Insulin Release and the Microtubular System of the Islets of Langerhans IDENTIFICATION AND CHARACTERIZATION OF TUBULIN-LIKE PROTEIN

By WILLIAM MONTAGUE

King's College Hospital Medical School, Denmark Hill, London SE5 8RX, U.K.

and SIMON L. HOWELL and IRENE C. GREEN

School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, Sussex, U.K.

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1. Incubation of islets of Langerhans *in vitro* in the presence of colchicine produced a progressive inhibition of the insulin-secretory response to glucose, which was dependent on the time of incubation. 2. The uptake of [3 H]colchicine by islet cells was a rapid process, equilibrium being reached in less than 30 min. Part of the colchicine taken up was bound to protein material, which was recovered largely in a post-microsomal supernatant fraction prepared from the islets. In contrast with this rapid uptake, the binding of colchicine by islet-cell proteins in intact islets or in islet homogenates was a slow process, and equilibrium was not reached for 60–90 min. After an initial 30 min delay, the time-course of the binding of [3 H]colchicine to islet-cell proteins paralleled that for the inhibitory effect of colchicine on insulin release. 3. Some purification of the colchicine-binding material present in islet homogenates could be achieved by precipitation of the protein with 2 mM-CaCl₂ (2.8-fold). However, ion-exchange chromatography on DEAE-Sephadex produced a further 27-fold purification on elution with 0.6 M-NaCl. 4. Colchicine-binding protein prepared from islets by ion-exchange chromatography showed an intrinsic association constant for colchicine of 1.4 μ M and an apparent molecular weight on gel filtration of 110000. 5. These results suggest that colchicine-binding protein in islet cells closely resembles tubulin extracted from other tissues. The delayed effectiveness of colchicine in inhibiting insulin secretion is not due to poor penetration of colchicine into the cells but rather to slow binding of the alkaloid to islet-cell tubulin. It seems likely that, as in other tissues, this binding prevents polymerization of the tubulin into microtubules, and thus interferes with the release process.

Several studies have shown that the alkaloids colchicine, vinblastine and vincristine inhibit the insulin secretory response of the pancreatic B-cell to stimulation by glucose, amino acids and theophylline (Lacy *et al.*, 1968; Malaisse *et al.*, 1971; Malaisse-Lagae *et al.*, 1971; Lacy *et al.*, 1972). These alkaloids have been shown to disrupt microtubular function in a wide variety of cell types (Eigsti & Dustin, 1955; Malawista, 1965; Borisy & Taylor, 1967; Bensch & Malawista, 1969) and to inhibit hormone release from several endocrine tissues (Williams & Wolff, 1970; Kraicer & Milligan, 1971; Poisner & Bernstein, 1971). Thus the inhibitory effects of *Vinca* alkaloids on insulin secretion have been taken to indicate that functional microtubules might be an essential component in the sequence of events leading to the release of insulin. Although ultrastructural studies of pancreatic B-cells have demonstrated the presence of microtubules (Lacy *et al.*, 1968; Malaisse-Lagae *et al.*, 1971), no studies have yet been reported on the biochemical characteristics of microtubules in islets, or on their interaction with *Vinca* alkaloids,

which may form the basis of the inhibitory effects of these agents on insulin secretion.

The purpose of the present work was to study the role of microtubular function in insulin release at the biochemical level, and to determine whether the inhibitory effect of colchicine on insulin release was mediated through the microtubular system of the B-cell. In addition, the present paper reports results of attempts to isolate and partially characterize microtubular protein (tubulin) from islet cells by the use of [3 H]colchicine, an agent that binds specifically to tubulin, and thus provides the basis for a quantitative assay of tubulin in intact islet cells, in subcellular fractions and in extracts that have been subjected to a variety of purification procedures.

Experimental

Reagents

The following reagents were purchased from Sigma (London) Chemical Co., Kingston-on-Thames,

Surrey KT2 7BH, U.K.: colchicine, $^2\text{H}_2\text{O}$, GTP, EGTA [ethanedioxybis(ethylamine)tetra-acetate], cytochrome *c*, bovine serum albumin and ovalbumin. 3-Isobutyl-1-methylxanthine was from Searle Co., Chicago, Ill., U.S.A. Sephadex G-200, DEAE-Sephadex A-50 and Dextran Blue were from Pharmacia (G.B.) Ltd., Ealing, London W5 5SS, U.K. Vinblastine sulphate (Velbe) was from Eli Lilly Co., Basingstoke, Hants., U.K. DEAE-cellulose paper (DE 81) and glass-fibre discs (GF/C) were from Whatman Ltd., Maidstone, Kent, U.K. [^3H]Colchicine (specific radioactivity 2Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

All other reagents were of the purest grade available commercially and were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K.

