Insulin-secreting activity of the traditional antidiabetic plant *Viscum album* (mistletoe)

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Abstract

Viscum album (mistletoe) has been documented as a traditional treatment of diabetes. In acute 20-min tests, 1–10 mg/ml aqueous extract of mistletoe evoked a stepwise 1·1- to 12·2-fold stimulation of insulin secretion from clonal pancreatic B-cells. This effect was abolished by 0·5 mM diazoxide and prior exposure to extract did not alter subsequent stimulation of insulin secretion induced by 10 mM L-alanine, thereby negating a detrimental effect on cell viability. The insulin-releasing effect of mistletoe extract was unaltered by 16·7 mM glucose, L-alanine (10 mM), 3-isobutyl-1-methylxanthine (IBMX) (1 mM), or a depolarising concentration of KCl (25 mM). The ability of extract to enhance insulin secretion did not depend upon the use of heat during extract preparation and was not mediated by lectins. These results demonstrate the presence of insulin-releasing natural product(s) in *Viscum album* which may contribute to the reported antidiabetic property of the plant.

Journal of Endocrinology (1999) **160,** 409–414

Introduction

Before the introduction of insulin in 1922 the treatment of diabetes mellitus relied heavily on dietary measures which included the use of traditional plant therapies. Many traditional plant treatments for diabetes exist (Bailey & Day 1989, Swanston-Flatt *et al.* 1991, Gray & Flatt 1997*a*). However, few have received scientific or medical scrutiny and the World Health Organization has recommended that traditional plant treatments for diabetes warrant further evaluation (World Health Organization 1980).

Viscum album (mistletoe) is a member of the Loranthacaea family. It has been reported to have a number of medicinal properties including the ability to lower blood pressure, slow the heart beat, stimulate the immune system, relax spasms and exert sedative, diuretic and anti-cancer effects (Bown 1995). A tea prepared from leaves of mistletoe is used traditionally to treat diabetes in the West Indies (Peters 1957). This treatment has been shown also to relieve the diabetic symptoms of severely hyperglycaemic streptozotocin-diabetic mice, including polydipsia, hyperphagia and body weight loss (Swanston-Flatt *et al.* 1989).

An antidiabetic agent could exert a beneficial effect in the diabetic situation by enhancing insulin secretion and/or by improving/mimicking insulin action (Gray & Flatt 1997*a*). The present studies were undertaken to investigate the possible presence of natural product(s) in an aqueous extract of mistletoe which stimulates insulin secretion.

Materials & Methods

Plant material

Dried mistletoe (leaf and stem) was obtained from a commercial source (The Health Food Centre, Bull Ring Shopping Centre, Birmingham, UK), homogenised to a fine powder and then stored at room temperature (20 ± 2 °C) in opaque screwtop jars until use. An aqueous extract of mistletoe was prepared by a method of infusion as described previously (Gray & Flatt 1997*b*). In brief, 1 g powdered material was placed in 40 ml boiling (distilled) water, then removed from the heat source and allowed to infuse for 15 min. This suspension was filtered (Whatman no. 1) and the volume readjusted to 40 ml with distilled water. For *in vitro* studies 1 ml aliquots of extract were brought to dryness under vacuum (Savant speedvac; Savant Instrumentation Inc, Framingdale, NY, USA), stored at -20 °C and reconstituted for use in incubation buffer. To allow for any variation in potency of different batches of extract, incubations within a single experiment using clonal insulin-secreting cells were always conducted using the same batch of extract. The dried material was confirmed botanically to be *Viscum album*.

  Printed in Great Britain
0022–0795/99/0160–409
Online version via http://www.endocrinology.org

