Serotoninergic Activation and Inhibition: Effects on Carbohydrate Tolerance and Plasma Insulin and Glucagon

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Glucose and arginine infusion tests were performed on 12 healthy volunteers (8 males, 4 females) before and after serotoninergic activation [oral administration of L-5-hydroxytryptophan (5-HTP-) for 6 days] and serotoninergic inhibition (oral treatment with D,L-p-chloropenylalanine for 6 days). 5-HTP treatment markedly increased urinary 5-hydroxyindoleacetic acid excretion, increased the mild hyperglycemic effect of arginine infusion, and lowered the glucose disposal rate constant. The adverse effect of serotoninergic activation on glucose tolerance is not sufficiently explained by the observed changes in insulin and glucagon secretion during the fasting state and after intravenous glucose and arginine infusions. Serotoninergic inhibition did not affect the carbohydrate tolerance of normal individuals. The results of this work supports the idea that excessive indoleamine production is probably the main cause for carbohydrate intolerance in carcinoid tumors.

SEROTONIN, dopamine, and, to a lesser degree, norepinephrine have been identified in the mammalian pancreatic islets by the use of fluorescent microscopy and cytochemical techniques.[1,2] Autoradiographic techniques have demonstrated that those mammalian species whose islets do not ordinarily fluoresce for biogenic amines retain the ability to produce these amines. The islets take up the monoamine precursors dopa and 5-hydroxytryptophan (5-HTP), converting them to dopamine and serotonin.[3,4] The importance of these observations in the normal physiologic state has been under recent investigation as there is significant species variation in the uptake of monoamines and their precursors into the pancreatic islets.[5]

Studies of the effect of exogenously administered biogenic amines on carbohydrate metabolism have constituted the first step in the investigation of the role of these compounds in normal islet physiology. The administration of pharmacologic doses of serotonin (5-hydroxytryptamine; 5-HT) to fasting dogs exerted a hyperglycemic effect.[6] Both in vitro and in vivo experiments have suggested that this effect is a result of an inhibitory action of serotonin on both glucose-dependent and non-glucose dependent insulin release mechanisms.[7-9] Further evidence for this effect is found in studies of human subjects with a diagnosis of carcinoid syndrome. Carbohydrate intolerance is observed in those

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carcinoid patients with high serum serotonin levels but is absent in those with normal levels.[10] Furthermore, a study of nonobese maturity-onset diabetics found them to have statistically higher fasting serum serotonin levels than a matched control group.[11]

The abovementioned facts suggest that excessive levels of serotonin, either in blood or tissues or in both, can lead to deterioration of carbohydrate tolerance. To test this hypothesis in human subjects, carbohydrate tolerance and related hormone responses were compared in healthy volunteers during the control state, during activation of the serotoninergic system by oral administration of 5-HTP (the immediate precursor of serotonin[12-14]), and during inhibition of tryptophan hydroxylation by oral administration of p-chlorophenylalanine (p-CPA, Fenclonine).[15,16] The results show that marked serotoninergic activation by 5-HTP impairs carbohydrate tolerance. Serotoninergic inhibition by p-CPA causes no net change in glucose tolerance.

MATERIALS AND METHODS

Human Subjects

Twelve healthy volunteers (8 males, 4 females), free of endocrine disorders and without a history of diabetes mellitus in the immediate family, gave written informed consent and were admitted to the Clinical Research Center of the University of Minnesota Hospital. Each subject had a physical examination and the following laboratory studies before and after each study period: complete blood count, urinalysis, serum creatinine, urea nitrogen, bilirubin, alkaline phosphatase, glutamic-oxalacetic transaminase (SGOT), and a chest x-ray. The mean age of the group was 23.3 ± 1.2 yr (SD); the mean weight was 67.4 ± 9.4 kg, or 103.8% ± 9.4% ideal weight (Metropolitan Life Insurance tables). After 3 days of supervised diet, containing at least 250 g carbohydrate and 80 g protein/day, an oral glucose tolerance test (glucose load, 1.75 g/kg) was performed. As Table 1 shows, the plasma glucose values were normal for the whole group (Conn and Fajans criteria).

