Specificity of Serotonin Inhibition of Insulin Release from Golden Hamster Pancreas

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SUMMARY

Five-hydroxytryptamine (serotonin) inhibited basal and stimulated insulin release from pieces of golden hamster pancreas. Five-hydroxyindole acetic acid was a less potent inhibitor of insulin release than was serotonin, while 5-hydroxytryptophan and tryptamine had no effect on this process. In contrast, L-tryptophan potentiated high glucose-mediated insulin release. Thus there was an absolute structural requirement for a hydroxyl group in the five position of the indole ring, and a relative requirement for an amine group in the alkyl chain for these indole compounds to be inhibitors of insulin release. In further studies, methysergide maleate, a specific serotonin inhibitor, blocked this action of serotonin. Methysergide maleate also stimulated glucose-mediated insulin release suggesting that endogenous serotonin may influence in vitro insulin secretion. Thus the inhibitory effect of serotonin on insulin secretion appears to be quite specific. DIABETES *19*:475-79, July, 1970.

Recently we reported that 5-hydroxytryptamine (serotonin) was a potent inhibitor of in vitro insulin release.[1] With use of pieces of golden hamster pancreas, it was possible to show that serotonin suppressed not only basal insulin secretion, but the insulin secretion provoked by glucose (3 mg./ml.), tolbutamide (1 mg./ml.) or dibutyryl cyclic AMP (1 mg./ml.). A species difference in serotonin action was noted, in that serotonin had no inhibitory effect on insulin secretion from mouse pancreas in vitro.[2]

The present report describes the specificity of this inhibition of insulin release. In figure 1 is depicted the biosynthetic and metabolic pathways for serotonin. The structural specificity for serotonin's effect was established by examining the activity of these serotonin precursors and metabolic products on influencing insulin secretion. The action of methysergide maleate, a specific serotonin antagonist, on high glucose (3 mg./ml.) mediated insulin release in the presence and absence of serotonin, was evaluated and indicated that the inhibition of insulin release is a specific effect of serotonin.

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MATERIAL AND METHODS

A golden hamster in vitro pancreas system previously described[2,3] was used in the present studies. In this system each piece of pancreas undergoes two sequential fifteen-minute incubation periods. The first incubation is carried out in basal media (initial incubation) while the second incubation is carried out in media containing the test substance added to basal media (treatment incubation). The basal media is Krebs-Ringer bicarbonate buffer with 0.005 M pyruvate, 0.005 M fumarate, 0.005 M glutamate, 60 mg./100 ml. glucose and 400 mg./100 ml. bovine serum albumin. At the conclusion of each incubation, aliquots from the incubation media are placed in chilled tubes and directly assayed for insulin by a radioimmunoassay technic. Net insulin release is expressed as the difference in insulin release between the treatment and initial incubations (μU./mg. pancreas/fifteen minutes). In a series of studies carried out in which the pancreas pieces were incubated in basal media in two consecutive incubation periods, it was observed that the insulin release in the second incubation differed from that in the first incubation by —10.7 per cent (standard deviation ± 25.0 per cent). Thus the insulin release in the second period was usually slightly less than that in the first.

In the studies in which the effect of various agents on high glucose (3 mg./ml.) stimulated insulin release were investigated, the insulin release from high glucose alone was compared to the insulin release from high glucose plus the agent by the Student *t* test.

Serotonin creatinine sulfate, 5-hydroxyindole acetic acid (cyclohexylammonium salt), L-5 hydroxytryptophan, L-tryptophan and tryptamine were purchased from Cal Biochemical. Methysergide maleate (Sansert) was a gift from Sandoz Pharmaceuticals.

