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3-Hydroxykynurenine, 3-Hydroxyanthranilic Acid, and *o*-Aminophenol Inhibit Leucine-Stimulated Insulin Release from Rat Pancreatic Islets (42010)

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Abstract. Individual islets were isolated from rat pancreas to study the effects of tryptophan and its metabolites on leucine-stimulated release of insulin. 3-Hydroxykynurenine, 3-hydroxyanthranilic acid, and *o*-aminophenol were inhibitors at concentrations below 10 m*M* whereas tryptophan, kynurenine, kynurenic acid, xanthurenic acid, and anthranilic acid were ineffective inhibitors at concentrations up to 10 m*M*. A structure–activity analysis of these metabolites demonstrated that vicinal aromatic hydroxy and amino groups with their concomitant electron donating properties are required for inhibition of insulin release. Inhibition of islet insulin release by the three kynurenine metabolites may be involved in the depressed insulin levels found in vitamin B_6-deficient rats by other workers.

Malaisse *et al.* (1) proposed that glucose may stimulate insulin release by acting as a metabolic fuel for the pancreatic islet rather than or in addition to activation of a specific glucoreceptor. To support this argument, they (2) showed that the amino acid leucine, which causes insulin release from the islet, acts as a metabolic fuel and stimulates islet glutamate dehydrogenase (3), NADH formation (4), ADP phosphorylation (4), and the accumulation of calcium (4). Alternatively, insulin secretion may depend on a "signal" as well as the metabolism of a nutrient (5).

Plasma insulin levels are decreased in rats that are deficient in Vitamin B_6 (6). Lack of this vitamin causes the accumulation and excretion (7) of 3-hydroxykynurenine, a tryptophan metabolite, because the vitamin product pyridoxal phosphate is then unavailable for kynureninase activity which normally breaks down 3-hydroxykynurenine (8). This suggests the possibility that tryptophan and/or kynurenine metabolites may be involved in insulin suppression.

We have isolated pancreatic islets from the rat and investigated the effects of tryptophan and its kynurenine metabolites on the release of insulin from leucine-stimulated islets.

Materials and Methods. Islets were isolated from the pancreas of 120-g male Sprague–Dawley rats (Flow Laboratories, Dublin, Va.) using a modification of the methods of Malaisse *et al.* (9) and Lacy and Kostianovsky (10). Rats were killed by decapitation. The pancreas was removed and excised from adhering fat tissue, small blood vessels, and lymph nodes. Pancreatic tissue was minced in ice-cold Hanks' balanced salt solution (11) (Gibco Laboratories, Grand Island, N.Y.) previously gassed with water-pumped 95% O_2/5% CO_2 and adjusted to pH 7.4 with saturated sodium bicarbonate. Collagenase from *Clostridium histolyticum* (Accurate Chemical and Scientific Corp., Westbury, N.Y.) was added to digest collagen and separate individual islets from the minced pancreatic tissue. The islets were suspended in Eagle's basal medium (12) (Gibco Laboratories) which contained 100 mg/dl glucose, Hanks' salts, no glutamine, bovine serum albumin, 5 mg/ml (Sigma Chemical Company, St. Louis, Mo.), and 25 m*M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer adjusted to pH 7.4 with sodium hydroxide.

Ten intact islets, capable of insulin release, 50 μunit of insulin/90 min/islet (cf. (3)), were handpicked twice from the above solution with the aid of a dissecting microscope and a 75-μl pipet. They were placed in ice-cold Eagle's solution and stored on ice for 30 min prior to addition of the secretogogues (10 m*M* L-leucine and 10 m*M* L-glutamine or 200 mg/dl glucose) and the test compounds. Tryptophan, 3-hydroxyanthranilic acid, and 3-hydroxy-DL-kynurenine were purchased from Mann Research Laboratories, New

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York, and Sigma Chemical Company. Kynurenic acid, kynurenine, xanthurenic acid, and anthranilic acid were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. *o*-Aminophenol was bought from Matheson Company, East Rutherford, New Jersey. The suspended islets were gassed (95% O_2/5% CO_2) in Erlenmeyer flasks, stoppered, and incubated 30–90 min at 37°C in a Dubinoff shaker. The reaction was stopped by being placed on ice, centrifuged, and an aliquot was removed from the supernatant for insulin analysis.

Insulin analyses (13, 14) were performed through the courtesy of Dr. William Blackard, Clinical Research Center, Medical College of Virginia, following the double-antibody radioimmunoassay method (15) utilizing ^{125}I-labeled insulin as a tracer. Crystalline rat insulin (Novo Laboratories, Copenhagen, Denmark) was used for preparation of standards.

Statistical analyses. A two-tailed Student *t* test was used to ascertain levels of significance (16). Probability values of 0.05 or less were considered significant.

