Reversal by methysergide of inhibition of insulin secretion by prostaglandin E in the dog.

R P Robertson, R J Guest

J Clin Invest. 1978;62(5):1014-1019. https://doi.org/10.1172/JCI109205.

Research Article

These studies were designed to examine whether interrelationships exist between serotonin and prostaglandin E (PGE) during regulation of insulin secretion in dogs in vivo. In our studies serotonin was found to inhibit insulin responses to intravenous glucose. This inhibition was not reversed by complete adrenergic blockade provided through combined phentolamine and propranolol pretreatment. This property of serotonin is similar to that of PGE which also inhibits glucose-induced insulin secretion in vivo independently of adrenergic activity. To investigate whether these effects of serotonin and PGE are related, studies with methysergide (a serotonin antagonist) and indomethacin (a PGE synthesis inhibitor) were performed. Methysergide reversed the effects of both PGE and serotonin. In contrast, indomethacin did not diminish the inhibitory effect of serotonin upon insulin secretion. It is hypothesized that endogenous serotonin may play a role in the inhibitory effect of PGE upon insulin secretion in dogs in vivo.

Reversal by Methysergide of Inhibition of Insulin Secretion by Prostaglandin E in the Dog

R. PAUL ROBERTSON and ROBERT J. GUEST, *Division of Clinical Pharmacology, Department of Medicine, University of Washington School of Medicine and Veterans Administration Hospital, Seattle, Washington 98108*

ABSTRACT These studies were designed to examine whether interrelationships exist between serotonin and prostaglandin E (PGE) during regulation of insulin secretion in dogs in vivo. In our studies serotonin was found to inhibit insulin responses to intravenous glucose. This inhibition was not reversed by complete adrenergic blockade provided through combined phentolamine and propranolol pretreatment. This property of serotonin is similar to that of PGE which also inhibits glucose-induced insulin secretion in vivo independently of adrenergic activity. To investigate whether these effects of serotonin and PGE are related, studies with methysergide (a serotonin antagonist) and indomethacin (a PGE synthesis inhibitor) were performed. Methysergide reversed the effects of both PGE and serotonin. In contrast, indomethacin did not diminish the inhibitory effect of serotonin upon insulin secretion. It is hypothesized that endogenous serotonin may play a role in the inhibitory effect of PGE upon insulin secretion in dogs in vivo.

INTRODUCTION

Prostaglandin E (PGE)[1] is synthesized by pancreatic islets of Langerhans (1) and inhibits insulin secretion in vivo (2, 3). This effect appears to be independent of the alpha adrenergic nervous system because it persists during blockade of alpha adrenergic receptors (2–4). Serotonin has also been identified within the islet (5, 6) and has also been shown to inhibit insulin secretion (7–9), but it is unclear whether or not this effect of serotonin is related to alpha adrenergic activity. There has been no published work assessing potential interactions between serotonin and PGE during regulation of insulin secretion in vivo, but it seems reasonable to hypothesize that such interactions exist. This contention arises from the facts that both serotonin and PGE are produced in the islet, both inhibit in vivo insulin secretion, both have effects that are similar in other tissues (e.g., intestinal smooth muscle), and both have been postulated to play a role in the abnormal insulin secretion characteristic of diabetes mellitus (10, 11).

To evaluate whether serotonin-PGE interactions exist in the islet, several questions were addressed. (*a*) Is the inhibitory effect of serotonin on insulin secretion independent of alpha-adrenergic activity? Because this has been shown for PGE, one would predict that this would be so for serotonin also if serotonin and PGE actions were linked. (*b*) Is the inhibitory effect of PGE on insulin secretion dependent upon intact serotoninergic activity? This dependency should be demonstrable if serotonin mediates PGE action on β cells. (*c*) Or, is the inhibitory effect of serotonin dependent upon endogenous PGE synthesis? If PGE mediates serotonin action on β cells, one would predict diminution of serotonin effect during inhibition of PGE synthesis.

Our approach to the first question was to compare the inhibitory effects of serotonin in the presence of an intact adrenergic input with its effects during complete adrenergic blockade with phentolamine and propranolol. The second question was approached by using the serotonin antagonist, methysergide, to determine whether PGE inhibition of insulin secretion could be reversed by blocking the effects of endogenous serotonin. In addition, the effect of PGE infusion upon systemic serotonin levels was assessed. The third question was studied by using a potent inhibitor of PGE synthesis, indomethacin, to determine whether serotonin effects could be diminished by blocking endogenous PGE production.

