Effects of monensin on metabolism of isolated rat islets of Langerhans

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1. Monensin, a univalent ionophore, is a carboxylic acid produced by *Streptomyces cinnamonensis*. It will complex various alkali-metal ions, but most readily binds Na^+ . Because of interest in the possible role of Na^+ in the regulation of insulin secretion, we examined its effects on several aspects of the metabolism of isolated rat islets of Langerhans. 2. The ionophore inhibited glucose-stimulated insulin release in a concentration-dependent manner, completely inhibiting secretion evoked by 20 mM-glucose at concentrations as low as 0.1 μM in static incubations. In perfusion experiments, both phases of insulin release were equally affected. 3. Monensin (0.1 μM) had no significant effect on glucose oxidation as measured by the generation of $^{14}\text{CO}_2$ from [^{14}C]glucose. 4. Monensin increased the rate of $^{22}\text{Na}^+$ efflux from preloaded islets and net $^{22}\text{Na}^+$ uptake over 30 min, in the absence of changes in islet volume or extracellular space. The ionophore increased the Rb^+/K^+ permeability of islet cells, as shown by its inhibition of $^{86}\text{Rb}^+$ retention and stimulation of $^{86}\text{Rb}^+$ efflux. 5. At 0.1 μM , monensin abolished glucose-stimulated $^{45}\text{Ca}^{2+}$ uptake by islets during 5 min incubations, and stimulated $^{45}\text{Ca}^{2+}$ efflux from preloaded islets perfused with Ca^{2+} -free medium, even in the complete absence of extracellular Na^+ . 6. Studies of the uptake of ^{14}C -labelled 5,5-dimethyloxazolidine-2,4-dione showed that 0.1 μM -monensin increased net intracellular pH from 7.05 to 7.13. 7. Monensin has widespread, complex, effects on the secretory responses and ion handling by the B cells, which are difficult to interpret in terms solely of actions as a Na^+ ionophore.

A role of Na^+ in the regulation of insulin secretion has been suspected since the original observation that its omission from incubation media led to inhibition of glucose-stimulated insulin secretion (Milner & Hales, 1968). Removal of extracellular Na^+ also inhibits insulin biosynthesis (Pipeleers *et al.*, 1973) and increases $^{45}\text{Ca}^{2+}$ uptake by islet cells (Hellman *et al.*, 1971). Glucose uptake and utilization are decreased in similar conditions (Ashcroft *et al.*, 1972; Matschinsky & Ellerman, 1973; Hellman *et al.*, 1974). More direct evidence that an increase in B-cell Na^+ concentration may stimulate insulin release was provided by studies with veratridine, a Na^+ -channel activator, which stimulates insulin secretion (Lowe *et al.*, 1976; Donatsch *et al.*, 1977).

Monensin is a carboxylic ionophore with a greater affinity for Na^+ than for any other physiological univalent cation (Pressman, 1968, 1973), and is capable of transporting metal ions or protons across artificial or biological membranes. In view of its apparent specificity for Na^+ , it was

hoped that this agent might be useful in further elucidating the role of this ion in the insulin-secretory process. Preliminary reports of part of this work have appeared in abstract form (Smith *et al.*, 1982; Smith & Howell, 1983).

Experimental

Isolation and incubation of islets of Langerhans

Islets were isolated by a standard collagenase-digestion procedure (Howell & Taylor, 1966) from 150–250 g inbred male Wistar rats (WAG), obtained from Charing Cross Hospital Medical School, London, U.K. Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932) was used throughout the isolation, and the islets were then preincubated for 30 min in a similar buffer at 37°C. For static incubation experiments, groups of three islets were distributed into tubes containing 1 ml of buffer with the addition of 2.8 mM- or 20 mM-glucose and 0.5 mg of bovine serum albumin/ml. For perfusion experiments, groups of islets were

transferred to a 10 μm -mesh nylon filter in a Swinnex filter holder (Millipore Corp.) and perfused with Krebs–Ringer bicarbonate buffer with a Gilson HP8 peristaltic pump (Gilson, Villiers-le-Bel, France) at a flow rate of 1 ml/min.

