The Traditional Plant Treatment, *Sambucus nigra* (elder), Exhibits Insulin-Like and Insulin-Releasing Actions In Vitro[1]

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ABSTRACT *Sambucus nigra* (elder) has been documented as a traditional treatment of diabetes. In the present study, an aqueous extract of elder (AEE, 1 g/L) significantly increased 2-deoxy-glucose transport, glucose oxidation and glycogenesis of mouse abdominal muscle in the absence of added insulin (2 × 2 factorial design). in acute 20-min tests, 0.25–1 g/L AEE evoked a stepwise stimulation of insulin secretion from clonal pancreatic β-cells. The insulin releasing effect of AEE (0.5 g/L) was significantly potentiated by 16.7 mmol/L of glucose and significantly reduced by 0.5 mmol/L of diazoxide. AEE did not further enhance insulin secretion in cells stimulated by 10 mmol/L of L-alanine, 1 mmol/L of 3-isobutyl-1-methylxanthine or a depolarizing concentration of KCl (25 mmol/L). Prior exposure of clonal pancreatic beta-cells to AEE did not alter subsequent stimulation of insulin secretion induced by 10 mmol/L of L-alanine, thereby precluding a detrimental effect on cell viability. The insulinotropic action of AEE was partially dependent upon use of heat during extract preparation. Activity of AEE was heat-stable, acetone-insoluble and unaltered by prolonged exposure to acid/alkali (0.1 mol/L of HCl and NaOH). However, activity was significantly decreased 41% by dialysis to remove components with molecular mass <2000 Da. Sequential extraction with solvents revealed activity in both methanol and water fractions, indicating a cumulative effect of more than one extract constituent. Known constituents of elder, including lectin, rutin and the lipophilic triterpenoid (lupeol) and sterol (β-sitosterol), did not stimulate insulin secretion. The results demonstrate the presence of insulin-releasing and insulin-like activity in the traditional antidiabetic plant, *Sambucus nigra*. J. Nutr. 130: 15–20, 2000.

KEY WORDS: • *elder (Sambucus nigra)* • *streptozotocin-diabetic mice* • *insulin* • *traditional plant treatments* • *diabetes*

Approaches to the control and prevention of hyperglycemia are central to the management of diabetes mellitus (Herman and Crofford 1997). The development of new dietary adjuncts and novel antidiabetic agents, which reinstate a normal metabolic environment, thereby reducing the long-term complications associated with diabetes is required. Such agents would both ideally stimulate the secretion and improve the action of insulin (Bailey and Flatt 1995).

Throughout the world, many traditional plant treatments for diabetes exist and therein lies a hidden wealth of potentially useful natural products for diabetes control (Bailey and Day 1989, Gray and Flatt 1997*a*, Swanston-Flatt et al. 1991). Despite this, few traditional antidiabetic plants have received scientific or medical scrutiny, and the World Health Organization (1980) recommended accordingly that this area warrants further evaluation.

Recent studies showed that aqueous extracts of agrimony (*Agrimony eupatoria*), lucerne (*Medicago sativa*), coriander (*Coriandrum sativum*), eucalyptus (*Eucalyptus globulus*), edible mushroom (*Agaricus campestris*) and mistletoe *Viscum album*) enhanced insulin secretion and mimicked the effect of insulin on glucose metabolism in vitro (Gray and Flatt 1997b, Gray and Flatt 1998a, b, c, Gray and Flatt 1999a, b). Such dual pancreatic and extrapancreatic actions would prove to be an important advance on existing therapies used to treat and control diabetes, such as oral hypoglycemic drugs (which act either by enhancing insulin secretion or by improving the action of insulin). These combined findings illustrate the enormous potential of plants for use as possible dietary adjuncts and the discovery of natural products for diabetes therapy.

Sambucus nigra (elder) was reported to have a number of potentially useful medicinal attributes including diuretic, diaphoretic, purgative and hemostatic properties (Chiej 1988, Lust 1986). In addition, it was advocated as an effective traditional remedy for diabetes in Europe (Atkinson 1979, Palaiseul 1983). The present study was undertaken to evaluate the possible presence of antidiabetic components in elder flower. Effects on glucose transport and metabolism by isolated abdominal muscle and on insulin secretion by clonal β-cells were studied to further evaluate the potential for natural products in elder for the treatment of diabetes.

