Effects of taxol and nocodazole on insulin secretion from isolated rat islets of Langerhans

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Taxol, a promotor of microtubule polymerization, and nocodazole, which induces microtubule depolymerization, used at concentrations known to be specific for these effects in other cell types, were each shown to inhibit glucose-stimulated insulin secretion from isolated rat islets of Langerhans. These findings suggest that the dynamic regulation of microtubule polymerization-depolymerization in pancreatic B cells may be important for insulin secretion via the microtubule-microfilamentous system.

Much of the evidence for the involvement of microtubules in the intracellular transport of insulin-storage granules prior to their secretion from the pancreatic B cell relies on the use of antimitotic drugs, e.g. colchicine or vinblastine, which inhibit the insulin-secretory process. The specificity of some of these agents has occasionally been questioned since the drugs have been found in some conditions to affect other aspects of B-cell metabolism (for review see Howell & Tyhurst, 1982). The case for the involvement of microtubules in insulin secretion would be correspondingly strengthened if other agents which are known to interfere with microtubule function were also shown to alter rates of insulin secretion. We report here that taxol, an antimitotic agent derived from the western yew plant, which promotes polymerization of cytoplasmic microtubules (Schiff & Horwitz, 1979, 1980; de Brabander et al., 1980), and nocodazole, a drug which causes disintegration of microtubules to their constituent subunits (de Brabander et al., 1976), each has profound effects on insulin secretion from isolated rat islets of Langerhans.

Materials and Methods

Isolation of islets
A bicarbonate-buffered medium (Gey & Gey, 1936) containing 5.5 mM glucose and gassed to pH 7.4 with 95% O₂ : 5% CO₂ was used throughout. Islets were isolated from male WAG rats (150-200 g body wt.) by a collagenase digestion procedure (Howell & Taylor, 1966). Taxol was kindly provided by Dr. J. Douros, Drug Synthesis in
Chemistry Branch, National Cancer Institute, NIH, Maryland, U.S.A. Nocodazole (methyl-5-(2-thienylcarbonyl)-1H-benzimidazol-2-yl carbamate) was from Sigma Ltd., Poole, Dorset, U.K.

**Insulin secretion in static incubations**

After isolation the islets were preincubated in medium containing 5.5 mM glucose for 30 min at 37°C with or without the addition of taxol (0-20 μM) or nocodazole (0-20 μM). At the end of this period they were distributed into medium containing 1 mg/ml albumin (Armour Pharmaceuticals Ltd., Eastbourne, Sussex) with the following additions: 5.5 mM glucose, 20 mM glucose, or 20 mM glucose + 0.1 mM IBMX, with or without taxol or nocodazole. This protocol allowed the islets to be pre-exposed to the drugs for 30 min before their effects on secretion were tested. After 60 min incubation at 37°C, samples of medium were removed for assay of their insulin content, and islets fixed for electron microscopy.

**Insulin secretion in perifusion**

Isolated islets were pre-exposed to the drugs during a 30-min static incubation before testing in the perifusion system. For this purpose islets were placed on a 10-μM-mesh nylon filter (Plastock Ltd., Birkenhead) in a Swinnex Filter holder (Millipore Corp.) and perfused at 37°C using a Gilson HP8 peristaltic pump (Anachem Ltd., Luton, Beds.). The flow rate was 1 ml/min and fractions of 1 ml were collected and stored at -20°C for immunoassay at a later date.

**Insulin immunoassay**

The insulin content of media was determined by radioimmunoassay, utilizing guinea-pig anti-insulin serum kindly donated by Dr. W. Montague, University of Leicester, rat insulin standards purchased from Novo Laboratories, Denmark, and 125I-labelled bovine insulin iodinated in our laboratory by a chloramine-T procedure (Hunter & Greenwood, 1962).

**Electron microscopy**

After incubation, the islets were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer for 60 min, post-fixed in 2% OsO₄ in similar buffer, dehydrated in ethanol, and embedded in epoxy resin by a standard procedure. Thin sections were stained with saturated uranyl acetate in 50% ethanol and examined in an AEI EM6B electron microscope.

**Results and Discussion**

Both taxol and nocodazole have profound effects on insulin secretion from isolated rat islets of Langerhans when used at concentrations which in other systems appear to exert specific effects on microtubule assembly in the case of taxol (Schiff & Horwitz, 1979, 1980), or disassembly in the case of nocodazole (de Brabander et al., 1976). Thus in static incubations both agents significantly altered the secretory responses to both 20 mM glucose (Fig. 1a,b) and 20 mM
Fig. 1. Effect of taxol (a) and nocodazole (b) on basal (5 mM glucose) and 20-mM-glucose-stimulated insulin release from isolated rat islets of Langerhans in static incubations. Islets were pre-incubated with the same concentration of drug for 30 min in 5 mM glucose before testing their secretory responses. * indicates value significantly different from 20 mM control (P < 0.05). Means ± S.E.M. of 8 observations are shown.
Fig. 2. Effects of 10 μM taxol (a) and 2 μM nocodazole (b) on basal (5 mM glucose) and 20-mM-glucose-stimulated insulin secretion from isolated rat islets of Langerhans in a perifusion system. Islets were pre-incubated with the same concentration of each drug for 30 min in 5 mM glucose before testing their secretory responses. Means ± S.E.M. for 4 experiments are shown.
Fig. 3. (Above and overleaf) Ultrastructure of islet cells after exposure to 10 μM taxol for 90 min (a) or 2 μM nocodazole (b). Increased numbers of microtubules (arrows) were observed in taxol-treated cells (c). The curved arrow shows a microtubule in direct contact with the membrane of an insulin-storage granule. (a) x approx. 10 700 (b) x approx. 13 400 (c) x approx. 40 000.

glucose in the presence of isobutyl methylxanthine (results not shown) without significantly affecting their rate of release seen at a non-stimulatory (5 mM) glucose concentration. Perifusion experiments showed that 10 μM taxol and 2 μM nocodazole each significantly inhibited glucose-induced insulin secretion provided that the islets were pre-exposed to the compound for a 30-min period before the perifusion was started (Fig. 2a,b). In the case of nocodazole this almost complete inhibition lasted only 11 min after stimulation by glucose and was followed by a progressive escape from the effects of the inhibitor; the reason for this is not clear. Ultrastructural studies showed that neither taxol nor nocodazole had deleterious effects on the structure of the pancreatic A, B, or D cells of the islets (Fig. 3a,b), while there were indications of increased numbers of cytoplasmic microtubules in those islets which had been exposed to taxol (Fig. 3).

Thus taxol and nocodazole, two agents which interfere specifically with microtubule function in somatic cells by completely different mechanisms, both inhibit rates of glucose-induced insulin secretion. This further strengthens the hypothesis that the dynamic regulation of microtubule polymerization-depolymerization may be of critical importance in the intracellular transport of insulin-storage granules before their ultimate secretion by exocytosis.
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References

Gey GO & Gey MK (1936) Amer. J. Cancer 27, 45-76.
Schiff PB, Grant J and Horwitz SB (1979) Nature 277, 665-667