Insulin secretion in vitro

A glucose-responsive clonal insulin-secreting cell line, BRIN-BD11, produced by electrofusion of immortal RINm5F cell with New England Deaconess Hospital rat pancreatic B-cell, was used to evaluate insulin secretion (McClenaghan *et al.* 1996*a*, Gray & Flatt 1997*b*). This cell line responds to a wide variety of insulinotrophic stimuli including glucose, amino acids, hormones, neurotransmitters and drugs (McClenaghan *et al.* 1996*a,b*, 1998, McClenaghan & Flatt 1998). The appropriateness of BRIN-BD11 cells for screening of antidiabetic plant materials and characterisation of novel insulin-releasing natural products has been described elsewhere (Gray & Flatt 1997*a,b*, 1998). Cells were seeded at a concentration of $0{\cdot}2 \times 10^6$ cells/well in 24-well plates (Falcon, NJ, USA) cultured in RPMI-1640 containing 11·1 mM glucose, 10% foetal calf serum and antibiotics (50 000 IU/l penicillin–streptomycin) to allow attachment overnight prior to acute tests. Cells were washed thrice with Kreb's–Ringer bicarbonate buffer (KRB; 115 mM NaCl, 4·7 mM KCl, 1·28 mM $CaCl_2$, 1·2 mM KH_2PO_4, 1·2 mM $MgSO_4$, 24 mM $NaHCO_3$, 10 mM Hepes-free acid, 1 g/l bovine serum albumin, 1·1 mM glucose; pH 7·4) and preincubated for 40 min at 37 °C. Unless otherwise stated, cells were then incubated for 20 min with 1 ml KRB at 1·1 mM glucose in the absence and presence of plant extract, diazoxide (an established opener of K^+–ATP channels) and other test agents. Following incubation, aliquots were removed from each well and stored at − 20 °C for insulin assay (Flatt & Bailey 1981). To evaluate the importance of heat during extract preparation, aqueous extracts of mistletoe were prepared by normal method of infusion (normal extract) or by cold infusion (cold extract; plant material placed in cold water, allowed to stand for 15 min, then filtered as before) and cold extract subsequently brought to the boil and allowed to infuse for 15 min (heated extract). Modified aqueous extract was freshly reconstituted in KRB and effect on insulin secretion evaluated at a concentration equivalent to 1 mg/ml compared with normal extract.

Haemagglutination assay of lectin presence in aqueous extract

Serial dilutions of aqueous extract of mistletoe were performed using phosphate buffered saline (PBS; 137 mM NaCl, 2·68 mM KCl, 14·07 mM KH_2PO_4, 204 mM Na_2HPO_4; pH 7·4) in V-shaped bottom microtitre wells to which an equal volume of freshly prepared 2% erythrocyte suspension (human Rh O − or pooled rat; in PBS) was added. Wells were incubated for 1 h at room temperature and the titre read visually and being equal to the dilution in the last well to show agglutination (as manifested by an evenly distributed layer of cells over the whole well bottom). The haemagglutinin activity of normal, cold and heated extract was examined and for each a titre value was obtained.

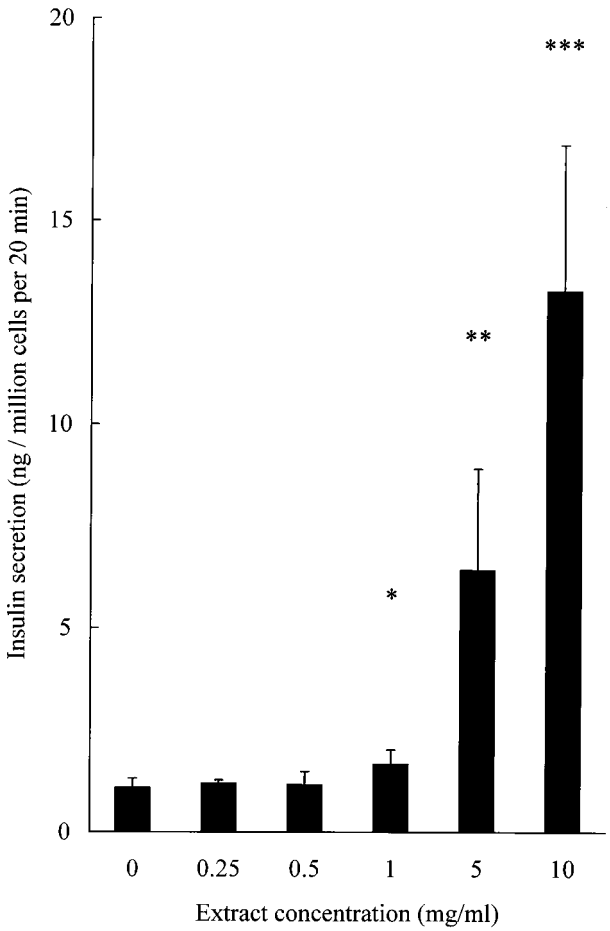

Figure 1 Effects of aqueous extract of mistletoe on insulin secretion. Values are means for groups of between four and six observations with their standard errors indicated by vertical bars. *P<0·05, **P<0·01, ***P<0·001 compared with control incubations without extract.