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MATERIALS AND METHODS

Plant material. Dried elder flowers were obtained from a commercial source (The Health Food Center, Birmingham, United Kingdom). Flowers were homogenized to a fine powder and stored at room temperature (20 ± 2°C) until use. Aqueous extract of elder (AEE)[3] was prepared by 15 min infusion of the powdered material at 250 g/L. In brief, 1 g of powdered material was placed in 40 mL of boiling water, 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 AEE were brought to dryness under vacuum (Savant Speedvac; Savant Instrumentation Inc., Framingdale, NY) and stored at −20°C until use. AEE doses are expressed as total plant material weight (rather than dry residue) per L of water. To account for possible differences in potency, test and control incubations within a single experiment using isolated muscle or insulin-secreting cells were always conducted using the same batch of extract. This allowed for variation in potency of different batches of extract apparent in the same insulin release experiments.

Glucose transport and glucose metabolism in vitro. Recently weaned postprandial male mice (3–5 wk) derived from a colony maintained at Aston University, Birmingham, United Kingdom (Flatt and Bailey 1981) were killed by cervical dislocation and each freshly excised abdominal muscle was dissected into eight individual pieces (~10–20 mg). Incubations were performed using Krebs-Ringer bicarbonate buffer supplemented with 2 g/L of insulin-free bovine serum albumin (KRB-BSA; 118 mmol/L of NaCl, 25 mmol/L of $NaHCO_3$, 5 mmol/L of KCl, 1.28 mmol/L of $CaCl_2$, 1.18 mmol/L of $MgSO_4$, 1.17 mmol/L of KH_2PO_4). In order to replicate by mouse, pieces of muscle from each mouse were designated to each of the treatment groups.

Glucose uptake was determined as described previously (Gray and Flatt 1997*b*). In brief, muscle pieces were incubated at 30°C for 30 min in 1 mL KRB-BSA supplemented with 2 mmol/L of sodium pyruvate, 3.7 Bq/L of 2-deoxy-D-[1-^{3}H]glucose, 0.37 Bq/L L-[1-^{14}C]glucose in the presence and absence of 10^{-8} mol/L of human insulin and 0.5 g/L of AEE (2 × 2 factorial design). After incubation, tissue was hydrolyzed using 1 mol/L of NaOH and counted for ^{3}H and ^{14}C radioactivity. The extracellular fluid volume of the muscle was determined from the amount of the nontransported L-[1-^{14}C]glucose, and this was taken into account in the calculation of tissue 2-deoxy-D-[1-^{3}H]glucose uptake, expressed as nBq/(mg wet muscle · h).

Oxidative glucose metabolism to CO_2 and incorporation of glucose into glycogen were determined as described previously (Gray and Flatt 1998*a*). In brief, muscles were incubated at 37°C for 60 min in 1 mL of KRB-BSA supplemented with 10 mmol/L of glucose, 18.5 Bq/L D-[U-^{14}C]glucose in the presence and absence of 10^{-8} mol/L human insulin and 0.5 g/L of AEE (2 X 2 factorial design). Following incubation, carbon dioxide was captured onto a NaOH saturated filter paper and muscles were removed for glycogen analysis (Gray and Flatt 1999a). ^{14}C Radioactivity of the filter paper was counted and CO_2 production was expressed as nmoles CO_2/(mg wet muscle · h). The incorporation of glucose into glycogen was expressed as nmoles glucose equivalents/(mg wet muscle · h).

This concentration of insulin (10^{-8} mol/L) was chosen to represent a large but submaximal stimulation of glucose uptake and oxidation within this preparation (O'Harte et al. 1997).

All animal studies were performed in accordance with the Animals (Scientific Procedures) Act, 1996.

