Flatt, 1997; Oubre include the treatment of diarrhoea, dysentery and general debility, Asparagus adscendens in India. Anecdotal uses of Indian folklore its origins can be traced back to the oldest mound musali in India and Sutaid musk in Pakistan. According to et al. (Bailey & Day, 1989; Swanston-Flatt in the potential of plant treatments for diabetes across the world cine (MacLennan regions. However, the recent explosion in the area of herbal medi- Napoleon treatment of diabetes. Diabetes mellitus is a complex metabolic disorder in which the control of blood glucose is of paramount importance (De Fronzo et al. 1992; Lilloja et al. 1993). Regimes that counter hyperglycaemia, including diet, oral anti-diabetic drugs and insu- lin form the cornerstone of available therapy (Clark, 1998; Laws, 2001). Through attempting to restore a near normal metabolic environment, such treatments help decrease the incidence of the long-term complications of the disease (Mandrup-Poulsen, 1998). Despite their usefulness, however, these treatments do not normalise blood glucose levels nor prevent the risk of devel- oping diabetic complications (UKPDS, 1995; Amos et al. 1997). As the incidence of diabetes increases rapidly across the globe there is an urgent need to expand the range of effective palliatives available to sufferers. Man has long turned to plants as a source of new and innova- tive medicines (Day, 1990). In 1980, 75% of the world’s popu- lation relied mainly upon plant medicines (Weragoda, 1980). Until recently this figure was mainly confined to developing regions. However, the recent explosion in the area of herbal medicine (MacLennan et al. 1996; Astin, 1998; Brevoort, 1998) has led to a renaissance of nutritional, clinical and scientific interest in the potential of plant treatments for diabetes across the world (Bailey & Day, 1989; Swanston-Flatt et al. 1991a,b; Gray & Flatt, 1997; Oubre et al. 1997). Asparagus adscendens is commonly referred to as Shweta musali in India and Sutaid musk in Pakistan. According to Indian folklore its origins can be traced back to the oldest moun- tain ranges in India. Anecdotal uses of Asparagus adscendens include the treatment of diarrhoea, dysentery and general debility, it is also known as a galactagogue, demulcent and tonic (Shinwari & Khan, 2000). The present study is the first to con- sider the anti-diabetic potential of Asparagus adscendens, and has investigated its effect on glucose uptake and insulin secretion at cellular level, and on starch digestion in vitro.

Asparagus adscendens: Diabetes: Insulin

Materials and methods

Plant material

Dried rhizome of Shweta musali (Asparagus adscendens) was obtained from a commercial supplier in Delhi, India, during the winter season. Rhizome was homogenised to a fine powder and stored in opaque screw-top jars at room temperature (20 ± 2°C) until use. For in vitro work, a decoction was prepared by bringing 25 g/l of material to the boil in water. Once boiling, the suspension was removed from the heat and allowed to infuse over 15 min. The suspension was filtered (Whatman no. 1 filter paper) and the volume adjusted so the final concentration was 25 g/l. Aliquots (1 ml) of the filtered plant solution were brought to dryness under vacuum (Savant Speedvac; Savant Instrumenta- tion Inc., New York, USA). Dried fractions were stored at −20°C until required. Fractions were reconstituted in incubation buffer for subsequent experiments as required.

Insulin secretion in vitro

Insulin release was determined using monolayers of BRIN-BD11 clonal pancreatic cells (McClenaghan et al. 1996). This cell line is

Abbreviations: [Ca2+], intracellular calcium concentration; IBMX, 3-isobutyl-1-methylxanthine; KRB buffer, Krebs Ringer bicarbonate buffer.

