Adrenergic Modulation of Pancreatic A, B, and D Cells

α-ADRENERGIC SUPPRESSION AND β-ADRENERGIC STIMULATION OF SOMATOSTATIN SECRETION, α-ADRENERGIC STIMULATION OF GLUCAGON SECRETION IN THE PERFUSED DOG PANCREAS

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ABSTRACT The effects of adrenergic substances on pancreatic insular secretions were studied in a completely isolated canine pancreas with exclusion of the duodenum from the perfusion circuit. To ensure adequate blockade, blockers were infused before agonists. A dose range of β-receptor blockade was tested, and putative α-adrenergic effects were confirmed by combined α- and β-adrenergic receptor blockade.

β-Adrenergic agonism (2 ng/ml isoproterenol) induced a mean integrated increase of 79±20% in somatostatin secretion, whereas glucagon and insulin secretion were increased by 185±45 and 495±146%, respectively. The stimulations of D, A, and B cells were abolished by propranolol.

α-Adrenergic agonism (10 ng/ml epinephrine) after β-adrenergic blockade) moderately decreased somatostatin (−37±7%) secretion, moderately increased glucagon (91±19%), and markedly decreased insulin (−85±3%) release. Similar effects on D-, A-, and B-cell secretion were induced with 2 ng/ml epinephrine or 10 ng/ml norepinephrine after β-adrenergic blockade. The α-adrenergic effects on the D and A cell were abolished by either phentolamine or by phenoxybenzamine.

This study showed that there are indeed α-adrenergic receptors on A cells and that the secretion of glucagon, a "stress" hormone, was stimulated either by α- or β-adrenergic receptor agonism. D-cell secretion, like that of the B cell, was inhibited by α-adrenergic agonism and was stimulated by β-adrenergic agonism. However, β-adrenergic-induced changes in D-cell secretion were smaller in magnitude than those of B-cell secretion.

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INTRODUCTION

Although there is general agreement (1) concerning adrenergic influence on the B cell, there is only preliminary information (2, 3) regarding adrenergic control of the D cell and no consensus (1, 4) about adrenergic modulation of the A cell. For example, it has been proposed that the effects of α-adrenergic agonism on A-cell secretion are (*a*) stimulatory in ducks (5), rats (6), and man (7, preliminary report); (*b*) inhibitory in man (8), a study not confirmed by others (9); or (*c*) not evident in dogs (10). It has also been suggested that β-adrenergic agonism either inhibits glucagon secretion in man (7) and ducks (5), or stimulates glucagon in man (8), rats (11), or dogs (10).

The present studies were designed to examine the effects of α- and β-adrenergic agonism on insular A, B, and D cells in the isolated perfused canine pancreas. To avoid possible flaws in previous studies, particular attention was devoted to ensuring adequate agonism and appropriate antagonism, including the use of combined α- and β-adrenergic blockade.

METHODS

Fasting male dogs of mixed German shepherd breed were anesthetized with thiopentone, intubated, and ventilated with room air with a Harvard positive pressure respirator (Harvard Apparatus Co., Inc., Millis, Mass.). Only healthy animals with normal hemoglobin and leukocyte counts, serum amylase, and glucose, and freedom from worms and parasites were accepted for this study.

The operative procedure was carried out and the perfusion system established according to Iversen and Miles (12) with a major modification: the exclusion of the duodenum from the perfusion circuit. In the modified procedure, after the left and

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right gastric arteries were cut, the main pancreatic duct was dissected and cannulated with polyethylene tubing. A homemade parallel bar clamp was then applied to the mesentery as close as possible to the pancreas. The clamp was tightened between the duodenum and pancreas and readjusted if discoloration was seen in the pancreas. The portal vein was catheterized (No. 10 Bardic), and the tip of the catheter was advanced toward the pancreatoduodenal vein. When the tip was ≅1 cm from the pancreatoduodenal vein, a ligature around the portal vein was tightened immediately behind the holes of the catheter and the distal end of the portal vein ligated close to the liver. The proximal end of the portal vein was then secured, ligated, and cut. The celiac artery was secured with a curved clamp, and a right angle clamp was applied across the celiac artery as close as possible to the aorta. The influx cannula was then placed in the celiac artery and secured by a ligature. The time interval between clamping the celiac artery and commencing perfusion was 20–30 s. By such a procedure, the duodenum was completely separated from the perfusion circuit. The completeness of exclusion, as well as the presence of adequate pancreatic perfusion, was confirmed by arteriography of the pancreas in many early studies.

The pancreas preparation was removed from the dog and placed in a closed perfusion chamber, maintained at a temperature of 37°C, containing a Krebs-Ringer buffer. Oxygenation was achieved by bubbling the gas mixture (5% CO_2 and 95% O_2) through a heated reservoir containing the medium. The pancreatic influx P_{O_2} was 450–500 mm Hg and efflux P_{O_2} was 150–200 mm Hg. The duodenum was catheterized in its distal part, and the secretions collected outside the perfusion chamber. The buffer was replaced by fresh warmed buffer throughout the experiment, the difference in input and output being a measure of leakage from the preparation.

The pancreas was perfused without recirculation with a synthetic medium consisting of a Krebs-Ringer buffer, with electrolyte concentrations found in dog plasma. The perfusate contained 4% dextran (Macrodex mol wt 70,000, Pharmacia Fine Chemicals, Piscataway, N. J.), 0.2% bovine albumin (Sigma Chemical Co., St. Louis, Mo.), and fumarate, pyruvate, and glutamate each at a concentration of 5 μM. The perfusate also contained 88 mg/dl glucose and 19 amino acids at a final concentration of 1 mM in the proportions used by Pagliara et al. (13). A constant flow rate of 18–20 ml/min was achieved with a perfusion pressure of between 30 and 40 mm Hg. Flow rate and pressure were constant throughout the experiment, even during α-adrenergic agonism.