Insulin secretion in vitro. Insulin secretion was evaluated using BRIN-BD11 cells, produced by electrofusion of immortal RINm5F cell with New England Deaconess Hospital rat pancreatic beta-cell (Gray and Flatt 1998*b*, McClenaghan et al. 1996). Secretory characteristics of these cells are described elsewhere (McClenaghan et al. 1996). Cells were seeded at a concentration of 0.2×10^6 cells/well in 24-well plates (Falcon, NJ) cultured in RPMI-1640 containing 11.1 mmol/L of glucose, 10 g/L of fetal calf serum and antibiotics (50,000 IU/L of penicillin-streptomycin) to allow attachment overnight prior to acute tests. Cells were washed thrice with KRB (115 mmol/L of NaCl, 4.7 mmol/L of KCl, 1.28 mmol/L of $CaCl_2$, 1.2 mmol/L of KH_2PO_4, 1.2 mmol/L of $MgSO_4$, 24 mmol/L of $NaHCO_3$, 10 mmol/L of hepes free acid, 1 g/L of BSA, 1.1 mmol/L of 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 of KRB at 1.1 mmol/L of glucose in the absence and presence of plant extract, diazoxide (an established opener of β-cell K^+-ATP channels) and other test agents as indicated. Following incubation, aliquots were removed from each well and stored at -20°C for insulin assay (Flatt and Bailey 1981). Cell viability following incubations was evaluated by modified neutral red assay (Hunt et al. 1987).

To investigate the importance of heat during extract preparation, AEE was 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). Modified aqueous extract was freshly reconstituted in KRB and effect on insulin secretion evaluated at a concentration equivalent to 1 g/L compared with AEE.

Further studies on the nature of the insulin-releasing component(s) involved exposure of the AEE to heat, overnight dialysis, acid-alkali or acetone treatment. Heat: AEE was boiled for 1 h immediately after preparation. Dialysis: AEE was dialyzed overnight (Spectra/Por molecular weight cut-off 2000 Da; Spectrum, Los Angeles, CA) against Millipore water at 4°C. Acid-alkali treatment: aliquots of AEE were added to 5 mol/L of HCl or 5 mol/L of NaOH to produce 0.1 mol/L of HCl or 0.1 mol/L of NaOH, allowed to stand at room temperature overnight, then neutralized. Acetone treatment: 1 mL of AEE (1 g/L) was added to 10 mL ice-cold acetone, allowed to stand for 30 min and centrifuged (800 × *g*, 5 min) to obtain acetone-soluble and acetone-insoluble fractions. Aliquots of AEE and modified aqueous extracts were dried under vacuum. All modified aqueous extracts were freshly reconstituted in KRB and effects on insulin secretion at a concentration equivalent to 1 g/L were compared with AEE.

In another series of experiments, elder flowers were subjected to sequential extraction by increasingly polar solvents. 0.25 g of plant material was placed in 5 mL of hexane, agitated for 15 min, centrifuged (950 × *g*, 5 min). The precipitate was dried under vacuum and extracted with a further 5 mL of hexane and centrifuged (as before). The extraction supernatants were pooled, filtered (Whatman no. 1) and the volume adjusted to 10 mL with hexane. The extraction precipitate (dried under vacuum) was subsequently extracted (as above) with 2 × 5 mL vol of ethyl acetate, then methanol and finally with water. All extract fractions were freshly reconstituted in KRB, and effects on insulin secretion at a concentration equivalent to 1 g/L were compared with AEE.

A final experimental series was undertaken to evaluate potential insulin-releasing actions of known chemical constituents isolated from elder flower (Shoaib et al. 1972, Willuhn and Richter 1997). These included *S. nigra* lectin, rutin, lupeol, β-sitosterol, tannic acid and choline chloride (Sigma Chemical Ltd., Poole, Dorset, United Kingdom). The concentration range tested (0.5, 5, 25 and 50 mg/L) assumed that these constituents represented at most 1% of the dried extract (Shoaib et al. 1972, Willuhn and Richter 1997). Lupeol and β-sitosterol were dissolved at 50 g/L in chloroform, and all natural products were tested using KRB supplemented with 1.1 mmol/L of glucose. We confirmed that the solvent did not influence insulin release at the dilutions used.

Statistical analyses. Data were evaluated using Student's unpaired *t* test, one-way or two-way ANOVA 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 the SD were significantly different (Bartlett's test for homogeneity of variances), data were transformed ($\log_{10}[\times]$).

[3] Abbreviations used: AEE, aqueous extract of elder; KRB, Kreb's-Ringer bicarbonate buffer; KRB-BSA, Kreb's Ringer bicarbonate buffer supplemented with 2 g/L insulin-free bovine serum albumin.

