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CALCIUM MOBILIZATION, PROSTAGLANDIN E_2 AND α_2-ADRENOCEPTOR MODULATION OF GLUCOSE UTILIZATION AND INSULIN SECRETION IN PANCREATIC ISLETS

SUZANNE G. LAYCHOCK* and SUNA BILGIN

Department of Pharmacology and Toxicology, The Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0524, U.S.A.

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Abstract—α_2-Adrenoceptor agonists inhibit glucose-stimulated insulin release and glucose utilization in pancreatic islets. In isolated pancreatic islets of the rat, the Ca^{2+} channel agonists CGP-28392 and BAY-K-8644 increased insulin release in the presence of clonidine. Neither CGP-28392 nor BAY-K-8644 antagonized the effect of clonidine on glucose utilization. The Ca^{2+} ionophore, ionomycin, also did not affect glucose utilization in the presence or absence of clonidine. Glucagon partly reversed the effects of clonidine on insulin release, and it potentiated glucose-stimulated insulin release in the absence of clonidine. Glucagon reversed the effects of clonidine on glucose utilization. Amiloride antagonized the effects of clonidine on insulin secretion but did not enhance markedly glucose utilization in the presence or absence of clonidine. Carbamylcholine and arecoline reversed the effects of clonidine on glucose utilization and partly reversed the effects on insulin release in the absence of extracellular Ca^{2+}. Prostaglandin (PG) E_2, but not $PGF_{2\alpha}$, inhibited glucose utilization in a time- and concentration-dependent manner. PGE_2 also inhibited glucose-stimulated insulin release. Pertussis toxin blocked both actions of PGE_2. The cyclooxygenase inhibitor indomethacin did not affect insulin release or glucose utilization in the presence of clonidine. Thus, elevated intracellular Ca^{2+} levels antagonize the effects of clonidine on insulin release, whereas other mediators appear to be required to alter glucose utilization.

The cellular mechanisms accounting for the actions of α_2-adrenoceptors are varied and include an opening of K^+ channels and hyperpolarization; an inhibition of voltage-sensitive Ca^{2+} channels and reduced entry of Ca^{2+} during depolarization; and a mechanism independent of membrane current interference which occurs at a step(s) between Ca^{2+} entry and exocytosis [1]. In the pancreatic islet, α_2-adrenoceptor stimulation suppresses insulin release, cyclic AMP formation, and intracellular Ca^{2+} levels [2–5]. Since the inhibitory effects of α_2-adrenoceptor stimulation on insulin release are not overcome by the addition of cyclic AMP or the elevation of intracellular Ca^{2+} [4–6], pancreatic islet α_2-adrenoceptors affect intracellular Ca^{2+} levels and a mechanism distal to Ca^{2+} entry. Stimulation of α_2-adrenoceptors inhibits glucose utilization in the islet by a mechansim independent of adenylate cyclase inhibition [7, 8] since analogues of cyclic AMP and forskolin do not reverse the inhibition. However, there is a partial reversal of the effects of α_2-adrenoceptor stimulation on insulin release by cyclic AMP [4, 8], suggesting that insulin release and glucose utilization may be partially uncoupled in the islet.

Glucose stimulation of the pancreatic islet is a voltage-regulated process evoking an influx of Ca^{2+}, which modulates stimulus–secretion coupling [9]. Islet muscarinic cholinergic receptor stimulation also elicits Ca^{2+} influx and promotes secretion [9]. Antagonism of the α_2-adrenoceptor inhibition of glucose utilization and insulin release by carbamylcholine has been demonstrated [7, 8], although whether this antagonism is linked to changes in Ca^{2+} mobilization is not known. Moreover, the purported inhibition of guanine nucleotide binding regulatory (G) proteins by pertussis toxin antagonizes the glucose utilization response to clonidine and promotes the response to carbamylcholine [7]. Since Ca^{2+} channels are modulated by inhibitory G-proteins in some tissues [10–12], a similar G-protein-Ca^{2+} link in islets may be a part of the transduction mechanism of the α_2-adrenoceptor. The present studies were undertaken to determine whether alterations in Ca^{2+} flux in islets modulate α_2-adrenoceptor effects on glucose utilization and insulin release. A link between G-proteins, glucose utilization, and insulin release was also investigated for the effects of prostaglandins in islets.

MATERIALS AND METHODS

Materials. D-[5-^{3}H]Glucose was from American Radiolabeled Chemicals (St Louis, MO); pertussis toxin was from List Biological Laboratories (Campbell, CA); collagenase was from Serva (Heidelberg, West Germany); BAY-K-8644 was a gift from Dr. A. Scriabine, Miles Laboratories, Inc. (New Haven, CT); prazosin hydrochloride was a gift from N. Belcher, Pfizer Inc. (Groton, CT); clonidine, glucagon, bovine serum albumin (BSA; fraction V), and amiloride hydrochloride were from the Sigma Chemical Co. (St Louis, MO); CGP-28392

* Correspondence: Dr S. G. Laychock, Box 524 MCV Station, Medical College of Virginia, Richmond, VA 23298-0524.

was a gift from Dr H. Brunner, CIBA-GEIGY (Basle, Switzerland); ionomycin was from Behring Diagnostics (La Jolla, CA); indomethacin was obtained from Merck Sharp & Dohme Research Laboratories (Rahway, NJ); prostaglandin (PG) E_2 and $PGF_{2\alpha}$ tromethamine salt were from the Upjohn Co. (Kalamazoo, MI).