Biochemical methods

Insulin and glucagon were measured by radioimmunoassay. Immunoreactive insulin (IRI)[1] was assayed by a charcoal separation technique (14). Porcine insulin, which is chemically and immunologically identical to canine insulin, was used for the standard. ^{125}I-Insulin was purchased from New England Nuclear (Boston, Mass.).

Immunoreactive glucagon (IRG) was measured by the charcoal separation method according to the instructions given to purchasers of 30-K antiserum. ^{125}I-Glucagon was purchased from Nuclear Medical Labs., Inc. (Dallas, Texas) and was repurified on Sephadex G-25 (Pharmacia Fine Chemicals) so that the nonspecific binding was <10% of the total counts.

The radioimmunoassay used for somatostatin (IRS) was a modification (15) of the method of Patel and Reichlin (16). The 1-tyrosine analogue of somatostatin was iodinated with ^{125}I and used as label. Cyclic somatostatin (kindly provided by Ayerst, McKenna, and Harrison Ltd., Montreal, Canada) was used as the standard. The assay sensitivity was 1–2 pg/incubation tube; i.e., ≅10 pg/ml of added sample or standard. Glucose was measured by a glucose oxidase method (Sigma Chemical Co.). There was no interference of glucose estimation by the high dextran concentrations.

Experimental Procedure

Samples were taken every minute from the efflux. Influx samples were collected at intervals for glucose measurements. Timing of sample collection was based on a 60-s lag period (determined with sodium fluorescein or methylene blue).

Aprotinin was added to the tubes collecting the efflux to produce a final concentration of 500 U/ml. The samples were transferred immediately to a refrigerator at 4°C and subsequently frozen at −35°C.

The substances to be examined were added to the perfusate by means of constant infusion syringes. The infusion pumps were adjusted to speeds that added 0.1–0.5 mg/min to the overall flow, which was 18–20 ml/min. Perfusate only was infused at the same rate during the rest periods between infusions of the experimental substances so that the flow rate remained identical throughout the study.

The pancreas was perfused for 30–40 min before the infusion of catecholamines or blockers. Several permutations were used to determine the effects of *dl*-isoproterenol, *l*-epinephrine, and *l*-norepinephrine (hereafter called catecholamines for convenience) and of receptor blockade, including *dl*-propranolol, phentolamine (CIBA Corp., Summit, N. J.), phenoxybenzamine hydrochloride (a gift from Schering Corp., Kenilworth, N. J.), and atropine sulfate. The duration of the catecholamine infusions was invariably 10 min.

Type A experiment. After equilibration, the catecholamine was infused. After cessation of the catecholamine infusion there was a rest period of 25 min, then a blockade with either an α- or β-receptor antagonist (single receptor blockade) was initiated and was continued for the remainder of the perfusion experiment. The same catecholamine was infused 15–25 min after blockade began. In a few studies, the catecholamine was infused twice with intervening rest periods of 25 min before the commencement of receptor blockade.

Type B experiment. Single receptor blockade was continued for the entire perfusion experiment. 15–25 min after blockade commenced, the catecholamine was then infused once.

Type C (combined receptor blockade) experiments. (*a*) Propranolol was infused for the entire perfusion experiment. After 25–30 min of β-adrenergic blockade, the catecholamine was infused, followed by a 25- to 30-min rest period. α-Adrenergic receptor blockade was added and continued for the remainder of the perfusion. The catecholamine was then infused 20–25 min after α-adrenergic blockade began. (*b*) Propranolol was infused for the entire perfusion experiment. After 15 min, α-adrenergic antagonism was added and continued for the rest of the perfusion. The catecholamine was infused 20 min after α-adrenergic blockade began. Each series of experiments using blockade contained a mix of type A and B or of type C (1 and 2) permutations.

Statistical analysis of the results was performed by Student's two-tailed *t* test, using percent change of hormone sampled at each minute compared with the preagonism or preantagonism mean for minutes −5 to −1. Tested in this fashion, the zero-minute sample (shown in the figures) was not significantly different from the preagonism or preantagonism mean. The mean percent integrated change during agonism or antagonism was calculated as simple mean of the mean change

[1] *Abbreviations used in this paper*: IRG, immunoreactive glucagon; IRI, immunoreactive insulin; IRS, radioimmunoassay for somatostatin.

(usually 10 min; $n = 1$) for each experiment, and is reported as mean percent change in the text.

RESULTS

Effects of β-adrenergic agonism. As shown in Fig. 1, isoproterenol stimulated IRG, IRS, and IRI secretion. The stimulations of IRG (185±45%), IRS (79 ±20%), and IRI (495±146%) were abolished by propranolol, 2 and 6 μM (Fig. 1).