Radioisotope-uptake experiments

In Na^+ -uptake experiments, groups of islets were incubated in 0.4 ml of Krebs–Ringer bicarbonate with 25 μCi of $^{22}\text{NaCl}$ /ml, 30 μCi of $[\text{U-}^{14}\text{C}]\text{sucrose}$ /ml and 35 μCi of $^3\text{H}_2\text{O}$ /ml. For $^{86}\text{Rb}^+$ uptake, 15 μCi of $^{86}\text{Rb}^+$ /ml and 25 μCi of 6,6'(n)- ^3H sucrose/ml were used, and 1 μCi of $^{45}\text{CaCl}_2$ /ml together with 2 μCi of [6,6'(n)- ^3H]sucrose/ml were used for studies of Ca^{2+} uptake. In each case the islets were separated from the incubation medium by centrifugation through a mixture of dibutyl phthalate and dinonyl phthalate (10:3, v/v) as described by Wollheim *et al.* (1977) and modified by Yaseen *et al.* (1982). After freezing in liquid N_2 , the bottom of the tube containing the islet pellet was cut off and the tissue dissolved in 0.5 ml of 2M HClO_4 for 30 min. After neutralization with 0.2 ml of 1M-NaOH, 5 ml of scintillant (Ready-Solv HP; Beckman) was added and the sample counted for radioactivity in a Beckman LS 7500 liquid-scintillation spectrometer by using double- or triple-isotope programmes as appropriate.

Radioisotope-efflux experiments

Groups of islets were prelabelled by incubation with $^{22}\text{NaCl}$ (100 μCi /ml, for 30 min), $^{86}\text{RbCl}$ (100 μCi /ml, for 120 min) or $^{45}\text{CaCl}_2$ (100 μCi /ml, for 120 min) as appropriate, in Krebs–Ringer bicarbonate containing 2.8 mM-glucose and 0.5 mg of albumin/ml at 37°C. Groups of 100–300 islets were then transferred on nylon filters to perfusion chambers and perfused with Krebs–Ringer bicarbonate with various additions at a rate of 1 ml/min for 60 min. Samples (0.5 ml) of each fraction were collected and mixed with 3.5 ml of scintillation fluid [0.5% 2,5-diphenyloxazole in toluene/Triton X-100 (2:1, v/v)] for radioactivity counting in a Beckman LS 7500 scintillation spectrometer. Radioactivity remaining in the islets at the end of the perfusion was detected by dissolving the tissue in HClO_4 , neutralizing and counting as described above. The fractional outflow rate (FOR), as percentage of total content of radioisotope effluxed/min, was calculated with a computer program.

Insulin radioimmunoassay

Insulin content of media was determined by a standard immunoassay with guinea-pig anti-insulin serum (gift from Dr. W. Montague, University of Leicester), rat insulin standards and bovine insulin [^{125}I]iodinated in our laboratory by the method of Hunter & Greenwood (1962). Antibody-

bound insulin was separated by poly(ethylene glycol) precipitation and centrifugation (Desbuquois & Aurbach, 1971).

Glucose oxidation

The rate of production of $^{14}\text{CO}_2$ from $[\text{U-}^{14}\text{C}]\text{-glucose}$ was determined by a modification of the procedure described by Ashcroft *et al.* (1972). Islets were incubated in 20 mM-glucose containing 10 μCi of $[\text{U-}^{14}\text{C}]\text{glucose}$ /ml for 2 h. After incubation $^{14}\text{CO}_2$ was released by addition of 100 μl of 2M-HCl, and trapped with methylbenzethonium chloride (Hyamine hydroxide). After 60 min radioactivity was determined after addition of scintillant (Ready-solv HP, Beckman) in a Beckman LS 7500 liquid-scintillation spectrometer.

Intracellular pH determination

Groups of 100–150 islets were incubated in Krebs–Ringer bicarbonate/Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] medium (pH 7.4) in which the NaHCO_3 concentration was lowered to 5 mM and 10 mM-Hepes was added with 2.8 mM-glucose with or without monensin (0.1 μM) for 30 min. They were then reincubated in 500 μl of similar buffer containing 7 μCi of ^{14}C -labelled 5,5-dimethyloxazolidine-2,4-dione/ml and 12.5 μCi of [6,6'(n)- ^3H]sucrose/ml for 30 min at 37°C as described by Hellman *et al.* (1972). After this incubation, groups of 10 islets were separated from the medium by centrifugation through oil and their radioactivity content was determined as described above. The intracellular pH was determined as described by Waddell & Butler (1959).

