Selective Inhibitors of GTP Synthesis Impede Exocytotic Insulin Release from Intact Rat Islets*

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To investigate whether GTP concentrations can be a regulatory step in exocytotic hormone secretion, we treated isolated rat islets with mycophenolic acid (MPA) or mizoribine, two selective inhibitors of de novo GTP synthesis. When islets were cultured overnight in purine-free medium containing the drug, MPA reduced GTP levels by up to 81 ± 1%; guanine circumvented this block via the nucleotide “salvage” pathway. MPA concomitantly inhibited glucose (16.7 mM)-induced insulin secretion in batch-type incubations (or perfusions), by up to 68% at 50 μg/ml. Although the inhibition of secretion occurred over a similar concentration range as the reduction in total GTP content, the two variables were not directly correlated. However, the secretory effects also were prevented by adding guanine, but not hypoxanthine or xanthine, to the culture medium. Similar results for GTP content and insulin release were seen using mizoribine. Insulin content was modestly (~18%) reduced by MPA but indices of fractional release (release/insulin content) were also markedly impaired. Although MPA also reduced ATP levels more modestly (~39%) and increased UTP (+87%), these were not the cause of the secretory defect since adenine restored ATP and UTP nearly to normal, but did not alter the reduction in ATP content or insulin secretion. MPA also inhibited secretion induced by amino acid or by a phorbol ester but had virtually no effect on release induced by a depolarizing concentration of K+, suggesting that GTP depletion does not merely impede Ca++ influx or directly block Ca++-activated exocytosis. However, a severe reduction of GTP content did not prevent the pertussis toxin-sensitive inhibition of insulin release induced by epinephrine, suggesting that the function of heterotrimeric GTP-binding proteins is not limited by ambient GTP concentrations. Although these studies do not elucidate the exact site(s) in the exocytotic cascade which depend on intact GTP stores, they do provide the first direct evidence that GTP is required and can be rate-limiting for insulin release.

The roles of GTP in mammalian cells have received intense scrutiny. One such role is to replace GDP and thereby activate various GTP-binding proteins (G-proteins) such as heterotrimeric G-proteins in plasma membranes and low molecular weight G-proteins which partition between cytosolic and intracellular membranes. Such proteins may participate in signal transduction and stimulus-secretion coupling in cells capable of exocytotic secretion, including the pancreatic islet (1-5). Other actions of GTP (and/or its deoxy derivative) which may be relevant to exocytosis include effects on microtubule assembly (4); ATP synthesis (5-7); calcium mobilization (8); membrane insertion of tRNA, translocation of the nascent secretory product across the endoplasmic reticulum and its subsequent targeting, vectorial transport, and vesicular traffic (9, 10); possibly the regulation of protein kinase activity (11-13); potential direct effects on a plasma membrane K+ channel (14), in addition to more general effects on DNA, RNA, and protein synthesis (15-17) including insulin synthesis (10); glycoprotein formation; and the regulation of certain enzymes, including the control of gluconeogenesis (18, 19).

However, little data exist (20-22) on GTP levels in islets, and there have been no studies of which we are aware which assess whether GTP levels are rate-limiting for (and thus can be a site for the regulation of) exocytosis from endocrine organs. To assess this question, we treated islets with pharmacologic agents which selectively block the de novo synthesis of GTP at the level of inosine monophosphate dehydrogenase (EC 1.1.1.205, IMP dehydrogenase, Fig. 1) and examined the consequent effects on islet GTP levels, and on insulin content and secretion, in the presence and absence of exogenous purine bases used to provide a “salvage” pathway for nucleotide synthesis.

EXPERIMENTAL PROCEDURES

Materials—Mycophenolic acid and nucleotide standards, monobasic ammonium phosphate (for HPLC mobile phase) and purine bases were purchased from Sigma. Mizoribine, also referred to as bredinin (4-carbamoyl-1-β-D-ribofuranosylimidazol-5-olate, Refs. 23-25), tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, Refs. 26, 27), and ribavirin (1-β-D-ribofuranosylthiazole-3-carboxamide, Refs. 26, 27) were generous gifts of Dr. N. Kazmatani (Tokyo Women’s Medical College, Tokyo, Japan), Dr. D. English (Indiana University School of Medicine, Indianapolis, IN), and Dr. R. Greene (National Cancer Institute, and Chemotherapeutics Agents Repository, Rockville, MD). The diluents used for making stock solutions of these drugs were ethanol (for mycophenolic acid), water (for mizoribine, tiazofurin, and ribavirin), MeSO4 (for guanine or adenine), and 0.1 N NaOH (for hypoxanthine or xanthine), respectively. Pertussis (PTX) toxin was from List Biologic Laboratories (Campbell, CA) and was diluted 0.1 M monobasic sodium phosphate buffer containing 0.5 M NaCl. In all studies, identical types and amounts of

The abbreviations used are: HPLC, high performance liquid chromatography; MPA, mycophenolic acid; NTP, nucleoside triphosphate; PTX, pertussis toxin (from Bordetella pertussis); IMP, inosine monophosphate; TFA, 12-O-tetradecanoylphorbol-13-acetate.

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Polyethylene filters (31, 32), were placed in 15-ml conical centrifuge tubes containing 1 ml of acid alcohol (composed of 77% absolute ethanol, 22% water, and 1% concentrated HCl (v/v)), sonicated and left overnight at 4 °C. After 20 h, 1 ml of phosphate-buffered saline and 30 μl of 5 N NaOH were added to raise the pH to 8.0, and the contents were vortexed after removing the glass tube but leaving the medium in the centrifuge chamber. Tubes were centrifuged for 5 min (×2000 rpm); the supernatant was removed, diluted (1:10) in radioimmunoassay buffer (phosphate-buffered saline) to a final dilution of 1:20, and assayed.

Insulin content of media or of islets was measured by radioimmunoassay as described (31-32).

**Determination of Nucleotides**—Islets were cultured overnight (as described above and in Ref. 32) except that 200 islets for each condition were cultured in individual dishes containing various experimental substances, so that islets could be rapidly extracted the next day without the need for time-consuming counting and aliquoting (during which the NTP contents might change). The next morning islets were placed (using siliconized Pasteur pipettes) into siliconized borosilicate tubes at 2-4 °C and were allowed to settle for 5 min. Culture medium was then removed and islets were resuspended in 1 ml of Krebs-Ringer buffer containing 0.5% bovine serum albumin (3.3 mM glucose) and were transferred to a 1.5-ml microcentrifuge tube. They were rapidly (1 min) spun down at 4 °C (×1000 rpm) to lightly pellet the islets. All but 20–50 μl of Krebs-Ringer buffer was removed and discarded. To this was added 290 μl of 0.5 M trichloroacetic acid, containing sufficient internal standard (CTP and UTP) to yield a final concentration of 0.8 nmol/100 μl. Islets were lightly vortexed and then sonicated (2 × 20 s) on ice using a Branson 450 sonifier (Danbury, CO). All debris was pelleted at 4 °C (5 min at 10,000 rpm), and the 300 μl of supernatant was transferred to a fresh microfuge tube. Samples were extracted four times using 0.75 ml of ether (to remove the trichloroacetic acid) and vigorous vortexing. The ether phase was discarded. Any residual ether was allowed to evaporate at 4 °C, and then samples were directly analyzed by HPLC or frozen at −20 °C for future analysis within 2 weeks. There was no difference in results between the two handling procedures.