Preparation and incubation of islets. Isolated pancreatic islets of the rat were prepared by a collagenase technique, as previously described [8]. Islets were incubated in Krebs–Ringer bicarbonate (KRB) buffer supplemented with 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes) (16 mM), glucose (2.8 mM, unless otherwise specified), 0.01% BSA, pH 7.4, after equilibration with O_2–CO_2 (19 : 1) at 37°. Islets were preincubated for 20 min in a gyro-rotatory water bath (150 rpm), 37°, under an atmosphere of O_2–CO_2, and then the buffer was replaced with fresh buffer (0.3 ml) containing or lacking clonidine and prazosin, or prostaglandins, as indicated, for a 20-min preincubation. At the end of this 20 min, [^{3}H]glucose (10 mM, 0.1 mCi/mmol) was added to the islets in order to determine total glucose utilization; agents present during the preincubation were present throughout the remainder of the incubation. The effects of agents other than those present during the preincubation were determined by addition of the agents at 25 min (time zero) after the start of the incubation with labeled glucose. The agents were added at time zero in order to allow the islets to achieve a linear rate of glucose utilization prior to exposure to agents which might alter metabolism [8]. Reported values for glucose utilization are in excess of glucose utilized from 0 to 25 min. The ^3H_2O formed from the labeled glucose was quantitated and converted to picomoles glucose utilized, as previously described [8]. The time-related release of insulin from islets was determined by radioimmunometrically measuring insulin in aliquots of buffer removed from the islets at various times, as previously described [8]. In experiments to determine the effects of pertussis toxin, islets were preincubated with the toxin (1.5 μg/ml) for 90 min prior to the addition of PGE_2 or buffer in paired batches of islets. The incubation was continued for 20 min, and then glucose was added and insulin release was determined, or [^{3}H]glucose was added to determine glucose utilization, after 2 hr.

Statistical analysis. Statistical analyses were performed on values obtained from paired islet samples using Student's *t*-test (two-tailed), or a one-way or two-way analysis of variance employing an unweighted means analysis for unequal cell sizes. Values are the means ± SE for different numbers (N) of experimental determinations. When percentage values are reported, statistical evaluation was performed using real data values.

RESULTS

Effects of Ca^{2+} channel agonists. Clonidine has been shown previously to inhibit glucose-stimulated insulin release and glucose utilization in pancreatic islets [2–5, 8]. Since it has been reported that clonidine reduces the Ca^{2+} level in islets [5, 13, 14], the effects of Ca^{2+} channel agonists on the glucose utilization responses to clonidine were investigated. The Ca^{2+} channel agonists CGP-28392 and BAY-K-8644 stimulated insulin release in the presence of clonidine and a sub-maximal secretagogic glucose concentration (10 mM) during 60 min (Fig. 1A). However, BAY-K-8644 did not induce a significant increase in hormone release until 60 min after exposure of the islets to the agent (Fig. 1A). Neither CGP-28392 nor BAY-K-8644 potentiated insulin release in the presence of 10 mM glucose alone (Fig. 1B).

Glucagon also activates Ca^{2+} channels, adenylate cyclase, and insulin secretion [15–18]. Glucagon stimulated insulin release in clonidine-treated islets within 30 min (Fig. 1A). However, in the presence of clonidine, neither glucagon nor CGP-28392 nor BAY-K-8644 completely restored insulin release. On the other hand, glucagon was a potent insulin secretagogue and potentiated the insulin release response to glucose in the absence of clonidine (Fig. 1B). Compared to control basal insulin release values with 2.8 mM glucose (313 ± 130 μU insulin/ml/25 islets), BAY-K-8644 (20 μM; 363 ± 161 μU insulin/ml/25 islets), CGP-28392 (20 μM; 413 ± 86 μU insulin/ml/25 islets), and glucagon (1 μM; 380 ± 74 μU insulin/ml/25 islets) did not affect significantly insulin release during 60 min.

Glucose utilization in these experiments was characteristically inhibited by clonidine; however, the presence of BAY-K-8644 or CGP-28392 did not affect significantly glucose utilization in the presence of the α_2-adrenoceptor agonist (Fig. 2). CGP-28392 did not affect significantly control glucose utilization values (Fig. 2). Similarly, glucose (10 mM) utilization with the Ca^{2+} ionophore, ionomycin (1 μM), was not significantly different in the absence (90 ± 9%) or presence of clonidine (1 μM) (107 ± 12%) compared to respective controls in four experiments.

In contrast to the lack of effect of the Ca^{2+} channel agonists on glucose utilization, glucagon stimulated glucose utilization and negated the effect of clonidine (Fig. 2). On the other hand, glucagon did not affect control glucose utilization (Fig. 2).