Sources of reagents

All reagents were of AnalaR grade, or the purest grade commercially available. Rat insulin standards were obtained from Novo Laboratories, Basingstoke, Hants., U.K. Monensin (sodium salt) was obtained from Calbiochem, Bishops Cleeve, Herts., U.K. $^{22}\text{NaCl}$ (carrier-free) and 5,5-dimethyl[2- ^{14}C]oxazolidine-2,4-dione (sp. radioactivity 40–60 Ci/mmol) were obtained from New England Nuclear, Dreieich, West Germany; $^{45}\text{CaCl}_2$ (sp. radioactivity 10–40 mCi/mg of Ca), $^3\text{H}_2\text{O}$ (sp. radioactivity 5 mCi/ml), $^{86}\text{RbCl}$ (sp. radioactivity 1–8 mCi/mg of Rb), $[\text{U-}^{14}\text{C}]\text{sucrose}$ (sp. radioactivity 5–15 mCi/mmol) and [6,6'(n)- ^3H]sucrose (sp. radioactivity 5–15 mCi/mmol) were obtained from Amersham International, Amersham, Bucks., U.K.

Results

Effects of monensin on insulin secretion

Monensin was found to be a potent inhibitor of 20 mM-glucose-stimulated insulin secretion, but

appeared to have no significant effect on basal (2.8 mM-glucose) insulin release. At concentrations of 0.1 μM -monensin or above, glucose-stimulated insulin release was completely inhibited (Fig. 1). The apparent K_i for the inhibitory effect of monensin in these static incubations was calculated as approx. 1–2 nM.

In perfusion experiments when monensin (10 μM) was administered simultaneously with 20 mM-glucose, no glucose-stimulated insulin secretion was observed. Monensin (10 nM) induced a 50% decrease in glucose-stimulated insulin release in perfusion when the islets were exposed to 20 mM-glucose and 10 nM-monensin at the same time (Fig. 2). If the islets were exposed to monensin (10 nM) 20 min before exposure to 20 mM-glucose, the inhibition of glucose-stimulated insulin release was even more profound (Fig. 2); the first phase of insulin secretion was almost completely abolished and the second phase was decreased by 80%.

The reversibility of these effects was investigated. Islets were exposed to 0.1 μM -monensin and 2.8 mM-glucose in static incubation for 20 min, washed four times with fresh monensin-free medium and transferred to perfusion chambers. They were then perfused with 2.8 mM-glucose for 1 h after exposure to 0.1 μM -monensin, before challenge with 20 mM-glucose; glucose-stimulated insulin secretion was not restored (results not

shown). Thus monensin inhibition of insulin secretion does not appear to be readily reversible at this concentration.

Effects on glucose oxidation

Monensin at 0.1 μM , a concentration which completely inhibited glucose-stimulated insulin secretion in static incubations (Fig. 1), had no significant effect on glucose oxidation compared with control islets incubated in the absence of monensin. The rates of glucose oxidation were 78.2 ± 12.7 and 72.5 ± 11.9 pmol/2 h per islet in the presence and absence of 0.1 μM -monensin respectively ($P > 0.8$; 15 observations in each case). Higher concentrations of monensin have not been tested.

Effects on Na^+ handling

At a concentration of 10 μM , monensin increased net $^{22}\text{Na}^+$ retention by islets by 2.5-fold, from 94.4 ± 2.5 to 238.4 ± 9.5 pmol/islet ($P < 0.001$) after 30 min incubation (Table 1). At 10 μM the ionophore increased net $^{22}\text{Na}^+$ retention by 1.5-fold, to 148.8 ± 11.5 pmol/islet ($P < 0.01$). Increasing the glucose concentration from 2.8 to 20 mM had no significant effect on the net $^{22}\text{Na}^+$ content of the islets ($P > 0.9$). There were no significant changes in islet volume or ratio of intra- to extra-cellular

Fig. 1. Effect of different concentrations of monensin on basal ($\square$) and glucose-stimulated ($\square$) secretion. The numbers of observations are in parentheses. Vertical lines represent $\pm$ S.E.M. The P values above the bars are relative to the high-glucose control.