**HPLC** was carried out using a modification of the procedure of Hoenig and Matschinsky (21). A LiChrosorb-NH2 anion-exchange column (3.7 × 300 mm) was used without a guard column, and separation from acinar tissue and debris on Ficoll gradients, as previously described (28-32). Islets were cultured overnight (32) in RPMI 1640 medium (containing 10% fetal calf serum, 11.1 mM glucose, 100 units/ml penicillin, and 100 μg/ml of streptomycin), except in studies involving freshly isolated islets (in Table I); the details for the isolation, culture, and incubation of these islets for batch-type, static assessments of insulin release have also been published (28-32). The incubation medium was Krebs-Ringer bicarbonate buffer, pH 7.4, containing 0.5% bovine serum albumin and 3.3 or 16.7 mM glucose (as indicated), gassed with 9.5% O2, 5% CO2. Except where indicated otherwise, static, batch-type incubations were for 45 min so as to include contributions from both first and second phase secretion. Secretion was corrected for the mean recovery of the two exogenous nucleotides studied in islets.

**GTP and Exocytotic Insulin Release**

**Fig. 1. Schematization of the pathways for synthesis of the NTPs studied in islets.** The pathways themselves have only been formally studied in non-islet cells, and their existence in islets is only presumptive but compatible with the data reported herein. Several cofactors, modulators, and intermediate steps have been omitted for the sake of clarity. Additional abbreviations: OPRT, orotate phosphoribosyltransferase; ODC, orotic acid decarboxylase; PRPP, phosphoribosylpyrophosphate; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; NMPK, nucleoside monophosphate kinase; NDPK, nucleoside diphosphokinase; APRT, adenine phosphoribosyltransferase; AK, adenylate (AMP) kinase; XMP, xanthosine monophosphate.

The relevant dienues (s) were added to all control tubes. HPLC columns were purchased from Alttech (Deerfield, IL); HPLC water was purchased from Fisher (Itasca, IL). RPMI 1640 medium was purchased from GIBCO.

**Isolation and Treatment of Pancreatic Islets for Studies of Insulin Release**—Intact pancreatic islets were isolated from adult male Sprague-Dawley rats using collagenase digestion and separation from acinar tissue and debris on Ficoll gradients, as previously described (21-24% of buffer B was used but the exact proportions of buffers A and B were corrected for the mean recovery of the two exogenous nucleotides, so that islets could be rapidly extracted the next morning in order to maximize separations, which were affected by changes in columns or new batches of solvent, and even by evaporation in pH. Separations were achieved by isocratic elution at 1.0–1.3 ml/min. Representative retention times are shown in Fig. 2A. One-hundred μl of sample (comprising the extract of about 70 islets) was usually injected, but 50–150 μl gave equivalent results in quantitation of the content of ATP, UTP, and GTP. All samples were analyzed using the mean of duplicate or triplicate injections. Since preliminary studies indicated that ATP and CTP were not detectable in islets under these conditions, both nucleotides were added as samples as internal standards, and results were corrected for the mean recovery of the two exogenous nucleotides. A standard curve was routinely run each day covering the relevant range (0.2-1.0 nmol) of ITP, CTP, GTP, ATP, and UTP.

**Recoveries of CTP and ITP were 99 ± 1% (n = 54) and 88 ± 1% (n = 53), respectively.** Intra-assay coefficients of variation for injection was low when replicate injections were made on separate days. For example, when 3 aliquots from single samples were injected in different columns (varying between 50 and 100 μl) on 3 sequential days, coefficients of variation averaged 5.5 ± 1.3% (n = 26; range of NTP = 0.44–12.79 pmol/islet). However, coefficients of variation were lower for NTP levels above 2 pmol/islet (2.7 ± 0.5%) than for very suppressed levels under 0.6 pmol/islet (15 ± 2%).

**Data Presentation and Statistical Analysis**—Insulin secretion is expressed as microunits/10 islets/unit time (mean ± S.E.) for static incubations and as micromolars/ml of perfusate for perfusions. Increments in the value for the value for the basal stimulated conditions (minus) the mean value for basal release (i.e. at 3.3 mM glucose) in that same study. Insulin content is expressed...
During a 45-min incubation period, control islets exposed to 16.7 mM glucose responded with a 5–11-fold increase in insulin release. An inhibitory effect of MPA was clearly visible by 2 μg/ml (Fig. 3), and insulin secretion fell in a log (MPA)-linear (insulin) fashion from 0.5 through 50 μg/ml of MPA with an EC50 of 13.2 μg/ml (Fig. 3). The inhibitory effect of 25 μg/ml MPA (~54 ± 2%) was seen in each of 27 separate experiments, MPA (25 μg/ml) also inhibited the effect of a submaximal glucose concentration (9.8 mM) by 64% (data not shown). Mere removal of MPA (from both the preincubation and incubation media) did not reverse these inhibitory effects.

**TABLE I**

| Condition                                      | Insulin release (microunits-incubation period) |
|------------------------------------------------|-----------------------------------------------|
| MPA present in 45-min preincubation and 15-min incubation periods (freshly isolated islets) |                                               |
| Control (3.3 mM glucose)                       | 94 ± 3 (3)                                    |
| Control (3.3 mM glucose + MPA)                 | 66 ± 7 (3)                                    |
| 16.7 mM glucose                               | 481 ± 54 (5)                                  |
| 16.7 mM glucose + MPA                         | 416 ± 26 (5)                                  |
| MPA present in 45-min preincubition and 45-min incubation periods (freshly isolated islets) |                                               |
| Control (3.3 mM glucose)                       | 78 ± 16 (3)                                   |
| Control (3.3 mM glucose + MPA)                 | 76 ± 1 (3)                                    |
| 16.7 mM glucose                               | 1054 ± 47 (5)                                 |
| 16.7 mM glucose + MPA                         | 1060 ± 82 (4)                                 |
| MPA present in 45-min incubation period only (overnight-cultured islets) |                                               |
| Control (3.3 mM glucose)                       | 64 ± 10 (3)                                   |
| 50 mM K⁺                                     | 491 ± 26 (8)                                  |
| 16.7 mM glucose                               | 439 ± 37 (4)                                  |
| Control (3.3 mM glucose + MPA)                 | 82 ± 12 (3)                                   |
| 50 mM K⁺                                           | 545 ± 39 (4)                                  |
| 16.7 mM glucose                               | 442 ± 60 (3)                                  |

