, most likely to be a closely related gymnemic acid. GS4, F2 and F43 were dissolved directly in the aqueous incubation medium (Gey & Gey 1936) used for the insulin secretion and trypan blue uptake experiments.

Insulin secretion

Groups of three islets were incubated in 600 μl physiological salt solution (Gey & Gey 1936), in the absence or presence of GS4, for 1 h at 37 °C, after which time a sample of the supernatant was removed for the measurement of insulin release. Groups of 30 000 MIN6, 100 000 HIT-T15 and 200 000 RINm5F β-cells were seeded into 96-well plates, left to adhere overnight, then pre-incubated in a glucose-free medium for 2 h before a 1-h incubation in glucose-free medium in the absence or presence of GS4. The insulin content of the supernatants

Table 1 GS4-stimulated insulin release from β-cell lines. Cells were incubated in a physiological salt solution (zero glucose) either in the absence of GS4 or supplemented with 0·125–0·5 mg/ml GS4 for 1 h at 37 °C, and insulin released into the supernatant was measured by radioimmunoassay. Data are means ± S.E.M., $n=5$–9

	Insulin release (ng/10^6 cells per h)		
	MIN6	RINm5F	HIT-T15
GS4 (mg/ml)			
0	22·3 ± 1·35	0·24 ± 0·02	2·5 ± 0·58
0·125	63·9 ± 0·92***	0·79 ± 0·05*	5·11 ± 0·86*
0·25	65·6 ± 0·55***	4·07 ± 0·21***	20·71 ± 1·43***
0·5		8·06 ± 0·48***	32·33 ± 2·75***

*$P<0·05$, ***$P<0·001$, compared with secretion in the absence of GS4.

was determined by radioimmunoassay (Jones *et al.* 1988). All experiments were performed with six to nine replicates per treatment group.

Membrane integrity

After incubation in the absence or presence of GS4, islets and β-cells were exposed to the membrane-impermeant dye, trypan blue (0·1% w/v) for 15 min at 4 °C or 37 °C. The presence of dye within cells was determined by light microscopy and the numbers of stained and unstained cells in a field were counted to obtain an estimate of the percentage of cells taking up the dye. MIN6 cells that had been incubated in the presence of the semi-purified GS4 extracts, F2 and F43, were also incubated with trypan blue as described above.

Statistical analysis

Data were analysed by one-way analysis of variance or Student's *t*-tests, as appropriate. Differences between experimental and control samples were considered significant at $P<0·05$.

Results

Isolation of GS4, F2 and F43

GS4, a smooth greenish-brown powder, was obtained in 2·5% w/w yield from dried *G. sylvestre* leaves. Chromatography of 10 g GS4 yielded 5·9 g F2, and 88 mg F43 were obtained from 5·4 g F2. F2 and F43 were both obtained as light-brown powders.

Effects of GS4 on insulin secretion from β-cells and islets

Exposure of β-cells to GS4 resulted in a dose-related increase in insulin release (Table 1). In the case of MIN6 β-cells, insulin release was stimulated at concentrations as low as 31 μg/ml GS4 (252 ± 15·8% basal, $P<0·001$). GS4 (0·2 mg/ml) caused significant ($P<0·001$) increases in the release of insulin from isolated rat islets at both substimulatory (2 mM glucose: 0·18 ± 0·02 ng/islet per h; +0·2 mg/ml GS4: 0·86 ± 0·18 ng/islet per h, $n=6$) and stimulatory (10 mM glucose; 594 ± 59% increase; 20 mM glucose: 259 ± 43% increase, $n=6$) concentrations of glucose. Higher concentrations of GS4 caused progressively increased insulin release from islets such that in the absence of any other stimuli (2 mM glucose), the release rate at 2 mg/ml GS4 was 16·4 ± 1·3 ng/islet per h ($n=6$, $P<0·001$ compared with 2 mM glucose).