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derived from electrophoresis of NEDH (New England Deaconess Hospital) rat pancreatic β cells and rat insulinoma RINm5F cells. BRIN-BD11 cells were grown in RPMI-1640 tissue culture medium containing 11.1 mmol glucose/l, 10 % fetal calf serum and antibiotics (50,000 IU penicillin–streptomycin/l), and maintained at 37°C in an atmosphere of 5 % CO₂ and 95 % air. Prior (24 h) to acute experiments, cells were harvested and seeded in twenty-four-well plates at a density of 1·0 × 10⁵ cells per well. Following overnight attachment, culture medium was removed and cells were preincubated with 1 ml Krebs Ringer bicarbonate (KRB) buffer for 40 min at 37°C. KRB buffer contained 115 mM-NaCl, 47 mM-KCl, 1·28 mM-CaCl₂, 1·2 mM-MgSO₄, 1·2 mM-KH₂PO₄, 25 mM-HEPES and 8·4 (w/v) NaHCO₃ (pH 7·4 with NaOH) supplemented with 1·1 mM-glucose and 1 % bovine serum albumin. Test solution consisted of KRB buffer supplemented with glucose, aqueous plant extract and various modulators of insulin secretion such as diazoxide (K⁺-ATP channel opener), verapamil (voltage-dependent Ca²⁺ channel blocker), 3-isobutyl-1-methylxanthine (IBMX; phosphodiesterase inhibitor, nylurea receptor component of K⁺-ATP channel blocker), increasing cellular cyclic AMP), KCl (inducing membrane depolarisation and Ca²⁺ influx) and tolbutamide (binds to sulphonylurea receptor component of K⁺-ATP channel leading to closure, depolarisation and consequent Ca²⁺ influx). All tests were performed at 5·6 mM-glucose unless specified. Cells were incubated for 20 min at 37°C with test solution, after which a 900 µl aliquot was collected from each well and stored at −20°C for subsequent determination of insulin concentration (Flatt & Bailey, 1981). Cell viability was subsequently assessed using a modified neutral red assay (Hunt et al., 1987). Following 20 min incubation with test agent, cells were washed three times with KRB buffer, and incubated for 2 h with 1 ml neutral red solution (50 µl neutral red solution dissolved in 50 µl dimethyl sulphoxide) and made up to 200 ml with KRB. After washing (as described earlier), 1 ml distilled water–ethanol–glacial acetic acid (49:50:1) was added and plates were gently agitated for 15 min. Absorbance of each well was read at 540 nm and means and their standard errors were calculated. Results were expressed as a percentage of control (incubations performed in the absence of test agent) giving percentage cell viability after 20 min exposure to test agent.

Intracellular calcium concentration and membrane potential studies

Changes in membrane potential and intracellular calcium concentration ([Ca²⁺]i) were determined fluorimetrically (Miguel et al. 2003) using monolayers of BRIN-BD11 cells. The fluorescent probes are internalised by living cells during preincubation and emit characteristic fluorescence in relation to changes in cellular membrane potential or [Ca²⁺]i. Cells were seeded into ninety-six-well plates (black walls, clear bottom; Costar, Roskilde, Denmark) at a density of 1·0 × 10⁵ viable cells per well and allowed to attach overnight in culture. The cells were washed once with KRB buffer (115 mM-NaCl, 4·7 mM-KCl, 1·28 mM-CaCl₂, 1·2 mM-KH₂PO₄, 1·2 mM-MgSO₄, 10 mM-NaHCO₃ and 0·1 % (w/v) bovine serum albumin, pH 7·4) supplemented with 5·6 mM-glucose, 20 mM-HEPES and 500 µM-probenecid. The cells were incubated at 37°C for 1 h with either membrane potential assay kit or Ca²⁺ assay kit (Molecular Devices), prepared with the same washing buffer, to a final volume of 200 µl. Fluorimetric data were acquired using a FlexStation scanning fluorimeter with integrated fluid transfer workstation (Molecular Devices). Excitation, emission and cut-off filter were set to 530, 565 and 550 nm for membrane potential, and 485, 525 and 515 nm for Ca²⁺. The FlexStation was set to run for 10 min, collecting data at a 2·5 s interval (six readings per well). Test solutions (50 µl at 5-fold concentration) were transferred at 60 s from the start of readings at a rate of 78 ml/s.