Methods

Source of tissue. Islets of Langerhans were prepared by the collagenase digestion of pancreatic tissue taken from female Sprague-Dawley rats or from male guinea pigs of the Duncan-Hartley strain. The technique has been described in detail by Howell & Taylor (1968). In all experiments islets were isolated and incubated in a bicarbonate-buffered medium (Gey & Gey, 1936) supplemented with 2mM-glucose. In experiments where insulin secretion was to be measured bovine serum albumin (2mg/ml) was also added to the incubation medium.

Measurement of insulin secretion. Isolated islets were preincubated for 30 min at 37°C in medium with 2mM-glucose. The incubation medium for insulin-secretion studies contained 12mM-glucose alone or with various concentrations of colchicine. Groups of three islets were placed in 0.6ml of incubation medium in tubes (50mm×11mm) which were capped after gassing with O_2+CO_2 (95:5). The tubes were incubated for 30, 60 or 90 min at 37°C with shaking. Samples of the incubation medium were removed at the end of the incubation periods, diluted with potassium phosphate buffer, pH 6.5, and assayed for insulin by the double-antibody technique of Hales & Randle (1963).

Protein determination. Protein was measured by the method of Lowry *et al.* (1951), with crystallized bovine serum albumin as standard.

Assay for colchicine-binding activity. The assay system used to measure colchicine-binding activity in islet cells was based on that described by Borisy (1972). The assay depends on the observation that colchicine-binding protein but not free colchicine is retained by DEAE-cellulose. Portions (0.2ml) of samples to be analysed were incubated at 37°C for 90 min with 0.2ml of potassium phosphate buffer (pH 6.5; 20mM) containing GTP (0.1mM), sucrose (0.3mM), $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ (5mM), EGTA (0.5mM)

and colchicine (3×10^5 c.p.m. per tube; $3\mu\text{M}$). At the end of the incubations the samples were filtered through a stack of four DEAE-cellulose paper discs (2.5cm diam.) and the discs were washed with $5\times 2\text{ml}$ of the ice-cold phosphate buffer. Blank values were obtained by using portions of the sample buffer alone in the incubation. After filtration the discs were placed in counting vials, 10ml of scintillator was added and radioactivity was measured as described below. Owing to the presence of impurities in The Radiochemical Centre [^3H]colchicine, which gave high blank values, it was necessary to purify the colchicine before use. This was accomplished by filtration of the colchicine through a stack of four DEAE-cellulose filter-paper discs (2.5cm diam.). The impurity was retained on the paper and the colchicine was washed through with 2ml of the potassium phosphate buffer (50mM, pH 7.4), collected and stored at -20°C .

Determination of colchicine uptake by islets. Islets isolated from rat pancreas were incubated at 37°C in groups of 30 in 0.6ml of bicarbonate-buffered medium (Gey & Gey, 1936) containing colchicine (0.1mM) and [^3H]colchicine (2×10^6 c.p.m./ml). At various time-intervals after the start of the incubation the medium was removed from the islets and the islets were washed quickly with 10ml of cold (0°C) medium containing colchicine (0.1mM). This was done either by resuspending the islets twice in 5ml of buffer and allowing them to settle through the buffer or by filtration of the islets on to glass-fibre membranes (2.5cm diam.) and washing them on the membrane. The results obtained with the two techniques were identical, and since the filtration technique was the easiest to perform it was used as a routine. After filtration the membranes with attached islets were transferred to counting vials and 10ml of scintillator was added before determination of radioactivity.

Determination of colchicine binding in intact islets. Islets were incubated as described above for the determination of colchicine uptake. At the end of the period of incubation the medium was removed from the islets which were then washed by resuspension in 5ml of the ice-cold bicarbonate buffer containing colchicine (0.1mM). The islets were then homogenized in 1ml of potassium phosphate buffer (pH 6.5, 20mM) containing sucrose (0.3mM), $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ (5mM) and EGTA (0.5mM). The homogenate was then filtered through a stack of DEAE-cellulose membranes as described above, to separate protein-bound colchicine from free colchicine, and the membranes were then transferred to counting vials, 10ml of scintillator was added and radioactivity determined.