**RESULTS**

Effects of Mycophenolic Acid (MPA) on Glucose-induced Insulin Release and on Insulin Content: Time and Concentration Dependence—If freshly isolated islets were exposed to MPA (25 μg/ml) only during a static, 45-min batch-type incubation period or during a 45-min preincubation plus an incubation period of 15 or 45 min, no effect on glucose (16.7 mM)-induced insulin release was seen (Table I). Similar results were seen using islets cultured overnight (in the absence of MPA) (Table I). In contrast, if islets were cultured (×18 h) in RPMI 1640 medium containing MPA, a consistent inhibition of glucose-induced secretion was seen (Fig. 3). During a 45-min incubation period, control islets exposed to 16.7 mM glucose responded with a 5–11-fold increase in insulin release. An inhibitory effect of MPA was clearly visible by 2 μg/ml (Fig. 3), and insulin secretion fell in a log (MPA)-linear (insulin) fashion from 0.5 through 50 μg/ml of MPA with an EC50 of 13.2 μg/ml (Fig. 3). The inhibitory effect of 25 μg/ml MPA (~54 ± 2%) was seen in each of 27 separate experiments. MPA (25 μg/ml) also inhibited the effect of a submaximal glucose concentration (9.8 mM) by 64% (data not shown). Mere removal of MPA (from both the preincubation and incubation media) did not reverse these inhibitory effects.

**Fig. 2.** HPLC system used for the quantitation of islets NTP content. Panel A, standards (approximately 0.2 nmol each), indicating the minutes and seconds for the peak elution times of each. Panel B, chromatogram from the extract of ~35 control islets; panel C, chromatogram from the extract of ~35 islets cultured 18–20 h in 25 μg/ml MPA, showing the decline in ATP and GTP, and the increase in UTP, with the two internal standards (CTP and TTP) flanking these nucleotides. $A_{254}$, absorbance at 254 nm; AUFS, absorbance units full scale.

**Fig. 3.** Effect of MPA concentration (on the abscissa) during an 18–20-h overnight period on incremental, glucose (16.7 mM)-induced insulin release (on the ordinate) expressed % of control (no MPA present). Data are expressed as the mean (plus or minus S.E.) of (n) separate experiments, with each single data point therein being comprised of multiple (four to nine) observations for both control tubes and for those containing each concentration of MPA.
**TABLE II**

**Effects of inosine monophosphate dehydrogenase inhibitors on fractional insulin release**

Inosine monophosphate dehydrogenase inhibitors ("experimental agent") were present during the overnight culture period, the periods required for washing and picking islets and the preincubation period, but were absent during the 45-min incubation period. Fractional release was calculated as (microunits secreted/45 min)/(microunits secreted + residual insulin content) and expressed as % per 45 min. The % inhibition is calculated as 100−([stimulated release−basal release]/stimulated release−basal release)×100. Numbers in parentheses = number of observations; mean ± S.E.

| Experimental agent | Glucose 3.3 mM (Control) | Glucose 3.3 mM (Experimental) | Glucose 16.7 mM (Control) | Glucose 16.7 mM (Experimental) | % inhibition, p |
|--------------------|--------------------------|-----------------------------|--------------------------|-------------------------------|-----------------|
| Mycophenolic acid, 25 μg/ml | 0.74 ± 0.12 (6) | 0.74 ± 0.02 (2) | 6.16 ± 0.38 (8) | 3.26 ± 0.22 (10) | −54%, p < 0.001 |
| Mycophenolic acid, 50 μg/ml | 0.82 ± 0.17 (3) | 0.78 ± 0.08 (4) | 6.57 ± 0.61 (7) | 2.47 ± 0.25 (8) | −71%, p < 0.001 |
| Mizoribine, 75 μg/ml | 0.71 ± 0.16 (3) | 0.43 ± 0.07 (3) | 6.08 ± 0.45 (7) | 3.02 ± 0.15 (7) | −82%, p < 0.001 |
| Ribavirin, 250 μM | 0.80 ± 0.17 (3) | 0.70 ± 0.10 (4) | 6.00 ± 0.64 (8) | 3.60 ± 0.27 (9) | −44%, p < 0.01 |

**TABLE III**

**Effects of inosine monophosphate dehydrogenase inhibitors on islet insulin content**

Content was assessed after an 18–20-h culture period in the presence of the test agent (or its diluent) followed by a 45-min incubation period and so were pooled. Data are expressed as microunits of insulin/10 islets. Numbers in parentheses = numbers of observations; mean ± S.E.

| Control | Inhibitor | % change, p |
|---------|-----------|-------------|
| Mycophenolic acid, 25 μg/ml | 5492 ± 173 (13) | 4806 ± 151 (9) | −12%, p < 0.02 |
| Mycophenolic acid, 50 μg/ml | 4346 ± 172 (6) | 7073 ± 298 (10) | −21%, p < 0.001 |
| Mizoribine, 75 μg/ml | 7104 ± 332 (10) | 7127 ± 234 (13) | −15%, p < 0.05 |
| Ribavirin, 250 μM | 8375 ± 527 (11) | 7127 ± 234 (13) | −15%, p < 0.05 |

* Not significant.

For example, when MPA (25 μg/ml) was present, glucose-induced insulin secretion fell by 49%, leaving a residual insulin secretion of 411 ± 24 microunit/45 min (n = 12); this figure rose only nonsignificantly (to 482 ± 35; n = 11; p = not significant) when MPA (which had been present during the overnight culture period) was excluded from the preincubation and incubation media (each = 45 min). Therefore, for all future studies, inosine monophosphate dehydrogenase inhibitors were excluded from the incubation medium in order to preclude any artifactual effects on the insulin radioimmunoassay.

To examine the possibility that these effects on release were merely secondary to reductions in the insulin content of islets, the latter parameter was directly assessed, and fractional release was calculated. Glucose (16.7 mM) induced a 8–10-fold increase in fractional insulin release from control islets (Table II). MPA caused a small reduction in insulin content (cf. Table III); the inhibition was 18 ± 3% at 25 μg/ml MPA (n = 13 experiments) and 19 ± 4% at 50 μg/ml MPA (n = four experiments). However, fractional release was also inhibited by MPA (Table II) to a degree (and at MPA concentrations) identical to those data expressed as absolute rates of release. These findings indicate that MPA reduced the effects of glucose on secretion principally by inhibiting exocytosis, not insulin stores (see "Discussion").

Effects of Other Inhibitors of Inosine Monophosphate Dehydrogenase on Insulin Release—Mizoribine also induced a dose-dependent reduction in glucose-induced insulin release, +5% (no inhibition) at 2 μg/ml; −18% at 10 μg/ml (p = not significant); −37% at 40 μg/ml (p < 0.01); −26% at 60 μg/ml (p < 0.02); and −47% at 75 μg/ml (p < 0.001) (Tables II and IV). Mizoribine did not reduce insulin content (Table III); consequently, fractional secretion was also inhibited (Table II). Tiazofurin or ribavirin also inhibited absolute insulin release (Table V) as well as fractional secretion (Table II). They inhibited release by 42 ± 4% (n = three experiments) at 250 μM and by a mean of 49% (two experiments) at 350 μM. In some experiments (cf. Table III), these two agents appeared to reduce insulin content slightly, but this was not a consistent finding (−10 ± 6%; n = five experiments). Consequently, fractional release fell by 37 ± 11%.