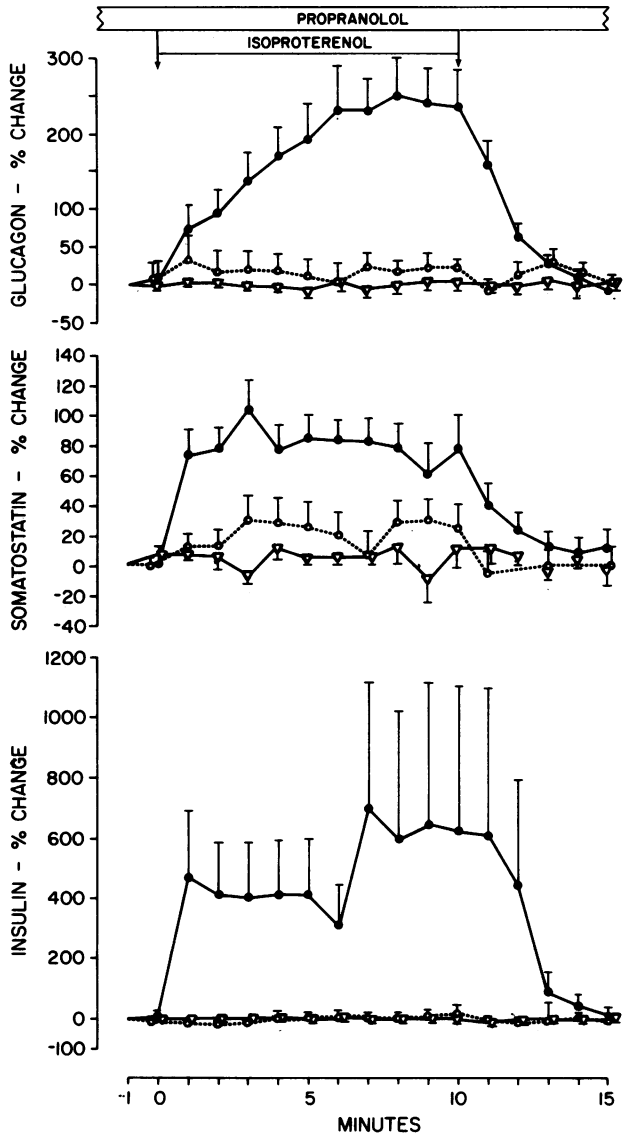

FIGURE 1 Effect of isoproterenol. Mean percentage change of glucagon, somatostatin, and insulin (±SEM) during 2 ng/ml isoproterenol ($n = 8$), ●; isoproterenol after β-adrenergic blockade with 2 μM propranolol ($n = 5$), ○; and isoproterenol after 6 μM propranolol ($n = 6$), ▽. The zero time sample (immediately before agonism) was derived from preagonism mean of −5 to −1 min. During the 10-min infusion of isoproterenol alone, mean changes were significant ($2P < 0.05$) for glucagon (last 9 min), somatostatin (10 min), and insulin (first 6 min). After blockade, the changes were not significant.

The effects of α-blockade with 3 μM phenoxybenzamine on the responses to isoproterenol were tested, measuring only IRG and IRI ($n = 6$, $p = 4$; i.e., six experiments, four pancreases). The monophasic IRG response (218±14%) was similar to that obtained with isoproterenol alone and the IRI response (787±70%) was prompt.

Epinephrine, 2 ng/ml, was infused after α-adrenergic blockade with 3 μM phenoxybenzamine. There was a monophasic IRG response (Fig. 2, Table I) and a prompt increase in IRI (Table I).

Effects of α-adrenergic agonism on glucagon secretion. After 2 or 4 μM propranolol, the infusion of 2 ng/ml epinephrine induced a significant increase in IRG secretion (Table I). In two-thirds of the individual experiments, the mean increase for each experiment in IRG exceeded 50%. After combined β- plus α-adrenergic blockade (either 2 μM propranolol plus 3 μM phenoxybenzamine, or 4 μM propranolol plus 4 μM phentolamine), 2 ng/ml epinephrine failed to increase IRG release (Table I).

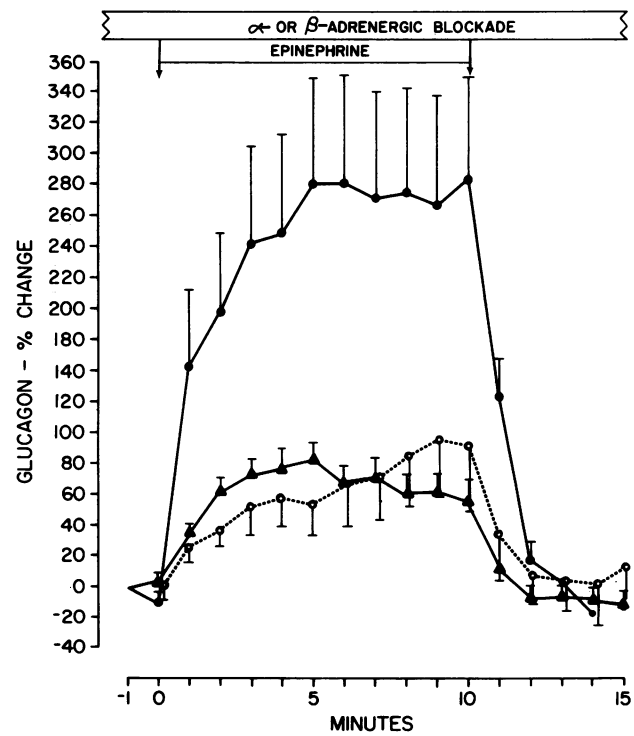

FIGURE 2 Effect of 2 ng/ml epinephrine ($n = 8$), ●; of 2 ng/ml epinephrine after α-adrenergic blockade with 3 μM phenoxybenzamine ($n = 7$), ○; and of 2 ng/ml epinephrine after 2 μM propranolol ($n = 13$), ▲. Mean percentage change of glucagon (±SEM). Table I gives the mean preagonism concentrations (±SEM) for the same experiments. During the 10-min infusion of epinephrine alone or after blockade, all mean increases were significant ($2P < 0.05$).