Fig. 2. Effect of monensin on the dynamics of insulin release induced by 20mM-glucose

The glucose concentration was increased from 2.8 to 20mM at 20min. Monensin was either absent throughout (shaded area) or present at 10 μ M (●) or 10nM (■) for 20–60min, or 10nM-monensin was present at 0–60min (▲). The numbers of observations are given in parentheses.

Table 1. Effect of monensin and 20mM-glucose on net $^{22}\text{Na}^+$ uptake by islets

Islets were incubated with $^{22}\text{NaCl}$, $[\text{U-}^{14}\text{C}]\text{sucrose}$ and $^3\text{H}_2\text{O}$ at 37°C for 30min. They were separated from incubation medium by centrifugation through a mixture of dibutyl phthalate and dinonyl phthalate (10:3, v/v), and the radioactivity associated with the tissue was determined by liquid-scintillation spectrometry with a triple-isotope programme. Islet volume was calculated as equivalent to the $^3\text{H}_2\text{O}$ space, and extracellular volume as the $[\text{U-}^{14}\text{C}]\text{sucrose}$ space. The calculated intracellular Na^+ concentration assumes complete equilibration of the $^{22}\text{Na}^+$ with the Na^+ content of the islet. Results are given as means $\pm$ S.E.M. for the numbers of observations shown; significance of differences was determined by Student's *t* test.

Glucose (mM)	Monensin	Islet volume (nl)	Extracellular space (% of islet volume)	Net $^{22}\text{Na}^+$ uptake (pmol/islet)	Intracellular Na^+ concn. (mM)	No. of observations	Significance of change in islet Na^+ content compared with control
2.8	–	2.66 ± 0.16	38.1 ± 1.9	94.4 ± 2.5	57.3 ± 1.5	93	–
20.0	–	2.71 ± 0.21	39.3 ± 3.2	98.7 ± 5.9	60.0 ± 3.6	48	$P > 0.9$
2.8	10nM	2.69 ± 0.23	40.4 ± 2.8	148.8 ± 11.5	92.8 ± 7.2	48	$P < 0.01$
2.8	10 μ M	2.67 ± 0.12	42.2 ± 2.3	238.4 ± 9.56	154.5 ± 6.2	60	$P < 0.001$

space after incubation with monensin at either concentration.

Monensin (10 μ M) evoked an initial 3-fold increase, and an overall 2-fold increase, in the rate of $^{22}\text{Na}^+$ efflux from islets which had been preloaded with $^{22}\text{Na}^+$ by incubation for 30min. Monensin (10nM) increased the rate of efflux by 1.5-fold, but did not evoke a peak of efflux as did 10 μ M-monensin (Fig. 3). In similar conditions, changing the glucose concentration of the perfusion medium from 2.8 to 20mM evoked no significant change in $^{22}\text{Na}^+$ efflux from the islets ($P > 0.9$; results not shown).

Effects on Rb^+ handling

Monensin (10nM) decreased net $^{86}\text{Rb}^+$ accumulation within islet cells compared with controls (Fig. 4). The inhibition of net $^{86}\text{Rb}^+$ uptake produced by monensin increased with time from 26% after 1h ($P < 0.001$) to 57% after 2h ($P < 0.001$).

Monensin at 10 μ M and 10nM increased $^{86}\text{Rb}^+$ efflux from preloaded perfused islets. At 10 μ M, monensin increased the rate of efflux by 2-fold within 5 min of coming into contact with the islets, and this rate of efflux was then largely sustained

Fig. 3. Effect of monensin on $^{22}\text{Na}^+$ efflux from preloaded islets

Monensin at 10 nM (Δ) or 10 μM ($\blacksquare$) was introduced at 11 min. The glucose concentration was 2.8 mM throughout, and the control islets were perfused in the absence of monensin (shaded area). The numbers of observations are given in parentheses.