TABLE 1

Effect of aqueous extract of elder (AEE) on glucose uptake and glucose metabolism by isolated mouse abdomen muscle during in vitro incubations in the absence and presence of 10^{-8} mol/L insulin

	Control	Insulin	AEE	Insulin + AEE
		10^{-8} mol/L	*1 g/L*	
Glucose uptake, *nBq/(mg · h)*	2.86 ± 0.18 (6)	5.65 ± 0.57*** (6)	4.78 ± 0.77* (6)	5.85 ± 0.38*** (6)
Glucose oxidation, *nmol/(mg · h)*	0.42 ± 0.03 (10)	0.63 ± 0.06** (10)	0.62 ± 0.06** (10)	0.74 ± 0.08** (10)
Incorporation of glucose into glycogen, *nmol/(mg · h)*	0.18 ± 0.02 (10)	0.36 ± 0.06** (10)	0.29 ± 0.05* (10)	0.41 ± 0.07** (10)

[1] Values represent means ± SEM of the number of observations given in parentheses.
[2]* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control incubations in the absence of added insulin and AEE.

RESULTS

Glucose transport and glucose metabolism in vitro. AEE (1 g/L) increased glucose uptake (70%), glucose oxidation (50%) and glycogenesis (70%) during incubations without insulin but did not significantly alter the stimulatory effect of 10^{-8} mol/L of insulin (**Table 1**).

Insulin secretion in vitro. AEE (0.25–1 g/L) exerted a dose-dependent stimulatory effect on insulin secretion from BRIN-BD11 cells at 1.1 mmol/L of glucose (**Fig. 1**). At concentrations of 5 g/L of AEE and above, cell viability during the test period was significantly diminished ($P < 0.001$) as evaluated by modified neutral red assay (Hunt et al. 1987) (data not shown). Such an effect was not evident at concentrations of 1 g/L of AEE and below. The presence of 0.5 mmol/L of diazoxide inhibited the stimulatory effect of AEE (**Fig. 2***A*), also indicating that the enhancement of insulin release was not a mere consequence of cellular damage. Consistent with lack of toxicity at lower concentrations, prior exposure of BRIN-BD11 cells to 0.5 g/L of AEE for 20 min did not alter the subsequent insulin secretory response to 10 mmol/L of L-alanine (Fig. 2*B*).

Insulin-releasing effect of 1 g/L of AEE was significantly potentiated by the presence of high (16.7 mmol/L) glucose (**Fig. 3***A*). In contrast, the extract did not augment insulin release in cells stimulated by 10 mmol/L L-alanine, 1 mmol/L of 3-isobutyl-1-methylxanthine (IBMX), or depolarized by combination of 16.7 mmol/L of glucose and 25 mmol/L of KCl (Fig. 3*B-D*).

Temperature of extraction had only a modest effect on

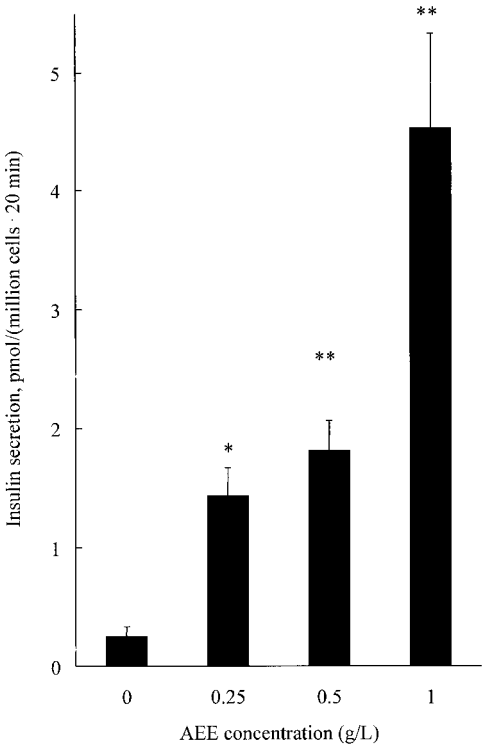

FIGURE 1 Effects of aqueous extract of elder (AEE) on in vitro insulin secretion from clonal pancreatic β-cells. Values are means ± SEM, $n = 6$. *$P < 0.05$, **$P < 0.01$ compared with control incubations without AEE.