Effects of MPA on Secretion Induced by Other Islet Agonists, on Basal Insulin Release, and on Phasic Insulin Secretion—The amino acid α-ketoisocaproic acid (20 mM), a phorbol ester activator of protein kinase C (12-O-tetradecanoylphorbol-13-acetate, 2 μM) or a depolarizing concentration of K+ (50 mM) induced increases in insulin release to levels 9–22 times basal rates of release. MPA (25 μg/ml) reproducibly inhibited insulin secretion induced by amino acid or by TPA (Table VI). The average inhibitions were 47 ± 2% (n = three separate experiments) and 44 ± 9% (three experiments) for these two agonists, respectively.

In contrast, the effect of depolarizing concentrations (50 mM) of K+ was inhibited much less (−15 ± 5%); the mean incremental response was 458 ± 23 microunits/10 islets in control islets versus 391 ± 36 microunits/10 islets in MPA-pretreated islets (n = six experiments; p < 0.05). However, this decrement was not statistically significant within most individual experiments (cf. Table VI and Fig. 4). This small effect on K+-induced release is probably explicable by the equally modest reduction by MPA of insulin content, since in a preliminary study in which absolute rates of release were inhibited 13% by MPA, fractional insulin release was not impeded by MPA (glucose, 3.3 mM = 0.50 ± 0.10%, n = 3; 50 mM K+ = 6.86 ± 0.35%, n = 5; MPA, 25 μg/ml at 3.3 mM glucose = 0.60 ± 0.15%, n = 3; K+ plus MPA = 8.10 ± 0.65, n = 5). The insignificant effects on K+-induced secretion in these batch-type incubations closely paralleled those seen in perfusions (see below).

MPA and other inosine monophosphate dehydrogenase inhibitors also usually reduced basal rates of insulin release (cf. Tables IV–VI). However, this inhibition was less marked than the inhibition of glucose, amino acid, or TPA. Furthermore, this effect on basal release lacked the clear MPA dose de-
GTP and Exocytotic Insulin Release

TABLE IV
Effects of guanine and other purine bases on the inhibition of insulin release by inosine monophosphate dehydrogenase inhibitors

| Data are expressed as insulin released at 3.3 mM glucose | Inhibitor alone | Inhibitor plus base | Degree of reversal* |
|----------------------------------------------------------|-----------------|---------------------|---------------------|
| Control 3.3 mM glucose/16.7 mM glucose                  | 39 ± 5 (3)      | 39 ± 5 (3)          | 49                  |
| MPA 25 µg/ml plus guanine                               | 40 ± 7 (3)      | 39 ± 13 (3)         | 41 ± 7 (2)          |
| MPA 50 µg/ml plus guanine                               | 50 ± 7 (4)      | 55 ± 7 (3)          | 412 ± 34 (5)        |
| MPA 25 µg/ml plus adenine                               | 60 ± 1 (2)      | 17 ± 2 (2)          | 104 ± 8 (3)         |
| MPA 25 µg/ml plus xanthine or hypoxanthine              | 586 ± 38 (5)    | 197 ± 25 (4)        | 251 ± 32 (5)        |
| Mizoribine, 60 µg/ml plus guanine                        | 47 ± 8 (3)      | 39 ± 3 (2)          | 211 ± 24 (4)        |
| Mizoribine, 75 µg/ml plus guanine                        | 432 ± 18 (5)    | 322 ± 32 (5)        | 44 ± 9 (3)          |

* Reversal = 100 - [% inhibition in presence of base]/(% inhibition in absence of base), where % inhibition is calculated as in Table II.

^ Bases were included in the incubation medium.

^ Bases were excluded from the incubation medium.

TABLE V
Effects of tiazofurin and ribavirin on glucose-induced insulin release

| Condition | Insulin |
|-----------|---------|
| 3.3 mM glucose | 70 ± 6 (3) |
| 16.7 mM glucose | 505 ± 34 (8) |
| 3.3 mM glucose + tiazofurin, 250 µM | 60 ± 7 (4) |
| 16.7 mM glucose + tiazofurin, 250 µM | 304 ± 20 (8) |
| 3.3 mM glucose + ribavirin, 250 µM | 44 ± 5 (4) |
| 16.7 mM glucose + ribavirin, 250 µM | 273 ± 31 (9) |

 lost when expressed as fractional secretion (cf. Table II, and data in preceding paragraph). Thus, fractional release basally in MPA-pretreated islets was 110 ± 14% of control (n = nine experiments, p = not significant) at 25 µg/ml MPA and 37 ± 14% (n = three experiments, p = not significant) at 50 µg/ml. These findings suggest that the effects of MPA on basal release (like those on K+−induced secretion) may have resulted at least in part from the modest reduction in insulin content induced by MPA. Compatible with this was the finding that, in preliminary studies, guanine did not seem to consistently prevent the reduction induced by MPA in basal or K+−induced insulin release (data not shown).

To examine effects of MPA on phasic insulin release, cultured islets were also perfused (Fig. 5). Glucose (16.7 mM) induced peak increases in rates of insulin release from control islets during the first phase (0–5 min) or the second phase (6–45 min) of secretion of 7- and 9-fold, respectively. In each of four separate perfusions, islets cultured in the presence of MPA demonstrated clear inhibition of both phases of secretion (Fig. 5). The average inhibition of (incremental) areas under the curves for the entire 45-min perfusion period was 63 ± 8%; this figure closely matches the data from the batch-type incubations described above. First-phase release was inhibited by 86 ± 6% (Fig. 5, inset). In contrast, when 50 mM K+ was added after 45 min at 16.7 mM glucose, the incremental insulin response was inhibited insignificantly by MPA (−11 ± 5%, Fig. 5), as had been observed in static incubations.