This work was presented in part at the Western Society for Clinical Research Meeting, Carmel, Calif. 4 February 1977, and the Annual Meeting of the American Society for Clinical Investigation in Washington, D. C., 2 May 1977.

Received for publication 27 December 1977 and in revised form 9 June 1978.

[1] *Abbreviations used in this paper*: 5-HIAA, 5-hydroxy indole acetic acid; PGE, prostaglandin E.

J. Clin. Invest. © The American Society for Clinical Investigation, Inc., 0021-9738/78/1101-1014 $1.00
Volume 62 November 1978 1014–1019

METHODS

Experimental method. Mongrel dogs were fasted for 36 h and systemically anesthetized with pentobarbital at 8 a.m. An endotracheal tube was used for assisted ambient air ventilation. A rectal thermometer and heating pad were used to control body temperature. 30 min were allowed to elapse to establish basal conditions before beginning the experiments. Venous blood was drawn through a three-way stopcock from a femoral vein catheter kept patent when not in use with a slow infusion of 0.85% sodium chloride solution. Samples were collected at 15, 20, 25, and 30 min after the beginning of the basal period, and at 2, 3, 4, 5, 7, 10, 15, 30, 45, and 60 min after each glocuse injection. In all of the studies to be described, intravenous glucose was injected as a 2-g pulse (4 ml of 50% glucose solution) in <3s. Insulin responses to these glucose pulses were expressed as the mean of the insulin increments over control observed at 4, 5, and 7 min after the pulse. Unless otherwise indicated, all infusions other than the glucose injections were given through a catheter placed in a femoral vein opposite to the one used for sample collection. Levels of plasma insulin and glucose were determined by previously published methods (12, 13). Serotonin levels were assayed commercially by Bio-Science Laboratories, Van Nuys, Calif., and levels of 5-hydroxy indole acetic acid (5-HIAA) were assessed by conventional methodology (14). Statistical comparisons were performed by Student's *t*, Wilcoxon, and Mann-Whitney U tests.

Experimental design. To determine the effect of serotonin upon insulin secretion, glucose was injected before and again at the beginning of the last hour of a 2-h serotonin (2 mg/min) infusion into the thoracic aorta.

To determine whether the effects of serotonin are dependent upon the adrenergic nervous system, intravenous phentolamine (0.2 mg/min) and propranolol (40 μg/min) were infused simultaneously for 3 h and 45 min to provide alpha and beta-adrenergic blockade. An intravenous glucose pulse was given 45 min after the beginning of the phentolamine and propranolol infusion. 60 min later, a 2-h infusion of serotonin was superimposed; the glucose pulse was repeated at the beginning of the 2nd h of serotonin infusion.

To demonstrate the effect of PGE_1 upon insulin secretion, glucose was injected before and again at the beginning of the last hour of a 2-h PGE_1 (10 μg/min i.v.) infusion. During PGE infusion in two dogs, multiple samples were collected for whole blood serotonin and urine 5-HIAA.

To assess whether PGE_1 effects are dependent upon intact serotoninergic activity, intravenous methysergide was infused at various doses. Methysergide was infused (4–4,000 μg/min i.v.) for 3 h and 45 min. An intravenous glucose pulse was given 45 min after the beginning of the methysergide infusion. 60 min later a 2-h infusion of PGE_1 (10 μg/min i.v.) was superimposed upon the methysergide infusion. Another glucose pulse was given at the beginning of the 2nd h of the PGE_1 infusion. A separate subgroup of animals was treated identically except that serotonin (2 mg/min infused into the thoracic aorta) rather than PGE_1 was used. This dose of serotonin was chosen because it inhibits glucose-induced insulin secretion to a degree similar to that seen with intravenous PGE_1 infusions given at a rate of 10 μg/min (see Results, Table I). A second serotonin antagonist, cyproheptadine, was infused (20 μg/min i.v.) for 1 h and 45 min to determine its effects on insulin responses to a 2-g glucose pulse given at the 45th min.