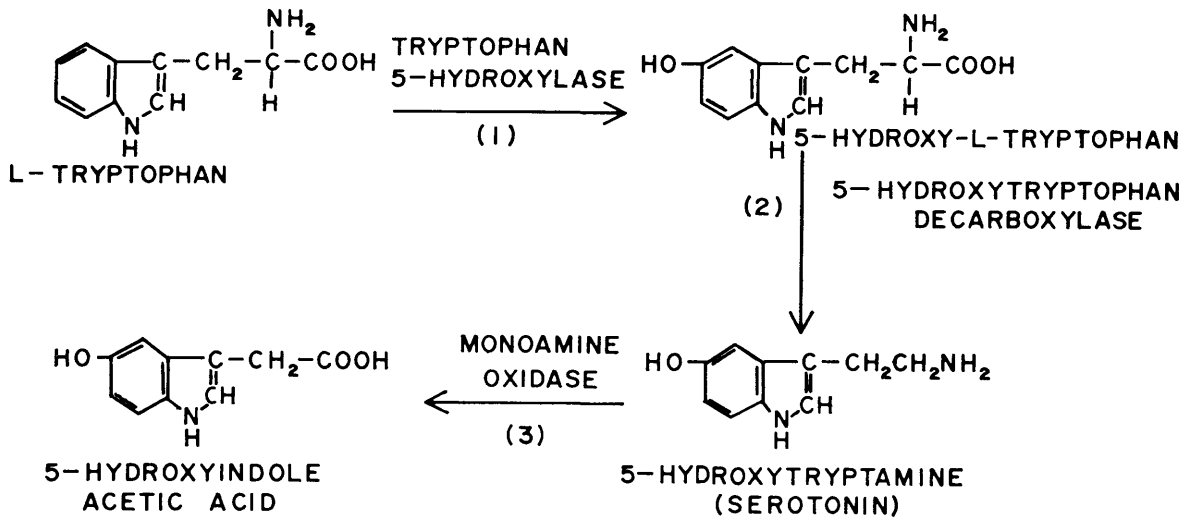

FIG. 1. Biosynthesis and metabolism of serotonin.

RESULTS

Structural specificity of serotonin inhibitory action on insulin release

Table 1 summarizes the pertinent studies with serotonin analogues. L-tryptophan and L-5 hydroxytryptophan in concentrations of 10^{-4} M did not affect glucose-mediated insulin secretion (Experiment 1). Although L-5 hydroxytryptophan was also without effect at a tenfold greater concentration, L-tryptophan potentiated glucose-mediated insulin secretion at this greater concentration (Experiment 2). Five-hydroxyindole acetic acid did not significantly affect glucose-mediated insulin secretion at 10^{-4} M concentration (Experiment 3). At a tenfold greater concentration 5-hydroxyindole acetic acid significantly inhibited glucose-mediated insulin secretion (Experiment 3). Five-hydroxyindole acetic acid (10^{-3} M) had no significant effect on basal insulin secretion (basal release, —0.74 ± 0.32 μU./mg. pancreas/fifteen minutes; 5-hydroxyindole acetic acid, —0.64 ± 1.27).

Experiment 4 demonstrates that the hydroxyl group in the five position of the indole ring is necessary for the inhibitory effect on insulin secretion, as tryptamine in both 10^{-4} and 10^{-3} M concentration was

TABLE 1

Effect of serotonin precursors and metabolites on glucose-mediated insulin release from hamster pancreas in vitro. Incubations were carried out in Krebs Ringer bicarbonate buffer with bovine serum albumin 400 mg./100 ml., and fumarate, glutamate, and pyruvate 0.5 mmoles/100 ml. In parentheses are the number of experimental observations. TP is L-tryptophan. 5-HTP is L-5-hydroxytryptophan. 5-HIAA is 5-hydroxyindole acetic acid.