Effects of agents that alter Ca^{2+} fluxes. Amiloride inhibits Na^+/H^+ exchange, reduces intracellular pH, increases Ca^{2+} flux, and has been reported to inhibit or potentiate glucose-stimulated insulin release in pancreatic islets [19, 20]. In agreement with published reports [19, 20], amiloride did not affect basal levels of insulin release (Fig. 3A). Insulin release with amiloride (0.1 mM) and glucose (10 mM) together (5399 ± 1371 μU insulin/ml/25 islets) was not significantly different from release values with glucose (10 mM) alone (5031 ± 1638 μU insulin/ml/25 islets) after 60 min. When islets were inhibited by clonidine, however, insulin release in the presence of amiloride was significantly higher than clonidine-treated control values (Fig. 3A). Insulin release with glucose (10 mM), clonidine and amiloride was not significantly different from insulin release values with glucose stimulation alone (Fig. 3A). A higher concentration of amiloride (1 mM) did not further enhance insulin release over 0.1 mM amiloride-treated release values with clonidine (data not shown), and 1 mM amiloride reduced glucose (10 mM)-stimulated insulin release by 29 ± 10%.

Amiloride did not affect glucose utilization mark-

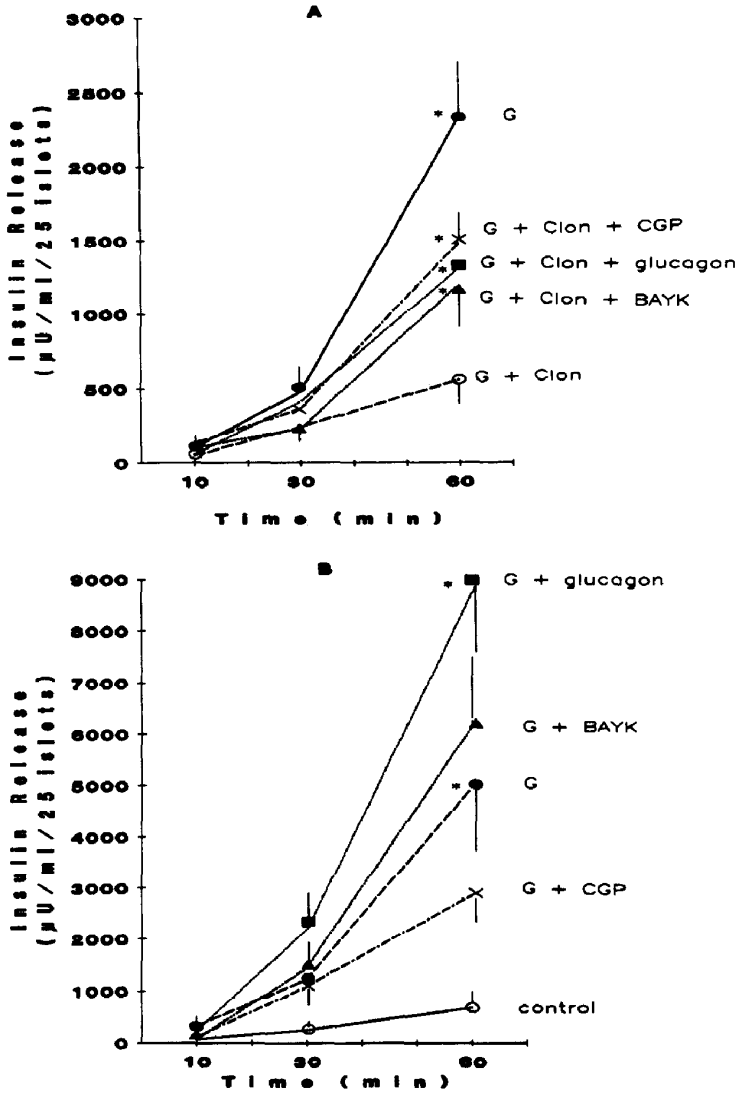

Fig. 1. Effects of altered Ca^{2+} channel activity on insulin release. Islets were preincubated in the presence (A) or absence (B) of clonidine (Clon; 1 μM) for 20 min, and then BAY-K-8644 (BAYK; 20 μM), CGP-28392 (CGP; 20 μM), or glucagon (1 μM) was added to the islets, as indicated. These additions were followed by the addition of glucose (G) (10 mM) to all samples in (A) and (B) except the control (2.8 mM glucose). Insulin release was determined at the times indicated. In (A), values are the means ± SE for eight independent experimental determinations, and an asterisk (*) denotes values significantly different ($P < 0.01$) from clonidine-treated values. In (B), values are means ± SEM for four or five independent experimental determinations, and an asterisk (*) denotes glucose-stimulated values significantly different ($P < 0.01$) from control, and glucagon-treated sample values significantly different ($P < 0.05$) from glucose-stimulated sample values; G + BAYK and G + CGP values were not different from G values. P values were determined by two-way analysis of variance.

edly. Amiloride-treated islet glucose utilization was no different from control, and amiloride did not increase significantly the rate of glucose utilization in the presence of clonidine (Fig. 3B). A higher concentration of amiloride (1 mM) also did not affect glucose utilization in the presence or absence of clonidine (data not shown). Prazosin was included in these experiments (Fig. 3) to void any interaction of α_1-adrenoceptor activity with α_2-adrenergic inhibition. Experiments conducted in the absence of prazosin provided results similar to those described above.