TABLE I

Effect of Epinephrine or Norepinephrine after α- and(or) β-Adrenergic Blockade on the Secretion of Glucagon, Somatostatin, and Insulin

		Glucagon				Somatostatin				Insulin			
Agonist*	Antagonist‡	Pre-agonism mean§	No. of experiments/ No. of pancreases	Δ%‖	2P	Pre-agonism mean	No. of experiments/ No. of pancreases	Δ%	2P	Pre-agonism mean	No. of experiments/ No. of pancreases	Δ%	2P
		pg/ml				*pg/ml*				*μU/ml*			
Epinephrine, 2 ng/ml		97±24	8/6	249±67	<0.01	84±18	8/6	−7±9	NS	274±8	8/6	−53±9¶	<0.001
Epinephrine, 2 ng/ml	Propranolol, 2 μM	79±15	13/11	64±11	<0.001	160±43	5/5	−23±4	<0.01	230±68	13/11	−77±2	<0.001
Epinephrine, 2 ng/ml	Propranolol, 2 μM, + phenoxybenzamine, 3 μM	129±34	13/8	−11±5	<0.05	129±16	8/6	17±4	<0.01	164±24	13/8	5±8	NS
Epinephrine, 2 ng/ml	Phenoxybenzamine, 3 μM	154±26	7/5	64±24	<0.05					161±29	7/5	607±151	<0.01
Epinephrine, 2 ng/ml	Propranolol, 4 μM	156±61	6/6	84±24	<0.02	84±16	6/6	−27±4	<0.005	233±72	6/6	−82±5	<0.001
Epinephrine, 2 ng/ml	Propranolol, 4 μM, + phentolamine, 4 μM	115±47	5/5	−2±9	NS	142±40	5/5	11±5	NS	461±111	5/5	10±7	NS
Epinephrine, 10 ng/ml	Propranolol, 4 μM	127±48	6/6	91±19	<0.005	84±17	6/6	−37±7	<0.005	230±53	6/6	−85±3	<0.001
Epinephrine, 10 ng/ml	Propranolol, 4 μM, + phentolamine, 4 μM	111±40	5/5	−1±4	NS	164±38	5/5	8±2	<0.02	660±162	5/5	−36±7	<0.01
Epinephrine, 10 ng/ml	Propranolol, 4 μM, + phentolamine, 4 μM, + atropine sulfate, 50 μM	120±33	5/5	5±6	NS	167±49	4/4	1±4	NS	975±240	5/5	−34±8	<0.02
Norepinephrine, 10 ng/ml	Propranolol, 2 μM	166±100	6/6	68±16	<0.02					396±123	6/6	−75±1	<0.001
Norepinephrine, 10 ng/ml	Propranolol, 2 μM, + phenoxybenzamine, 3 μM	168±107	9/6	−6±8	NS					465±84	9/6	−14±5	<0.05

* Agonist infused for 10 min.

‡ Antagonist infusion commenced 15–25 min before agonist infusion.

§ The preagonism period is defined as the 5-min period preceding infusion of the agonist. The preagonism value for each experiment was derived from the mean of five 1-min samples from −5 to −1 min. The preagonism mean is derived from the individual preagonism values.

‖ Δ% is the mean integrated percentage of change during agonism. All data in individual experiments were converted into a percentage of change from the preagonism value. The percentage of change for each experiment (n = 1) was derived from the mean of 10 1-min samples from minutes 1 to 10 during agonism. The Δ%±SEM is derived from the individual percentage of change values.

¶ Mean of the last 9 min.

When the concentration of epinephrine was increased to 10 ng/ml and infused after blockade with 4 μM propranolol, there was again a significant increase in IRG (Fig. 3). After β-adrenergic blockade, individual infusions of 10 ng/ml epinephrine in each of six different pancreases induced a mean increase in IRG exceeding 50%. In contrast, no change in IRG release was induced by 10 ng/ml epinephrine either after combined α- and β-adrenergic blockade (4 μM propranolol plus 4 μM phentolamine, Fig. 3) or after

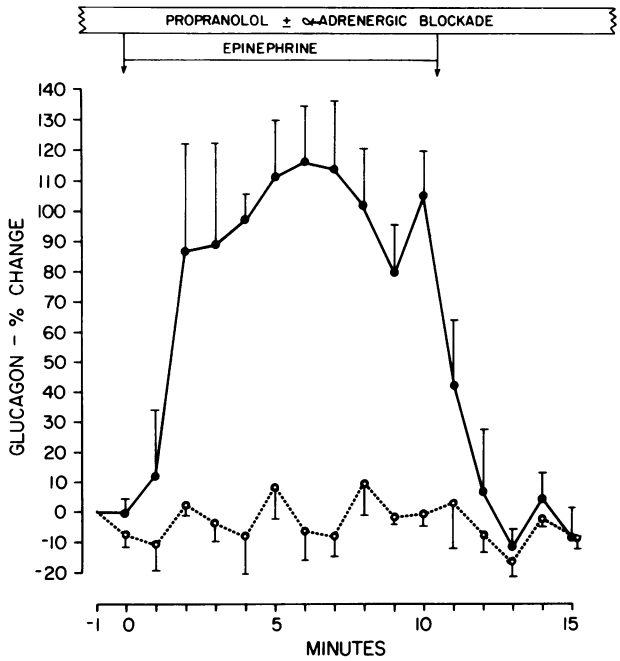

FIGURE 3 Effect of α-adrenergic agonism on mean percentage change of glucagon (±SEM). Comparison of effect of 10 ng/ml epinephrine after 4 μM propranolol (n = 6), ●; with responses to the same experiments plus α-receptor blockade using 4 μM phentolamine (n = 5), ○. During 10 min of α-adrenergic agonism, all mean increases were significant ($2P < 0.05$) except for the 1st min.

combined adrenergic blockade plus muscarinic blockade (50 μM atropine, Table I).

Infusion of 10 ng/ml norepinephrine after β-adrenergic blockade (2 μM propranolol) induced a significant increase in IRG release (Table I). This increase was abolished when 3 μM phenoxybenzamine was added to the β-adrenergic blockade.

Effects of α-adrenergic agonism on somatostatin secretion. After β-adrenergic blockade with 2 and 4 μM propranolol, the infusion of 2 ng/ml epinephrine induced decreases ($2P < 0.01$, <0.005, respectively) in IRS secretion (Table I) on the order of 23 and 27%, respectively. However, after α- plus β-adrenergic blockade, 4 μM phentolamine plus 4 μM propranolol, 2 ng/ml epinephrine failed to inhibit IRS release, or actually induced a small increase in IRS after 3 μM phenoxybenzamine plus 2 μM propranolol.