Effects of Exogenous Purines on Insulin Release and the

dependence seen with glucose-stimulated insulin release. For example, inhibition of basal secretion was not consistently seen at 2–10 µg/ml MPA whereas a similar degree of inhibition was seen at 25 µg/ml (−27 ± 5%, n = 25 experiments) as at 50 µg/ml (−21 ± 10%, n = five experiments). Furthermore, effects of MPA on basal insulin release were attenuated or

However, it should be noted that, unlike stimulated secretion, basal insulin release in vitro probably reflects variable amounts of nonsuppressible leakage, making quantitative assessments of the degree of inhibition somewhat unreliable.
Inhibition of Insulin Release Induced by MPA or by Mizoribine: Specificity and Time Course—Guanine (when added by itself during the culture period) had effects on glucose-induced insulin release which were small, inconsistent, and not clearly concentration-dependent: incremental responses to glucose were increased 23% at 5 μM guanine (n = one experiment), 9 ± 4% at 30 μM guanine (n = 6), 12% at 50 μM guanine (n = 1), and 29% at 100 μM guanine (n = 2) (Fig. 6). In contrast, provision of guanine (5–100 μM) during the culture period dose responsively prevented the inhibitory effects of MPA, 25 μg/ml (Fig. 6 and Table IV). Overall, provision of guanine (30–100 μM) restored secretion back to 78 ± 3% of high glucose plus guanine controls and virtually to 100% of high glucose (alone) controls (Fig. 6 and Table IV). In two additional experiments, guanine (50–100 μM) also partially but significantly restored insulin secretion in the presence of 50 μM K⁺ (from values of 31% of control back to 69% of control; data not shown). Guanine (30 μM) also prevented the inhibitory effects of mizoribine, 60 or 75 μg/ml, on secretion (Table IV). In each of two perfusion studies, co-culture with guanine (50 μM) reduced the inhibitory effects of MPA both on first-phase

Inhibition of Insulin Release Induced by MPA on insulin secretion induced by non-glucose agonists (glucose = 3.3 mM)

Data are expressed as microunits of insulin released over 45 min; numbers in parentheses = numbers of observations; mean ± S.E. Incremental responses were calculated as in Table II.

| Basal                                      | Control     | +MPA, 25 μg/ml | % inhibition by MPA |
|--------------------------------------------|-------------|---------------|---------------------|
| + α-Ketoisocaproic acid, 20 mM             | 44 ± 6 (6)  | 41 ± 1 (8)    | -44% (p < 0.001)    |
| + 12-O-tetradecanoylphorbol-13-acetate, 2 μM | 989 ± 49 (10)| 570 ± 172 (11)| -38% (p < 0.001)    |
| + 50 mM K⁺                                  | 524 ± 25 (10)| 337 ± 30 (12) |                     |
|                                            | 486 ± 22 (10)| 421 ± 38 (12) |                     |

* Not significant.

**Fig. 4.** Effects of pretreatment with MPA and/or pertussis toxin on insulin release induced by 16.7 mM glucose (open bars) or 50 mM K⁺ (cross-hatched bars in main panel) in the absence and presence of epinephrine (during the incubation period only). Data are from two separate experiments with identical results and are expressed as incremental insulin release: stimulated release [minus] corresponding control release (the latter parameter was nearly constant under the various experimental conditions). Data are expressed as microunits secreted in 45 min (static incubation period). Figures in parentheses indicate the number of observations (mean ± S.E.). The numbers in brackets indicate the % inhibition (compared to 16.7 mM glucose or 50 mM K⁺ alone in main figure, and compared to pertussis toxin alone at 16.7 mM glucose in the inset, except where indicated otherwise).

**Table VI**

|                             | Control     | +MPA, 25 μg/ml | % inhibition by MPA |
|-----------------------------|-------------|---------------|---------------------|
| Basal                       | 44 ± 6 (6)  | 41 ± 1 (8)    | -44% (p < 0.001)    |
| + α-Ketoisocaproic acid, 20 mM | 989 ± 49 (10)| 570 ± 172 (11)| -38% (p < 0.001)    |
| + 12-O-tetradecanoylphorbol-13-acetate, 2 μM | 524 ± 25 (10)| 337 ± 30 (12) |                     |
| + 50 mM K⁺                  | 486 ± 22 (10)| 421 ± 38 (12) |                     |

* Not significant.

**Fig. 5.** Effects of a culture period in the presence or absence of MPA (25 μg/ml) on insulin release from subsequently perfused rat islets. Main panel shows effects of increase from 3.3 to 16.7 mM glucose in the perfusate (first arrow) followed by the addition of 50 mM K⁺ (second arrow). Inset shows the inhibitory effects of MPA on incremental insulin release (increase over prestimulus insulin levels) in response to glucose at 0–5 min (first-phase release), at 0–45 min (largely second phase release), and in response to K⁺. Data are from four paired experiments * = p < 0.05, by paired t test; ns = not significant versus control.

**Fig. 6.** Effects of exogenous guanine (0–100 μM), provided during the overnight culture period, to prevent the effects of mycophenolic acid (25 μg/ml) to impair glucose (16.7 mM)-induced insulin release (eight bars on the right). The four bars on the left indicate that guanine alone had only small and variable direct effects on glucose-induced insulin release. The eight bars on the right indicate that MPA (25 μg/ml) reproducibly inhibited insulin release (open bars), an effect progressively abrogated by the inclusion of increasing concentrations of guanine. Data are expressed as incremental release (microunits insulin in 45 min at 16.7 mM glucose minus microunits in 45 min at 3.3 mM glucose), where 100% = no MPA or guanine in each individual study. Except where indicated otherwise in parentheses, values are from one or two separate experiments at each guanine concentration, each comprised of multiple observations.
concentration which totally restored insulin secretion (Table VII). All three changes were reproducibly inhibited insulin secretion; see above), caused a concentration-dependent reduction of GTP, beginning at 50.5 pg/ml and reaching apparent saturation at an inhibition by MPA (25 μg/ml) of glucose-induced insulin release in the absence and presence of adenine, respectively, each compared to its respective control (diluent or adenine alone, respectively).

and on second-phase insulin release stimulated by glucose, but did not alter K+-induced secretion (data not shown). In contrast, neither adenine, xanthine, nor hypoxanthine (30 μM), nor 150 μM adenine, had any restorative effects in batch type incubations (Table IV and Fig. 7). In preliminary studies, 150 μM adenine also failed to prevent the inhibitory effect of MPA on islet insulin content.

The effects of guanine were time-dependent. In a preliminary study, wherein the purine base (50 μM) was provided only during a preincubation period of 30 min, the inhibition of glucose-induced insulin release caused by MPA (~59%) was reduced (to ~39%) by guanine, whereas the addition of 50 μM guanine to the overnight culture period as well reduced the degree of inhibition considerably more (to 13% less than control).