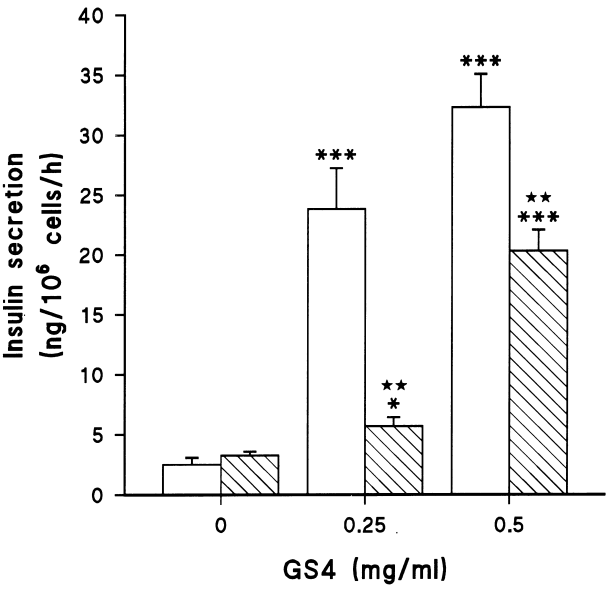

Figure 1 Effect of Ca^{2+} chelation on GS4-stimulated insulin release. HIT-T15 cells were incubated for 1 h at 37 °C in the absence (open bars) or presence (hatched bars) of 1 mM EGTA. Insulin released into the supernatant was measured by radioimmunoassay. Data are means ± S.E.M., $n=8$. *$P<0·05$, ***$P<0·001$ compared with appropriate control in the absence of GS4; **$P<0·01$ compared with secretion in the absence of EGTA.

Mode of action of GS4

As GS4 exerted stimulatory actions on all the β-cell lines tested and on whole islets, the HIT-T15 cell line was used as a representative β-cell population in which to examine the mechanisms through which GS4 increased insulin secretion. It was found that, when extracellular Ca^{2+} was chelated in the presence of 1 mM EGTA, the dose-dependent effects of GS4 were shifted to the right such that 0·25 mg/ml only had a small stimulatory effect, but 0·5 mg/ml still caused a large increase in insulin release (Fig. 1). However, EGTA significantly reduced insulin release at 0·5 mg/ml GS4 and at 0·25 mg/ml ($P<0·01$). Blockade of Ca^{2+} channels with the dihydropyridine blocker, isradipine, did not significantly inhibit the

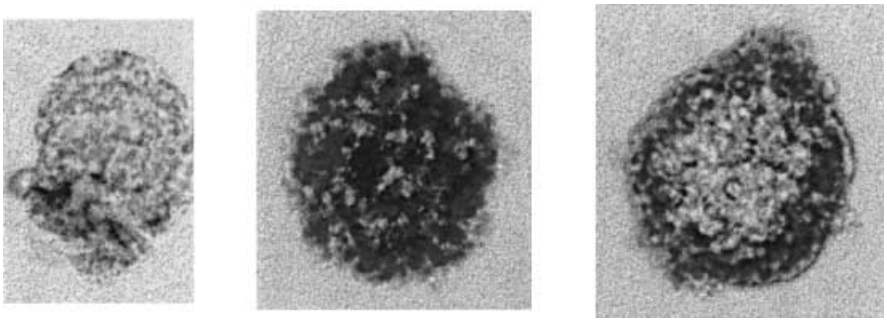

Figure 2 Effects of GS4 on trypan blue uptake by islet cells. Rat islets were incubated in a physiological salt solution supplemented with 0·1% (w/v) trypan blue at 37 °C (left), in the presence of 0·25 mg/ml GS4 and 0·1% (w/v) trypan blue at 37 °C (middle) or in the presence of 0·25 mg/ml GS4 and 0·1% (w/v) trypan blue at 4 °C (right).

stimulatory effects of 0·5 mg/ml GS4 on insulin release from HIT cells (control: 29·4 ± 2·7 ng/10^6 cells per h; +10 μM isradipine: 24·0 ± 1·9 ng/10^6 cells per h; n=8, P>0·1). Incubation of HIT-T15 β-cells at a range of temperatures indicated that GS4 (0·125 mg/ml) stimulated insulin release even at temperatures as low as 4 °C (37 °C: 6·1 ± 0·75 ng/10^6 cells per h; 30 °C: 5·1 ± 0·33 ng/10^6 cells per h; 24 °C: 4·3 ± 0·67 ng/10^6 cells per h; 4 °C: 5·7 ± 1·33 ng/10^6 cells per h; n=5–6).