After β-adrenergic blockade with 4 μM propranolol, a higher dose of epinephrine, 10 ng/ml, induced a 37% decrease ($2P < 0.005$) in mean IRS secretion (Fig. 4, Table I). However, after combined α- plus β-adrenergic blockade, the infusion of 10 ng/ml epinephrine failed to inhibit IRS and actually induced a small mean increase in IRS (Table I). With the addition of 50 μM atropine to the combined adrenergic blockade, the infusion of epinephrine caused no change in IRS release.

Effects of α-adrenergic agonism on insulin secretion. After β-adrenergic blockade with 4 μM propranolol, the inhibition of IRI release (−82±5%) induced by 2 ng/ml epinephrine was not different in magnitude from that induced by 10 ng/ml epinephrine (Fig. 5). However, after addition of 4 μM phentolamine to the β-adrenergic blockade, 2 ng/ml epinephrine failed to induce a change in IRI; whereas 10 ng/ml epinephrine still induced a significant inhibition of IRI secretion (−36±7%), and induced a similar inhibition of IRI (−37±8%) after addition of atropine to the combined adrenergic blockade (Table I). After β-adrenergic blockade with 2 μM propranolol, 10 ng/ml norepinephrine inhibited IRI secretion, and the addition of 3 μM phenoxybenzamine reduced but did not abolish this inhibitory effect (Fig. 5, Table I). In contrast, the α-adrenergic agonistic effects of 10 ng/ml epinephrine and of 10 ng/ml norepinephrine on IRG and IRS secretion were completely abolished by α-adrenergic blockade (Table I, Figs. 3 and 4).

Effects of combined α- and β-adrenergic agonism. 2 ng/ml epinephrine increased IRG, induced no change in IRS (−7±9%), and after a brief stimulation during the 1st min (10), significantly decreased IRI secretion (Table I). The mean integrated increase in IRG (249 ±67%) was significantly larger ($2P < 0.05$) than the mean increase induced by 2 ng/ml epinephrine after β-adrenergic blockade (64±11 and 84±24%) or after α-adrenergic blockade (64±24%). Fig. 2 shows the minute-to-minute changes for comparison of the effect of combined vs. separate α- and β-adrenergic agonism on IRG secretion.

All infusions of 10 ng/ml epinephrine and 10 ng/ml norepinephrine (three pancreases each) induced an increase in IRG and a decrease in IRI secretion.

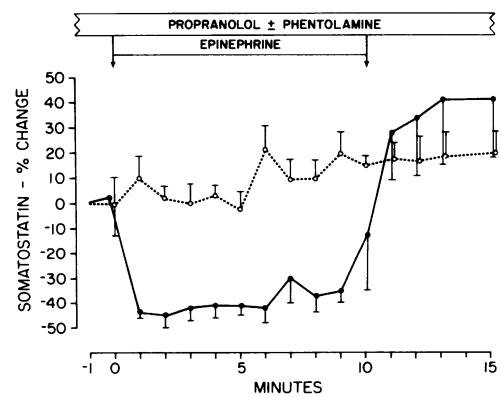

FIGURE 4 Effect of 10 ng/ml epinephrine after 4 μM propranolol (n = 6), ●, and of 10 ng/ml epinephrine after 4 μM propranolol plus 4 μM phentolamine (n = 5), ○. Mean percentage change of somatostatin (±SEM). Table I gives the mean preagonism concentrations for the same experiments. During α-adrenergic agonism the mean decreases were significant ($2P < 0.05$) from the 1st to the 9th min.

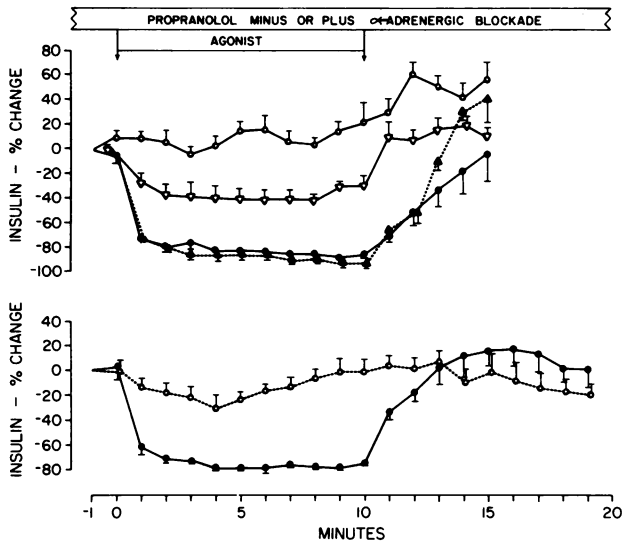

FIGURE 5 Effect of dose range of α-adrenergic agonism with 2 ng/ml epinephrine plus 2 μM propranolol, ●; 10 ng/ml epinephrine plus 4 μM propranolol, ▲; (upper panel) or 10 ng/ml norepinephrine plus 2 μM propranolol, ● (lower panel), compared with the addition of α-adrenergic receptor blockade, i.e., 2 ng/ml epinephrine after 4 μM propranolol plus 4 μM phentolamine, ○; 10 ng/ml epinephrine after 4 μM propranolol plus 4 μM phentolamine, ▽ (upper panel), and 10 ng/ml norepinephrine after 2 μM propranolol plus 3 μM phenoxybenzamine, ○ (lower panel). Mean percentage change in insulin (±SEM). Table I gives the mean preagonism concentrations for the same experiments. After combined α- plus β-adrenergic blockade, the mean decreases were significant ($2P < 0.05$) during infusion of 10 ng/ml epinephrine (2nd–10th min) and 10 ng/ml norepinephrine (4th–6th min).