Results and Discussion. The influence of tryptophan and its kynurenine metabolites on the release of insulin from leucine-stimulated pancreatic islets is presented in Table I. Tryptophan, kynurenine, kynurenic acid, xanthurenic acid, and anthranilic acid were ineffective at the highest concentrations listed after 90 min of incubation for altering the release of insulin from the isolated pancreatic islet. Salicyclic acid (6 m*M*) and *p*-aminobenzoic acid (7 m*M*) were also ineffective as inhibitors (data not shown). In contrast, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and *o*-aminophenol inhibited insulin release throughout the incubation period. This inhibition did not depend upon calcium ion chelation (3) by the three metabolites since the addition of 2 m*M* calcium chloride to the incubation medium did not prevent inhibition by 3-hydroxykynurenine (data not shown). 3-Hydroxykynurenine and 3-hydroxyanthranilic acid were equally effective in inhibiting the release of insulin from 300 mg/dl glucose-stimulated islets in the absence of added leucine and glutamine (Table II). The inhibition (ca. 36%) by 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and *o*-aminophenol (Table III) could have resulted from their potential as uncouplers (17) since 2.5 m*M* ethidium bromide which is an inhibitor and uncoupler of mitochondrial oxidative phosphorylation (18) inhibited islet release of insulin by 80% (data not shown).

TABLE I. EFFECT OF TRYPTOPHAN AND ITS METABOLITES ON INSULIN RELEASE FROM LEUCINE-STIMULATED PANCREATIC ISLETS

Metabolite	Incubation time (min)	Insulin release μunit/islet
	30	
Control, 0 m*M*		47 ± 3[a]
3-Hydroxyanthranilic acid, 8.4 m*M*		30 ± 2[b]
3-Hydroxy-DL-kynurenine, 3.5 m*M*		36 ± 3[b]
	60	
Control, 0 m*M*		72 ± 4
3-Hydroxyanthranilic acid, 8.4 m*M*		35 ± 3[b]
3-Hydroxy-DL-kynurenine, 3.5 m*M*		49 ± 1[b]
o-Aminophenol, 11.0 m*M*		45 ± 3[b]
	90	
Control, 0 m*M*		91 ± 3
3-Hydroxyanthranilic acid, 8.4 m*M*		57 ± 3[b]
3-Hydroxy-DL-kynurenine, 3.5 m*M*		58 ± 3[b]
	90	
Control, 0 m*M*		49 ± 5
Tryptophan, 9.8 m*M*		41 ± 2
Kynurenine, 9.6 m*M*		42 ± 3
Kynurenic acid, 10.6 m*M*		44 ± 1
Xanthurenic acid, 9.8 m*M*		43 ± 2
Anthranilic acid, 15.7 m*M*		42 ± 3

[a] Mean ± SEM of four observations in duplicate.
[b] $P < 0.05$.

3-Hydroxykynurenine, 3-hydroxyanthranilic acid, and *o*-aminophenol are closely related in structure by having in common a benzene nucleus with 3-hydroxy and 2-amino substitutions. Loss of a substituent destroyed the effectiveness of the inhibitor (Table I). Therefore inhibition by 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and *o*-aminophenol required an aromatic hydroxy group vicinal (adjacent) to an aromatic amino group. This geometry may be different from that of serotonin and epinephrine, inhibitors of insulin release from hamster pancreatic

TABLE II. COMPARISON OF 3-HYDROXYANTHRANILIC ACID AND 3-HYDROXYKYNURENINE INHIBITIONS OF INSULIN RELEASE FROM LEUCINE-STIMULATED AND GLUCOSE-STIMULATED PANCREATIC ISLETS

Metabolite	Insulin release (μunit/60 min/islet)
Leucine stimulus	
Control, 0 m*M*	99 ± 5[a]
3-Hydroxyanthranilic acid, 8.4 m*M*	49 ± 4[b]
3-Hydroxy-DL-kynurenine, 3.6 m*M*	74 ± 8[b]
Glucose stimulus	
Control, 0 m*M*	98 ± 5
3-Hydroxyanthranilic acid, 8.4 m*M*	50 ± 4[b]
3-Hydroxy-DL-kynurenine, 3.6 m*M*	68 ± 3[b]

[a] Mean ± SEM of 10 observations in duplicate.
[b] $P < 0.05$.

islets (19, 20), which required an aromatic hydroxy group situated many carbons (angstroms) away from an aliphatic amino group. This indicated to us that the conformations of the islet binding sites for the two groups of compounds may have different geometries. Although both groups of compounds may have different steric requirements for binding to islets, we speculate it is conceivable that both groups may involve free radical formation at the islet receptor site(s) as a "signal" to prevent insulin release. Both groups of compounds may be capable of donating electrons (we have calculated electron densities and solvation energies of similar model compounds (21)) and one group, serotonin and epinephrine, has been shown to form free radicals upon oxidation (22, 23).

TABLE III. INHIBITION OF INSULIN RELEASE FROM LEUCINE-STIMULATED PANCREATIC ISLETS BY 3-HYDROXYANTHRANILIC ACID, 3-HYDROXYKYNURENINE, AND *o*-AMINOPHENOL

Metabolite	Insulin release μunit/60 min/islet
Control, 0 m*M*	84 ± 6[a]
3-Hydroxyanthranilic acid, 3 m*M*	54 ± 2[b] (36)[c]
o-Aminophenol, 4 m*M*	49 ± 2[b] (42)
Control, 0 m*M*	99 ± 5
3-Hydroxy-DL-kynurenine, 4 m*M*	68 ± 3[b] (31)

[a] Mean ± SEM of six observations in duplicate.
[b] $P < 0.05$.
[c] Number in parentheses denotes percentage inhibition of control.

In conclusion, our observations that 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and *o*-aminophenol inhibited the release of insulin from leucine-stimulated pancreatic islets may offer some explanation for the depressed plasma insulin levels found in Vitamin B_6 deficiency (6).

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