Preparation of subcellular fractions. Subcellular fractions were prepared from rat islets of Langerhans as described in detail by Howell *et al.* (1969). Islets were homogenized in 1ml of sodium phosphate

buffer (10mM, pH6.5) containing 0.3M-sucrose. This and all subsequent steps were performed at 4°C. The homogenate was separated by differential centrifugation into nuclear material and debris (600g for 5min), a mitochondrial and secretory granule fraction (20000g for 10min) and microsomal pellet (105000g for 60min), and a post-microsomal supernatant fraction. Particulate material was resuspended in the homogenization buffer.

Subcellular distribution of colchicine-binding activity. Two methods were used to study the subcellular distribution of colchicine-binding activity in islets of Langerhans. Islets were either incubated for 90min in the presence of [3 H]colchicine (2×10^6 c.p.m./ml; 20 μ M), washed once with incubation buffer and then the subcellular fractions prepared, or subcellular fractions were prepared from freshly isolated islets and incubated for 90min in the presence of [3 H]colchicine (3×10^5 c.p.m./ml; 3 μ M). All fractions were filtered through DEAE-cellulose filter discs as described above to separate protein-bound colchicine from free colchicine and the radioactivity retained on the discs was measured after the addition of scintillator.

Precipitation of islet-cell colchicine-binding protein. A homogenate of 500 islets of Langerhans was prepared at 4°C in 1ml of phosphate buffer (pH6.5, 20mM) containing $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (5mM), GTP (0.1mM), EGTA (1mM) and sucrose (0.3M). It was centrifuged at 20000g for 10min and portions of the supernatant were treated with one of the following: vinblastine (final concn. 2mM); CaCl_2 (final concn. 20mM); or $(\text{NH}_4)_2\text{SO}_4$ (final concn. 45% satn.). The treated supernatants were left at 4°C for 30min before centrifugation at 20000g for 10min. The resulting supernatants and the pellets, which had been resuspended in phosphate buffer (containing 10mM-EGTA for the CaCl_2 -treated pellet) were dialysed against 1 litre of water for 4h at 4°C with three changes of the water. Samples were then removed and assayed for protein and colchicine binding.

Gel filtration. Gel filtration was performed at 4°C on columns (40cm $\times$ 1cm) of Sephadex G-200 equilibrated with 50mM-sodium phosphate buffer, pH7.4, containing 5mM- $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. Samples (0.4ml) were applied to the column and eluted with the equilibration buffer, 0.6ml fractions being collected. Each fraction was analysed for protein and colchicine-binding activity. For molecular-weight determinations the columns were calibrated by chromatography of Blue Dextran (2000000), ovalbumin (45000), aldolase (158000), cytochrome *c* (12500) and insulin (6000). Elution positions were determined by measurements of the E_{280} of the column effluent in each case.

Ion-exchange chromatography. Ion-exchange chromatography was performed on a column (2.0cm $\times$ 0.5cm) of DEAE-Sephadex A-50 equilibrated with

potassium phosphate buffer (20mM, pH6.5). Samples in phosphate buffer were applied to the column, which was then washed with 5ml of phosphate buffer followed by 5ml of buffer containing 0.3M-NaCl and finally with 5ml of buffer containing 0.6M-NaCl. Fractions (0.5ml) were collected and analysed for protein and colchicine-binding activity.

Radioactivity determination. Radioactivity was determined in a Beckman LS233 liquid-scintillation spectrometer. The scintillator used was toluene-Triton X-100-2,5-diphenyloxazole (140:60:1, v/v/w). Radioactivity on filter discs was determined without drying the discs. After scintillator had been added to the discs they were left overnight before the addition of 10 drops of water to clear the solution. The counting efficiency of protein-bound colchicine absorbed on to discs was 20%, and for colchicine in solution 30%.