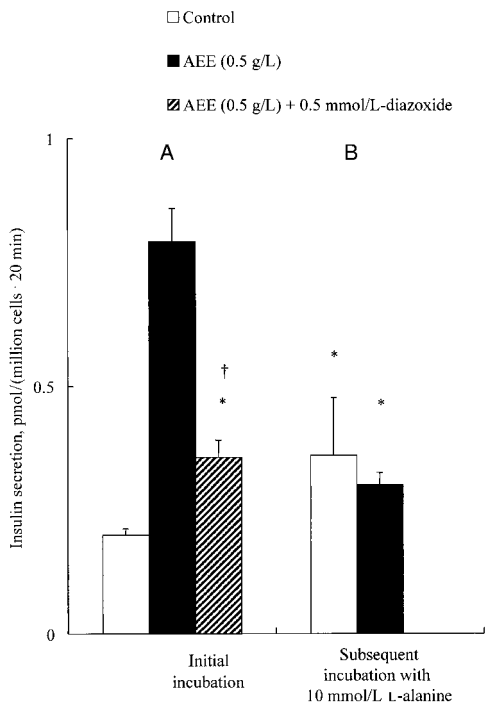

FIGURE 2 (*A*) Effects of aqueous extract of elder (AEE, 0.5 g/L) on in vitro insulin secretion from clonal pancreatic β-cells at 1.1 mmol/L of glucose (control) in the absence or presence of 0.5 mmol/L of diazoxide. (*B*) Effects of 20 min prior exposure to 1.1 mmol/L of glucose (control) or AEE (1 g/L) on the subsequent insulin secretory response to 10 mmol/L L-alanine. Values are means ± SEM, n = 6. *$P < 0.05$, **$P < 0.001$ compared with control incubations without extract. †$P < 0.001$ compared with incubation with extract.

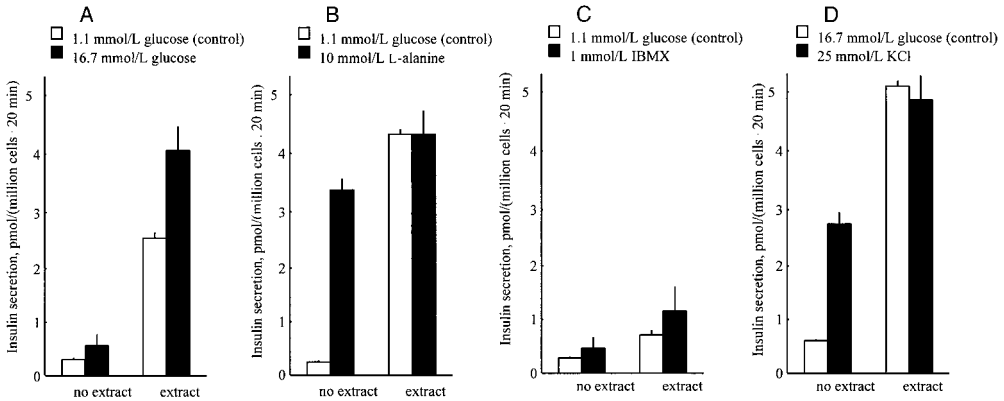

FIGURE 3 Effects of (*A*) glucose, (*B*) L-alanine, (*C*) IBMX or (*D*) KCl on the in vitro insulin releasing actions of aqueous extract of elder (AEE, 1 g/L) on clonal pancreatic β-cells. Values are means for groups of six observations with their SEM indicated by vertical bars. Two-way ANOVA revealed (*A*) AEE effect ($P < 0.001$), glucose effect ($P = 0.001$) and AEE-glucose interaction ($P < 0.05$); (*B*) AEE effect ($P < 0.001$), L-alanine effect ($P < 0.001$) and AEE-L-alanine interaction $P < 0.001$); (*C*) AEE effect ($P < 0.001$), IBMX effect ($P < 0.01$) and AEE-IBMX interaction (NS); (*D*) AEE effect ($P < 0.001$), KCl effect ($P < 0.001$) and AEE-KCl interaction ($P < 0.01$).

insulin-releasing activity, with cold infusion giving 85% of normal activity ($P < 0.05$). Activity of AEE was heat-stable, acetone-insoluble and resistant to prolonged exposure to an acid/alkali environment (**Table 2**). Overnight dialysis of AEE to remove components with a molecular mass < 2000 Da reduced the insulin-releasing activity by 41% ($P < 0.01$; Table 2). Although significantly less potent than AEE, the methanol and water fractions of elder (produced by sequential extraction) exhibited insulin-releasing activity (**Fig. 4**). However, activity in the ethyl acetate fraction was significantly reduced compared with AEE, and the hexane fraction did not alter basal insulin release (Fig. 4).

