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α_2-Adrenergic inhibition of pancreatic islet glucose utilization is mediated by an inhibitory guanine nucleotide regulatory protein

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The rate of glucose utilization in isolated pancreatic islets of the rat was inhibited by the α_2-adrenoceptor agonists clonidine and epinephrine. Yohimbine reversed the inhibition. α_1 or β-adrenoceptor agonists had little or no effect on glucose utilization. Stimulation of muscarinic receptors by carbamylcholine reversed the effect of clonidine. Pertussis toxin blocked the effect of clonidine on glucose utilization, and potentiated the response to carbamylcholine. 8-Bromo-cAMP did not affect glucose utilization in the presence of clonidine. Thus, α_2-adrenoceptors negatively modulate glucose utilization, and the effect is mediated by an inhibitory guanine nucleotide regulatory protein, but not by cAMP.

α-Adrenoceptor; Glucose utilization; N-protein; Clonidine; cyclic AMP; (Islets of Langerhans)

1. INTRODUCTION

The function of pancreatic islets of Langerhans is negatively modulated by α_2-adrenoceptor stimulation. α_2-Adrenoceptor agonists inhibit insulin release and adenylate cyclase activity, and suppress intracellular Ca^{2+} levels [1–3]. However, the addition of exogenous cAMP analogues or the stimulation of adenylate cyclase with forskolin does not reverse the effect of α_2-adrenergic stimulation on insulin release, even though intracellular Ca^{2+} levels are elevated [3–5]. Thus, α_2-adrenoceptors mediate cellular mechanisms in addition to, and distal to, adenylate cyclase.

Glucose is the primary physiological stimulus for insulin release, which is directly correlated with glucose utilization [6,7]. The present study was undertaken to determine whether the modulation of insulin release by the autonomic nervous system in pancreatic islets is mediated by changes in glucose utilization. The results of this study demonstrate that α_2-adrenoceptors exert specific effects on the rate of glucose utilization which are modulated by an inhibitory guanine nucleotide regulatory protein.

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2. MATERIALS AND METHODS

2.1. *Materials*

Pertussis toxin was obtained from List Biological Laboratories (Campbell, CA); D-[5-^{3}H]glucose was obtained from American Radiolabeled Chemicals (St. Louis, MO); collagenase from *Clostridium histolyticum* was from Serva (Heidelberg); clonidine, yohimbine, isobutylmethylxanthine, and carbamylcholine were obtained from Sigma (St. Louis, MO); prazosin hydrochloride was a gift from N. Belcher, Pfizer Inc. (Groton, CT); DL-phenylephrine HCl was from Winthrop Laboratories (New York, NY).

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2.2. *Methods*

Pancreatic islets were isolated from excised pancreata of male Sprague-Dawley rats by collagenase digestion as in [8]. Equal batches of isolated islets were incubated in Krebs-Ringer bicarbonate (KRB) buffer containing 16 mM Hepes, 1 or 10 mM D-[5-^{3}H]glucose, and 0.01% bovine serum albumin, and adjusted to pH 7.4 after equilibration with O_2-CO_2 (19:1) at 37°C. [5-^{3}H]Glucose is metabolized through glycolysis and ^3H_2O is produced. Quantitation of the ^3H_2O produced is converted into fmol glucose utilized, based upon the specific activity of the glucose in the incubation medium [7,9,10]. In this study, the rate of glucose utilization was determined between 25 and 45 min when glucose utilization is linear [9]. Adrenergic agents, including agonists and antagonists, were added 20 min prior to [^{3}H]glucose and were present throughout the incubation. Islets were preincubated with pertussis toxin for 90 min prior to [^{3}H]glucose, since there is a lag period of 60 min before the action of the toxin is expressed [11]; the toxin was also present throughout the remainder of the experiment. Carbamylcholine and 8-Br-cAMP were added to the islets after 25 min of incubation with [^{3}H]glucose. Islet DNA content was determined as described [9]. Statistical analysis of the data was carried out using a two-tailed Student's *t*-test (paired or unpaired).