Effects of GS4 on membrane integrity

Exposure of β-cells to GS4 resulted in a dose-related increase in the number of cells to which trypan blue dye gained access, with 98% of MIN6 cells, 95% of RINm5F cells and 88% of HIT-T15 cells taking up the dye at 0·25 mg/ml GS4. Islets incubated in the absence of GS4 excluded trypan blue, but those which had been incubated in the presence of 0·25 mg/ml GS4 showed substantial dye uptake, whether they were exposed to GS4 at 37 °C or at 4 °C (Fig. 2).

Effects of GS4 fractionation products on insulin release and membrane integrity

F2 and F43, fractions obtained by further purification of GS4, increased the rate of insulin release from MIN6 cells in the absence of any other stimulus (Table 2). However, F2 and F43 also caused a significant increase in the extent of trypan blue uptake by MIN6 cells (Table 2). In contrast, exposure of MIN6 cells to a maximal stimulatory combination of the nutrient secretagogue, 4α ketoisocaproate, and the protein kinase C activator, 4β phorbol myristate acetate, caused a significant increase in insulin secretion that was not accompanied by a loss of membrane integrity, as assessed by trypan blue uptake (Table 2).

Discussion

The results of the current study confirm previous observations that alcoholic extracts of the leaves of *Gymnema sylvestre* are capable of direct actions on pancreatic β-cells to increase the release of insulin (Shanmugasundaram *et al.* 1990). We found that GS4 caused a dose-related increase in insulin release from a variety of β-cell lines and rat islets in the absence of any other stimulus, and, although there were some minor differences in sensitivity to the extract, in all cases it caused a profound secretory response. In the case of islets, with which a concentration as high as 2 mg/ml was used, it was found that insulin release was in excess of 16 ng/islet per h. This high output, equivalent to ~50% of the islet insulin content, suggests that the effects of GS4 may not be physiological because, under these conditions, the insulin release would not be compensated for by sufficient insulin biosynthesis. Confirmation that the stimulatory effects of GS4 differed from those of other insulin secretagogues was provided by the observation that GS4 was able to stimulate insulin release at temperatures as low as 4 °C, whereas regulated physiological insulin secretion only occurs at temperatures in excess of 30 °C (Hedeskov 1980).

Table 2 Effect of GS4 fractionation products on insulin release and membrane integrity. MIN6 cells were incubated in a physiological salt solution for 1 h in the presence of the agents shown. A sample of the supernatant was removed for the measurment of insulin release, then the cells were incubated for a further 15 min at 37 °C in the presence of 0·1% (w/v) trypan blue. Data for insulin release are means ± S.E.M., n=6–8 and those for trypan blue uptake show the percentage range of cells to which the dye gained access, n=2

	Insulin release (ng/10^6 cells per h)	**Cells taking up trypan blue** (% total)
Treatment		
0 glucose	22·7 ± 3·3	5–10
0·5 mg/ml F2	85·4 ± 8·0***	95–100
0·5 mg/ml F43	41·4 ± 2·7***	50–60
0·5 mg/ml GS4	48·0 ± 4·7***	90–100
10 mM KIC +500 nM PMA	89·4 ± 7·3***	5–10

KIC, 4α ketoisocaproate; PMA, phorbol myristate acetate. ***P<0·001, compared with secretion at 0 glucose.