The possible involvement of established natural products of elder flower, effects of a number of predominant constituents were examined. *S. nigra* lectin, rutin, lupeol, β-sitosterol, tannic acid or choline did not effect insulin release from BRIN-BD11 cells when incubated over a wide range of concentrations (0.5–50 μg/mL). Insulin release by BRIN-BD11 cells at 1.1 mmol/L of glucose ($n = 6$ for each test) was consistently between 0.21–0.26 pmol/(10^6 cells·20 min) irrespective of the absence or presence of plant constituent.

TABLE 2

Effect of heat, dialysis, acid-alkali treatment and acetone treatment on ability of aqueous extract of elder (AEE) to enhance in vitro insulin secretion from clonal pancreatic β-cells

Test	Insulin secretion
	pmol/(10^6 cells · 20 min)
Control (without AEE)	0.27 ± 0.02
AEE	3.19 ± 0.25*
Boiled extract	3.27 ± 0.35*
Dialyzed extract	1.87 ± 0.18*†
Acid-exposed extract	2.76 ± 0.29*
Alkali-exposed extract	2.83 ± 0.36*
Acetone-insoluble extract	2.75 ± 0.18*
Acetone-soluble extract	0.65 ± 0.08*‡

[1] Values are means ± SEM, $n = 6$.

[2] $P < 0.001$ compared to control incubations; ‡$P < 0.01$ compared to incubations with AEE, ‡$P < 0.001$ compared to incubations with acetone-insoluble extract.

DISCUSSION

Although elder has been long advocated as an effective traditional plant treatment to counter the symptoms of diabetes (Atkinson 1979, Palaiseul 1983), scientific studies to evaluate its efficacy and possible mode of action are lacking. The present study reports for the first time that elder flowers contain water-soluble natural products which directly stimulate glucose metabolism by muscle and promote insulin secretion from clonal pancreatic β-cells.

The effect of elder extract on glucose transport and metabolism was evaluated in vitro using an insulin-sensitive muscle preparation (Gray and Flatt 1997*b*). AEE (1 g/L) significantly enhanced glucose uptake, glucose oxidation and glycogen syn-

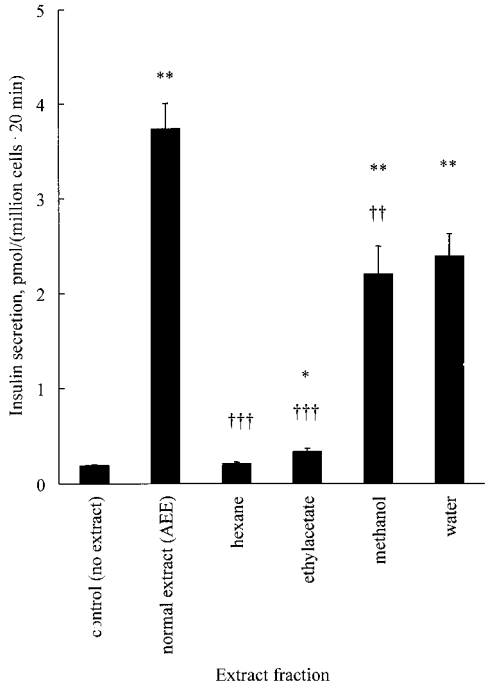

FIGURE 4 Effect of sequential extraction by solvents with increasing polarity on insulin-stimulating effect of elder in vitro on clonal pancreatic β-cells. All extracts were tested at concentrations equivalent to 1 g/L. Values are means for groups of six observations with their SEM indicated by vertical bars. *$P < 0.05$, **$P < 0.001$ compared to incubations without aqueous extract of elder (AEE); †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared to incubations with AEE.

thesis in a magnitude similar to 10^{-8} mol/L of insulin. Although this effect was observed in the absence of added insulin, it does not preclude involvement of residual insulin receptor binding within the muscle preparation. However, the lack of significant potentiation by AEE and insulin in combination implies that the extract is likely to act via pathways (at least terminally) that are utilized by insulin rather than entirely separate pathways. Notably, the effect of AEE on glucose uptake is different from that of the established antihyperglycemic drug, metformin, which enhances glucose uptake via insulin-mediated enhanced peripheral glucose uptake (Bailey and Puah 1986, Prager et al. 1986). The ability of AEE to enhance glucose uptake and oxidation in vitro is paralleled by findings for other aqueous plant extracts, including agrimony, lucerne, coriander, mushroom, mistletoe and eucalyptus (Gray and Flatt 1997b, Gray and Flatt 1998a,b,c, Gray and Flatt 1999*a,b*).