Adipocyte differentiation and glucose uptake in vitro

3T3-L1 fibroblasts obtained from the American Type Culture Collection (Manassas, Virginia, USA), were used to determine glucose uptake (Frost & Lane, 1985). Cells were seeded in twelve-well plates at a density of 1·0 × 10⁵ cells per well and fed every 2 d with Dulbecco’s Modified Eagle’s Medium supplemented with penicillin (50 U/ml), streptomycin (50 µl/ml) and fetal bovine serum (10 %, v/v). Cells were maintained at 37 ± 0·5°C and 5 % CO₂. Adipocyte differentiation was initiated 2–3 d post-conflueney by the addition of culture medium containing 1 µg/ml insulin, 0·5 mM-IBMX (in dimethyl sulphoxide) and 0·25 µM-dexamethasone (in ethanol). Cells were maintained in this medium for 3 d followed by a further 3 d in medium supplemented with insulin (1 µg/ml) alone. Following this period cells were returned to original medium until experiment (after 1–2 d). Cells were used at passages five to ten. On the morning of experiment, cells were incubated in serum-free Dulbecco’s Modified Eagle’s Medium for 2–3 h to establish basal glucose uptake. Cell monolayers were given three rapid washes with warmed PBS, prior to the addition of KRB buffer (116 mM-NaCl, 4·7 mM-KCl, 1·28 mM-KH₂PO₄, 1·2 mM-MgSO₄, 24 mM-NaHCO₃, 10 mM-HEPES, pH 7·4) for 15 min at 37°C. During the experiment, groups of cells (n 4) were incubated with and without aqueous plant extract (5 mg/ml), in the absence and presence of a stimulatory concentration of insulin (10⁻⁹ M). Extracts (5 mg/ml) were also tested in the presence of known enhancers of insulin action, metformin (1 mM), orthovanadate (500 mM) and molybdate (30 mM) and a further group of cells was incubated with insulin (10⁻⁸ M), which was considered the maximal stimulatory dose. Following 15 min exposure to test reagents, hexose uptake was initiated by the addition of 50 µl tritiated 2-deoxyglucose (0·5 µCi/well) plus glucose (50 mM final concentration). Hexose uptake was terminated after 5 min by three rapid washes with ice-cold PBS, after which cells were detached by the addition of 0·1 % sodium dodecyl sulphate and subsequently lysed. Scintillation fluid (8 ml) was added to solubilised cell suspensions and mixed thoroughly. Activity was measured on a Wallac 1409 Scintillation Counter (Wallac, Turke, Finland) and data are expressed as dpm.

Starch digestion

To assess in vitro starch digestion, 100 mg soluble starch (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 3 ml distilled water in the absence and presence of plant extract (50 mg/ml). Heat-stable α-amylase (40 µl, 0·01 %; from Bacillus leicheniformis; Sigma-Aldrich) was added and the solution vortexed and placed in a water-bath at 80°C for 20 min. The mixture was then diluted to 10 ml with distilled water. To 1 ml of this solution, 2 ml 0·1 m-sodium acetate buffer (pH 4·75) and 30 µl 0·1 % amylglucosidase from Rhizopus mould (Sigma-Aldrich) were added and placed in a water-bath at 60°C for 30 min. The
glucose released was measured on the Analox GM9 glucose analyser (Analox Instruments, London, UK). Incubations performed with 50 μg/ml acarbose (GlucoBay®; Bayer AG, Leverkusen, Germany) served as a positive control. Experiments were performed in triplicate.

Statistical analysis

All results are expressed as means and their standard errors for a given number of observations (n). Groups of data were compared statistically using unpaired Student’s t-test. When experiments included more than two test groups one-way ANOVA followed by Tukey’s post hoc test was used. Calculations were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). Results were considered significant if P<0.05.