To determine whether serotonin effects are dependent upon endogenous prostaglandin synthesis, dogs were given the prostaglandin synthesis inhibitor, indomethacin. Indomethacin was given at a dose of 25 mg orally four times daily for 2 days and 50 mg orally on the morning of the experiment. Glucose was injected before and at the beginning of the last hour of a 2-h serotonin infusion (2 mg/min infused into the thoracic aorta).

TABLE I

Reversal by Methysergide (METH) of PGE_1-Induced and Serotonin (SER)-Induced Inhibition of Insulin Secretion

	Inhibition of insulin	
	PGE_1	SER
	%	
PGE_1, mean±SE	71±16, n = 11	—
SER, mean±SE	—	63±13, n = 11
PGE_1 or SER + METH, 4 μg/min	79, 87	48, 81
PGE_1 or SER + METH, 10 μg/min	33, 64	38, 86
PGE_1 or SER + METH, 20 μg/min	30, 56	44, 56
PGE_1 or SER + METH, 40 μg/min	19	0
PGE_1 or SER + METH, 400 μg/min	0	16
PGE_1 or SER + METH, 1,000 μg/min	21	0
PGE_1 or SER + METH, 4,000 μg/min	0	0

PGE_1 (10 μg/min) and METH (at various doses) were infused via femoral veins and SER (2 mg/min) was infused via thoracic aorta. See text for calculations of percent inhibition of insulin secretion.

RESULTS

Effect of serotonin upon insulin secretion. Compared to control levels of circulating insulin (18±4, mean±SE; n = 11) there was an immediate and significant insulin response (35±8 μU/ml, mean 4–7 min Δ immunoreactive insulin; $P < 0.005$) after the initial glucose injection (Fig. 1). Insulin then returned to basal levels by 60 min. Serotonin caused a transient, but statistically nonsignificant, increase in insulin levels that was maximal 4 min after the onset of the serotonin infusion; by 60 min insulin levels were virtually identical to preserotonin control levels. After the second glucose injection, a significant insulin response (14±4, $P < 0.005$) was observed; however, this response was significantly less ($P < 0.005$) than that observed after the first glucose injection. Circulating glucose levels increased significantly during serotonin infusion (before serotonin = 127±21; after 1 h of serotonin = 198±70 mg/dl; $P < 0.005$).

Effect of serotonin upon insulin secretion in the presence of combined alpha- and beta-adrenergic block-

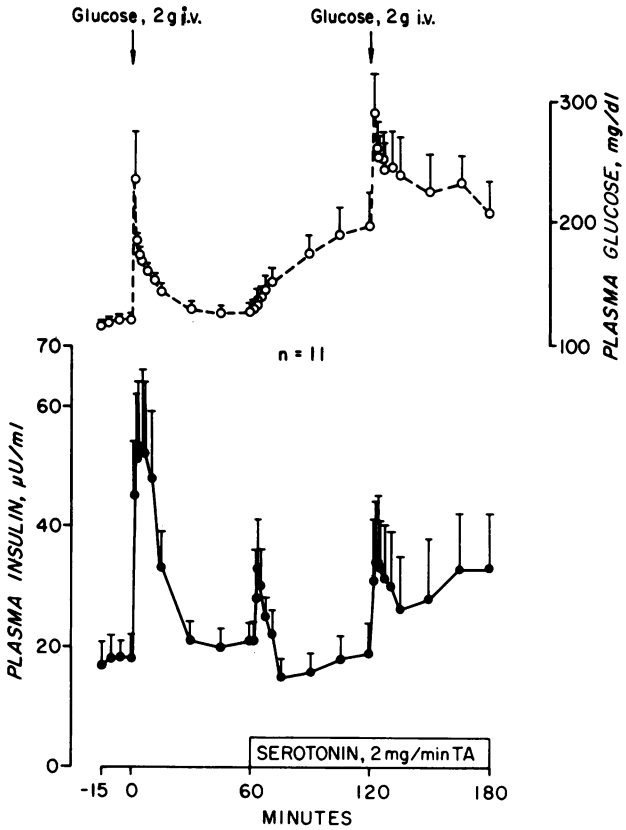

FIGURE 1 The effect of intravenous glucose pulses before and during an intrathoracic aortic (TA) infusion of serotonin upon insulin and glucose levels.