The stimulation of muscarinic receptors in islets evokes cell depolarization, increased intracellular Ca^{2+} levels, and insulin secretion [21–23]. Moreover, in the presence of extracellular Ca^{2+}, muscarinic receptor agonists antagonize the effects of clonidine on glucose utilization and insulin release [7, 8]. The muscarinic receptor agonists carbamylcholine and arecoline were studied for their effects on clonidine-inhibited insulin release and glucose utilization in the absence of extracellular Ca^{2+} to determine if Ca^{2+} influx affects these responses. In the absence of Ca^{2+}, clonidine inhibited glucose-stimulated glucose

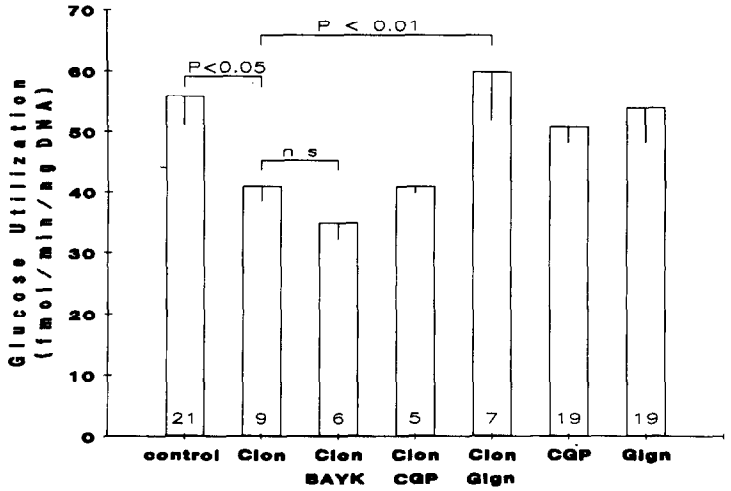

Fig. 2. Effects of altered Ca^{2+} channel activity on glucose utilization. Islets were preincubated in the presence or absence of clonidine (Clon) (1 μM) for 20 min, as indicated. Then, the islets were suspended in [^{3}H]glucose (10 mM) in the absence (control) or continued presence of clonidine, and BAY-K-8644 (BAYK) (20 μM), CGP-28392 (CGP) (20 μM), or glucagon (Glgn) (1 μM) was added to the islets at time zero. Glucose utilization was determined at 5, 20 and 60 min after time zero, and the mean ± SE rate of glucose utilization was determined from these values for the number of experimental determinations indicated at the base of each bar. P values were determined by one-way analysis of variance.

utilization, and carbamylcholine and arecoline reversed the effects of clonidine (Fig. 4A). The stimulatory effects of both muscarinic receptor agonists were evident within 5 min after addition of the drugs (Fig. 4A). Neither carbamylcholine nor arecoline affected glucose utilization in the presence of glucose alone (Fig. 4A).

Insulin release mimicked the response of glucose utilization to the muscarinic agonists. Although there was great variability among the secretory responses, both carbamylcholine and arecoline enhanced insulin release in the absence of Ca^{2+} (Fig. 4B). Moreover, both agonists stimulated insulin release in the presence of clonidine, and the insulin release values were not significantly different from values obtained in the absence of clonidine (Fig. 4B). Neither carbamylcholine nor arecoline evoked secretion in the presence of a basal concentration of glucose (2.8 mM) (data not shown).

Effects of prostaglandins. Increased Ca^{2+} levels in the islet can stimulate phospholipase A_2 activity [24, 25], and increase arachidonic acid availability and the synthesis of cyclooxygenase and lipoxygenase products [26, 27]. To determine if prostaglandins (PG) modulate glucose utilization, two predominant prostaglandins synthesized in islets, PGE_2 and $PGF_{2\alpha}$ [26, 27], were studied. In islets preincubated with prostaglandin, PGE_2 induced a concentration-related suppression of the rate of glucose utilization in the presence of 10 mM glucose (Fig. 5). Without the benefit of preincubation, however, the potency of PGE_2 was reduced and a higher concentration of the prostaglandin (10 μM) was required to induce an inhibition of glucose utilization (Fig. 5). $PGF_{2\alpha}$, on the other hand, did not affect glucose utilization with or without a preincubation period (Fig. 5). Under similar conditions, glucose (10 mM)-stimulated insulin secretion in islets pretreated with PGE_2 (1 μM) was 29 ± 3% ($P < 0.05$) of paired glucose-stimulated control values at 60 min.

Islets were also incubated for a longer period in the presence and absence of pertussis toxin to determine if a G-protein(s) mediates insulin secretory and glucose utilization responses. PGE_2 inhibited insulin release induced by 10 mM glucose; however, this inhibition was not observed when islets were treated with pertussis toxin (Table 1). PGE_2 did not affect significantly insulin release with a substimulatory glucose concentration (5.44 mM) (Table 1).

To correlate changes in insulin release with glucose utilization, islets were incubated with various concentrations of glucose in the presence or absence of pertussis toxin and PGE_2. During a 2-hr incubation with 10 mM glucose, PGE_2 (1 μM) inhibited glucose utilization by 69 ± 12% of control ($P < 0.05$). When islets were princubated with pertussis toxin (1.5 μg/ml) for 90 min prior to the determination of glucose utilization with PGE_2 (1 μM), glucose utilization (5.44 mM) with PGE_2 and pertussis toxin was 196 ± 35% of values with PGE_2 alone ($P < 0.01$). Glucose utilization with pertussis toxin alone was 111 ± 5% of control values.