Details of the Protocol

The protocol consists of five consecutive phases. Phase 1 consisted of a 6-day control period; an intravenous glucose tolerance test (IVGTT) was performed on day 4 of this phase and the intravenous arginine test (IVAT) was completed on the sixth day. The second phase consisted of 6 days of treatment with either 5-HTP or p-CPA. The actual order of drugs was alternated so that 50% of the group were given 5-HTP first and 50% received p-CPA first. Phase 3 consisted of a 6–8-wk drug-free interval during which the subjects were at home on their usual diets and with their usual full activity. Phase 4 was simply a repeat control period identical to phase 1. Phase 5 was equivalent to phase 2 but involved the administration of the other drug.

A total dose of 4500 mg of 5-HTP was given spread over a 6-day period, with a gradual increase from 250 mg/day to a maximum of 1000 mg/day in divided doses; the last 250-mg capsule was administered 1 hr before the last test of the protocol. The maximal dose (1000 mg, distributed as one capsule of 250 mg every 6 hr) was maintained during the final 3 days of the treatment periods. Possible effects of both medications were monitored.[13,15,17-19]

For the IVGTT, a large glucose load (50 g/1.73 sq m body surface) diluted as 25% solution in distilled water[20] was infused in 5–7 min through an indwelling catheter in one vein of the forearm, with the patient in a recumbent position; from the opposite forearm, venous blood samples

Table 1. Oral Glucose Tolerance Test (1.75 g/kg)

	Plasma Glucose (mg/dl)					
	0 min	30 min	60 min	90 min	120 min	180 min
Mean	89	131	126	112	101	94
SEM	2.0	5.3	8.8	8.0	5.5	3.3

Table 2. Urinary 5-Hydroxyindoleacetic Acid (mg/24 hr)

	Day 1	Day 2
5-HTP		
Before	2.9 ± 0.6	3.9 ± 0.8
After	538.0 ± 64.7	525.0 ± 73.4
t	7.93	4.68
p	<0.005	<0.01
p-CPA		
Before	3.5 ± 0.5	3.4 ± 0.4
After	1.4 ± 0.3	1.9 ± 0.4
t	3.10	2.63
p	<0.01	<0.02

Results show mean ± SEM for 12 subjects.

were drawn through another indwelling catheter. A standard 30-g dose of L-arginine monochloride, pH adjusted to 7.4, was infused as 12.5% solution in 30 min for the IVAT.[21] Preparation for the tests included a 250-g carbohydrate, 80-g protein diet, low in serotonin, for at least 72 hr, and 12 hr of fasting and bed rest.

Plasma glucose concentrations were measured by the O-toluidine method,[22] plasma insulin by a previously reported method,[23] and plasma glucagon by a modified assay[24] using dextran-coated charcoal and 30 K antibody, from Dr. Roger Unger. Fasting samples were also obtained on the last day of both control periods and both drug treatment periods, they were analyzed for serum cortisol by a competitive-binding assay.[25] Twenty-four-hour urine collections were obtained preceding the IVGTT and IVAT during control periods and just before ending each drug period. 5-hydroxyindolacetic acid (5-HIAA) was measured for each urine collection by a colorimetric method, using the addition of 2-mercaptoethanol to enhance the specificity of the nitrosonaphtol reaction for 5-hydroxyindoles.[26]

The glucose disposal rate constant (K value) was calculated by the method of least squares, as the slope of the linear decay of the logarithms of the absolute plasma glucose levels from 15 to 60 min; timing was from the start of the infusion.[27]

Table 3. Intravenous Glucose Tolerance Test: Before and After 5-HTP (*N* = 12)