ade with phentolamine and propranolol. After 45 min pretreatment with both phentolamine and propranolol, the first glucose pulse elicited an immediate insulin response (16±3 μU/ml, $P < 0.001$) that returned to basal by 60 min (Fig. 2). During the 1st h of the superimposed serotonin infusion there were no changes in circulating insulin. The second glucose pulse elicited an insulin response (8±3, $P < 0.01$) that was significantly less ($P < 0.025$) than the response observed after the first glucose pulse. During the control studies (Fig. 3) there was no change in circulating insulin during the 2nd h after the first glucose pulse, and there was no significant difference between the insulin responses after the two glucose pulses. Circulating glucose levels tended to increase but did not change significantly during infusion of serotonin in the presence of combined adrenergic blockade.

Effect of PGE_1 upon insulin secretion. There was an immediate and significant insulin response (30±10 μU/ml, $P < 0.005$) after the initial glucose injection; insulin then returned to basal levels by 60 min (Fig. 4). After 60 min of PGE_1 infusion, insulin levels fell significantly (−10±3 μU/ml, $P < 0.005$) compared to levels

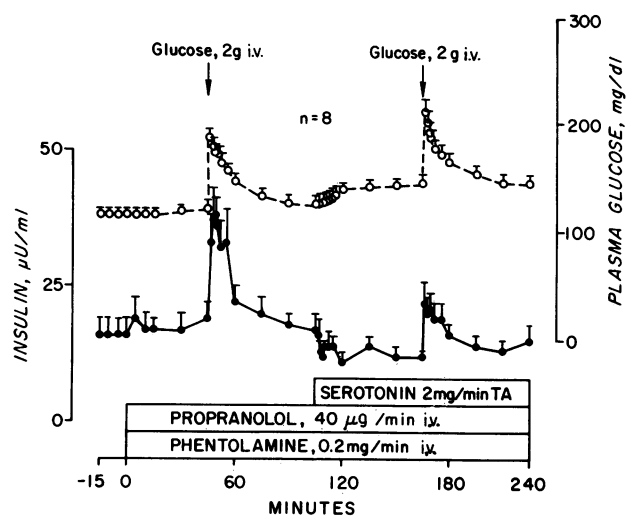

FIGURE 2 The effect of combined phentolamine and propranolol infusions upon insulin and glucose responses to glucose pulses and serotonin infusion.

observed 60 min after the first glucose injection. After the second injection, the insulin response (9±4 μU/ml, $P < 0.005$) was significantly less ($P < 0.01$) than the response observed after the first glucose pulse. There were no significant changes in circulating glucose during the 1st h of PGE_1 infusion. No changes in systemically circulating serotonin or urine 5-HIAA were observed during PGE infusion.

Effect of PGE_1 upon insulin secretion during serotonin blockade with methysergide. Methysergide infusion at a rate of 40 μg/min (Fig. 5) prevented a superimposed PGE_1 (10 μg/min) infusion from lowering insulin levels by the end of 60 min and from inhibiting the insulin reponse to an intravenous glucose pulse. Consequent investigation of a range of methysergide doses

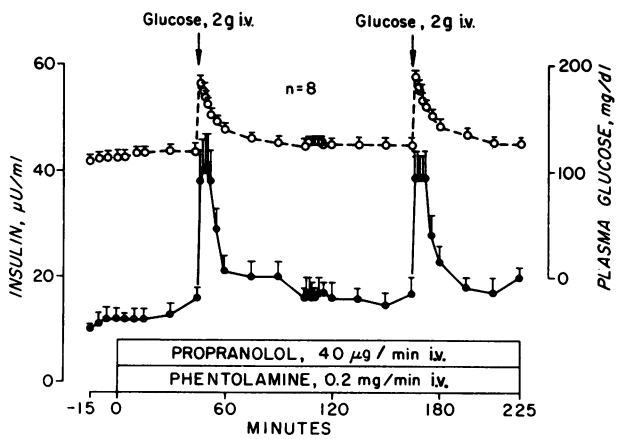

FIGURE 3 The effect of combined phentolamine and propranolol infusions upon insulin and glucose responses to glucose.