To determine if prostaglandins mediated the inhibitory effects of clonidine, an inhibitor of cyclooxygenase was added to islets. Ten micromolar indomethacin, a concentration sufficient to inhibit cyclooxygenase activity in islets [26, 28], was without effect on insulin release or glucose utilization in the presence of clonidine (1 μM) and 10 mM glucose (data not shown). In addition, 0.01 and 0.1 μM clonidine inhibited insulin release by 51 ± 10% and 33 ± 17% of glucose (10 mM)-stimulated release values respectively. Indomethacin (10 μM) did not alter significantly the inhibitory effects of the lower

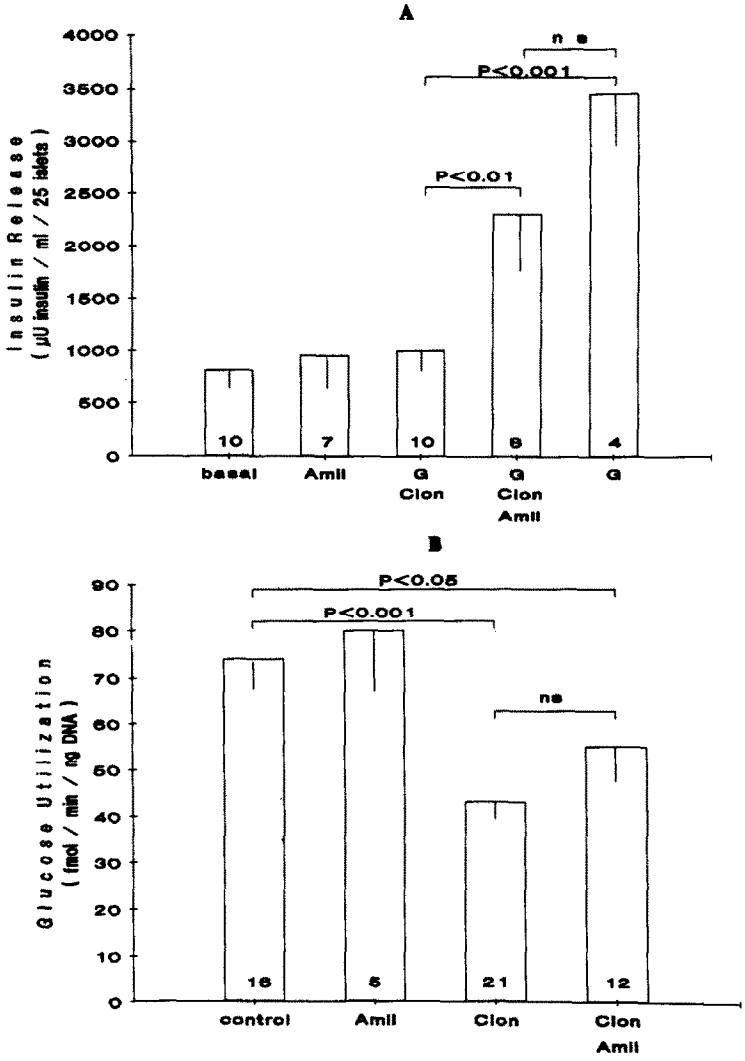

Fig. 3. Effects of amiloride on insulin release and glucose utilization. (A) Islets were preincubated in the presence or absence of clonidine (Clon) (1 μM) and prazosin (1 μM) for 20 min, and then the incubation was continued in the presence of glucose (basal; 2.8 mM) (G; 10 mM); amiloride (Amil; 0.1 mM) was added to the islets at time zero, as indicated. Insulin release was determined 60 min after time zero. (B) Following a 20-min preincubation with clonidine (1 μM) and prazosin (1 μM), islets were incubated with [^{3}H]glucose (10 mM); amiloride (Amil; 0.1 mM) was added to the islets at time zero, as indicated, and the incubation was continued for up to 60 min. P values were determined by one-way analysis of variance. The number of different determinations is shown at the base of each bar.

concentrations of clonidine, and insulin release with clonidine (0.1 μM), indomethacin (10 μM) and glucose (10 mM) was 51 ± 13% of release with glucose alone.

DISCUSSION

Although clonidine (0.3 μM or lower) has been reported to affect insulin release selectively [29], micromolar and submicromolar concentrations of clonidine and epinephrine have been demonstrated to reduce: (1) insulin release [2, 4, 8, 13, 30]; (2) glucose utilization [7, 8]; (3) adenylate cyclase activity [2, 30]; and (4) Ca^{2+} levels in beta cells of the islet [5, 12, 13]. The dihydropyridine derivative Ca^{2+} channel activators, CGP-28392 and BAY-K-8644, partially overcame the clonidine-induced inhibition of insulin release, although neither compound greatly affected insulin release from glucose-stimulated rat islets in the absence of clonidine. CGP-28392 induces insulin release due to an influx of Ca^{2+} [31]. BAY-K-8644 also stimulates Ca^{2+} influx in islets [32], but acts on the open Ca^{2+} channels induced by moderate depolarization with glucose or K^+ [33]. Thus, it appears that elevations in intracellular Ca^{2+} can antagonize or compensate for $\alpha_2$adrenergic actions. However, the Ca^{2+} effects may be indirect.