Incubations were performed with glucose-responsive BRIN-BD11 cells (McClenaghan et al. 1996) to investigate the possible effects of AEE on insulin secretion in vitro. This revealed a stepwise dose-dependent stimulation of insulin secretion by AEE at low (non-stimulatory) glucose concentration. Evaluation of cell viability using modified neutral red assay and the insulin-releasing action of L-alanine following exposure of clonal β-cells to extract argues against a simple cytotoxic action at or below 1 g/L of extract.

Studies to evaluate the possible mechanisms underlying the insulin-releasing action of elder indicated a similarity to the sulfonylurea class of drugs currently used for diabetes therapy (Bailey and Flatt 1995). These agents enhance insulin secretion by binding to sulfonylureas receptors on the β-cell, with subsequent closure of K^+-ATP channels, membrane depolarization and Ca^{2+} influx (Rorsman 1997). Diazoxide inhibits the stimulatory action of sulfonylureas by preventing closure of the K^+-ATP channels (Dunne et al. 1994). In the present study the insulin-releasing action of elder extract was inhibited by diazoxide. Importantly, the action of AEE was potentiated by 16.7 mmol/L of glucose, suggesting that β-cell glucose metabolism is able to augment the insulinotropic effect. Consistent with this view, L-alanine, which promotes insulin secretion through changes in Na^+ transport (Yada 1994), failed to augment the insulin-releasing effect of AEE. In contrast to sulfonylureas (Eliasson et al. 1996, Flatt et al. 1994), AEE failed to stimulate insulin secretion from beta-cells depolarized by 25 mmol/L of KCl, indicating absence of similar K^+-ATP channel-independent effects. Interestingly, the phosphodiesterase inhibitor IBMX which increases cyclic AMP and promotes insulin release (Sharp 1979) did not potentiate the insulin-releasing effect of AEE, raising the possibility that AEE itself may inhibit islet phosphodiesterase (Leibowitz et al. 1995).

Elder flowers contain a number of established natural products including lectin, the flavenoid rutin, choline tannin and lipophilic triterpenoid and sterol compounds such as lupeol and β-sitosterol (Shoaib et al. 1972, Willuhn and Richter 1997). It was therefore of interest to evaluate if any of these available compounds could account for the insulin-releasing action. Since *Agaricus bisporus* (mushroom) lectin documented insulinotropic activity (Ahmad et al. 1984a and 1984b; Ewart et al. 1975), it was particularly interesting to evaluate whether the lectin component of elder might be important. However, neither *S. nigra* lectin, rutin, lupeol, β-sitosterol, tannic acid or choline affected insulin release over a wide range of concentrations approximating to 1% of active plant extract. Thus, the chemical nature of potential antihyperglycemic component(s) of elder remains to be established. In terms of insulin-releasing activity, the present study indicates that the active principle(s) is heat-stable, acetone-insoluble and unaffected by altered pH environment. The approximate 40% decrease in the insulin-releasing activity of AEE following overnight dialysis (to remove components with a molecular mass $<$ 2000 Da) suggests some involvement of smaller molecules or ions in the insulin releasing effect. Sequential solvent extractions point toward a cumulative effect of more than one active constituent, likely to be more polar in nature. Other aqueous plant extracts have also been shown to enhance insulin secretion using this model (Gray and Flatt 1997b, Gray and Flatt 1998a,b,c, Gray and Flatt 1999a and 1999b). However, the precise mechanisms by which these extracts enhance insulin secretion and the chemical identity of biologically active component(s) remain to be elucidated.

In conclusion, the traditional antidiabetic plant elder contains water-soluble components capable of stimulating insulin secretion and enhancing muscle glucose uptake and metabolism. *Sambucus nigra* therefore represents a possible dietary adjunct for the treatment of diabetes and a potential source for the discovery of new orally active agent(s) for future diabetes therapy.

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