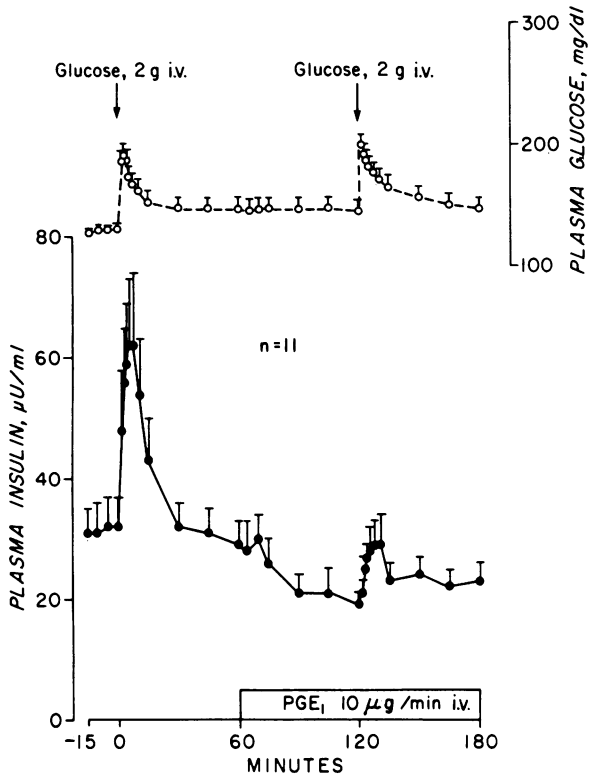

FIGURE 4 The effect of intravenous glucose pulses before and during PGE_1 infusion upon insulin and glucose levels.

(4–4,000 μg/min) revealed a dose-dependent reversal by methysergide of the PGE_1 inhibitory effect upon insulin responses to glucose pulses (Table I).

In the absence of methysergide, PGE_1 inhibited glucose-induced insulin responses by 71±16% (calculation: 100% − [(4–7 min Δ immunoreactive insulin, 2nd pulse/4–7 min Δ immunoreactive insulin 1st pulse) × 100], mean±SE; n = 11; Table I). Identical calculations for serotonin (2 mg/min intrathoracic aorta infusion, n = 11) in the absence of methysergide demonstrated that serotonin given at this rate inhibits glucose-induced insulin responses by 63±13% (Table I) which is not significantly different than the magnitude of the 71±16% inhibitory effect of PGE_1. Investigation of the same range of methysergide doses used to reverse PGE_1 effects also demonstrated reversal of serotonin inhibition of glucose-induced insulin responses (Table I). During the initial evaluation of cyproheptadine for use as a second serotonin antagonist, it was found that this drug itself partially inhibited glucose-induced insulin secretion (response = 10±2 μU/ml, n = 3; compare to responses to first pulse on Figs. 1, 4, 5, 6, and 7). Consequently, this drug could not be used to evaluate the serotonin specificity of the methysergide effect on PGE.

Effect of serotonin upon insulin secretion during inhibition of endogenous prostaglandin synthesis with indomethacin. In the dogs pretreated with indomethacin, there was an immediate insulin response (27±5, $P < 0.01$) after the first pulse of glucose (Fig. 6). There was no significant change in circulating insulin during the 1st h of the serotonin infusion. The insulin response (4±3) to the second glucose pulse was not statistically significant and was significantly less ($P < 0.005$) than the response to the first glucose pulse. In the control experiment (Fig. 7) there were no changes in circulating insulin during the 2nd h after the first glucose pulse and insulin responses to the first and sec-

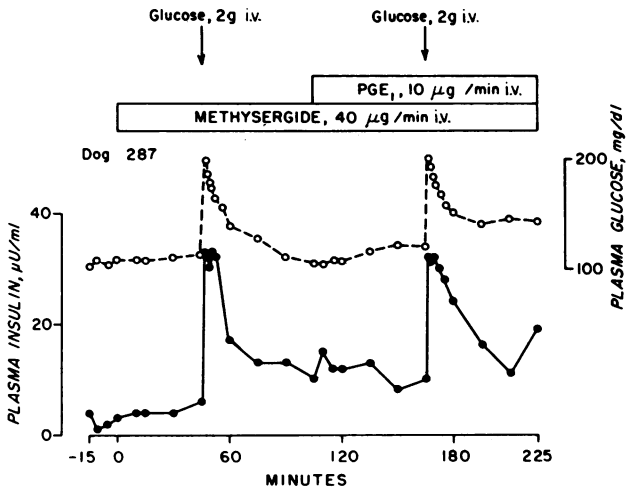

FIGURE 5 The effects of methysergide infusion upon insulin and glucose responses to glucose pulses and PGE_1 infusion.