Fig. 4. Effect of monensin on net $^{86}\text{Rb}^+$ uptake by islets. The glucose concentration was 2.8 mM in all instances. $\square$, Control; $\hatched$, 10 nM-monensin. The P values above the bars are relative to the control in each case ($n = 45$ in each group).

until the end of the experiment (Fig. 5). At 10 nM-monensin the rate of efflux of $^{86}\text{Rb}^+$ was increased to 1.5-fold the control rate within 5 min and then further increased, until after 18 min exposure the rate of efflux was almost the same as that observed in the presence of 10 μM -monensin (Fig. 5).

Effects of Ca^{2+} handling

As shown in Fig. 6, 20 mM-glucose stimulated $^{45}\text{Ca}^{2+}$ uptake by 4.2-fold compared with that in the presence of 2.8 mM-glucose ($P < 0.001$). Monensin inhibited glucose-stimulated $^{45}\text{Ca}^{2+}$ uptake in a concentration-dependent manner. Thus, in the presence of 10 nM-monensin, 20 mM-glucose caused a 2.5-fold stimulation in $^{45}\text{Ca}^{2+}$ uptake. The presence of 50 nM-monensin resulted in 20 mM-glucose inducing only a 1.2-fold increase in $^{45}\text{Ca}^{2+}$ uptake ($P < 0.05$), equivalent to over 86% inhibition of glucose-stimulated $^{45}\text{Ca}^{2+}$ uptake. With 0.1 μM -monensin there was no significant difference between $^{45}\text{Ca}^{2+}$ uptake at 2.8 mM- and 20 mM-glucose. Monensin had no significant effect on the basal rate of $^{45}\text{Ca}^{2+}$ uptake in 2.8 mM-glucose at any of the three concentrations tested.

Monensin increased the rate of $^{45}\text{Ca}^{2+}$ efflux at both 10 nM and 10 μM . At either concentration the rate of efflux increased until a peak rate was reached 4 min after the islets were exposed to monensin. When perfused with 10 μM -monensin, the peak was 2.4-fold the control rate in the absence of monensin ($P < 0.001$); after 13 min the

Fig. 5. Effect of monensin on $^{86}\text{Rb}^+$ efflux from preloaded islets

Monensin at 10 nM (Δ) or 10 μM ($\blacksquare$) was introduced at 30 min. The glucose concentration was 2.8 mM throughout, and the control islets were perfused in the absence of monensin (shaded area). The numbers of observations are given in parentheses.

rate of efflux had fallen back to 1.6-fold the control rate (Fig. 7). This rate was then maintained until the end of the experiment. Overall there was a 2.2-fold increase in the rate of $^{45}\text{Ca}^{2+}$ efflux,

the integrated values being 13.30 ± 0.73 and $28.84 \pm 1.39\%$ /islet per 19 min for the control and monensin-treated islets respectively ($P < 0.001$).

When the islets were perfused with medium containing 10 nM-monensin the peak rate of efflux was 1.5-fold the basal rate ($P < 0.01$). The rate of $^{45}\text{Ca}^{2+}$ efflux then fell, until after 13 min exposure

to monensin (10 nM) the rate of efflux was again within the basal range (Fig. 7).

Effects of monensin on Ca^{2+} efflux in the presence of 20 mM-glucose are shown in Fig. 8. Increasing the glucose concentration from 2.8 mM- to 20 mM-glucose decreased the rate of $^{45}\text{Ca}^{2+}$ efflux by 50% ($P < 0.01$). When monensin (0.1 μM)

Fig. 6. Effect of monensin on basal and glucose-stimulated $^{45}\text{Ca}^{2+}$ uptake measured over 5 min

Results for n observations are shown; the P values above the bars are relative to the 20 mM-glucose control. □, 2.8 mM-glucose; ■, 20 mM-glucose.

Fig. 7. Effect of monensin on $^{45}\text{Ca}^{2+}$ efflux from preloaded islets perfused in the complete absence of Ca^{2+} . Monensin at 10 nM (▲) or 10 μM (■) was introduced at 30 min. The glucose concentration was 2.8 mM throughout, and the control islets were perfused in the absence of monensin (shaded area). The values are means $\pm$ S.E.M. for six experiments.