The small latent effects of BAY-K-8644 may be due to the paucity of open Ca^{2+} channels in clonidine-inhibited islets [5]. CGP-28392 may have elicited a

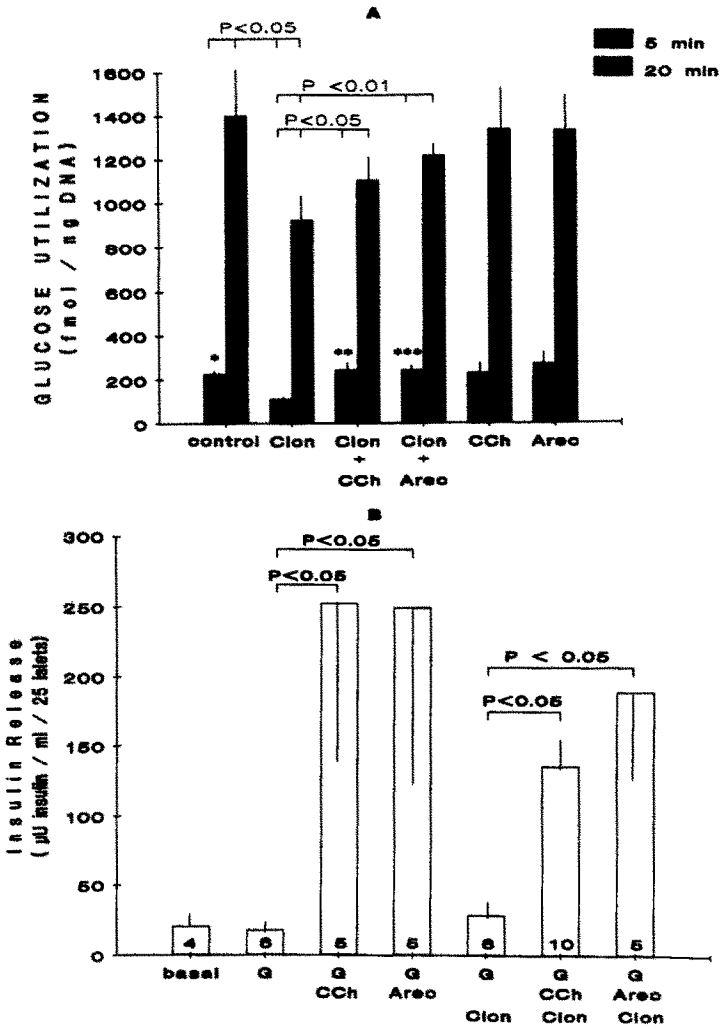

Fig. 4. Effects of carbamylcholine and arecoline on clonidine-inhibited glucose utilization and insulin release in the absence of Ca^{2+}. (A) Islets were preincubated in KRB buffer containing ethyleneglycolbis(amino-ethylether)tetra-acetate (EGTA) (0.5 mM) and no added $CaCl_2$, with clonidine (Clon; 1 μM) for 20 min. Then, [^{3}H]glucose (10 mM) was added to all samples to determine glucose utilization. Carbamylcholine (CCh; 10 μM) and arecoline (Arec; 10 μM) were added at time zero. Differences between treatment groups were determined by two-way analysis of variance; differences between 5-min values for treatment groups versus clonidine-treated samples [(*) $P < 0.05$, (**) $P < 0.02$, and (***) $P < 0.01$] were determined by Student's t-test (unpaired). Values are the means ± SE for six different experimental determinations. (B) Insulin release values for islets were determined after incubating the islets as described in (A) in the absence of Ca^{2+} and the presence of glucose (basal; 2.8 mM) (G; 10 mM) and the absence or presence of Clon (1 μM), with the addition or omission of CCh (10 μM) or Arec (10 μM), as indicated. Differences between treatment groups were determined by one-way analysis of variance. The number of experimental determinations is shown at the base of each bar.

stronger response than BAY-K-8644 based upon its ability to evoke prolonged hormone release in contrast to the monophasic release observed with BAY-K-8644 [31, 32]. The results agree with the observation in mouse beta cells that K^+-evoked increases in Ca^{2+} entry in clonidine-treated cells stimulates insulin release [5]. Perhaps these drugs did not evoke increases in glucose-stimulated insulin release in rat islets in the absence of clonidine because quantitation of the early phase of insulin release is difficult in static incubations of islets, and small initial changes in release may not have contributed remarkably to the pattern of insulin release in these studies.