Effects of blocking agents. (*a*) Propranolol, 2 μM ($n = 9$, $p = 6$), 4 μM ($n = 5$, $p = 4$), and 6 μM ($n = 4$, $p = 3$), infused for 20 min did not induce any significant change in mean integrated IRG, IRS, and IRI secretion except for a small mean decrease (−15±3%, $2P < 0.02$) in IRI, induced by 6 μM propranolol. (*b*) After blockade with 2 μM propranolol, the infusion of 3 μM phenoxybenzamine ($n = 10$, $p = 10$) for 30 min induced no change in IRG secretion and caused a brief mean integrated increase in IRI release (27±8%, $2P < 0.01$) for 10 min, but IRI secretion had returned to preinfusion levels by the 16th min. (*c*) After blockade with 4 μM propranolol, the infusion of 4 μM phentolamine ($n = 6$, $p = 6$) induced no changes in mean integrated IRG, IRS, or IRI release. In terms of minute-to-minute changes, mean IRI values increased by 29% after the 8th min, but this increase was significant ($2P < 0.05$) only at the 8th–10th min.

DISCUSSION

Effects of β-adrenergic receptor agonism. β-adrenergic agonism clearly stimulated IRG, IRS, and IRI secretion in the canine pancreas. By comparing the relative magnitude of stimulations of A-, D-, and B-cell secretions, the largest absolute and percentage mean change was shown by IRI secretion, and the smallest absolute and percentage mean stimulation was obtained for IRS secretion.

Effects of α-adrenergic receptor agonism. Our study showed that α-adrenergic agonism moderately stimulated IRG secretion, moderately inhibited IRS release, and markedly inhibited IRI secretion.

To verify that the effects observed were indeed the result of α-adrenergic agonism, and not secondary to nonspecific actions of antagonists or "breakthrough" of undesired (e.g., β-adrenergic) agonism, permutations of the following agents were explored: varying concentrations of epinephrine, use of norepinephrine, and abolition of α-adrenergic agonistic effects by phentolamine and by phenoxybenzamine. Assuming that reported (17) equilibrium dissociation constants (K_D) for *l*-epinephrine of 0.23 μM and for *l*-norepinephrine of 0.65 μM are valid for pancreatic insular cells, the α-adrenergic potency range ratio used in this study of 2 ng/ml epinephrine: 10 ng/ml norepinephrine: 10 ng/ml epinephrine was approximately 1:2:5. These ratios emphasize that epinephrine contains more than twice the quanta of α-adrenergic agonism than does an equimolar concentration of norepinephrine.

α-Adrenergic agonism and IRG secretion. In the studies with 2 or 4 μM propranolol, the possibility of potent β-agonistic breakthrough is most unlikely, and therefore the stimulation of glucagon secretion by epinephrine or norepinephrine was induced by α-adrenergic receptor agonism. This conclusion is confirmed by the complete abolition of the α-agonistic stimulation of IRG by phentolamine or by phenoxybenzamine. Each infusion (after β-adrenergic blockade) of 10 ng/ml epinephrine induced a >50% mean increase in IRG secretion, whereas only two-thirds of the infusions of 2 ng/ml epinephrine did so. As 2 ng/ml epinephrine represented the highest quanta of α-adrenergic agonism used by Iversen (10), it is possible that the sensitivity of his preparation, although adequate to detect B-cell responses to α-adrenergic agonism, was not sufficient to detect the α-receptors on the A cells. Although stimulation of mean integrated IRG secretion in our studies by maximal α-adrenergic agonism was ≅90%, that induced by maximal β-agonism exceeded 180%, suggesting that the ratio of β-adrenergic receptors to α-adrenergic receptors exceeds 2:1 on the A cells, assuming postreceptor phenomena are not important. The mean percent increase in IRG induced by 2 ng/ml epinephrine (249%) was significantly larger than that induced by relevant α-adrenergic agonism (64 or 84%) or by relevant β-adrenergic agonism (84%), suggesting the possibility of synergism when combined α- plus β-adrenergic stimulation occurred.

However, the variability of responses observed in different pancreases makes us cautious about overinterpreting quantitation as defined by mean percentage changes.

We have not resolved the variety of glucagon responses to α- and β-adrenergic agonism observed in various species under varying conditions (see Introduction). We cannot definitively explain why Iversen was unable to detect α-adrenergic receptors on the canine A cell (10), although it seems unlikely that the phenomenon would have been missed if, for example, 10 ng/ml epinephrine had been infused. Major technical differences between our study and that of Iversen, such as exclusion of the duodenum, thereby excluding duodenal glucagon (18), somatostatin (19), and the diluent effect of the perfusate traversing the duodenum (30–50% of the total flow), are possible, but unlikely, explanations for the difference in results.

Effects of α-adrenergic receptor agonism on IRS secretion. As the β-adrenergic blockade with propranolol (2 and 4 μM) was increased, so the statistical significance of mean integrated inhibition of D-cell secretion by α-adrenergic agonism improved. This is compatible with the fact that β-adrenergic agonism stimulated D-cell secretion. After phentolamine or phenoxybenzamine was added to the β-adrenergic blockade, epinephrine failed to inhibit IRS release, and in two of the four experimental series, actually caused a small mean increase in release. It is not clear whether the "increase" represents a statistical quirk, "nonspecific" effect, or a phenomenon of unrecognized importance. Epinephrine, 2 ng/ml, alone induced no significant change in IRS secretion, presumably because the sum of its α- and β-adrenergic components produced a combined "null" effect on IRS release. Our conclusions that α-adrenergic agonism inhibits D-cell secretion support those of Ipp et al. (3) who noted in a preliminary report that 10 ng/ml epinephrine inhibited IRS and IRI release, but when 10 ng/ml epinephrine was perfused with phentolamine (1.78 μM), the inhibition was reversed and a 50–100% increase in IRS and IRI secretion occurred.