Fig. 8. Effect of 20 mM-glucose and 0.1 μM -monensin on $^{45}\text{Ca}^{2+}$ efflux from preloaded islets in the absence of Ca^{2+} . The glucose concentration was raised from 2.8 mM- to 20 mM-glucose at 30 min. Monensin was introduced at 50 min (■). The values are means $\pm$ S.E.M. for six experiments: ■, 20 mM-glucose at 30 min; ■, 20 mM-glucose at 30 min and 0.1 μM -monensin at 50 min; ▲, 2.8 mM-glucose and no monensin throughout.

Fig. 9. Effect of monensin ($0.1 \mu\text{M}$) on Ca^{2+} efflux in the absence of Ca^{2+} and Na^+ . Na^+ -free medium was used from 30 min, and monensin ($0.1 \mu\text{M}$) was introduced at 46 min (■). The values are the means $\pm$ S.E.M. for six experiments. ■, Na^+ -free at 30 min; ■, Na^+ -free at 30 min and $0.1 \mu\text{M}$ -monensin at 46 min; ▲, 142 mM - Na^+ and no monensin throughout.

was introduced, the rate of efflux increased by 1.2-fold ($P < 0.05$). Unlike the experiments where monensin (10 nM) was introduced during perfusion with 2.8 mM -glucose, the increase in the rate of efflux was sustained (Fig. 8). However, the rate of $^{45}\text{Ca}^{2+}$ efflux in the presence of $0.1 \mu\text{M}$ -monensin and 20 mM -glucose was still significantly lower than that observed in the absence of monensin and presence of 2.8 mM -glucose (the integrated values from the point of exposure to monensin are 16.35 ± 1.20 and $12.17 \pm 0.96\%$ /islet per 12 min for the control and monensin-treated islets respectively; $P < 0.01$). Thus a concentration of monensin which completely inhibited glucose-stimulated insulin secretion only partially reversed the glucose-induced inhibition of $^{45}\text{Ca}^{2+}$ efflux.

The results of experiments in which Ca^{2+} efflux from preloaded islets was studied in Na^+ -free medium which contained 140 mM -choline chloride and 2.8 mM -glucose are presented in Fig. 9. The change to Na^+ -free medium resulted in a short-lived (4 min) but significant ($P < 0.05$) increase in $^{45}\text{Ca}^{2+}$ efflux, which at its peak was 1.4-fold the control rate. The rate of efflux then decreased rapidly and stabilized at about 60% of the control rate (the integrated values from 42 min were 15.58 ± 0.42 and $9.17 \pm 0.42\%$ /islet per 24 min, for the control and Na^+ -free perfused islets respectively; $P < 0.001$).

When monensin ($0.1 \mu\text{M}$) was introduced there was a significant increase in the rate of efflux ($P < 0.001$). The rate of $^{45}\text{Ca}^{2+}$ efflux rapidly increased and reached a peak after 4–5 min of exposure to monensin. The peak rate was twice the rate of efflux observed in the absence of Na^+ and absence of monensin ($P < 0.01$). The monensin-

induced increase in $^{45}\text{Ca}^{2+}$ efflux then declined, but was still significantly raised ($P < 0.01$) compared with the efflux in the absence of Na^+ and monensin (Fig. 9). Thus the increase in efflux evoked by monensin in the absence of Na^+ was greater than in the presence of extracellular Na^+ . Since monensin increases $^{45}\text{Ca}^{2+}$ efflux in the absence of extracellular Ca^{2+} and Na^+ , the $^{45}\text{Ca}^{2+}$ extrusion observed seems unlikely to be mediated by $\text{Na}^+-\text{Ca}^{2+}$ or $\text{Ca}^{2+}-\text{Ca}^{2+}$ exchange mechanisms across the cell membrane.

Estimation of intracellular pH

In the presence of 2.8 mM -glucose and, the absence of monensin, an intracellular pH value of 7.05 ± 0.02 was calculated for the islet cells by using the 5,5-dimethyloxazolidine-2,4-dione method. This value is in good agreement with the estimated intracellular pH value (7.05) reported by Hellman *et al.* (1972).

