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STIMULATION OF INSULIN SECRETION BY AN AQUEOUS EXTRACT OF GYMNEMA SYLVESTRE : ROLE OF INTRACELLULAR CALCIUM

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INTRODUCTION

Plant derived extracts have been used to treat Type 2 diabetes mellitus for several millennia and a number of tribes, including those in east-central India, have used Gymnema sylvestre leaves in their diet and as a folk-medicine for centuries. In the current study we have investigated the effects of a leaf extract of an organic Gymnema sylvestre (a virgin isolate designated OSA) on insulin secretion from mouse and human β–cells, and examined the dependence of the secretory response on extracellular Ca^{2+}. The name 'OSA' denotes Om Santal Adivasi, as a tribute to the Santal tribal people. The virgin isolate is proprietarily prepared by re-creating the Santal tribe's methods to retain the labile compounds of the active principles.

METHODS

Insulin secretion: For static incubations, groups of 20,000 MIN6 cells were seeded into 96 well plates, incubated with OSA for 30 minutes, then the supernatant was retrieved for measurement of insulin secretion by radioimmunoassay. For time-course studies, Liberase-isolated human islets were loaded into chambers and perifused at a rate of 0.5ml/min at 37°C with a physiological salt solution supplemented with OSA. Following collection of the perifusate samples at 2 minute intervals for 50 minutes, the insulin content was determined by radioimmunoassay.

Cell viability: Cells were incubated with 0.1% (w/v) Trypan blue for 15 minutes and non-viable (blue) cells were identified by light microscopy.

Single cell microfluorimetry: The effects of OSA on $[Ca^{2+}]i$ were determined by single cell microfluorimetry in which Fura 2-loaded MIN6 cells seeded on APES coated glass cover slips were illuminated alternately at 340nm and 380nm and emitted light was filtered at 510nm.

RESULTS

Figure 1. Effect of OSA on insulin secretion

OSA caused a concentration-dependent increase in insulin secretion from the MIN6 mouse insulin-secreting cell line at both 2mM glucose (left panel) and 20mM glucose (right panel). The left panel also shows data (red line) from Trypan blue uptake studies: OSA caused significant increases in Trypan blue uptake at concentrations ≥0.5mg/ml, so for all further experiments OSA was used at a concentration of ≤0.2mg/ml. Data are mean±SEM, n=10.

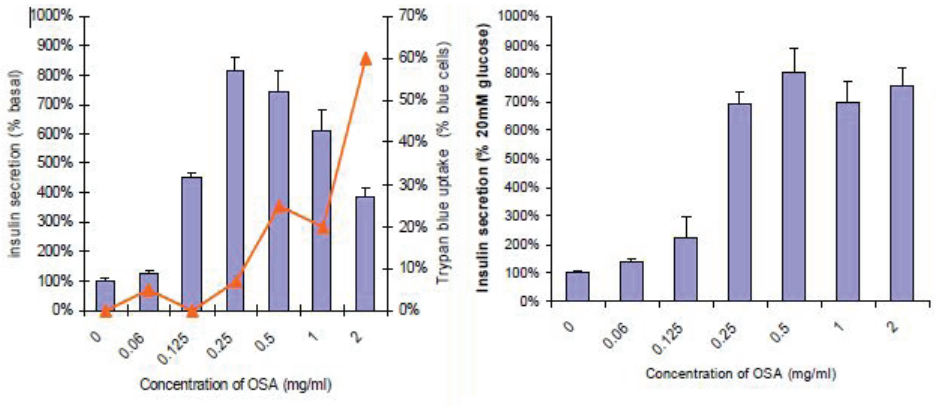

Figure 2. Time course of OSA effects

OSA (0.125mg/ml) caused a rapid stimulation of insulin secretion from perifused human islets at 2mM glucose (left panel), with a peak increase of 266±54% basal secretion. Secretion declined towards basal levels when OSA was removed. OSA also potentiated glucose-stimulated insulin secretion from human islets (right panel). The secretory response was rapid in onset, with an initial peak 4.4-fold higher than the 20mM glucose-induced secretory plateau, and this was followed by a sustained elevation in secretion. Data are mean±SEM, n=3.