Effects of α-adrenergic agonism on IRI secretion. The breakthrough inhibition of IRI release by 10 ng/ml norepinephrine and by 10 ng/ml epinephrine after combined adrenergic blockade with or without atropine is compatible with a dose potency ratio of α-adrenergic agonism for B cells similar to the theoretical values noted earlier. The absence of a simultaneous breakthrough α-agonistic effect on the A and D cells during the infusion of increasing quanta of α-adrenergic agonism suggests that α-adrenergic receptors are more numerous on the B cell than on either the A or the D cells. This could also explain why B-cell secretion was inhibited much more than was D-cell secretion by α-adrenergic agonism. As B-cell secretion has been used (10) as a "marker" for adrenergic agonism reaching the pancreatic islets, it is interesting that we could induce sufficient α-adrenergic agonism to inhibit the B cell without affecting A- and D-cell secretion, a phenomenon that could lead to the erroneous conclusion that A or D cells do not possess α-adrenergic receptors.

Choice of concentrations of antagonists and their independent effects. It seemed possible that previous techniques (10) infusing the reversible competitive antagonists, 1 μM propranolol or 1 μM phentolamine, simultaneously with agonist may have been deficient in terms of equilibration (20, 21) and(or) absolute dose. Failure to abolish the IRI response to isoproterenol (10) is strong evidence that the β-adrenergic blockade was incomplete in Iversen's study, but this does not explain his failure to find α-adrenergic receptors on the A cell.

There was no evidence that nonspecific effects of the propranolol interfered with the present study. 2 and 4 μM propranolol alone had no effect on A-, D-, and B-cell secretion. Although it is recognized that propranolol may block α-adrenergic receptors (22), the most recently reported K_D value for this phenomenon was 27 μM (17).

To cover the dose potency range used in our study for α-adrenergic agonism, we used the experimental curves of Langer and Trendelenburg (23) for phentolamine-norepinephrine dose ratios supplemented by the theoretical curves of Furchgott (22) to select a concentration of 4 μM phentolamine. This relatively high concentration of phentolamine appears to have been justified because there was still some breakthrough of α-adrenergic effects when 10 ng/ml epinephrine was infused. It is unlikely that nonspecific effects of phentolamine interfered with our basic results because infusion of phentolamine after β-adrenergic blockade induced no significant mean integrated change in A-, D-, or B-cell secretion, and analyzed as a minute-to-minute change, only a minor temporary increase in IRI release. Moreover, separate studies (results not presented) with 10 ng/ml epinephrine after blockade with 2 μM propranolol plus 1.5 μM phentolamine yielded changes in IRG, IRS, and IRI secretion very similar to those we report with 10 ng/ml epinephrine after 4 μM propranolol plus 4 μM phentolamine.

The 3-μM concentration of phenoxybenzamine was selected on the basis of preliminary studies. Although the phenoxybenzamine induced a rapid but brief minor mean increase in IRI secretion, this effect had subsided by the time of infusion of α-adrenergic agonist. That the selected phenoxybenzamine concentration was not unnecessarily high is indicated by the small but significant breakthrough of α-adrenergic agonistic effects on the B cell with 10 ng/ml norepinephrine during combined adrenergic blockade.

The effects of cholinergic blockade during infusion

of high concentrations of epinephrine were tested because of reports that phenylephrine, synephrine, and ephedrine at high concentrations may compete for muscarinic receptors (24) and because acetylcholine has been shown to stimulate glucagon (12) and inhibit somatostatin release (25).

Paracrine effects. Such effects (26) are difficult to either prove or disprove. It is possible that the potent inhibition of insulin secretion by α-adrenergic agonism could have caused the observed stimulation of glucagon release. The less potent inhibition of insulin secretion by α-adrenergic agonism (10 ng/ml epinephrine), after combined α- plus β-adrenergic antagonism, was not associated with a change in A-cell secretion. Our data are also compatible with the possibility that the inhibition of somatostatin release by α-adrenergic agonism might be a paracrine cause of the increased glucagon release. The slower rise of glucagon release toward maximum during β-adrenergic agonism compared with α-adrenergic agonism may be secondary to the stimulation of somatostatin release induced by the former agonism. However, there is no reason why the A- and D-cell response should not be interpreted in terms of adrenergic receptor theory, as is universally accepted with respect to B-cell secretion.

Physiologic significance. The concentrations of epinephrine used in our study are reported (10) as being within the physiologic range of circulating increases that occur in man. The physiologic concentrations of norepinephrine in the region of the receptor are unknown but are presumably very high locally after release from sympathetic nerve terminals adjacent to islet cells (1).

Physiologically, it would be advantageous that glucagon, a "stress hormone," be released in response to a stressful stimulus regardless of the predominant sympathetic or parasympathetic component of the autonomic response to that stimulus. An increase in IRG levels has been documented in a wide variety of stress situations (1, 4) ranging from trauma, severe infection, and burns to myocardial infarction and diabetic ketoacidosis. Our studies suggest that sympathetic stimulation would enhance IRG secretion whether the stimulus is predominantly α-adrenergic from local islet norepinephrine release or substantially β-adrenergic (increased levels of circulating epinephrine). Because acetylcholine increases insulin and glucagon secretion (12), it is likely that all forms of autonomic stimulation will increase glucagon release; whereas the secretion of insulin is restrained by the delivery of sufficient quanta of α-adrenergic agonism available to the receptors.

The significance of the D-cell responses to α- and β-receptor activation may become clearer when a physiologic role for pancreatic somatostatin is established.