Results

Insulin secretion studies

Asparagus adscendens exerted a concentration-dependent stimulatory effect on insulin secretion in BRIN-BD11 cells at 5·6 mM-glucose (Fig. 1). Cell viability remained 100 (SEM 4·0) % stimulatory effect on insulin secretion in BRIN-BD11 cells at presence of the extract (Table 1). Furthermore, the extract did not significantly increase insulin secretion in depolarised cells (Table 1). Aqueous extract of Asparagus adscendens (5 mg/ml) did not augment the insulin secretory activity of 200 μM-tolbutamide (P=0.09; Table 1). Similarly, IBMX (100 μM) retained its full insulinotropic effect in the presence of the extract (P=0.91; Table 1).

Intracellular calcium concentration and membrane potential studies

Aqueous extract of Asparagus adscendens (5 mg/ml) generated depolarisation of the β cell. This produced a biphasic increase of [Ca2+]i, comprising a short sharp increase, followed by a gradual sustained increase (Fig. 3(A)). The initial increase in [Ca2+]i, largely declined before the addition of verapamil (50 μM), but addition of the voltage-dependent calcium channel antagonist had a small effect on [Ca2+]i (Fig. 3(B)). Addition of diazoxide to cells previously exposed to extract produced a pronounced decrease in membrane potential level (Fig. 3(C)).

Glucose uptake

Asparagus adscendens (5 mg/ml) increased glucose uptake by 81 % in 3T3-L1 adipocytes (P<0.001; Table 2). This effect was at least equivalent in magnitude to that of insulin (10−9 M). The combined actions of extract and insulin did not exceed the

![Fig. 1](https://www.cambridge.org/core/submit/35d7f150b2703902aa035a3d4c9164b9)

**Fig. 1.** Effects of aqueous extract of Asparagus adscendens on insulin secretion. Values are means with their standard errors depicted by vertical bars (n 7). For details of procedures, see p. 576. Mean values were significantly different from those of the control group (no extract): **P<0.01, ***P<0.001.

<table>
<thead>
<tr>
<th>Test agent</th>
<th>Concentration (mmol/l)</th>
<th>Glucose (mmol/l)</th>
<th>aqAA (5 mg/ml)</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>–</td>
<td>5·6</td>
<td>–</td>
<td>0·62</td>
<td>0·07</td>
</tr>
<tr>
<td>–</td>
<td>5·6</td>
<td>+</td>
<td>1·41</td>
<td>0·11</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>16·7</td>
<td>–</td>
<td>0·68</td>
<td>0·06</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>16·7</td>
<td>+</td>
<td>4·53</td>
<td>0·35</td>
<td></td>
</tr>
<tr>
<td>Diazoxide</td>
<td>0·30</td>
<td>5·6</td>
<td>+</td>
<td>0·42</td>
<td>0·04</td>
</tr>
<tr>
<td>Verapamil</td>
<td>0·05</td>
<td>5·6</td>
<td>+</td>
<td>0·61</td>
<td>0·07</td>
</tr>
<tr>
<td>KCl</td>
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<td>6·18</td>
<td>0·48</td>
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<tr>
<td>30</td>
<td>16·7</td>
<td>+</td>
<td>7·68</td>
<td>0·80</td>
<td></td>
</tr>
<tr>
<td>IBMX</td>
<td>0·10</td>
<td>5·6</td>
<td>–</td>
<td>1·27</td>
<td>0·08</td>
</tr>
<tr>
<td>0·10</td>
<td>5·6</td>
<td>+</td>
<td>2·99</td>
<td>0·20</td>
<td></td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>0·20</td>
<td>5·6</td>
<td>–</td>
<td>0·90</td>
<td>0·04</td>
</tr>
<tr>
<td>0·20</td>
<td>5·6</td>
<td>+</td>
<td>1·51</td>
<td>0·22</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** Effects of glucose, diazoxide, verapamil, 3-isobutyl-1-methylxanthine (IBMX), tolbutamide and depolarising conditions on the insulinotropic actions of aqueous extract of Asparagus adscendens (aqAA) in BRIN-BD11 cells (n 5–7).