Effects of Inhibitors of Inosine Monophosphate Dehydrogenase on Islets Nucleotide Triphosphate (NTP) Levels—MPA led to a concentration-dependent reduction of GTP, beginning at ≤0.5 μg/ml and reaching apparent saturation at an inhibition of 80–92% (Table VII). This is the same range of concentrations over which insulin secretion was inhibited. MPA (25 μg/ml) caused a lesser inhibition (maximal inhibition = 43%) in ATP levels and a stimulation to 208% of control in UTP levels (Table VII). All three changes were largely, albeit not totally, prevented by provision of guanine (Fig. 8). Likewise, 75 μg/ml mizoribine (a concentration which reproducibly inhibited insulin secretion; see above), caused a guanine-sensitive reduction in ATP and GTP, and an increment in UTP, to 61 ± 1, 32 ± 1, and 201% of control, respectively (all p < 0.001) (Fig. 9). Guanine (30 μM), a concentration which totally restored insulin secretion (Table IV), restored GTP to 125 ± 9% of control, while leaving ATP still reduced (75 ± 2% of control, p < 0.001) and UTP still somewhat elevated (135 ± 6%, p < 0.01) (Fig. 9). In a preliminary experiment, islets cultured in the presence of guanine 50 μM (in the absence of MPA), had ATP, GTP, and UTP contents which were 98 ± 3, 121 ± 0.33, and 98 ± 3% of control. (Note that the slight rise in ATP content of control islets when cultured in the presence of guanine seems to match a slight augmentation of glucose-induced insulin release from control islets; Fig. 6.)

Since the effects of the inosine monophosphate dehydrogenase inhibitors not only reduced GTP, but also caused more minor changes in UTP and ATP, islets were cultured in the

### Table VII

| MPA conc, μg/ml | ATP | GTP | UTP |
|-----------------|-----|-----|-----|
| 0 (13)          | 12.60 ± 0.22 | 2.71 ± 0.08 | 3.16 ± 0.08 |
| 0.5 (2)         | 8.6 ± 0.20 | 9.22 ± 0.04 | 3.54 ± 0.14 |
| 1.0 (2)         | [-24 ± 2%] | [-60 ± 2%] | [+35 ± 5%] |
| 2.0 (4)         | [-43 ± 6%] | [-78 ± 5%] | [+45 ± 16%] |
| 25.0 (13)       | 5.6 ± 0.50 | 0.55 ± 0.07 | 5.31 ± 0.19 |
| 50.0 (5)        | [-36 ± 3%] | [-78 ± 3%] | [+87 ± 8%] |

FIG. 8. Effects of provision of adenine (150 μM), in the presence or absence of exogenous guanine (50 μM), on NTP content of islets (expressed as pmol/islet). Numbers in parentheses reflect numbers of observations. MPA reduced the content of GTP, ATP, and UTP (each p < 0.001). In the presence of guanine, levels of each approached control levels but GTP and ATP were still significantly reduced (by 26 and 14%, p < 0.01 and < 0.001, respectively), whereas UTP was still slightly elevated (+26%, p < 0.02).

FIG. 9. Effects of mizoribine (75 μg/ml) in the presence or absence of exogenous guanine (30 μM) on NTP content of islets (expressed as pmol/islet). Numbers in parentheses reflect numbers of observations. Mizoribine reduced each nucleotide (each p < 0.001). Values in the presence of mizoribine plus guanine indicated that GTP was slightly (25%, p < 0.05) greater than control values, ATP remained slightly (~23%, p < 0.001) reduced, and UTP remained somewhat (~26%, p < 0.01) elevated.

FIG. 7. Effects of provision of adenine (150 μM) on insulin release (main panel; numbers in parentheses = numbers of observations) or NTP content of islets (inset, n = 3 each). The open bars in the main panel are values at a substimulatory glucose concentration (3.3 mM); the shaded or cross-hatched bars are at 16.7 mM glucose. The figures in brackets in the main panel indicating Δ reflect the % inhibition by MPA (25 μg/ml) of glucose-induced insulin release in the absence and presence of adenine, respectively, each compared to its respective control (diluent or adenine alone, respectively).
presence of MPA (25 μg/ml) plus adenine (150 μM). The latter nearly totally vitiates the change in ATP and UTP, leaving GTP selectively inhibited (Fig. 7, inset). As indicated above, under these conditions of selective GTP depletion, insulin release was still inhibited (Table IV and Fig. 7).

Effects of MPA Pretreatment on the Inhibitory Effects of Epinephrine (Fig. 4)—Since the inhibitory effect of epinephrine on insulin secretion involves an inhibitory GTP-binding protein (G, and/or Go, see “Discussion”) we determined whether MPA could reduce GTP levels sufficiently to limit G, function.

MPA, as expected, significantly inhibited glucose (16.7 mM)-induced insulin secretion (by 60%, p < 0.001) and reduced K+ (50 mM)-induced release much less (−21%, p = not significant) (Fig. 4, B versus A). Epinephrine (5 μΜ) inhibited the effects of the two agonists by 96 and 79%, respectively (C versus A). We have previously ascertained that this effect involves an α, β-adrenergic mechanism (28). When epinephrine was added to islets which had been pretreated with MPA, the residual insulin release was nonetheless totally ineffective to inhibition by epinephrine (D versus B). Rats were then pretreated with pertussis toxin to determine whether the effect of epinephrine to inhibit the residual insulin release remaining after MPA pretreatment did, in fact, involve G, or a related G protein, at least in part. The rats received 1.5 μg of pertussis toxin intraperitoneally 48 and 24 h prior to sacrifice; 100 μg/ml of the toxin was also added to the overnight dishes used to culture the islets. Islets from rats pretreated with pertussis toxin showed potentiated glucose-induced release (E versus A-1). Furthermore, the inhibitory effect of epinephrine was reduced to 27% (F versus E) from a control value of 96% (C versus A). The effect of MPA was not altered by pertussis toxin pretreatment (−67%, G versus E; cf. to −60%, B-1 versus A-1). The addition of epinephrine to MPA-pretreated islets now led to only a blunted inhibition (−30%, H versus G), similar to that seen in epinephrine-exposed control islets from PTX-pretreated rats (−27%, F versus E), and, in fact, restored absolute insulin release to values (148 ± 23, condition H) identical to those (148 ± 18, condition B-1) seen with MPA alone, indicating that PTX pretreatment has obliterated all of the additional inhibitory effect of epinephrine.

DISCUSSION
The four pharmacologic agents used all inhibit inosine monophosphate dehydrogenase (17, 23–27, 34, 35) and are felt thereby to inhibit lymphocyte function and have immunosuppressive properties (25, 36–38). The concentrations of MPA used are similar to those attainable in vivo in mice or monkeys (34, 39) treated with MPA (or its prodrug RS 61443, Ref. 38). Since such agents require several hours to reduce cellular levels of GTP (17, 40), the lack of an acute effect of MPA (Table I) not only is compatible with its proposed mechanism of action but also documents the absence of short term, nonspecific toxic effects. Likewise, the lack of reversibility of MPA’s inhibitory effect on secretion after simple removal from the medium for up to 45 min prior to assessments of insulin release, in conjunction with the ability of guanine (but not other purine bases) to prevent (reverse) the effects of the test agents (MPA, mizoribine), supports a key role of guanine nucleotide availability in the mechanism(s) of action of this class of agents. The effects of mycophenolic acid and mizoribine to inhibit inosine monophosphate dehydrogenase in a wide variety of cells are quite selective whether given in vivo or in vitro (17, 23, 26, 27, 34, 35). However, MPA (35, 36) and possibly mizoribine (24) may also have smaller effects on GMP synthetase (Fig. 1), an action which would potentiate the inhibition of guanine nucleotide levels. Tiazofurin and ribavirin also act principally to inhibit inosine monophosphate dehydrogenase, although other minor effects have been observed (26, 27).