In insulin secreting permeabilized RINm5F cells, the stimulation of α_2-adrenoceptors inhibits Ca^{2+}-induced secretion in a GTP-dependent manner, suggesting that a locus of α_2-adrenergic inhibition is distal to Ca^{2+}-mediated responses [34]. Another locus of inhibition undoubtedly accounts for the inability of the Ca^{2+} channel agonists to reverse completely the effects of clonidine on secretion. Since neither the Ca^{2+} channel activators nor the Ca^{2+} ionophore ionomycin evoked changes in glucose utilization in clonidine-treated islets, glucose

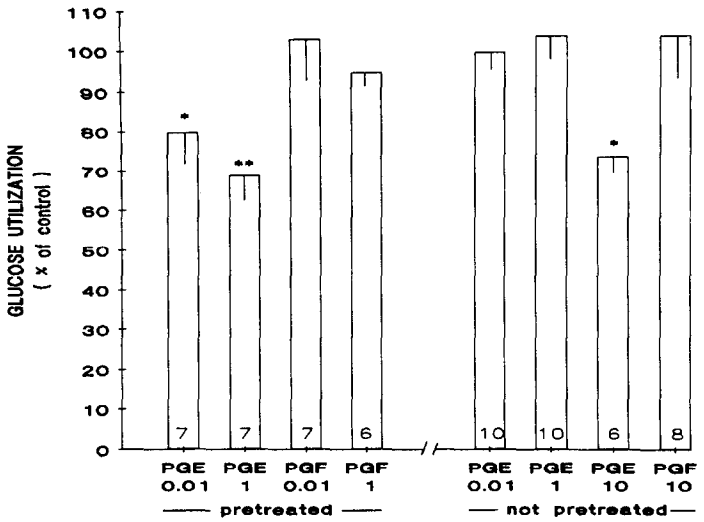

Fig. 5. Effects of prostaglandins on glucose utilization. Islets were preincubated in the absence (not pretreated) or presence of PGE_2 (PGE; 0.01 or 1 μM), or $PGF_{2\alpha}$ (PGF; 0.01 or 1 μM) (pretreated) for 20 min, as indicated. Then, the islets were incubated with [^{3}H]glucose (10 mM) to determine glucose utilization; where islets were pretreated, prostaglandins were present throughout the incubation. The islets not pretreated were exposed to the prostaglandins (0.01, 1 or 10 μM), as indicated, at time zero. The incubation was continued for 20 min after time zero, and the rate of glucose utilization was determined from values quantitated at 5 and 20 min after time zero. Values are the mean (± SE) percent of control values (44 ± 3 fmol/min/ng DNA) with 10 mM glucose, for the number of independent determinations shown at the base of each bar. Levels of significance [(*) $P < 0.05$; (**) $P < 0.01$] were determined by Student's *t*-test (paired).

Table 1. Insulin release from isolated pancreatic islets

	Insulin release (μU/ml/25 islets)			
	Control	PGE_2	PT	PT + PGE_2
Glucose (5.44 mM)	1401 ± 350 (10)	1874 ± 461 (10)	1855 ± 388 (9)	2135 ± 803 (9)
Glucose (10 mM)	4011 ± 801* (10)	1646 ± 279† (11)	2889 ± 628 (9)	3020 ± 546 (9)

Islets were preincubated in KRB buffer with 2.8 mM glucose in the presence or absence of pertussis toxin (PT; 1.5 μg/ml) for 90 min. Then PGE_2 (1 μM) was added to the islets for 20 min. The concentration of glucose was then increased to 5.44 or 10 mM, and insulin release was determined after 120 min. Values (mean ± SE) are minus insulin released during the preincubation period. The number of different determinations is shown in parentheses. P values were determined by Student's *t*-test (unpaired).

* $P < 0.01$ compared to control with 5.44 mM glucose.

† $P < 0.05$ compared to control with similar glucose concentration.

utilization is another pathway affected by α_2-adrenergic activity, independent of Ca^{2+}. Perhaps the persistent inhibition of glucose metabolism contributes to suppressed secretory activity even with elevated intracellular Ca^{2+} levels.

Glucagon was also investigated for its effects on clonidine-inhibited islets, since glucagon receptors on beta cells activate adenylate cyclase, increase cyclic AMP levels, promote Ca^{2+} entry in cells [15] and stimulate insulin release [16–18]. The most striking difference between glucagon and the Ca^{2+} channel activators in this study was the ability of glucagon to normalize glucose utilization in the presence of clonidine. Since 8-bromo-cyclic AMP and forskolin do not overcome the effects of clonidine on glucose utilization [7, 8], the combined elevation of cyclic AMP and Ca^{2+}, or perhaps the generation of a different messenger, contribute to changes in islet metabolism. The observation that glucagon did not affect glucose utilization in normal islets agrees with a previous report [35].

Glucagon also evoked insulin release in clonidine-inhibited islets and potentiated glucose-induced insulin release in normal islets. The effect of glucagon on insulin release is probably related to cyclic AMP and Ca^{2+}, since both of these agents can partly overcome the effects of clonidine on secretion [4, 8]. At the concentration of clonidine used in this study,

the agonist would be expected to partially inhibit cyclic AMP accumulation in response to glucagon [30]. The extent to which cyclic AMP and Ca^{2+} influx, as opposed to glucose utilization, contribute to the effect of glucagon on insulin release is not known. In the presence of clonidine, the Ca^{2+}-mediated mechanism of glucagon is likely to be blunted, and glucose metabolism may contribute significantly to insulin secretion. This hypothesis is supported by the ability of glucagon to potentiate the effects of glucose on insulin release in the absence of clonidine, when Ca^{2+} channel activity would be unimpeded. An investigation of these mechanisms is the subject of continuing studies.