Statistical analyses

Data were evaluated using Student's unpaired *t*-test, one-way analysis of variance (ANOVA) or two-way analysis of variance where appropriate. Groups were considered to be significantly different if P<0·05. When a significant F value was obtained for ANOVA the differences between all pairs were tested using Student–Newman–Keuls multiple comparisons test. If S.D.s were significantly different (Bartlett's test for homogeneity of variances) data were transformed ($\log_{10}[x]$).

Results

Aqueous extract of mistletoe (1–10 mg/ml) had a dose-dependent stimulatory effect on insulin secretion by clonal B-cells at 1·1 mM glucose (Fig. 1). It was confirmed that

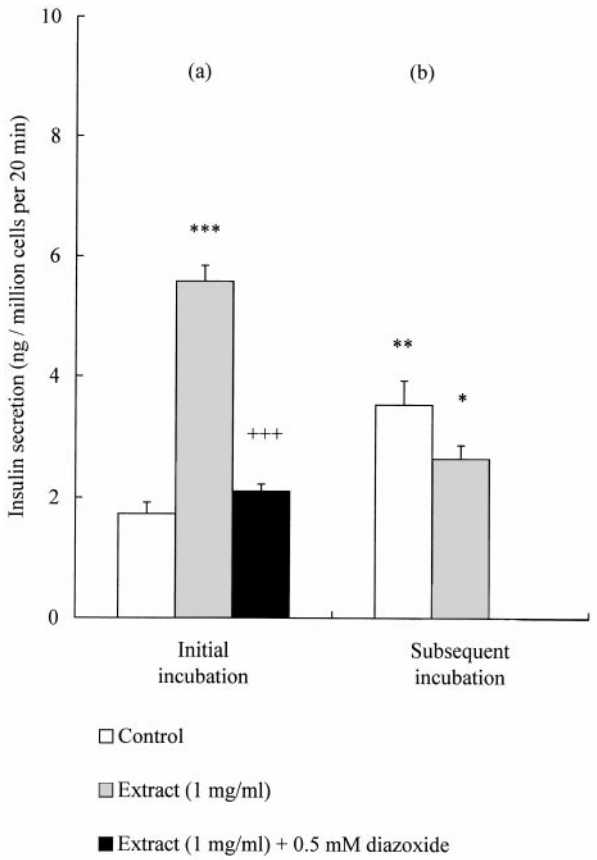

Figure 2 (*a*) Effects of aqueous extract of mistletoe (1 mg/ml) on insulin secretion at 1·1 mM glucose (□, control) in the absence (□) or presence (■) of 0·5 mM diazoxide. (*b*) Effects of 20 min prior exposure to 1·1 mM glucose (□, control) or extract (1 mg/ml) (□) on the subsequent insulin-secretory response to 10 mM L-alanine. Values are means for groups of six observations with their standard errors indicated by vertical bars. *P<0·05, **P<0·01, ***P<0·001 compared with initial control incubations without extract (*a*). +++P<0·001 compared with incubation with extract.

these concentrations of extract did not influence the viability of the cells during the test period as evaluated by modified neutral red assay (Hunt *et al.* 1987) (data not presented). In order that the effect of extract could be studied at submaximal levels of stimulation 1 mg/ml extract was used for subsequent investigations. Incubation with 0·5 mM diazoxide inhibited the stimulatory effect of the extract, indicating that the enhancement of insulin release was not a mere consequence of cellular damage (Fig. 2a). Consistent with this view, prior exposure of clonal B-cells to extract for 20 min did not alter the subsequent insulin-secretory response to 10 mM L-alanine (Fig. 2b).