3. RESULTS

Isolated pancreatic islets incubated with 10 mM [^{3}H]glucose had a higher rate of glucose utilization compared to islets with 1 mM [^{3}H]glucose (fig.1). The addition of the α_2-adrenoceptor agonist clonidine to islets incubated with 10 mM [^{3}H]glucose inhibited glucose utilization by 40% (fig.1). Yohimbine, an α_2-adrenoceptor antagonist, completely reversed the effects of clonidine on glucose utilization (fig.1). The β-adrenoceptor agonist, isoproterenol, did not affect glucose utilization (fig.1). Moreover, α_2-adrenoceptor stimulation by the physiological agonist epinephrine, in the presence of the α_1-adrenoceptor antagonist prazosin and the β-adrenoceptor antagonist propranolol, also inhibited glucose utilization (table 1A). The response to epinephrine was inhibited by yohimbine (table 1A). Glucose utilization in the presence of propranolol and prazosin (table 1A)

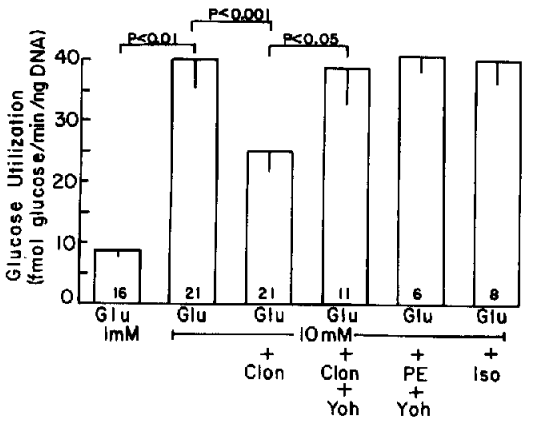

Fig.1. Effects of adrenergic agents on islet glucose utilization. Islets were incubated in buffer containing [^{3}H]glucose (Glu) at the concentration indicated, in the absence or presence of clonidine (Clon) (1 μM), yohimbine (Yoh) (10 μM), phenylephrine (PE) (1 μM), or isoproterenol (Iso) (10 μM). Values are means ± SE for the number of different experimental determinations shown at the base of each bar.

Table 1

Effect of adrenoceptor stimulation and 8-Br-cAMP on islet glucose utilization

Treatment		Rate of glucose utilization (fmol glucose/min per ng DNA)	(*n*)
A	Prop, Praz	44 ± 3	(16)
	Epi, Prop, Praz	34 ± 3[b]	(16)
	Epi, Prop, Praz, Yoh	42 ± 5	(5)
B	Praz	55 ± 11	(6)
	Clon, Praz	31 ± 3[a]	(13)
	Clon, Praz, 8-Br-cAMP	34 ± 3[a]	(10)

Islets were incubated in Krebs buffer containing 10 mM [^{3}H]glucose and the presence or absence of propranolol (Prop) (1 μM), prazosin (Praz) (1 μM), epinephrine (Epi) (1 μM), yohimbine (Yoh) (10 μM), clonidine (Clon) (1 μM), or 8-Br-cAMP (0.2 mM), as indicated. Values are the means ± SE for the number of different experimental determinations (*n*). *P* values ([a] <0.02, [b] <0.01) indicate the differences from Prop plus Praz-treated islets in (A), or from Praz-treated islets in (B), and were determined by Student's *t*-test

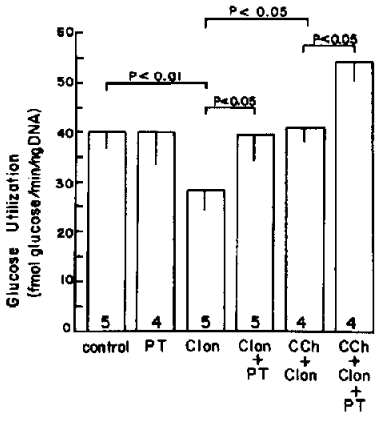

Fig.2. Effects of carbamylcholine and pertussis toxin on islet glucose utilization. Islets were incubated with 10 mM [^{3}H]glucose in the absence (control) or presence of pertussis toxin (PT) (1.5–3 μg/ml), clonidine (Clon) (1 μM), or carbamylcholine (CCh) (10 μM) plus isobutylmethylxanthine (0.1 mM), as indicated. Values are means ± SE for the number of different experimental determinations shown at the base of each bar.

was comparable to control values obtained in islets exposed to 10 mM glucose. Moreover, prazosin did not affect the response to clonidine (table 1B). In addition, 8-Br-cAMP did not alter the effect of clonidine on glucose utilization (table 1B). Moreover, the rate of glucose utilization in the presence of the α_1-adrenoceptor stimulus phenylephrine plus yohimbine was comparable to that observed in control islets (fig.1).