		−10*	−5	+5	+10	+15	+20	+30	+40	+50	+60
Plasma glucose (mg/dl)											
Before	Mean	81	84	276	340	321	306	246	194	154	134
	SEM	2.5	2.6	28.0	18.0	15.6	18.2	18.3	15.5	13.7	19.3
After	Mean	81	86	299	370	352	322	262	216	181	162
	SEM	2.3	2.3	39.3	20.7	14.6	11.4	36.5	38.0	10.8	12.8
	t	0	0.58	0.59	1.13	1.22	0.72	0.76	0.54	1.52	1.22
Plasma insulin (μU/ml)											
Before	Mean	14	16	84	78	82	71	58	50	43	43
	SEM	0.9	1.2	28	28	22.7	17.3	6.4	4.5	4.0	5.0
After	Mean	13	14	73	73	72	61	54	55	54	67
	SEM	0.9	1.4	6.1	18.3	18.4	8.9	6.1	7.3	6.9	11.3
	t	0.79	1.09	2.01	0.15	0.34	0.53	0.47	0.63	1.66	2.76
	p			<0.1							<0.05
Plasma glucagon (pg/ml)											
Before	Mean	187	191	163	130	138	123	120	120	120	110
	SEM	21.4	22.7	19.0	20.1	19.1	18.5	17.4	16.8	16.6	10.5
After	Mean	179	196	163	131	121	123	127	124	128	108
	SEM	22.8	28.5	18.6	17.9	18.0	18.9	19.0	20.0	20.1	21.4
	t	0.29	0.14	0	0.04	0.65	0	0.28	0.15	0.31	0.08

*Minutes before (−) or after (+) 5-HTP administration.

Table 4. Intravenous Glucose Tolerance Test: K Values With 5-HTP and p-CPA Treatment (N = 12)

Subject	5-HTP		p-CPA	
	Before	After	Before	After
M.C.J.	2.69	3.16	2.93	3.04
P.E.D.	2.53	2.72	2.49	2.42
S.D.S.	1.93	1.17	1.22	1.14
W.R.R.	2.93	2.43	2.62	2.34
D.E.E.	1.73	1.13	1.61	1.39
D.K.E.	2.33	1.87	1.94	1.84
P.A.H.	1.85	1.41	1.62	2.07
E.E.E.	1.43	1.24	1.76	1.49
E.M.R.	4.97	3.05	3.36	1.93
H.E.J.	2.24	2.07	2.51	2.73
C.E.W.	2.10	1.50	2.04	2.18
W.O.R.	1.84	2.03	1.67	2.29
Mean	2.38	1.98	2.15	2.07
SEM	0.28	0.22	0.19	0.17
t		2.27		0.51
p		<0.05		NS

Insulin secretion was expressed as the area under the plasma insulin curve above the fasting level (ΔI, μU/min/ml). Glucagon secretion was expressed as the area of the plasma glucagon curve below the fasting level for the IGVTT and above the fasting level for the IVAT (Δ glucagon, pg/min/ml). Plasma glucose elevation (ΔG, mg/min/dl) was expressed as the area under the plasma glucose curve above the fasting level. The insulinogenic index (ΔI/ΔG) was calculated by dividing the area circumscribed by the insulin curve by the corresponding area circumscribed by the glucose curve during the IVGTT.

Student's tests were used for analysis of the difference before/after each drug treatment; only p values lower than 0.05 were considered statistically significant.

RESULTS

Effects of 5-HTP

As expected, a striking elevation of urinary 5-HIAA followed the administration of 5-HTP (Table 2). Plasma glucose, insulin, and glucagon concentrations

Table 5. Intravenous Glucose Tolerance Test: 5-HTP and p-CPA Treatment (N = 12)