Results

Colchicine inhibits the release of insulin in response to glucose (12mM) from rat islets of Langerhans. The

Fig. 1. Effect of various concentrations of colchicine on insulin release from islets of Langerhans incubated *in vitro*

Groups of three islets were incubated for 60min with 12mM-glucose alone or with various concentrations of colchicine and the insulin release into the medium was determined as described in the Experimental section. Each value is the mean, $\pm$ S.E.M. (indicated by the bars), of 15 observations.

concentration-dependency of this effect of colchicine is shown in Fig. 1. A concentration of 0.1mM produced the maximum effect and was used in all studies with intact islets. The time-course for the inhibitory effect of this concentration of colchicine on insulin release is shown in Fig. 2. The alkaloid had no effect on release during the

Fig. 2. Time-course for the uptake and binding of colchicine and for its inhibitory effect on insulin release in islets of Langerhans

Groups of 30 islets were incubated for various periods of time with 0.1mM-colchicine and [3 H]colchicine (2×10^6 c.p.m.), and the uptake (O) and binding (Δ) of [3 H]-colchicine by the same islets was measured as described in the text. The inhibitory effect of this concentration of colchicine on insulin release in response to 12mM-glucose over the same time-periods was also determined and is plotted as percentage inhibition of the insulin released from control islets in the absence of colchicine (●). Each value is the mean of eight observations. The vertical bars represent the S.E.M. values.

Fig. 3. Time-course for the binding of [3 H]colchicine to islet-cell proteins

A homogenate was prepared from 300 rat islets and samples were incubated in the presence of [3 H]colchicine (3×10^5 c.p.m.; $3 \mu\text{M}$) for various time-periods as described in the Experimental section. At the end of the incubation free colchicine was separated from protein-bound colchicine by filtration through DEAE-cellulose filters and the radioactivity remaining on the discs was determined. Each value is the mean $\pm$ S.E.M. of six observations.

first 30min of incubation, but after 30min a progressive inhibitory effect was observed.

In contrast with its slow effect on insulin release, the uptake of colchicine by islet cells was a relatively rapid process, which saturated by 30min (Fig. 2). Part of the colchicine taken up was bound to islet-cell proteins. The binding of colchicine to islet-cell proteins, both in intact islets and in islet homogenates (Figs. 2 and 3), was a slow process, equilibrium not being reached until 60min. The amount of colchicine bound was linearly related to the concentration of islet-cell protein (Fig. 4).

When subcellular fractions were prepared from islets which had previously been incubated in the presence of [3 H]colchicine, 75% of the bound colchicine was recovered in a post-microsomal supernatant fraction and 15, 7 and 3% in the nuclear, granule and microsomal fractions respectively (Table 1). A similar subcellular distribution for the colchicine-binding activity was obtained when the subcellular fractions were prepared first and subsequently incubated in the presence of [3 H]colchicine (Table 1).

An attempt was made to purify and characterize the colchicine-binding material from islet cells by using a number of techniques that have been used to purify similar material from other tissues (Table 2). Since the major portion of the activity was in the post-microsomal supernatant fraction prepared from an islet-cell homogenate, the preparation of this fraction was the first stage of the purification. Various agents, including vinblastine,

Fig. 4. Relationship between tissue concentration and [3 H]colchicine binding

An homogenate of 400 rat islets of Langerhans was prepared in and diluted serially with phosphate buffer (20mM; pH 6.5) containing sucrose (0.3M). Samples of the resulting homogenates were incubated with [3 H]colchicine for 90min and the colchicine bound was determined as described in the text. Each point is the mean $\pm$ S.E.M. of six observations.

Table 1. Subcellular distribution of colchicine-binding activity in islets of Langerhans

Subcellular fractions were prepared from homogenates of islets which had previously been incubated for 90min either in the presence (fractions a) or absence (fractions b) of [³H]colchicine. Fractions (a) were assayed for protein-bound [³H]-colchicine as described in the text. Fractions (b) were incubated for 90min with [³H]colchicine and then the protein-bound colchicine was determined as described in the text. The results are given as the means of eight observations. The s.d. did not exceed the mean value by more than 10% for any observation.

Fraction	Protein content (μg)		[³ H]Colchicine bound (c.p.m.)		[³ H]Colchicine bound (c.p.m./μg of protein)	
	(a)	(b)	(a)	(b)	(a)	(b)
Homogenate	287	250	2372	2060	8.3	8.3
Nuclear pellet	87	108	352	337	4.0	3.1
Granule pellet	50	77	160	192	3.2	2.5
Microsomal pellet	23	18	61	81	2.6	4.5
Post-microsomal supernatant	66	67	1714	1374	25.9	20.4

Table 2. Purification of islet-cell colchicine-binding activity

The activity in an homogenate prepared from 1000 guinea-pig islets was purified by the procedures shown (see the text for details). Colchicine-binding activity and protein content were determined at each stage of the purification as described in the text. The results presented are the means of duplicate determinations performed on three separate occasions.