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REFERENCES

1. Woods, S. C., and D. Porte, Jr. 1974. Neural control of the endocrine pancreas. *Physiol. Rev.* **54**(3): 596–619.
2. Samols, E., G. Weir, R. Ramseur, J. Day, and Y. Patel. 1978. Modulation of pancreatic somatostatin by adrenergic and cholinergic agonism and by hyper- and hypoglycemic sulfonamides. *Metab. Clin. Exp.* **27**(9)(Suppl. 1): 1219–1221.
3. Ipp, E., R. E. Dobbs, K. McCorkle, V. Harris, and R. H. Unger. 1978. Further evidence for similar secretory responses of pancreatic somatostatin and insulin. *Clin. Res.* **26**(Suppl. 1): 33A. (Abstr.)
4. Gerich, J. E., M. A. Charles, and G. M. Grodsky. 1976. Regulation of pancreatic insulin and glucagon secretion. *Annu. Rev. Physiol.* **38**: 353–388.
5. Tyler, J. M., and H. Kajinuma. 1972. Influence of beta adrenergic and cholinergic agents in vivo on pancreatic glucagon and insulin secretion. *Diabetes.* **21**: 332. (Abstr.)
6. Harvey, W., G. Faloona, and R. Unger. 1972. Effect of adrenergic blockade on exercise-induced hyperglucagonemia. *Clin. Res.* **20**: 752A. (Abstr.)
7. Gerich, J. E., J. H. Karam, and P. H. Forsham. 1972. Reciprocal adrenergic control of pancreatic alpha- and beta-cell function in man. *Diabetes.* **21**: 332–333. (Abstr.)
8. Gerich, J. E., M. Langlois, C. Noacco, V. Schneider, and P. H. Forsham. 1974. Adrenergic modulation of pancreatic glucagon secretion in man. *J. Clin. Invest.* **53**: 1441–1446.
9. Walter, R. M., R. J. Dudl, J. P. Palmer, and J. W. Ensinck. 1974. The effect of adrenergic blockade on the glucagon responses to starvation and hypoglycemia in man. *J. Clin. Invest.* **54**: 1214–1220.
10. Iversen, J. 1973. Adrenergic receptors and the secretion of glucagon and insulin from the isolated, perfused canine pancreas. *J. Clin. Invest.* **52**: 2102–2116.
11. Luyckx, A. S., and P. J. Lafebvre. 1972. Role of catecholamines in exercise-induced glucagon secretion in rats. *Diabetes.* **21**: 334. (Abstr.)
12. Iversen, J., and D. W. Miles. 1971. Evidence for a feedback inhibition of insulin on insulin secretion in the isolated, perfused canine pancreas. *Diabetes.* **20**: 1–9.
13. Pagliara, A. S., S. N. Stillings, B. Hover, D. M. Martin, and F. M. Matschinsky. 1974. Glucose modulation of amino acid-induced glucagon and insulin release in the isolated perfused rat pancreas. *J. Clin. Invest.* **54**: 819–832.
14. Herbert, V., K-S. Lau, and C. W. Gottleib. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* **25**: 1375–1384.
15. Weir, G. C., P. C. Goltsos, E. P. Steinberg, and Y. C. Patel. 1976. High concentration of somatostatin immunoreactivity in chicken pancreas. *Diabetologia.* **12**: 129–132.
16. Patel, Y. C., and S. Reichlin. 1978. Somatostatin in hypothalamus, extrahypothalamic brain, and peripheral tissues of the rat. *Endocrinology.* **102**: 523–530.
17. Williams, L. T., D. Mullikin, and R. J. Lefkowitz. 1976. Identification of α-adrenergic receptors in uterine smooth muscle membranes by (^{3}H)dihydroergocryptine binding. *J. Biol. Chem.* **251**(22): 6915–6923.
18. Dobbs, R., H. Sakurai, G. Sasaki, G. Faloona, I. Valverde, D. Baetens, L. Orci, and R. Unger. 1975. Glucagon: Role

in the hyperglycemia of diabetes mellitus. *Science (Wash. D. C.).* **187:** 544–547.
19. Polak, J. M., A. G. E. Pearse, L. Grimelius, S. R. Bloom, and A. Arimura. 1975. Growth-hormone release-inhibiting hormone in gastrointestinal and pancreatic D cells. *Lancet.* **I:** 1220–1222.
20. Furchgott, R. F. 1967. The pharmacological differentiation of adrenergic receptors. *Ann. N. Y. Acad. Sci.* **139:** 553–570.
21. Potter, L. T. 1967. Uptake of propranolol by isolated guinea-pig atria. *J. Pharmacol. Exp. Ther.* **155:** 91–100.
22. Furchgott, R. F. 1972. Classification of adrenoceptors (adrenergic receptors). Evaluation from the standpoint of receptor theory. *In* Handbook of Experimental Pharmakology. H. Blaschko and E. Muschall, editors. Springer-Verlag, Berlin. **33:** 283–335.
23. Langer, S. Z., and U. Trendelenburg. 1969. The effect of a saturable uptake mechanism of the slopes of dose-response curves for sympathomimetic amines and on the shifts of dose-response curves produced by a competitive antagonist. *J. Pharmacol. Exp. Ther.* **167:** 117–142.
24. Furchgott, R. F. 1970. Pharmacological characteristics of adrenergic receptors. *Fed. Proc.* **29:** 1352–1361.
25. Samols, E., G. C. Weir, Y. C. Patel, S. W. Loo, and K. H. Gabbay. 1977. Autonomic control of somatostatin and pancreatic peptide secretion by the isolated perfused canine pancreas. *Clin. Res.* **25**(Suppl. 3): 499A. (Abstr.)
26. Samols, E., J. Tyler, and V. Marks. 1972. Glucagon-insulin interrelationships. *In* Glucagon. Molecular Physiology, Clinical and Therapeutic Implications. P. J. Lefebvre and R. H. Unger, editors. Pergamon Press Ltd., Oxford. 1st edition. **9:** 151–173.