† For details of procedures, see p. 576.
Fig. 3. (A), Effects of Asparagus adscendens (5 mg/ml) on intracellular Ca\(^{2+}\) (○) and membrane potential (○). (B), Effects of verapamil (50 μM) on intracellular Ca\(^{2+}\) levels in cells previously exposed to extract (●) (○, control). (C), Effects of diazoxide (300 μM) on membrane potential of cells previously exposed to extract (●). Cells were exposed to test agents as indicated by the arrows. For details of procedures, see p. 576. Results were normalised with a control group that received 5.6 mM-glucose during the full experimental time. Values are means with their standard errors depicted by vertical bars (n 6). RFU, Relative Fluorescence Unit.

Antidiabetic actions of Asparagus adscendens

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Table 2. Effects of aqueous extract of Asparagus adscendens (aqAA) on glucose uptake in 3T3-L1 adipocytes (n=5–7)†

<table>
<thead>
<tr>
<th>Test agent</th>
<th>Concentration (mmol/l)</th>
<th>Insulin (mol/l)</th>
<th>aqAA (5 mg/ml)</th>
<th>Insulin action (dpm × 10²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Mean SEM</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>42.4 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>10⁻²</td>
<td>–</td>
<td>76.7 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>10⁻²</td>
<td>+</td>
<td>70.8 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>10⁻⁴</td>
<td>–</td>
<td>83.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10⁻⁴</td>
<td>–</td>
<td>107.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10⁻⁹</td>
<td>+</td>
<td>79.6 ± 3.2</td>
</tr>
<tr>
<td>Metformin</td>
<td>0.5</td>
<td>10⁻⁹</td>
<td>+</td>
<td>73.5 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>10⁻⁹</td>
<td>+</td>
<td>89.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>10⁻⁹</td>
<td>0</td>
<td>–</td>
<td>84.5 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>10⁻⁹</td>
<td>0</td>
<td>–</td>
<td>89.0 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>10⁻⁹</td>
<td>0</td>
<td>–</td>
<td>89.7 ± 1.5</td>
</tr>
</tbody>
</table>

Mean values were significantly different (P<0.001; Table 3). For details of procedures, see p. 576.

Table 3. Effects of aqueous extract of Asparagus adscendens and acarbose on starch digestion (n=3)†

<table>
<thead>
<tr>
<th>Test conditions</th>
<th>Glucose liberated (%)</th>
<th>Reduction (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99.6 ± 1.6</td>
<td>–</td>
</tr>
<tr>
<td>Asparagus adscendens</td>
<td>79.5 ± 2.6</td>
<td>21***</td>
</tr>
<tr>
<td>Acarbose</td>
<td>4.7 ± 0.5</td>
<td>95***</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those of the control group: ***P<0.001.
† Aqueous extract of Asparagus adscendens (50 mg/ml) or acarbose (50 µg/ml) were incubated with 100 mg starch in the presence of digestive enzymes in vitro and the amount of glucose released was measured.
‡ Unsupplemented control incubations were considered 100%.

Discussion

Insulin secretion

Aqueous extract of the powdered rhizome of Asparagus adscendens, also known as Shweta musali, stimulated insulin secretion from BRIN-BD11 cells at levels of 5 mg/ml and above. Acute incubation did not affect viability at any of the doses tested, as assessed by neutral red assay (Hunt et al., 1987), confirming that the insulinotropic effect was physiological. Incubation with diazoxide abolished the stimulatory effect of extract, indicating an effect mediated through K⁺-ATP channels (Dunn & Peters, 1995). Modulation of Ca²⁺ handling by the β cell is an integral part of Asparagus adscendens-induced insulin secretion. This is confirmed by inhibition of secretion by verapamil, or removal of extracellular Ca²⁺. Like glucose and sulphfonylurea drugs, inclusion of IBMX enhanced insulin secretion in the presence of extract. Fluorometric determination of membrane depolarisation revealed a sustained depolarising phase in response to extract. This alteration in membrane potential was accompanied by a biphasic increase in intracellular Ca²⁺. The present findings are consistent with the profile of insulinotropic activity observed in the presence of Asparagus adscendens. Diazoxide had a marked effect on cellular fluorescence in the presence of extract; this corresponds to a repolarising effect, again implicating involvement of the K⁺-ATP channel in the actions of extract.