To determine whether the effects of clonidine on glucose utilization are mediated by the inhibitory guanine nucleotide binding regulatory protein (N_i), islets were incubated with pertussis toxin, an inhibitor of N_i. Pertussis toxin did not change glucose utilization compared to control values (fig.2). However, pertussis toxin completely blocked the inhibitory effects of clonidine on glucose utilization (fig.2). The muscarinic receptor agonist, carbamylcholine, reversed the inhibitory effect of clonidine on glucose utilization, and pertussis toxin enhanced this effect of carbamylcholine (fig.2). Since preliminary studies indicate that isobutylmethylxanthine augments the response to carbamylcholine (not shown), this phosphodiesterase inhibitor was included in the carbamylcholine experiments. Carbamylcholine (10 μM) did not significantly affect basal (10 mM) glucose utilization (not shown).

4. DISCUSSION

These results demonstrate that stimulation of α_2-adrenoceptors in the pancreatic islet suppresses glucose utilization. Although adenylate cyclase can be inhibited by α_2-adrenergic agonists, this response plays a minor role in insulin release since diminished cAMP levels alone do not correlate with the suppression of insulin secretion [3–5]. Since insulin release is directly modulated by glucose phosphorylation and metabolism, the present data suggest that α_2-adrenergic receptor stimulation reduces insulin release through the inhibition of glucose utilization. The response to α_2-adrenoceptor agonists is specific since neither α_1- nor β-adrenoceptor agonists affected glucose utilization, and the effect was inhibited by an α_2-, but not by an α_1-, adrenergic antagonist. Moreover, the inhibition of adenylate cyclase by clonidine probably did not mediate the effects of this agent on glucose utilization since 8-Br-cAMP did not alter the response to clonidine.

The experiments utilizing pertussis toxin demonstrate that α_2-adrenoceptor effects on glucose utilization are mediated by an inhibitory guanine nucleotide regulatory protein, N_o or N_i. Pertussis toxin ribosylates and inactivates certain N-proteins, and this toxin also reversed the effects of clonidine on glucose utilization, and restores insulin release [11]. However, the activation of adenylate cyclase or the addition of exogenous cAMP analogues does not antagonize the cellular inhibition by α_2-adrenergic agonists [4,5]. Therefore, it is likely that islet α_2-adrenoceptor-associated inhibitory N-protein has pluralistic functions, perhaps affecting glucose transport, or phosphodiesterase activity [12]. However, pertussis toxin did not change basal glucose utilization, suggesting a specific interaction with α_2-adrenoceptor-mediated cellular events.

Carbamylcholine reverses the inhibitory effect of clonidine on glucose utilization (fig.2) and potentiates the insulin secretory response after α-adrenoceptor antagonism [13]. Cholinergic stimulation of cells can also induce changes in polyphosphoinositide turnover, Ca^{2+} mobiliza-

tion, and cGMP levels [14,15]. It is not known which of these effects, or others, mediate the changes observed in glucose utilization with cholinergic stimulation. However, the enhanced cholinergic response in the presence of pertussis toxin suggests that carbamylcholine not only antagonizes the clonidine inhibition but also stimulates the production of a positive modulator of glucose utilization which fully expresses its effect in the presence of pertussis toxin.

In conclusion, this study is the first to demonstrate that in pancreatic islets α_2-adrenoceptors express an inhibitory action on the rate of glucose utilization. The effect is antagonized by cholinergic receptor stimulation, and mediated by an inhibitory guanine nucleotide regulatory protein through a mechanism that does not involve cAMP.

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