		K	ΔG (mg/min/dl)	ΔI (μU/min/ml)	ΔI/ΔG	ΔGlucagon (pg/min/ml)
5-HTP						
Before	Mean	2.38	8,806	2.614	0.30	−3,615
	SEM	0.28	1,030	687	0.04	1,009
After	Mean	1.98	10,089	2,760	0.27	−3,425
	SEM	0.22	1,383	537	0.01	1,112
	t	2.27	0.74	0.19	0.75	0.13
	p	<0.05	NS	NS	NS	NS
p-CPA						
Before	Mean	2.15	9,542	2,769	0.29	−4,039
	SEM	0.19	715	593	0.04	993
After	Mean	2.07	9,822	3,242	0.33	−4,036
	SEM	0.17	685	577	0.03	978
	t	0.51	0.28	0.57	0.80	0.002
	p	NS	NS	NS	NS	NS

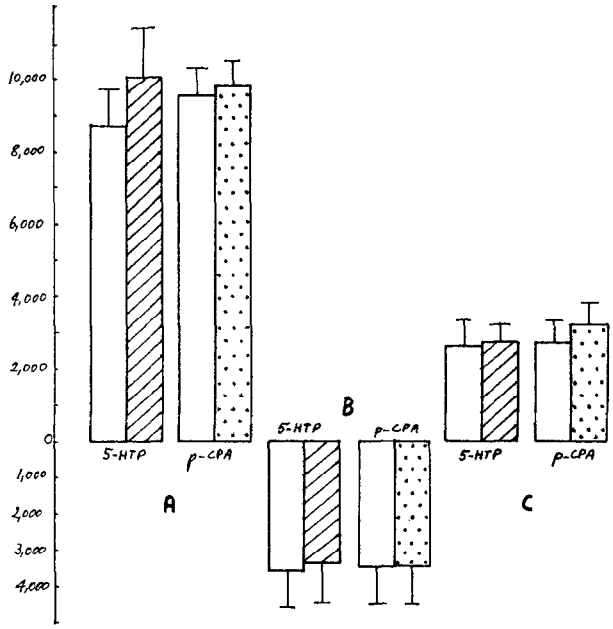

Fig. 1. Effects of 5-HTP and p-CPA during IVGTT. (A) ΔG (mg/min/dl). (B) ΔGlucagon (pg/min/ml). (C) ΔI (μU/min/ml). Bars show mean area and brackets SEM.

during IVGTT and before and after 5-HTP treatment are presented in Table 3. Table 4 shows K values for the IGVTT before and after the treatment. Table 5 and Fig. 1 depict changes in glucose disposal rate, plasma glucose elevation, insulin and glucagon secretion, and insulinogenic index for the IVGTT before and after each treatment. Although all the mean plasma glucose values after

Table 6. Intravenous Arginine Test: Before and After 5-HTP (*N* = 12)

		−15*	−5	+15	+30	+45	+60	+90	+120
Plasma glucose (mg/dl)									
Before	Mean	80	82	99	102	85	73	74	81
	SEM	1.6	1.1	1.2	1.3	7.3	2.5	2.2	1.9
After	Mean	81	84	104	106	92	76	78	86
	SEM	1.9	2.1	2.2	3.8	8.5	3.0	1.9	0.99
	t	1.97	1.56	3.12	1.27	1.27	0.77	1.87	4.48
	p	<0.1		<0.01				<0.1	<0.001
Plasma insulin (μU/ml)									
Before	Mean	15	13	64	89	60	22	10	11
	SEM	1.2	0.8	18.4	16.2	8.1	2.4	0.9	1.5
After	Mean	14	13	72	116	100	24	11	11
	SEM	1.6	1.7	19.2	33.3	50.4	6.3	1.3	1.1
	t	0.50	0	1.80	1.49	1.00	0.30	0.63	0.00
	p			<0.1					
Plasma glucagon (pg/ml)									
Before	Mean	176	188	605	750	686	468	252	195
	SEM	22.1	23.5	70.3	71.6	73.7	59.6	28.5	22.8
After	Mean	207	204	697	810	644	404	248	207
	SEM	32.6	33.2	89.3	95.9	75.4	25.2	27.1	26.0
	t	1.86	1.13	1.80	1.19	0.67	1.56	0.10	0.34
	p	<0.1		<0.1					

*Minutes before (−) or after (+) 5-HTP administration.