Amiloride was also investigated for its effects on clonidine-treated islets since it can increase Ca^{2+} flux and insulin release in normal islets [20]. Amiloride evoked modest changes in insulin release with glucose and clonidine, without evoking changes in basal or control glucose-stimulated insulin release. Although amiloride can compete with clonidine receptor binding [36] and perhaps enhance islet activity in this manner, this seems a less plausible explanation if one considers that a lower concentration of amiloride was as, or better, able to evoke secretion than a millimolar concentration of the drug. Amiloride also did not affect significantly the rate of glucose utilization in the presence or absence of clonidine, suggesting that antagonism of the actions of clonidine was molecular rather than receptor-directed. These results support the hypothesis that changes in Ca^{2+} flux affect secretion but not glucose utilization.

Since agents which stimulate Ca^{2+} mobilization in cells can stimulate phospholipase A_2, and thereby increase arachidonic acid availability and metabolism, the major cyclooxygenase metabolites of arachidonic acid in islets, PGE_2 and to a lesser extent $PGF_{2\alpha}$, were investigated for effects on glucose utilization. Prostaglandins, including PGE_2, inhibit insulin release in response to glucose in some studies [37, 38], and inhibitors of cyclooxygenase promote secretion of hormone [39, 40]. In the present study, PGE_2 inhibited glucose utilization in a concentration-dependent manner, consistent with the inhibition of insulin release, whereas $PGF_{2\alpha}$ was without effect. Moreover, the response to PGE_2 was time dependent, requiring a period of preincubation with the tissue in order to affect glucose utilization. Without the benefit of preincubation, higher concentrations of PGE_2 were required to elicit inhibition.

However, it is unlikely that the inhibition of insulin release or glucose utilization by clonidine was due to enhanced prostaglandin biosynthesis, since indomethacin did not affect the responses to clonidine. Dunlop *et al.* [41] reported that indomethacin does not affect glucose utilization.

The mechanism of inhibition of insulin secretion and glucose utilization by clonidine and PGE_2 may involve a similar mechanism, since pertussis toxin pretreatment of islets antagonized the inhibitory response to both agents [7, 42]. Pertussis toxin also reversed the inhibitory effect of PGE_2 on islet insulin release. Pertussis toxin also blocked the inhibitory action of PGE_2 on insulin release from hamster insulinoma cells [37]. Since cyclic AMP generation is without effect on glucose utilization [8], the guanine nucleotide binding regulatory protein affected by pertussis toxin may pertain to the regulation of another enzyme(s) or cation regulatory system involved in glucose utilization in islets.

Since carbamylcholine and arecoline antagonize the effects of clonidine on insulin release and glucose utilization in islets in the presence of extracellular Ca^{2+} [7], the ability of these agents to influence glucose utilization in the absence of extracellular Ca^{2+} was determined. Both muscarinic cholinergic receptor agonists elicited slight increases in glucose-stimulated insulin release under Ca^{2+}-free conditions. In the absence of Ca^{2+}, mouse islets also respond to a combined acetylcholine–glucose stimulus with an early peak of insulin release [21]. In addition, carbamylcholine and arecoline antagonized the effects of clonidine on glucose utilization and insulin release. The responses of islets to cholinergic receptor stimulation include an increase in voltage-regulated Ca^{2+} influx, as well as an increase in intracellular Ca^{2+} mobilization as a result of phospholipase C stimulation and inositol phosphate production [21–23, 43]. Moreover diglyceride formed through phospholipid hydrolysis can stimulate protein kinase C [44]. The mechanism for cholinergic receptor-stimulated reversal of the clonidine responses may lie in the combined effects of Ca^{2+} mobilization and protein kinase C activation since these data indicate that Ca^{2+} mobilization alone is not sufficient to affect glucose utilization. However, other investigators suggest that α_2-adrenoceptor actions affect insulin release mechanisms distal to Ca^{2+}, cyclic AMP or protein kinase C [6].

In summary, these results demonstrated that Ca^{2+} entry in islets can evoke insulin release in the presence of clonidine, although Ca^{2+} is not an effective modulator of glucose utilization. Receptor agonists which stimulate Ca^{2+} mobilization and protein kinase C activity antagonized the effects of clonidine on insulin release and glucose utilization, perhaps through changes in G-protein activity or the generation of another cellular messenger such as cyclic GMP. The reversal of the actions of clonidine by the pharmacologic agents used in this study may be due to fortuitous compensatory cell stimulatory mechanisms which overcome the effects of clonidine on independent pathways. Glucagon is also an effective modulator of glucose utilization and insulin release; however, the mechanism for the antagonism of clonidine's actions remains to be determined. PGE_2 shares the inhibitory action of clonidine in suppressing glucose utilization and insulin release, and in the sensitivity of the response to pertussis toxin. Thus, prostaglandin synthesis may represent a physiological mechanism modulating insulin release through changes in cyclic AMP [34] and glucose utilization mediated by a G-protein. The specific locus of the inhibition of glucose utilization by α_2-adrenoceptor stimulation and prostaglandin remains to be determined.

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