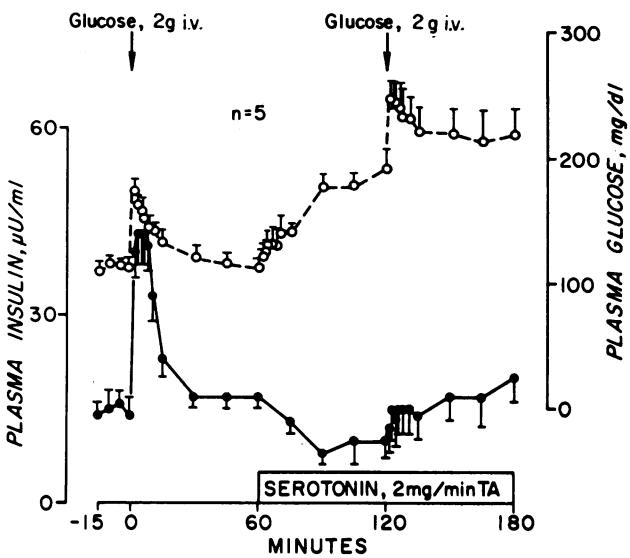

FIGURE 6 The effect of oral indomethacin (pretreatment) upon insulin and glucose responses to glucose pulses and serotonin infusion.

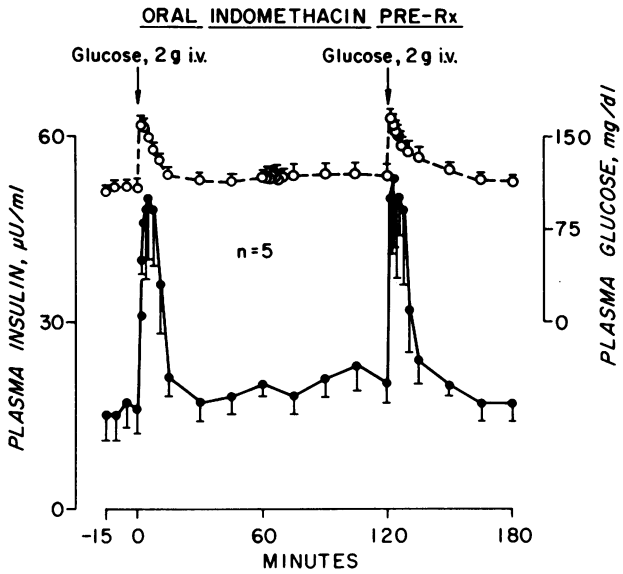

FIGURE 7 The effect of oral indomethacin upon insulin and glucose responses to glucose pulses.

ond intravenous glucose pulses were not significantly different. Circulating glucose levels increased significantly ($P < 0.02$) from 118±8 to 193±16 after 60 min of serotonin infusion.

DISCUSSION

The data provided by these studies demonstrate that (*a*) serotonin inhibits insulin secretion in dogs in vivo; (*b*) this effect is independent of adrenergic activity; (*c*) the inhibitory effect of PGE upon insulin secretion can be prevented by an antagonist of serotoninergic activity; and (*d*) the inhibitory effects of serotonin are not diminished by blockade of endogenous PGE synthesis. These observations allow several conclusions to be drawn. First, serotonin and PGE are similar in that their inhibitory effects upon insulin secretion are independent of adrenergic input. This finding distinguishes these two substances from the only other extensively studied inhibitors of insulin secretion—somatostatin and catecholamines. The effects of the latter substances are reversed by adrenergic blockade (15, 16), whereas the effect of PGE (2, 4) and serotonin (shown in this investigation) are not. Second, serotonin and PGE also have in common that their inhibitory effect upon insulin secretion in the dog in vivo are prevented during blockade of serotoninergic activity by methysergide. Third, use of indomethacin to inhibit PGE synthesis failed to diminish the effect of serotonin on the islet. This dose of indomethacin has been shown to reach sufficient circulating levels (17) to markedly inhibit PGE synthesis in dog tissues such as spleen, brain, kidney, and platelets (18).