Although the current studies were not designed to directly characterize the pathways of NTP synthesis in pancreatic islets, and their interruption by these pharmacologic probes, the data are compatible with the following schema. Inhibition of GMP synthesis by MPA or mizoribine (Fig. 1) reduces cellular GTP levels. There is a secondary, more modest reduction in ATP (since GTP is used to transphosphorylate ADP to ATP via nucleoside diphosphate kinase and is also required in the adenylosuccinate synthetase step, Fig. 1) (5–7). This inhibition of ATP content is not a direct effect of MPA on ATP synthesis (35) and, in fact, in many cells (17, 27, 26, 27, 34, 36–42) there is little change or even a modest rise in ATP, attendant presumably on the accumulation and redirection of its precursor, IMP (Fig. 1). At the same time, in MPA-treated islets, as in other cells (36, 40), UTP increases, possibly as a result of an accumulation of phosphoribosylpyrophosphate and/or of IMP (Fig. 1); the latter can act as a phosphoryl donor in nucleoside metabolism (43), which might favor the formation of UTP. Provision of guanine, by activating the “salvage pathway” (HGPRT, Fig. 1) bypasses the block induced by MPA (or mizoribine), thereby reversing all of the secondary defects (cf. 15, 23, 26, 27, 34, 36, 37). Provision of adenine bypasses the secondary block at the adenylosuccinate synthetase reaction induced by a decline in GTP, a cofactor for this enzyme (5) (Fig. 1) and repletion ATP (but not GTP) levels (cf. Ref. 42). UTP is also restored, possibly due to phosphoribosylpyrophosphate consumption by adenine phosphoribosyltransferase. Thus, MPA reduces GTP and to a lesser degree ATP and elevates UTP. Co-culture with guanine reverses all three defects, whereas co-culture with adenine creates a selective deficiency of GTP. We emphasize that while this formulation is compatible with and probably explains the data observed, its accuracy in pancreatic islets remains to be established by formal enzymatic analysis.

The conclusion most compatible with the insulin secretory and nucleotide data is that GTP content or concentration plays a critical, heretofore overlooked role in signal transduction and/or exocytosis in islets. Similar conclusions have recently been reached using mast cells (46, 47), basophilic leukemia cells (42), or paramecium (48). In mast cells, “wash-out” of small cytosolic molecules blocks exocytosis, a phenomenon reversed by providing 300 μM GTP. The latter apparently is permissive for secretion, acting at a step distal to

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1. We (28) and others (3) have observed previously that PTX does not totally prevent epinephrine’s inhibitory effect. We have not attempted to verify that our experimental protocol achieved maximal ADP-ribosylation of the PTX substrate but only that epinephrine still was able to inhibit secretion despite a marked cellular depletion of GTP, and to verify that the major part of that inhibition was PTX-sensitive, and therefore involved, in all likelihood, an inhibitory GTP-binding protein.
calcium transients (47). Using basophilic leukemia cells, Wilson et al. (42) reported that MPA reduced GTP levels by more than 60% and reduced serotonin release by 50%. These effects were accompanied by little or no reduction in phospholipase C activation, but Ca\(^{2+}\) influx was reduced. In paracrineum, microinjection of GTP (but not its nonhydrolyzable analogs) was needed to support exocytosis (48). In the current studies, four different inhibitors of GTP synthesis reduced insulin secretion, an effect which was reversed by guanine (which also prevented decrements in GTP levels). Since increased ambient glucose levels can augment islet GTP concentrations and the GTP/GDP ratio (20-22), it is possible that both increments and decrements in GTP content could regulate islet function. Although effects of increments in GTP were not specifically addressed in the current studies, this speculation is compatible with the observation that, in control studies, guanine supplementation of the culture medium caused small increments in both GTP content and insulin release.

However, other explanations for the data should be considered. ATP content fell modestly with MPA or mizoribine provision and this effect also was largely prevented by guanine. ATP is felt to be important for glucose-induced insulin release (49). However, the modest decrement in ATP (up to 14% with MPA and 39% with mizoribine) may not be sufficient to explain the marked degree of secretory inhibition (49). Furthermore, provision of adenine with MPA restored ATP levels nearly to basal values yet did not prevent the inhibition of insulin release induced by MPA. The latter finding cannot be explained by a small, residual reduction in ATP (−14%) since an equivalent or greater reduction (−14 to −25%) in ATP content was seen with either MPA plus guanine or with mizoribine plus guanine (Figs. 8 and 9, respectively), conditions wherein insulin secretion was normalized essentially completely. Nonetheless, the finding that a decrement in GTP seems to produce a decline in islet ATP content supports the conclusion from other studies that GTP is felt to be important for glucose-induced insulin release for several reasons. K\(^{+}\) (50 mM)-stimulated and basal insulin release are both dependent on a similar, newly synthesized pool of insulin to a degree at least as great as (53), if not greater than (55), the one on which high (16.7 mM) concentrations of glucose are dependent. Yet the former types of secretion were largely refractory to inhibition by MPA, in contrast to release induced by high glucose levels. This is the opposite of what would be expected if MPA selectively inhibited synthesis of a small but readily released (and therefore critical) “pool” of insulin. Nonetheless, the newly synthesized, readily releasable pool of insulin has been estimated to comprise only ~33% of total insulin stores (53). It remains possible that partial blockade of this pool contributes to the small but detectible inhibition of insulin content induced by the inhibitors (mizoribine had no effect).

The blockade by MPA of the secretory effects of an amino acid or of a phorbol ester also precludes the possibilities that glucose transport, glucose metabolism, or plasma membrane receptors are the sole or principal target(s) of GTP depletion. The virtual absence of inhibition by MPA of depolarization (K\(^{+}\))-induced release excludes a direct inhibition of Ca\(^{2+}\) entry via voltage-sensitive Ca\(^{2+}\) channels, or blockade of the direct effects of Ca\(^{2+}\) on exocytosis, as likely effects of GTP depletion (at least to the degree achieved in the current studies). This finding is in accord with the failure of exogenous GTP (up 1 mM) to augment Ca\(^{2+}\)-induced secretion from normal islet cells (56), HIT cells (57), or RIN mβF cells (58) which have been permeabilized and therefore are presumably GTP-depleted. Furthermore, the findings using K\(^{+}\) as agonist further abrogate the possibility of an effect of MPA solely on insulin content. However, a role for GTP in the potentiation of submaximal intracellular Ca\(^{2+}\) levels or in “Ca\(^{2+}\)-independent” exocytosis (56) is not excluded and, indeed, exogenous GTP doubles insulin release in permeabilized islet cells at basal Ca\(^{2+}\) levels (53). The blockade of TPA-induced insulin release raises the speculation that protein kinase C activation or effects might have been inhibited by GTP depletion. Interestingly, GTP-utilizing protein kinases have recently been described (11, 12). These findings using TPA suggest at least one site of action of GTP distal to phospholipase C activation, possibly a G-protein/GTPase which is phosphorylated by protein kinase C. However, further studies will be needed to address these possibilities.