The presence of 16·7 mM glucose or 10 mM L-alanine did not significantly enhance the insulin-releasing effect of mistletoe extract (1 mg/ml; Fig. 3a and b respectively). The action of mistletoe extract (1 mg/ml) was not potentiated by 1 mM 3-isobutyl-1-methylxanthine (IBMX), which increases cyclic AMP in insulin-secreting cells (Sharp 1979) (Fig. 3c). Furthermore, unlike the stimulatory effect of 16·7 mM glucose alone, the action of mistletoe extract was not augmented by further depolarisation of the cell by 25 mM KCl (Fig. 3d). As shown in Fig. 4, the temperature of extraction did not alter the insulin-releasing effect of mistletoe extract.

No observed haemagglutinin activity was observed in the normal aqueous extract of mistletoe (titre value 0, n=3). Haemagglutinin activity was present in extract produced by cold infusion (cold extract), as indicated by titre values for human and rat erythrocytes (8 and 4 respectively, n=3). The activity of this cold extract was subsequently lost by heating (to produce heated extract) (titre value 0, n=3).

Discussion

Despite being long advocated as an effective traditional treatment for diabetes (Peters 1957), few scientific studies have attempted to evaluate the efficacy and possible mode of action of mistletoe. In one of the few studies reported (Swanston-Flatt *et al.* 1989) chronic administration of mistletoe (6·25% by weight of diet, 1 g/400 ml infusion in place of drinking water) ameliorated symptoms of polydipsia, hyperphagia and body weight loss in severely hyperglycaemic streptozotocin-diabetic mice. However, a significant decrease of plasma glucose was not clearly demonstrable in this insulin-deficient model (Swanston-Flatt *et al.* 1989), which indicates that leaf and stem of mistletoe contain water soluble natural product(s) which directly stimulate insulin secretion from clonal B-cells.

Diazoxide inhibits glucose and sulphonylurea-induced insulin secretion by preventing the closure of voltage dependent K^+–ATP channels, thus preventing membrane depolarisation and Ca^{2+} influx, an initial key step in insulin secretion (Rorsman 1987). In the present studies, diazoxide abolished the insulin-releasing effects of mistletoe extract on clonal B-cells. Mistletoe extract is therefore likely to act at an early stage of the insulin-secretory pathway before Ca^{2+} influx. However, the action of the extract was not potentiated by glucose or L-alanine. These characteristics are reminiscent of sulphonylureas, but unlike members of this family of established antidiabetic drugs which appear to exert additional K^+–ATP channel independent effects (Eliasson *et al.* 1996), mistletoe extract did not augment insulin secretion from depolarised B-cells exposed to 25 mM KCl. Interestingly, the phosphodiesterase inhibitor, IBMX, did not potentiate the insulin-releasing effect of mistletoe extract, raising the possibility that the extract may itself inhibit islet phosphodiesterase (Leibowitz *et al.* 1995). It is noteworthy that the viability of clonal B-cells was maintained in the presence of mistletoe