Experiment	Additions to the modified Krebs bicarbonate buffer	Insulin release (μU./mg./15 min.)	Significance from glucose (3 mg./ml.)
1	Glucose 3 mg./ml.	7.3±2.44 (6)	
	Glucose 3 mg./ml. + TP 10^{-4} M	7.6±1.23 (6)	N.S.
	Glucose 3 mg./ml. + 5-HTP 10^{-4} M	6.4±0.93 (6)	N.S.
2	Glucose 3 mg./ml.	5.6±2.03 (6)	
	Glucose 3 mg./ml. + 5-HTP 10^{-3} M	8.8±1.25 (6)	N.S.
	Glucose 3 mg./ml. + TP 10^{-3} M	20.6±3.52 (6)	$p<0.01$
3	Glucose 3 mg./ml.	12.1±1.92 (11)	
	Glucose 3 mg./ml. + 5-HIAA 10^{-4} M	9.2±0.86 (6)	N.S.
	Glucose 3 mg./ml. + 5-HIAA 10^{-3} M	4.9±0.79 (11)	$p<0.01$
4	Glucose 3 mg./ml.	14.8±2.40 (6)	
	Glucose 3 mg./ml. + tryptamine 10^{-4} M	12.8±3.25 (6)	N.S.
	Glucose 3 mg./ml. + tryptamine 10^{-3} M	18.7±4.71 (6)	N.S.
5	Glucose 3 mg./ml.	7.9±1.08	
	Glucose 3 mg./ml. + serotonin 10^{-4} M (5-hydroxytryptamine)	0.9±1.04 (6)	$p<0.01$

without effect on glucose-mediated insulin secretion. Five-hydroxytryptamine (serotonin) 10^{-4} M, as previously reported, inhibits both glucose-mediated insulin secretion (Experiment 5) and basal insulin secretion.[1,2]

Effect of serotonin inhibitor methysergide maleate on in vitro insulin release

Figure 2 demonstrates that methysergide maleate blocked the inhibition by serotonin of glucose-mediated insulin secretion ($p < .01$). Figure 3 demonstrates that even in the absence of exogenous serotonin, methysergide maleate significantly potentiated glucose-mediated insulin secretion. In other experiments the effect of methysergide maleate on basal insulin secretion was studied. Basal insulin secretion alone was -0.39 ± 0.25 μU./mg./fifteen minutes, while in the presence of methysergide maleate basal insulin secretion was 0.64 ± 0.44 μU./mg./fifteen minutes (eight observations/group). This difference just missed significance at the 5 per cent confidence level.

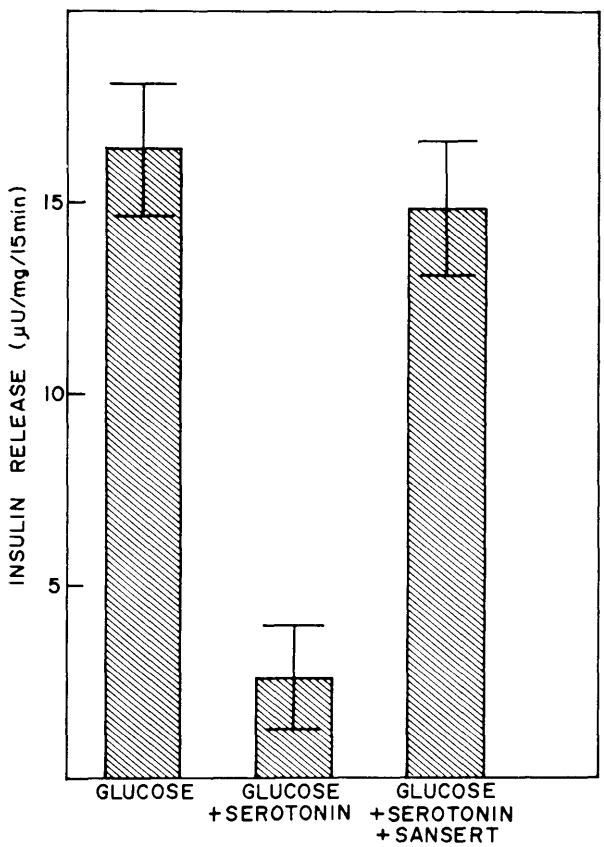

FIG. 2. Methysergide maleate (Sansert) block of serotonin inhibition of glucose (3 mg./ml.) mediated insulin release in golden hamster pancreas in vitro. The bars represent the means and the brackets the S.E.M. of six observations. Serotonin and Sansert were present at 10^{-4} M.