If depletion of GTP stores is proposed as the mechanism whereby MPA and the other three inosine monophosphate dehydrogenase blockers reduce secretion, it is important to
deal with the dose-response characteristics of these two parameters. MPA reduced GTP levels and insulin release over a similar concentration range (0.5 through 50 μM/ml). However, the decline in GTP had nearly reached an apparent plateau by 1–2 μg/ml, whereas insulin release fell further at higher levels of MPA (Fig. 3). There are several possible reasons for this apparent discrepancy. The extraction and HPLC techniques employed measure total (free plus bound) GTP levels in the whole cells. As has been discussed by others (6, 16, 59–61), there is reason to believe that nucleotides are compartmentalized. Furthermore, free, rather than total, nucleotide levels may be the relevant parameter. Thus, a very small but functionally important decline of free GTP levels in a critical pool could easily go undetected in the presence of a background level of ~1 mM GTP, much of which may be bound. Indeed, when GTP levels were examined at various levels of MPA only within individual studies, there was evidence of a further decline in GTP levels between 2 and 25 or 50 μg/ml MPA (data not shown). Note that the data in Table VII are pooled from five separate experiments. Additionally, it should be noted that GTP levels were measured in intact islets, of which only 70–80% are insulin-secreting cells. This fact may obscure the presence of closer relationships between GTP content in β cells and insulin release.

Such issues may be relevant to whether a reduction in GTP levels could become rate-limiting for the function of GTP-binding proteins. This possibility has often been dismissed since free GTP levels in the nanomolar to lower micromolar range have usually been found to be sufficient for the function of high affinity, low Kₜ G-proteins, including those in islets (1, 42, 62–67). However, such levels may be achieved only in the presence of much higher levels of total GTP. The exogenous provision of at least 10–100 μM of GTP was required for maximal G-protein action (1, 2, 41, 52, 68) in several cell systems, including islets (1, 2, 57). The evidence has been summarized which suggests that free concentrations of GTP could become rate limiting for the function of GTP-binding proteins (60). For example, in cultured rat kidney cells, MPA or virazole (another inosine monophosphate dehydrogenase inhibitor) reduced by 50–70% the effect of isoproterenol or prostaglandin E₁ to elevate cyclic AMP levels under conditions where the GTP content of cell was inhibited 80% (similar to the current studies) (40); guanosine reversed these effects. In C6 glialoma cells, MPA blunted the cyclic AMP response to isoproterenol in cells, homogenates, or membrane fractions; GTP reversed the inhibition (61). In Ehrlich ascites tumor cells, similar results were seen using epinephrine as agonist (41). Thus, it remains theoretically possible that GTP depletion impairs signal transduction in islets via a mechanism involving a classical GTP-binding protein.

To begin to examine this issue functionally, we pretreated islets with MPA to reduce (but not obliterate) glucose- and K⁺-induced secretion. We then determined whether the residual secretion remained inhibitable by epinephrine. Since epinephrine inhibits secretion largely via pertussis toxin-sensitive G proteins, we tested the possibility that MPA-treated islets might be resistant to epinephrine’s inhibitory effect. However, epinephrine obliterated all residual secretion. This inhibition was reversed (by 70–73%) by pertussis toxin. Since free GTP levels of ≤1–10 μM are nearly maximal for epinephrine’s effect to activate a high affinity GTPase and to inhibit adenylate cyclase in islets (1, 62) the simplest conclusion would be that MPA is not able to reduce free GTP levels sufficiently to block the function of G (or, by extrapolation, that of other heterotrimeric G proteins). However, it should be noted that, in other studies, GTP levels of 100 μM or higher were needed for epinephrine (or somatostatin) to inhibit secretion from permeabilized HIT cells in a pertussis toxin-sensitive fashion (2, 57). Thus, it remains possible that GTP levels during MPA exposure could become regulatory for a putative low affinity, high Kₜ GTPase which, we speculate, might be directly involved in exocytosis (i.e. “Gₜ”). Even some low affinity GTPases may be subject to regulation (72). This speculation is in accord with the finding of Howell et al. (46) who observed that the effect of GTP on exocytosis in permeabilized mast cells reached saturation only at 500 μM GTP. However, this possibility is not directly addressed by the current studies and can only be investigated when the GTP-binding proteins involved in physiologic insulin release are all identified and characterized. Furthermore, GTP serves a number of roles, only some which involve its interaction with GTP-binding proteins or GTPases involved in signal transduction (see Introduction). For example, levels of GTP above 1 mM may be needed for maximal synthesis of inositol-containing phospholipids (73). It has recently been suggested that a reduction in cellular content of phosphatidylinositol may impede exocytosis (74). Clearly, additional studies will be required to address this possibility and to ascertain the exact step(s) in islets whereby GTP depletion reduces exocytosis.

Finally, a word of caution is indicated with regard to the clinical uses of inosine monophosphate dehydrogenase inhibitors. MPA, ribavirin, and other inosine monophosphate dehydrogenase inhibitors are currently undergoing clinical testing for use in the treatment of human immunodeficiency virus (HIV)-associated diseases (43), cancer, inflammatory diseases, and for use as immunosuppressive agonists in the treatment of several human disease states, including renal or pancreatic transplantation (75) and, conceivably, for the immunotherapy of newly diagnosed type I diabetes mellitus. Levels of MPA achieved during therapy with its prodrug RS-61443 in humans (75) are similar to those used in the current study. Therefore, it may now be possible to assess the effects of nucleotide metabolism on islet function in vivo in humans. More cogently, the inhibition of insulin release in vitro induced by this class of pharmacologic agents suggests that islet function and glucose tolerance should be closely monitored during their use in vivo.

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6 This figure uses the average islet GTP content in our studies of 3 pmol (a figure very similar to the GTP content of freshly isolated islets in the studies of others, Refs. 21, 22) and an average islet volume of ~3 nl. An 80% reduction of GTP content by MPA would therefore reduce average GTP concentrations (bound plus free) only to ~200 μM. However, free GTP concentrations in untreated cells are probably much lower and have been estimated in other cells to be only 25–150 μM (60). A significant decrement in free GTP might make this parameter rate-limiting.

6 Note that we (28) and others (3) have observed that a fraction of epinephrine’s effect seems to be PTX-insensitive and that a variable percentage of epinephrine’s inhibitory effects in islets may persist in the apparent absence of GT (1, 2, 50, 69). Recently, we have observed the presence of such a high Kₜ (~725 μM) specific GTPase in islet secretory granules and homogenates in addition to more traditional GTPases (A. Kowluru and S. Metz, unpublished observations).
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