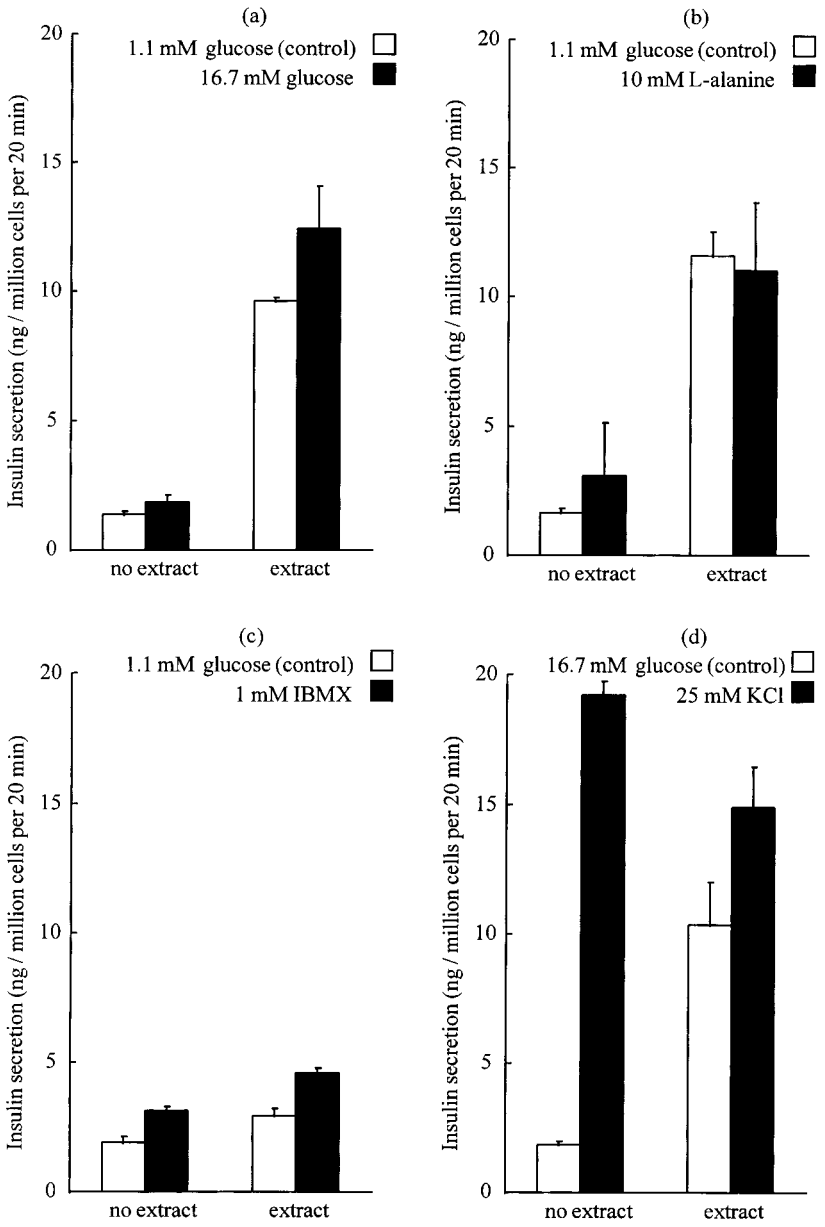

Figure 3 Effects of (*a*) glucose, (*b*) L-alanine, (*c*) IBMX, or (d) KCl on the insulin-releasing actions of aqueous extract (1 mg/ml) of mistletoe. Values are means for groups of six observations with their standard errors indicated by vertical bars. Two-way analysis of variance revealed (*a*) extract effect ($P<0{\cdot}001$), glucose effect ($P<0{\cdot}05$) and extract–glucose interaction (NS); (*b*) extract effect ($P<0{\cdot}001$), L-alanine effect ($P<0{\cdot}001$) and extract–L-alanine interaction (NS); (*c*) extract effect ($P<0{\cdot}001$), IBMX effect ($P<0{\cdot}001$) and extract–IBMX interaction (NS); (*d*) extract effect ($P<0{\cdot}001$), KCl effect ($P<0{\cdot}001$) and extract–KCl interaction ($P<0{\cdot}001$).

extract as determined by modified neutral red assay. In addition, previous exposure to extract did not diminish the subsequent insulin-secretory response to L-alanine, providing further evidence that the action of mistletoe extract was not due to a detrimental cellular effect.

The ability of lectins isolated from mushrooms (*Agaricus campestris*, *Agaricus bisporus*) to enhance insulin release by isolated rat islets of Langerhans has been documented (Ewart *et al.* 1975, Ahmad *et al.* 1984*a*,*b*). However, heating the *Agaricus campestris* lectin to 100 °C for 3 min