The presence of $0.1 \mu\text{M}$ -monensin caused a significant increase in intracellular pH to 7.13 ± 0.03 ($n = 168$, $P < 0.05$). Since the volume of an islet and the proportion of that volume which is extracellular space is known, it can be calculated that this represents a loss of 14×10^6 – 16×10^6 protons per islet. Since there are some 5000 cells in an islet, this suggests that approx. 3000 protons per cell may be lost during exposure to $0.1 \mu\text{M}$ -monensin.

Discussion

These studies have demonstrated that monensin is a very potent inhibitor of insulin secretion. At a concentration found to inhibit glucose-stimulated

insulin secretion completely ($0.1\ \mu\text{M}$), the ionophore was found to have no significant effect on the rate of glucose oxidation as indicated by the generation of $^{14}\text{CO}_2$ from $[^{14}\text{C}]\text{glucose}$. Monensin increased the $^{22}\text{Na}^+$ content of islet cells within 30 min exposure, which is sufficient to achieve isotopic equilibrium (Sehlin & Täljedal, 1974; Kawazu *et al.*, 1978). In other studies, increasing the intracellular Na^+ concentration, either indirectly by the removal of extracellular K^+ or the addition of ouabain (Milner & Hales, 1967; Hales & Milner, 1968; Howell & Taylor, 1968), or directly by the use of veratridine (Lowe *et al.*, 1976; Donatsch *et al.*, 1977; Pace, 1979; Pace & Blaustein, 1979) or a Na^+ ionophore, dianemycin (Corkey & Mayhew, 1974), was not found to inhibit glucose-stimulated insulin secretion, and was in general found to be stimulatory. It therefore seems unlikely that the inhibitory effect of monensin on insulin release is due solely to its ability to increase intracellular Na^+ concentrations. Monensin also increased the rate of efflux of $^{22}\text{Na}^+$ from preloaded islets, an effect possibly attributable to an increase of $^{23}\text{Na}^+$ entering the cells and displacing intracellular $^{22}\text{Na}^+$. In this study, increasing the extracellular glucose concentration from 2.8 to 20 mM had no effect on either the net $^{22}\text{Na}^+$ uptake or the rate of ^{22}Na efflux. This is in conflict with the findings of Kawazu *et al.* (1978), conceivably because the latter authors examined the effect of glucose on $^{22}\text{Na}^+$ handling in islets by comparing the effect of 16.7 mM-glucose with that of zero glucose. It is possible that any effect of glucose is caused by a change from zero to 2.8 mM-glucose, which would not have been observed in the present study. Sehlin & Täljedal (1974) were also unable to show a convincing effect of increasing the glucose concentration from 3 to 20 mM on net $^{22}\text{Na}^+$ uptake.

The inhibitory effects of monensin on glucose-stimulated insulin secretion were similar to those of valinomycin, reported by Henquin & Meissner (1978). Since valinomycin is a highly specific K^+ ionophore, the effects of monensin on K^+ fluxes were also examined by using net uptake and efflux studies with the more convenient analogue $^{86}\text{Rb}^+$. Monensin altered $^{86}\text{Rb}^+$ handling by islet cells, inhibiting net $^{86}\text{Rb}^+$ accumulation by islets, and increasing the rate of efflux of $^{86}\text{Rb}^+$ from preloaded islets in a cumulative manner. A comparable increase in K^+ permeability elicited by valinomycin was found to result in hyperpolarization of the B-cell membrane (Henquin & Meissner, 1978). Thus monensin might be expected to cause hyperpolarization in B cells, and such an effect has been reported by Tarvin *et al.* (1981), Pace & Sachs (1982) and Pace *et al.* (1983). Those workers suggested that the Na^+-H^+ antiporter activity of