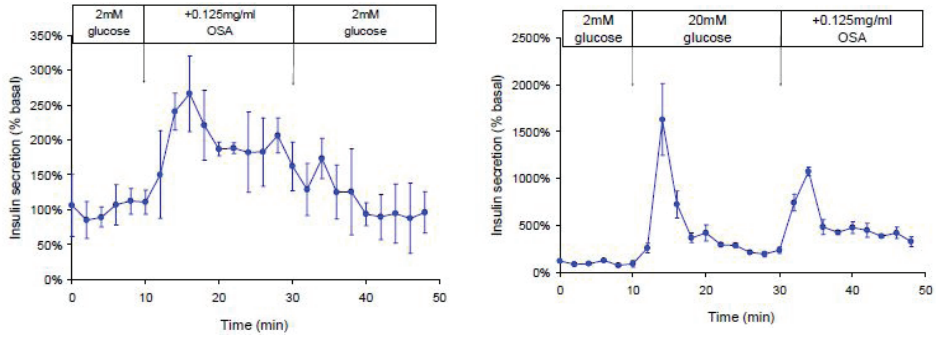

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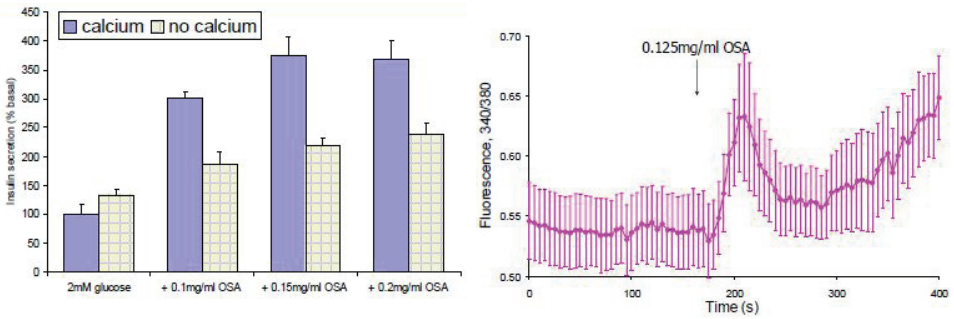
Figure 3. Calcium-dependence of OSA effects

Left panel: Effect of removal of extracellular Ca^{2+}
OSA (0.1-0.2mg/ml) stimulated insulin secretion from MIN6 cells both in the presence (filled bars) and absence (hatched bars) of extracellular Ca2+, but the secretory response was significantly ($P<0.01$) reduced by removal of extracellular Ca^{2+}. Data are mean+SEM, n=6

Right panel: Effect of OSA on intracellular Ca^{2+}
OSA (0.125mg/ml) caused a rapid, transient increase in $[Ca^{2+}]i$ in Fura 2-loaded MIN6 cells, followed by a sustained rising phase. Data are mean±SEM, n=10 cells.



SUMMARY

- OSA (Om Santal Adivasi), a proprietary and patented virgin isolate from organic Gymnema sylvestre leaves, stimulated insulin secretion from the MIN6 mouse insulin-secreting cell line at both sub-stimulatory (2mM) and maximal stimulatory (20mM) glucose concentrations. Concentrations of OSA <0.5mg/ml stimulated secretion without compromising cell viability (Figure 1).
- OSA also stimulated insulin secretion from isolated human islets at 2 and 20mM glucose. Its effects were rapid in onset and reversible (Figure 2).
- The stimulatory effect of OSA partially persisted in the absence of extracellular Ca2+, and it was able to stimulate increases in intracellular Ca2+ in Fura 2-loaded MIN6 cells (Figure 3).

CONCLUSION

Our data indicate that the OSA extract of Gymnema sylvestre contains one or more insulin secretagogue components. At least part of the effect of OSA is dependent on elevation in intracellular Ca^{2+}, but OSA may also exert direct effects on exocytosis in the absence of extracellular Ca^{2+}. The capacity of low concentrations of OSA to cause a reversible stimulation of insulin secretion from human islets suggests that further research should be performed to determine its usefulness as a therapeutic agent in individuals with Type 2 diabetes mellitus.