Stage	Protein content (μg)	[³ H]Colchicine bound (c.p.m.)	[³ H]Colchicine bound (c.p.m./μg of protein)	Degree of purification	Recovery (%)
(1) Homogenate	2500	20660	8.3	1	100
(2) 105 000g supernatant	674	13 740	20.4	2.5	66
(3) CaCl ₂ precipitation	127	7309	57.6	6.9	35
(4) DEAE-Sephadex	4	6266	1564	188	30

Fig. 5. Ion-exchange chromatography of colchicine-binding protein from islets of Langerhans

Material obtained at stage (3) of the purification procedure shown in Table 2 was applied to a column (2cm×0.5cm) of DEAE-Sephadex and eluted with 5ml of phosphate buffer (pH 6.5; 10mM), followed by 5ml of the phosphate buffer containing 0.3M-NaCl and finally with 5ml of the phosphate buffer containing 0.6M-NaCl. Fractions (1ml) were collected and assayed for colchicine binding (—) and protein (----) (see the text for details).

Fig. 6. Chromatography on Sephadex G-200 of colchicine-binding protein from islets of Langerhans

The supernatant fraction from an homogenate of 400 rat islets of Langerhans was applied to a column (40cm×1cm) of Sephadex G-200 and eluted with phosphate buffer (50mM; pH 6.5). Fractions (1ml) were collected and assayed for protein (----) and colchicine binding (—) (see the text for details).

Ca²⁺ and (NH₄)₂SO₄ (Wilson *et al.*, 1970; Weisenberg *et al.*, 1968; Bhattacharyya & Wolff, 1974) have been shown to precipitate colchicine-binding protein extracted from other cells. All three agents appeared to precipitate colchicine-binding protein, although the degree of purification was small (approx. twofold) and recoveries were low (approx. 50%). The precipitates obtained with vinblastine and (NH₄)₂SO₄

Fig. 7. Effect of colchicine concentration on the binding of colchicine to protein extracted from islets of Langerhans

Colchicine-binding activity of the purified colchicine-binding protein (stage 4) was determined as described in the text with various concentrations of $[^3\text{H}]$ colchicine. The results are the means of six observations and have been plotted as described by Scatchard (1949) to allow an estimate of the binding constant of the protein for colchicine.

were small and were found to be difficult to handle. Since calcium phosphate co-precipitated with the colchicine-binding protein in the procedure using CaCl_2 precipitation, the precipitate was easy to collect. This procedure was therefore used as the second step of the purification procedure. The material precipitated by Ca^{2+} was dissolved in potassium phosphate buffer (20mM, pH 6.5) containing 10mM-EGTA and dialysed against water, before application to a column of DEAE-Sephadex. The major portion of the islet-cell protein was eluted from the column at low ionic strength (0–0.3M-NaCl). Colchicine-binding activity was eluted after the major protein peaks with 0.6M-NaCl (Fig. 5). The use of DEAE-Sephadex gave a marked increase in the degree of purification with little loss of activity (Fig. 5 and Table 2). The partially purified material from the DEAE-Sephadex step ran as a single peak when chromatographed on Sephadex G-200 in a position corresponding to a molecular weight of 110000. A molecular weight of 110000 was also obtained for the colchicine-binding activity present in the supernatant fraction of an islet-cell homogenate (Fig. 6). The association constant for colchicine of the partially purified material was $1.4\mu\text{M}$ (Fig. 7).

Discussion

Colchicine has been shown to affect a large number of cellular functions, including secretion, which depend either directly or indirectly on microtubules (for review see Olmsted & Borisy, 1973). The effects of the alkaloid appear to result from its

binding to microtubule subunits, thereby preventing their polymerization into microtubules (Wilson *et al.*, 1974). Colchicine inhibits the release of insulin in response to glucose from islets of Langerhans and it has been assumed that this effect is related to a disruption of the microtubular system in the B-cell (Lacy *et al.*, 1968; Malaisse *et al.*, 1971). However, studies designed to define the mode of action of colchicine in islet cells in biochemical terms have so far been lacking. In the present study we have investigated the uptake and binding of colchicine by islets of Langerhans and have attempted to characterize the colchicine-binding protein in islet cells. Since islets of Langerhans of the rat and guinea pig consist of 70–80% B-cells and only 20–30% A-cells the results obtained have been assumed to relate to the B-cell.