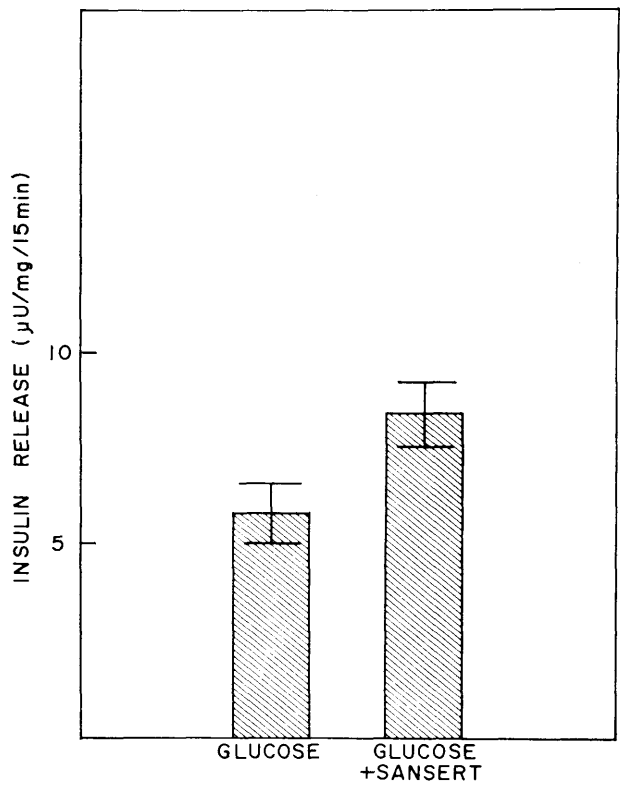

FIG. 3. Potentiation of glucose (3 mg./ml.) mediated insulin release from golden hamster pancreas in vitro by methysergide maleate (Sansert) 10^{-4} M. The bars represent the means and the brackets the S.E.M. of nine observations. The difference is significant at $p < 0.05$.

DISCUSSION

Serotonin exerts a number of epinephrine-like effects on the regulation of fat and carbohydrate metabolism. It stimulates glycolysis and glycogenolysis in the liver fluke, by producing an increase in cyclic 3′5′ AMP.[4] In mammalian tissues, serotonin stimulates both hepatic glycogenolysis,[5] and adipose tissue lipolysis.[6] It also alters thyroid tissue metabolism, in a manner similar to epinephrine.[7] Accordingly, it is not unexpected that serotonin inhibits pancreatic insulin secretion as does epinephrine.[8,9] Indeed, it is possible that in some species, such as the golden hamster, serotonin might be a physiologically more important regulatory amine. Its inhibitory activity is quite specifically related to its structure.

L-tryptophan in appropriate concentration (10^{-3} M) markedly enhanced glucose-mediated insulin secretion from hamster pancreas. Although they did not study tryptophan, Edgar et al. have noted similar enhancement of glucose-mediated insulin release by arginine, lysine, leucine and histidine.[10] In the serotonin-sensitive clam

heart system, Greenberg found that L-tryptophan was completely inactive in up to 10^{-3} M concentration.[11] The potentiating effect of L-tryptophan on insulin release in the present system may be due to structural similarities it shares with the other L-amino acids that also potentiate insulin release.