monensin leads indirectly to an increase in K^+ permeability and hyperpolarization, and reported that ouabain had no effect on the monensin-induced hyperpolarization. They proposed that monensin causes a rise in cytosolic pH, which has in turn been shown to affect $^{86}\text{Rb}^+$ efflux (Henquin, 1981) and $^{45}\text{Ca}^{2+}$ efflux (Herchuelz *et al.*, 1982; Lebrun *et al.*, 1982). In the present study it was found, by using the equilibration of $[^{14}\text{C}]\text{-dimethylloxazolidinedione}$, that monensin did indeed increase the intracellular pH of islets, after 1 h of exposure by 0.08 pH unit, from 7.05 to 7.13. Finally, monensin could cause hyperpolarization by the mobilization of intracellular Ca^{2+} , which would in turn activate Ca^{2+} -sensitive K^+ channels (Matthews, 1975). Such a mechanism has been demonstrated by Henquin (1979), when high concentrations of intracellular Ca^{2+} produced by the ionophore A23187 were found to increase the efflux of $^{86}\text{Rb}^+$ from preloaded islets. Monensin was found to mobilize intracellular Ca^{2+} in the present study, and so it is possible that the ionophore increases the K^+ permeability of B cells by all of these mechanisms. Such a hyperpolarization would be expected to result in inhibition of Ca^{2+} uptake (via voltage-sensitive Ca^{2+} channels), and this was observed in the present experiments.

Monensin also increased the rate of efflux of $^{45}\text{Ca}^{2+}$ from preloaded islets perfused with Ca^{2+} -free medium, consistent with a report that veratridine, which also increases the intracellular Na^+ concentration, increased $^{45}\text{Ca}^{2+}$ efflux from preloaded islets (Richardson *et al.*, 1980). Increasing the extracellular glucose concentration from 2.8 to 20 mM caused a 50% decrease in the rate of $^{45}\text{Ca}^{2+}$ efflux, as reported by others (Malaisse *et al.*, 1973; Bukowiecki & Frienkel, 1976), possibly by an effect on the $\text{Na}^+-\text{Ca}^{2+}$ exchange mechanism (Herchuelz *et al.*, 1980). Experiments performed with Ca^{2+} -free medium also showed that the removal of extracellular Na^+ decreased the rate of $^{45}\text{Ca}^{2+}$ efflux to 60% of the control value (in the presence of 142 mM- Na^+), presumably owing to an inhibition of the $\text{Na}^+-\text{Ca}^{2+}$ exchange mechanism. When monensin ($0.1\ \mu\text{M}$) was introduced, there was a significant increase in the rate of efflux (Fig. 9), suggesting that the monensin-induced efflux of $^{45}\text{Ca}^{2+}$ observed in the presence of extracellular Na^+ may not depend on an influx of this ion.

Carpinelli *et al.* (1980) proposed that changes in the intracellular pH affect the rate of Ca^{2+} extrusion via the $\text{Na}^+-\text{Ca}^{2+}$ and $\text{Ca}^{2+}-\text{Ca}^{2+}$ exchange mechanisms, H^+ competing with Ca^{2+} . It is difficult to reconcile the monensin-stimulated efflux of $^{45}\text{Ca}^{2+}$ with this model, since neither of these Ca^{2+} -exchange mechanisms would be able to operate in the absence of extracellular Na^+ and

Ca^{2+} . Since monensin is lipid-soluble, it may permeate rapidly through the cells and tend to dissipate the Na^+ , K^+ and H^+ gradients across organelle membranes as well as across the cell membrane, as shown in the present study. The secretory granules have been demonstrated to have a low intragranular pH (Hutton, 1982). The pH gradient across the granule membrane can be detected by the fluorescence of 9-aminoacridine (Abrahamsson & Gylfe, 1980; Pace & Sachs, 1982), and monensin has been demonstrated to dissipate that gradient (Pace & Sachs, 1982). It has been proposed that a chemiosmotic mechanism, dependent on the relatively high intragranular H^+ concentration, is responsible for the exocytotic process (Pollard *et al.*, 1977; Grinstein *et al.*, 1982). The dissipation of the pH gradient across the granule membrane would decrease the potential of the granule to attract anions (Pollard *et al.*, 1977) or accumulate cations in exchange for protons (Grinstein *et al.*, 1982), resulting in a decrease in osmotic swelling of the granules and inhibition of the secretory process.

In summary, it is clear that monensin has complex and interrelated effects on Na^+ , K^+ , H^+ and Ca^{2+} handling in pancreatic islet cells, some or all of which lead to profound inhibitory effects on insulin secretion. It is clearly unsafe to use this agent in intact cell systems in the belief that its effects are solely those of a specific Na^+ ionophore.

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