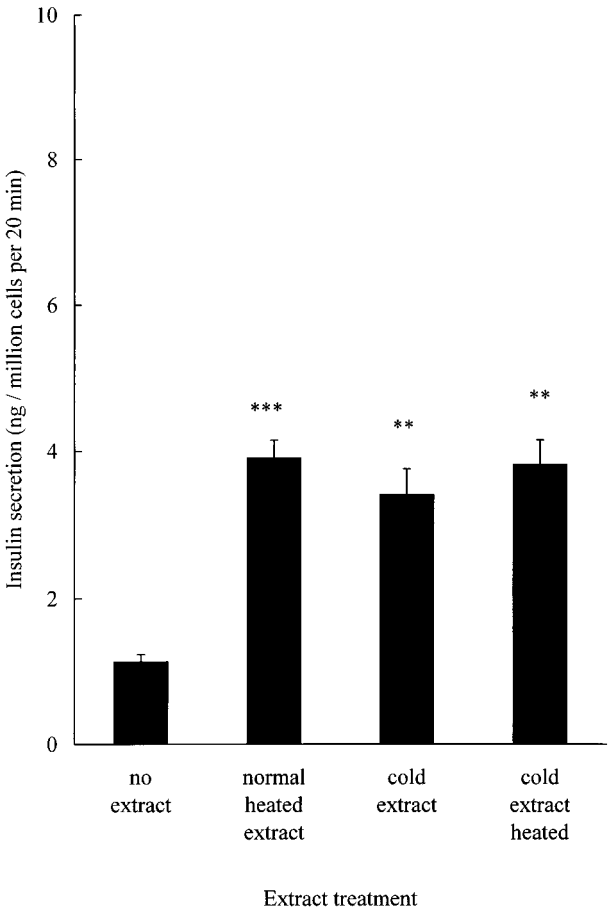

Figure 4 Effect of extraction temperature on ability of mistletoe extract to enhance insulin secretion. Values are means for groups of six observations with their standard errors indicated by vertical bars. $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$ compared with incubations without extract.

abolished its activity (Ewart *et al.* 1975). We have previously shown that the anti-hyperglycaemic activity of *Agaricus campestris* is associated with insulin-releasing and insulin-like activity not involving lectins (Gray & Flatt 1998). In the present studies, we have shown that lectins present in a cold aqueous extract of mistletoe are not present in a hot (normal) aqueous extract. In addition, extraction of the natural product(s) responsible for the enhancement of insulin secretion *in vitro* neither requires nor is destroyed by heat. These observations indicate that the insulin-releasing activity of mistletoe extract is not mediated by lectins. Leaves and twigs of mistletoe have been reported to contain β-amyrin, tyramine, quercitin, syringin and flavoyedorinin A and B (Duke 1985). However, the presence of these compounds in mistletoe extract and the possible involvement of these or other natural products in the insulin-releasing action remains to be established.

European mistletoe (*Viscum album*) is an evergreen, semiparasitic plant found on the branches of deciduous trees in Europe and Northern Asia. The antidiabetic properties of the African mistletoe (*Loranthus bengwengis*) were investigated in streptozotocin-diabetic rats and found to be highly dependent on the host plant species (Obatomi *et al.* 1994). It is possible that the activity of European mistletoe is also related to its host plant species. This aspect together with isolation and chemical characterisation of the natural product(s) responsible for the stimulation of insulin secretion merit further study. Glucose-sensitive insulin secreting cells such as BRIN-BD11 clearly facilitate screening and isolation of bioactive agents which following chemical characterisation can be evaluated further with human islets and clinical studies.

In conclusion the present study provides evidence for the first time for water soluble, heat-resistant insulin-releasing components in mistletoe. Thus, in addition to representing an advocated plant treatment for diabetes, mistletoe represents a source of potential new oral hypoglycaemic agent(s) as yet to be isolated and identified.

Acknowledgements

These studies were supported by the Department of Health and Social Services, Northern Ireland and Northern Ireland Developmental Research Funding. The authors thank Dr P Kwasowski (University of Surrey, Guildford, UK) for guidance on lectin studies.

References

Ahmad N, Bansal AK & Kidwai JR 1984*a* Effect of PHA-B fraction of *Agaricus bisporus* lectin on insulin release and $^{45}Ca^{2+}$ uptake by islets of Langerhans *in vitro*. *Acta Diabetologica Latina* **21** 63–70.

Ahmad N, Khan MM, Rastogi AK & Kidwai JR 1984*b* Effect of age on *Agaricus bisporus* PHA-B stimulated insulin release and $^{45}Ca^{2+}$ uptake *in vitro* by islets of Langerhans. *Acta Diabetologica Latina* **21** 349–355.

Bailey CJ & Day C 1989 Traditional treatments for diabetes. *Diabetes Care* **12** 553–564.