Evidence that GS4 was acting at a physical, rather than a physiological, level was provided by estimates of uptake of the membrane impermeant dye, trypan blue. Cells or islets that had been incubated in the absence of GS4 for 1 h showed low levels of trypan blue uptake, indicative of an intact plasma membrane capable of excluding dye entry. However, increasing concentrations of GS4 caused progressively more damage to the β-cells, such that, for all β-cell types, trypan blue gained access to virtually all cells when used in a concentration of 0·25 mg/ml. Similar results were obtained with whole islets, which consist of clusters of around 3–5000 cells, and dye uptake was observed at both 4 °C and at 37 °C. At 4 °C, the dye gained access to an outer mantle of cells, and at 37 °C more islet cells became permeable to trypan blue. The loss of membrane integrity after exposure to GS4 may have been mediated by gymnemic acids, a complex mixture of saponin glycosides, known to be present in the extract (Suttisri *et al.* 1995). Glycosides such as saponin and digitonin have long been used experimentally to permeabilise cellular membranes, including those of cells within islets of Langerhans (Biden *et al.* 1984, Colca *et al.* 1985), and it is known that their effects are independent of temperature (Schulz 1990) and that they cause loss of large cytosolic proteins when used in high concentrations (Ahnert-Hilger & Gratzl 1988). The likelihood that membrane damage resulted from the presence of glycosides was borne out experimentally using GS4 subfractions enriched in gymnemic acid saponins (F2 and F43), which also caused increased release of insulin from MIN6 cells concomitant with increased uptake of trypan blue dye.

Thus the ability of GS4 to stimulate insulin release at 4 °C and its effects on β-cell plasma membrane integrity are indicative of a mode of action in which GS4 causes insulin to leak from effectively permeabilised β-cells. There also appears to be a regulated component to the stimulatory effects of GS4, as the increase in insulin release was sensitive to chelation of extracellular Ca^{2+} by EGTA. However, the increase in insulin release still occurred after isradipine-induced channel blockade, indicating that it did not result from Ca^{2+} influx through voltage-operated Ca^{2+} channels. Moreover, in the presence of sufficiently high concentrations of GS4, there was still a substantial release of insulin, despite the presence of EGTA, suggesting that GS4 can affect the secretory apparatus independently of changes in Ca^{2+}.

In summary, the current data suggest that GS4 increases insulin release *in vitro* by two mechanisms: 1) the major mode of action is through permeabilisation of the β-cell plasma membranes, most likely resulting from the high saponin glycoside content of the extract, leading to unregulated loss of insulin from the cells; 2) there is also a Ca^{2+}-sensitive component, and at least part of this release of insulin may be dependent on channel-independent Ca^{2+} influx into the β-cells, perhaps through the pores formed by plasma membrane disruption. Thus, although extracts of *G. sylvestre* have been shown to reduce hyperglycaemia *in vivo*, the effects of GS4 on β-cells described herein suggest that its suitability as a potential novel treatment for NIDDM can not be assessed by direct measurements of islet and β-cell function *in vitro*. However, it is worth bearing in mind that the membrane-damaging effects of GS4 are unlikely to be observed *in vivo*, as sugars in the saponins will be hydrolysed off within the gastrointestinal tract, so islets are more likely to be exposed to the aglycone moiety. There is, as yet, no information on the effects of aglycones on insulin secretion *in vitro* or *in vivo*, but these components of *G. sylvestre* merit further investigation, particularly in light of the enhanced insulin concentrations observed *in vivo* after GS4 administration (Shanmugasundaram *et al.* 1990).

Acknowledgements

We are grateful to Elizabeth Redmond, Sudharshini Selvarajan and Anita Johansen for technical assistance, and to Dr A D Kinghorn, College of Pharmacy, University of Illinois, Chicago for providing the facilities for some of the fractionation work. We also thank the Government of Kuwait for a scholarship for H A-M.

References

Ahnert-Hilger G & Gratzl M 1988 Controlled manipulation of the cell interior by pore-forming proteins. *Trends in Pharmacological Sciences* **9** 195–197.

Baskaran K, Ahamath BK, Shanmugasundaram KR & Shanmugasundaram ERB 1990 Antidiabetic effect of a leaf extract from *Gymnema sylvestre* in non-insulin-dependent diabetes mellitus patients. *Journal of Ethnopharmacology* **30** 295–305.

Biden TJ, Prentki M, Irvine RF, Berridge MJ & Wollheim CB 1984 Inositol 1,4,5-trisphosphate mobilizes intracellular Ca^{2+} from permeabilized insulin-secreting cells. *Biochemical Journal* **223** 467–473.