In contrast to L-tryptophan, L-5 hydroxytryptophan had no effect on insulin secretion in any of the concentrations studied. This is surprising, for one might expect that even if inactive, it might exert an effect if the pancreatic tissue converted it to serotonin. Such a metabolic conversion has been demonstrated in many tissues.[12] Either hamster pancreas is unable to form serotonin from precursors, or the incubation time (fifteen minutes) in the present study was so short that the glucose stimulated insulin secretion before adequate amounts of L-5 hydroxytryptophan could be decarboxylated to serotonin. In the serotonin-sensitive clam heart system, L-5-hydroxytryptophan had about 1/100,000 the potency of serotonin.[11]

Five-hydroxyindole acetic acid (5-HIAA) is usually considered a biologically inert excretory product of serotonin.[13] It has been reported, however, to have 1/10,000 the potency of serotonin on the isolated estrous rat uterus. This oxytocic effect of 5-HIAA is not blocked by the serotonin antagonist bromlyseric acid diethylamide.[14] In the clam heart system 5-HIAA has only 1/20,000 the potency of serotonin.[11] In the present studies 5-hydroxyindole acetic acid had an inhibitory effect on glucose-mediated insulin secretion, but this was only noted with 5-hydroxyindole acetic acid in at least a tenfold greater concentration than serotonin.

In the hamster pancreatic system, tryptamine has no effect on insulin secretion. This amine has been reported to have one tenth the potency of serotonin on the clam heart system,[11] and 1/400 the potency of serotonin on the isolated rat stomach strip preparation.[15]

These studies demonstrate the structural specificity of indole compounds for the inhibition of pancreatic insulin release. The absence of activity by tryptamine indicates an absolute requirement for a hydroxyl group in the five position of the indole ring. The inhibitory activity of 5-HIAA shows that a primary amine group in the alkyl chain is not absolutely essential, but since serotonin is at least ten and possibly fifty times as active, the amine group greatly enhances activity. The failure of 5-hydroxy L-trypotophan to exert an effect may be related to steric hindrance of having both the carboxyl and amino group on the alkyl side chain.

The effect of methysergide maleate in blocking the inhibitory action of serotonin on insulin release makes it highly unlikely that serotonin is acting through the same receptors as epinephrine, since this blocker has been shown to be a specific antagonist to serotonin and not epinephrine in other systems.[16]

The observation that methysergide maleate potentiates glucose-mediated insulin release indicates that endogenous serotonin in pancreatic β cells may play some role in the physiologic control of insulin release in the golden hamster. While this would be consistent with the reported observations that serotonin is present within the insulin granule of the β cell of some species,[17] it is highly speculative as it is not yet known whether serotonin is present in the insulin granule of the β cell of the golden hamster.

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REFERENCES

1 Feldman, J. M., and Lebovitz, H. E.: Inhibition of insulin secretion by serotonin. Diabetes *18*: Sup. 1, 326, 1969.

2 Feldman, J. M., and Lebovitz, H. E.: Serotonin inhibition of in vitro insulin release from golden hamster pancreas. Endocrinology *86*:66, 1970.

3 Feldman, J. M., and Lebovitz, H. E.: Biological activities of tolbutamide and its metabolites: A dissociation of insulin-releasing and hypoglycemic activity. Diabetes *18*:529-37, 1969.

4 Stone, C. A., and Mansour, T. E.: Phosphofructokinase from liver fluke fasciola hepatica. I. Activation by adenosine 3′5′-phosphate and by serotonin. Molec. Pharmacol. *13*:161-76, 1967.

5 Levine, R. A., Pesch, L. A., Klatskin, G., and Giarman, N. J.: Effect of serotonin on glycogen metabolism in isolated rat liver. J. Clin. Invest. *43*:797-809, 1964.

6 Bieck, P. Stock, K., and Westerman, E.: Lipolytic action of serotonin in vitro. Life Sci. *5*:2157-63, 1966.

7 Maayan, M. L., Suzuki, H., Lennox, S. L., and Ingbar, S. H.: In vitro effects of serotonin on thyroid iodine and intermediary metabolism. Program of 51st meeting of The Endocrine Society, p. 66, 1969.

8 Coore, H. G., and Randle, P. J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. *93*:66-78, 1964.

9 Porte, D., Jr., Graber, A., Kuzuya, T., and Williams, R. H.: The effect of epinephrine on immunoreactive insulin levels in man. J. Clin. Invest. *45*:228-36, 1966.