It has been a general finding that the inhibitory effect of colchicine on insulin release is dependent on a long time of exposure of the islet cells to colchicine (Malaisse *et al.*, 1971; Lacy *et al.*, 1972). In the experiments reported here no effect of colchicine was observed during the first 30min of incubation, although a progressive inhibitory effect was observed at 60 and 90min (Fig. 2). The inhibitory effect of colchicine on secretion from other tissues such as the thyroid has also been shown to require a prolonged incubation of the tissue with the alkaloid (Williams & Wolff, 1972).

It is clear from the present experiments that the delay in the onset of the effect of colchicine on insulin secretion is unlikely to be due to poor or delayed penetration of colchicine into islets, since this appears to be a rapid process (Fig. 2). A comparable rapid uptake of colchicine has been demonstrated in the liver, spleen and pancreas (Gillespie, 1971), and thyroid (Williams & Wolff, 1970). In contrast with the rapid uptake of colchicine by tissues such as islets, the binding of colchicine to tissue proteins may be a relatively slow process, equilibrium not being reached for at least 60min (Fig. 3; Owells *et al.*, 1972; Williams & Wolff, 1972; Wilson & Meza, 1973). Fig. 2 shows that after 60 or 90min incubation the increasing effectiveness of colchicine as an inhibitor of insulin secretion closely parallels its rate of binding to islet protein. Presumably the binding that has occurred during the first 30min of incubation is insufficient to affect secretory function significantly.

The similarity in the time-course for the binding of colchicine to islet proteins and its effect on secretion (Fig. 2) suggested that the two might be related. These observations prompted an investigation into the characteristics of the colchicine-binding material in islets and a comparison of its properties with those of microtubule subunit protein (tubulin) in other tissues. Colchicine-binding activity has been largely localized in the soluble fraction

of homogenates from several tissues, including brain (Weisenberg *et al.*, 1968), liver, pancreas and spleen (Gillespie, 1971), and thyroid (Williams & Wolff, 1970), although significant activity has also been demonstrated in membrane fractions from liver and brain (Wilson, 1970; Stadler & Franke, 1972, 1974).

The colchicine-binding activity in islets of Langerhans appeared to be predominantly confined to the post-microsomal supernatant fraction, and material from this fraction was used in studies to determine the nature of the colchicine-binding material in islet cells.

Colchicine-binding protein has been isolated and purified from a number of mammalian tissues, including brain (Weisenberg *et al.*, 1968; Bryan & Wilson, 1971; Eipper, 1972) and thyroid (Williams & Wolff, 1970; Bhattacharyya & Wolff, 1974) and has been identified as microtubule subunit protein (tubulin). The colchicine-binding protein isolated from islets of Langerhans has many of the characteristics of tubulin isolated from other tissues. Thus the protein is found largely in the post-microsomal supernatant fraction prepared from tissue homogenates and it has a molecular weight of 110000. The protein binds colchicine slowly; equilibrium is not reached for at least 60 min at 37°C. The binding constant for colchicine is in the micromolar range. The protein is precipitated by vinblastine and Ca^{2+} ions. It binds to DEAE-cellulose and DEAE-Sephadex and is eluted only with high salt concentrations (0.6M-NaCl), under conditions that are known to result in elution of tubulin. Thus it seems likely that the colchicine-binding protein isolated from islets of Langerhans may be the microtubular subunit protein of islet cells. Colchicine is believed to exert many of its effects by binding to microtubule subunits, thus preventing their polymerization to form microtubules (Wilson *et al.*, 1974) and the results of the present study suggest a similar mode of action in the B-cell. Vinblastine and vincristine precipitate large amounts of protein (tubulin) in a crystalline form within B-cells (Malaisse-Lagae *et al.*, 1971), presumably rendering it unavailable for polymerization into microtubules, and both of these agents inhibit insulin secretion (Malaisse *et al.*, 1971; Lacy *et al.*, 1972). By contrast, treatment with $^2\text{H}_2\text{O}$, which may stabilize intact microtubules (preventing their breakdown into subunits), also inhibits rates of insulin secretion (Malaisse-Lagae *et al.*, 1971; Lacy *et al.*, 1972). Each of these agents therefore alters the equilibrium between subunits and intact microtubules in the B-cell and affects rates of secretion. It would be of interest to determine whether similar changes in equilibrium can be elicited by physiological agents that are capable of altering rates of insulin secretion.

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