Billington DC, Perron-Sierra F, Picard I, Beaubras S, Duhault J, Espinal J & Challal S 1994 Total synthesis of novel conduritol related compounds capable of modulating insulin release. *Bioorganic and Medicinal Chemistry Letters* **4** 2307–2312.

Colca JR, Wolf BA, Comens PG & McDaniel ML 1985 Protein phosphorylation in permeabilized pancreatic islet cells. *Biochemical Journal* **228** 529–536.

Gey GO & Gey MK 1936 Maintenance of human normal cells in continuous culture: preliminary report; cultivation of mesoblastic tumors and normal cells and notes on methods of cultivation. *American Journal of Cancer* **27** 45–76.

Hedeskov CJ 1980 Mechanism of glucose-induced insulin secretion. *Physiological Reviews* **60** 442–509.

Henquin JC 1980 Tolbutamide stimulation and inhibition of insulin release: studies of the underlying ionic mechanisms in isolated rat islets. *Diabetologia* **18** 151–160.

Jones PM, Stutchfield J & Howell SL 1985 Effects of Ca^{2+} and a phorbol ester on insulin secretion from islets of Langerhans permeabilised by high voltage discharge. *FEBS Letters* **191** 102–106.

Jones PM, Salmon DMW & Howell SL 1988 Protein phosphorylation in electrically permeabilised islets of Langerhans. Effects of Ca^{2+}, cyclic AMP, a phorbol ester and noradrenaline. *Biochemical Journal* **254** 397–403.

Jones PM, Mann FM, Persaud SJ & Wheeler-Jones 1993 Mastoparan stimulates insulin secretion from pancreatic β-cells by effects at a late stage in the secretory pathway. *Molecular and Cellular Endocrinology* **94** 97–104.

Khare AK, Tondon RN & Tewari JP 1983 Hypoglycaemic activity of an indigenous drug (*Gymnema sylvestre, 'Gurmar'*) in normal and diabetic persons. *Indian Journal of Physiology and Pharmacology* **27** 257–258.

Miyatake K, Takenaka S, Fujimoto T, Kensho G, Upadhaya S, Kirihata M, Ichimoto I & Nakano Y 1993 Isolation of conduritol A from *Gymenma sylvestre* and its effect against intestinal glucose absorption in rats. *Bioscience, Biotechnology and Biochemistry* **57** 2184–2185.

Schulz I 1990 Permeabilizing cells: some methods and applications for the study of intracellular processes. *Methods in Enzymology* **192** 280–300.

Shanmugasundaram KR & Panneerselvam C 1981 The insulinotropic activity of *Gymnema sylvestre* R.Br. an Indian medical herb used in controlling diabetes mellitus. *Pharmacological Research Communications* **13** 475–486.

Shanmugasundaram KR, Panneerselvam C, Samudram P & Shanmugasundaram ERB 1983 Enzyme changes and glucose utilisation in diabetic rabbits: the effects of *Gymnema sylvestre*, R.Br. *Journal of Ethnopharmacology* **7** 205–234.

Shanmugasundaram ERB, Gopinath KL, Shanmugasundaram KR & Rajendran VM 1990 Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given *Gymnema sylvestre* leaf extracts. *Journal of Ethnopharmacology* **30** 265–279.

Srivastava Y, Bhatt HV, Prem AS, Nigam SK & Verma Y 1985 Hypoglycemic and life-prolonging properties of *Gymnema sylvestre* leaf extract in diabetic rats. *Israel Journal of Medical Sciences* **21** 540–542.

Sturgess NC, Cook DL, Ashford MLJ & Hales CN 1985 The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* **2** 474–475.

Suttisri R, Lee I & Kinghorn AD 1995 Plant derived triterpenoid sweetness inhibitors. *Journal of Ethnopharmacology* **47** 9–26.

Yoshikawa K, Nakagawa M, Arihara S & Matssra K 1992 Antisweet natural products V. Structures of gymnemic acids VIII-XII from *Gymnema sylvestre* R. Br. *Chemical and Pharmaceutical Bulletin* **40** 1779–1782.

Received 13 November 1998
Revised manuscript received 22 April 1999
Final manuscript received 22 June 1999
Accepted 22 June 1999