Bown D 1995 *The Royal Horticultural Society Encyclopaedia of Herbs and their Uses*. London: Dorling Kindersley Ltd.

Duke JA 1985 *Handbook of Medicinal Herbs*. Florida: CRC Press.

Eliasson L, Renström E, Ämmälä C, Berggren P-O, Bertorello AM, Bokvist K, Chibalin A, Deeney JT, Flatt PR, Gabel J, Gromado J, Larsson O, Lindström P, Rhodes CJ & Rorsman P 1996 PKC-dependent stimulation of exocytosis by sulfonylureas in pancreatic B-cells. *Science* **271** 813–815.

Ewart RBL, Kornfeld S & Kipnis DM 1975 Effect of lectins on hormone release from isolated rat islets of Langerhans. *Diabetes* **24** 705–714.

Flatt PR & Bailey CJ 1981 Abnormal plasma glucose and insulin responses in heterozygous lean (*ob*/+) mice. *Diabetologia* **20** 573–577.

Gray AM & Flatt PR 1997*a* Nature's own pharmacy: the diabetes perspective. *Proceedings of the Nutrition Society* **56** 507–517.

Gray AM & Flatt PR 1997*b* Pancreatic and extra-pancreatic effects of the traditional anti-diabetic plant, *Medicago sativa* (lucerne). *British Journal of Nutrition* **78** 325–334.

Gray AM & Flatt PR 1998 Insulin-releasing and insulin-like activity of *Agaricus campestris* (mushroom). *Journal of Endocrinology* **157** 259–266.

Hunt SM, Chrzanowska C, Barnett CR, Brand HN & Fawell JK 1987 A comparison of *in vitro* cytotoxicity assays and their application to water samples. *Alternatives to Laboratory Animals* **15** 20–29.

Leibowitz MD, Biswas C, Brady EJ, Conti M, Cullinan CA, Hayes NS, Manganiello VC, Saperstein R, Wang L, Zafian PT & Berger J 1995 A novel insulin secretagogue is a phosphodiesterase inhibitor. *Diabetes* **44** 67–74.

McClenaghan NH & Flatt PR 1998 Engineering cultured insulin-secreting pancreatic B-cell lines. *Journal of Molecular Medicine* (In press).

McClenaghan NH, Barnet CR, Ah-Sing E, Abdelwahab YHA, O'Harte FPM, Yoon T-W, Swanston-Flatt SK & Flatt PR 1996*a* Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. *Diabetes* **45** 1132–1140.

McClenaghan NH, Barnett CR, O'Harte FPM & Flatt PR 1996*b* Mechanisms of amino acid induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic B-cell line. *Journal of Endocrinology* **151** 349–357.

McClenaghan NH, Flatt PR & Bailey CJ 1998 Insulin-releasing action of the novel antidiabetic agent BTS 67582. *British Journal of Pharmacology* **123** 400–404.

Obatomi DK, Bikomo EO & Temple VJ 1994 Anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology* **43** 13–17.

Peters G 1957 Ubersichten insulin-ersatzmittel pflanzlichen ursprungs (Review of insulin substitutes from vegetable sources). *Deutsche Medicizin Wochenschrift* **82** 320–322.

Rorsman P 1987 The pancreatic β-cell as a fuel sensor: an electrophysiologists viewpoint. *Diabetologia* **40** 487–495.

Sharp GWG 1979 The adenylate cyclase–cyclic AMP system in islets of Langerhans and its role in the control of insulin release. *Diabetologia* **16** 287–297.

Swanston-Flatt SK, Day C, Bailey CJ & Flatt PR 1989 Evaluation of traditional plant treatments for diabetes: studies in streptozotocin-diabetic mice. *Acta Diabetologica Latina* **26** 51–55.

Swanston-Flatt SK, Flatt PR, Day C & Bailey CJ 1991 Traditional dietary adjuncts for the treatment of diabetes mellitus. *Proceedings of the Nutrition Society* **50** 641–651.

World Health Organization 1980 *Second Report of the WHO Expert Committee on Diabetes Mellitus*. Technical Report Series 646, p 66. Geneva: World Health Organization.

Received 27 July 1998
Accepted 23 October 1998