Despite the fact there was no statistical significance observed following concurrent incubation of Asparagus adscendens with tolbutamide and IBMX, there was a trend in augmenting insulin secretion. Insulin secretion was greatly enhanced when incubations were performed at 16.7 mM-glucose, indicating the action of the extract was glucose-dependent. The extract failed to significantly enhance insulin secretion in depolarised cells, suggesting that it does not operate through K⁺-ATP channel-independent mechanisms. The failure of verapamil to prevent biphasic Ca²⁺ increase and abolish insulin secretion may dispute the present finding, although differences in kinetics between the two types of experiment could be relevant.

Glucose uptake

The ability of Asparagus adscendens to stimulate cellular glucose transport was examined using 3T3-L1 adipocytes. 3T3-L1 adipocytes have been extensively used in studies investigating insulin-mediated glucose transport (Foyt et al., 2000) and displays all components of the insulin receptor and signal transduction cascade. Asparagus adscendens had a direct effect on glucose uptake in 3T3-L1 adipocytes. At a concentration of 5 mg/ml, its stimulatory actions were similar to that of insulin (10⁻⁹ m) alone. Although not statistically significant, the small number of experiments performed suggested an 18% increase in Asparagus adscendens-induced insulin-mediated glucose uptake. However, the extract clearly failed to augment the effects of orthovanadate or molybdate. This is suggestive of a shared, at least in part, mechanism of action. The rapid effect of Asparagus adscendens on glucose uptake may involve an effect on the redistribution of glucose transporters. However, further work would be needed to clarify this.

Starch digestion

Using an in vitro model, consisting of the digestive enzymes α-amylase and α-glucosidase, the potential of Asparagus adscendens to retard starch digestion was assessed by its effect on glucose liberation from starch. Incubation with the therapeutic α-glucosidase inhibitor, acarbose, validated the method, showing complete inhibition of glucose release liberation at a dose of 50 mg/ml. Asparagus adscendens produced a significant 21% reduction in starch digestion, representing inhibitory effects on α-amylase and/or α-glucosidase activity. Despite, the in vitro inhibitory effects of Asparagus adscendens on starch digestion, this could be partially weighed down in vivo by release of...
excessive amounts of amylase during the digestive process. Due to the lack of substantial, reliable information on the chemical composition of the extract, mechanistic actions are purely speculative. Possible explanations include the presence of alkaloids, which are commonly found in plants. Several alkaloid compounds, including castanospermine from the seeds of Castanospermum australe (Rhinehart et al. 1987), have demonstrated α-glucosidase inhibitory action (Day, 1990). 1-Deoxynojirimycin (moronoline), of which the therapeutic agent miglitol is a derivative, was originally isolated from mulberry root bark (Mori cortex) (Yoshikuni, 1998). A recent study has also identified a plant extract, which inhibits α-glucosidase enzymes (Kurihara et al. 2003). Several hydroxyflavonoid compounds have been isolated from marjoram leaves, each exhibiting glucosidase inhibitory activity (Kawabata et al. 2003).

In conclusion, the present study has shown that aqueous extract of Asparagus adscendens stimulates both the secretion and action of insulin as well as inhibiting starch digestion. The plant’s ability to influence insulin secretion and action in vivo depends entirely on soluble active principle(s) in the extract being absorbed via the gut. Future work directed at the purification and characterisation of active components may reveal new agents for diabetic therapy.

Acknowledgements
This study was supported by the University of Ulster Research Strategy Funding.

References