These data are compatible with the hypothesis that serotonin may play a role in the inhibitory effect of PGE upon the secretory function of dog pancreatic β cells. One can only speculate about the source of serotonin for this hypothetical sequence of events. No increase of systemic serotonin or its major metabolite was found during PGE infusion. However, it is of interest that canine pancreatic α cells contain serotonin (6) and that PGE has been shown to stimulate α cell secretion in vivo (19). Although it is not yet known whether these facts have any relevance to regulation of islet secretion, they could conceivably provide a basis for a functional inter-relationship between α and β cells. An alternate interpretation of our findings is that the actions of serotonin and PGE on β cells are not related, but that methysergide occupied a receptor that is common to both of these substances or blocks a mechanism distal to their receptors. Regarding the former point, however, there is no precedent for direct stimulation of serotonin receptors by PGE nor is this likely in view of the structural dissimilarity of the serotonin and PGE molecules. Attempts to examine this question further by studying a second serotonin antagonist, cyproheptadine, were thwarted by the finding that this drug itself partially inhibited glucose-induced insulin secretion, a phenomenon that has been observed previously (20).

It should be noted that the changes observed in circulating glucose during serotonin infusion were absent in the dogs pretreated with combined adrenergic blockade. Whether or not this difference in glycemia confounds the interpretation of the insulin data is an important consideration. This potential problem can be resolved by recalling that hyperglycemia of this magnitude and duration as a result of glucose infusion (21) inhibits subsequent insulin responses to intravenous glucose pulses. The preservation of the inhibitory effect of serotonin in the absence of hyperglycemia (as in the combined adrenergic blockade experiments) clearly indicates that this effect of serotonin is independent of and distinct from inhibitory effects of adrenergic stimulation and hyperglycemia.

Although a substantial amount of work has been performed by different investigators dealing with the role of serotonin in the regulation of insulin secretion, there have been few reported experiments in vivo studying serotonin itself. Federspil et al. (7) reported that injection of serotonin into the pancreatic artery of anesthetized dogs stimulated insulin secretion but that a fourfold greater dose failed to have any effect. Similar results were reported by Lechin et al. (8) who used the portal vein for serotonin injections. No experiments involving adrenergic blockade were performed in either of these studies. The effects of serotonin upon insulin secretion as a result of intravenous glucose stimulation has not been previously studied in dogs or humans, but

Quickel et al. (9) reported studies in white rabbits and white mice in vivo in which intravenous glucose injections were given during treatment with serotonin. In both species serotonin inhibited glucose-induced secretion. A study by Lundquist et al. (22) described use of L-5-hydroxytryptophan, a serotonin precursor, in mice in vivo. This serotonin precursor resulted in inhibition of insulin secretion induced by sulphonylura and L-isopropylnoradrenaline, but not that induced by glucose.

Our observation that methysergide can reverse inhibition of insulin secretion by PGE gives rise to new considerations regarding the data of Quickel et al. (10) which provided the interesting demonstration that methysergide augments insulin secretion in diabetic humans. These studies were interpreted as evidence for the existence of an endogenous pancreatic biogenic monoamine mechanism that inhibits insulin release in adult-onset diabetic patients. However, in light of the experiments described herein, it is conceivable that these ameliorative effects in diabetic subjects might also be related to reversal of inhibition by endogenous PGE of β-cell function. This possibility is consonant with our previous demonstrations that PGE inhibits glucose-induced insulin secretion in humans and that sodium salicylate, an inhibitor of endogenous PGE production, restores absent acute insulin responses and augments second phase insulin secretion in diabetics (11).

ACKNOWLEDGMENTS

We wish to thank Mr. Howard Beiter for his skillful technical assistance.

This work was supported by U. S. Veterans Administration Research and Education Program MRIS no. 7511.