10 Edgar, P., Rabinowitz, D., and Merimee, T. J.: Effects of amino acids on insulin release from excised rabbit pancreas. Endocrinology *84*:835-43, 1969.

[11] Greenberg, M. J.: Structure-activity relationship of tryptamine analogues on the heart of venus mercanaria. Brit. J. Pharmacol. *15*:375-88, 1960.

[12] Udenfriend, S., Weissbach, H., and Bogdanski, D. F.: Increase in tissue serotonin following administration of its precursor 5-hydroxytryptophan. J. Biol. Chem. *224*:803-10, 1957.

[13] Bianchine, J. R., and Eade, N. R.: The effect of 5-hydroxytryptamine on the cotton pellet local inflammatory response in the rat. J. Exp. Med. *125*:501-10, 1967.

[14] Gessner, P. K., McIsaac, W. M., and Page, I. H.: Pharmacological actions of some methoxyindole-alkylamines. Nature *190*:179-80, 1961.

[15] Vane, J. R.: The relative activities of some tryptamine analogues on the isolated rat stomach strip preparation. Brit. J. Pharmacol. *14*:87-98, 1959.

[16] Franchamps, A., Doepfner, W., Weidmann, H., and Cerletti, A.: Pharmakologische Charakterisierung Von Deseril Einem Serotonin-Antagonisten. Schweiz. Med. Wschr. *90*: 1040-46, 1960.

[17] Jaim-Etcheverry, Guillermo, and Zieher, Luis Maria: Electron microscopic cytochemistry of 5-hydroxytryptamine (5-HT) in the beta cells of guinea pig endocrine pancreas. Endocrinology *83*:917-23, 1968.

Artificial Sweeteners—Possible Photosensitizers

A few individuals develop a dermatitis on those parts of the body exposed to sunlight when consuming large amounts of cyclamates. The skin condition disappears when cyclamate is removed from the diet.

When substances like artificial sweeteners are used by very large numbers of people, a variety of untoward reactions might be anticipated. One of those seen in a small percentage of the people consuming cyclamates is increased sensitivity of the skin to sunlight. The erythema and subsequent pruritis are apparent only on those parts of the body that are exposed directly to the sun.

Some of the early reports of this reaction came from Japan. There, T. Kobori and H. Araki (*J. Asthma Res.* 3:213, 1966) described a severe recurrent photodermatitis which had existed for two years in a thirty-five-year-old woman employed as a candy maker. How cyclamates were discovered as a causative agent of the dermatitis is not described. However, when this woman stopped taking cyclamates, her skin condition rapidly improved. The Japanese investigators said that eleven similar cases had been seen in their outpatient department. They also stated that the cyclamates are "very widely used in various candy, cans, and juices."

A more complete description of such a patient was given by S. I. Lamberg (*J. Am. Med. Assn.* 201:747, 1967). This patient was a forty-year-old housewife who had no previous skin disease and whose only other complaint was moderate obesity. About a month before seeking medical attention, she had noticed a burning and pruritic eruption on her face, back of the neck, and arms. This condition had become so troublesome that it interfered with sleep.

Of the possible allergenic substances to which this woman might have been exposed, the one most closely associated in point of time with the development of the skin condition was consumption of large amounts of diet soft drinks. In her efforts to lose weight, she had consumed an average of six twelve-ounce cans of the soft drinks, which contained cyclamates, each day. She had not eaten other foods containing artificial sweeteners.

Within two days after she discontinued the soft drinks, her pruritis disappeared and the skin began to heal. When she reinitiated consumption of soft drinks, the dermatitis reappeared. Eruption of the skin and pruritis usually began about six hours after exposure to the sun.

It was calculated that on the basis of her intake of soft drinks this woman was consuming the equivalent of seventy-two tablets of the combination calcium cyclamate and saccharin per day. When she was completely free of any dermatological symptoms, consumption of these tablets caused her skin symptoms to reappear within four days.

The photosensitivity was attributable to cyclamates and not to saccharin, which was exonerated when the patient abstained from any artificial sweeteners for a

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