REFERENCES

1. Hamamdzic, M., and K. U. Malik. 1977. Prostaglandins in adrenergic transmission of isolated perfused rat pancreas. *Am. J. Physiol.* **232:** E201–E209.
2. Robertson, R. P., D. J. Gavareski, D. Porte, Jr., and E. L. Bierman. 1974. Inhibition of in vivo insulin secretion by prostaglandin E_1. *J. Clin. Invest.* **54:** 310–315.
3. Robertson, R. P. 1974. In vivo insulin secretion: prostaglandin and adrenergic interrelationships. *Prostaglandins.* **6:** 501–508.
4. Sacca, L., G. Perez, F. Rengo, I. Pascucci, and M. Condorelli. 1975. Reduction of circulating insulin levels during the infusion of different prostaglandins in the rat. *Acta Endocrinol.* **79:** 266–274.
5. Lundqvist, I., F. Sundler, R. Håkanson, L.-I. Larsson, and L. G. Heding. 1975. Differential changes in the 5-hydrotryptamine and insulin content of guinea-pig B-cells. *Endocrinology.* **97:** 937–947.
6. Cegrell, L. 1968. The occurrence of biogenic monoamines in the mammalian endocrine pancreas. *Acta Physiol. Scand.* **314**(Suppl.): 1–60.
7. Federspil, G., D. Casara, S. Pedrazzoli, N. Sicolo, and C. Scandellari. 1974. In vivo studies on 5-hydroxytryptamine and insulin secretion in dogs and in man. *Diabetologia.* **10:** 13–17.
8. Lechin, F., E. Coll-García, B. van der Dijs, F. Peña, A. Bentolilla, and C. Rivas. 1975. The effect of serotonin (5-HT) on insulin secretion. *Acta Physiol. Lat. Am.* **25:** 339–346.
9. Quickel, K. E., Jr., J. Feldman, and H. E. Lebovitz. 1971. Inhibition of insulin secretion by serotonin and dopamine: species variation. *Endocrinology.* **89:** 1295–1302.
10. Quickel, K. E., Jr., J. Feldman, and H. E. Lebovitz. 1971. Enhancement of insulin secretion in adult onset diabetics by methysergide maleate: evidence for an endogenous biogenic monoamine mechanism as a factor in the impaired insulin secretion in diabetes mellitus. *J. Clin. Endocrinol. Metab.* **33:** 877–881.
11. Robertson, R. P., and M. Chen. 1977. A role for prostaglandin E in defective insulin secretion and carbohydrate intolerance in diabetes mellitus. *J. Clin. Invest.* **60:** 747–753.
12. Technicon Autoanalyzer Methodology. Method File, Review. 11 February 1960. Technicon Instruments Corp., Tarrytown, N. Y.
13. Morgan, C. R., and A. Lazarow, 1963. Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes.* **12:** 115–126.
14. Tietz, N. W., 1976. Fundamentals of Clinical Chemistry. W. B. Saunders Co., Philadelphia. 818–823.
15. Smith, P. H., S. C. Woods, and D. Porte, Jr. 1976. Phentolamine blocks the somatostatin-mediated inhibition of insulin secretion. *Endocrinology.* **98:** 1073–1076.
16. Porte, D., Jr. 1967. A receptor mechanism for the inhibition of insulin release by epinephrine in man. *J. Clin. Invest.* **46:** 86–94.
17. Palmér, L., L. Bertilsson, G. Alván, M. Orme, F. Sjöquist, and B. Holmstedt. 1974. Indomethacin: quantitative determination in plasma by mass fragmentography including pilot pharmokinetics in man. *In* Prostaglandin Synthesis Inhibitors, H. J. Robinson and J. R. Vane, editors. Raven Press, Inc., New York. 91–97.
18. Flower, R. J. 1974. Drugs which inhibit prostaglandin biosynthesis. *Pharmacol. Rev.* **26:** 33–67.
19. Sacca, L., and G. Perez. 1976. Influence of prostaglandins on plasma glucagon levels in the rat. *Metabolism.* **25:** 127–130.
20. Rickert, D. E., and L. J. Fischer. 1975. Cyproheptadine and beta cell function in the rat: insulin secretion from pancreas segments in vitro. *Proc. Soc. Exp. Biol. Med.* **150:** 1–6.
21. Porte, D., Jr., and A. A. Pupo. 1969. Insulin responses to glucose: evidence for a two pool system in man. *J. Clin. Invest.* **48:** 2309–2319.
22. Lundqvist, L., R. Ekholm, and L. E. Ericson. 1971. Monoamines in the pancreatic islets of the mouse. *Diabetologia.* **7:** 414–422.