Table 7. Intravenous Arginine Test: 5-HTP and p-CPA Treatment (N = 12)

		ΔG (mg/min/dl)	ΔI (μU/min/ml)	Δ Glucagon (pg/min/ml)
5-HTP				
Before	Mean	255	2,645	31,155
	SEM	70	505	5,775
After	Mean	642	3,890	28,837
	SEM	92	1,645	5,563
	t	3.34	0.72	0.29
	p	0.01	NS	NS
p-CPA				
Before	Mean	275	2,187	30,156
	SEM	45	320	4,584
After	Mean	343	2,342	30,538
	SEM	5	1,668	5,199
	t	1.51	0.09	0.05
	p	NS	NS	NS

5-HTP were higher, differences in mean plasma glucose and plasma glucose elevations were not significant. Baseline and glucose-stimulated insulin levels as well as the insulinogenic index remained unchanged with the exception of a significant rise in plasma insulin concentration at 60 min, possibly associated with the hyperglycemic trend. Baseline plasma glucagon levels and the supressive effect of hyperglycemia on plasma glucagon remained unmodified after 5-HTP therapy. Mean K value was significantly lower after 5-HTP (2.38 ± 0.28 before 5-HTP, 1.98 ± 0.22 after 5-HTP; $p < 0.05$). In addition, the individual K values after 5-HTP were lower in 9 of 12 cases. Table 6 shows the effects of 5-HTP administration on plasma glucose, insulin, and glucagon concentrations. Table 7

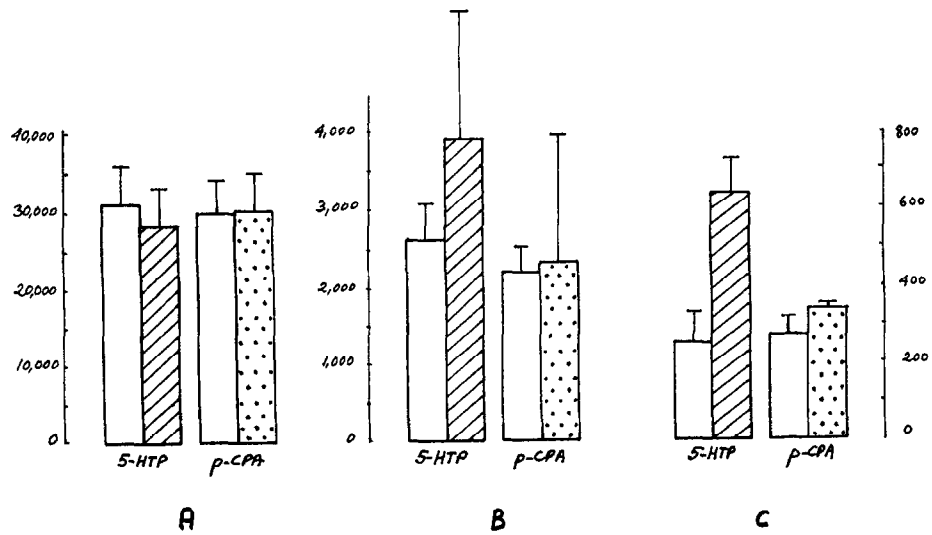

Fig. 2. Effects of 5-HTP and p-CPA during IVAT. (A) ΔGlucagon (pg/min/ml). (B) ΔI (μU/min/ml). (C) ΔG (mg/min/dl). Bars show mean area and brackets SEM. There was a significant increase in plasma ΔG after 5-HTP therapy ($p < 0.01$).

and Fig. 2 depict the changes in plasma glucose elevation and insulin and glucagon secretion for the IVAT before and after each treatment.

The administration of 5-HTP potentiated the hyperglycemia after arginine infusion with a significant increase of the area of plasma glucose elevation, without affecting fasting plasma glucose or insulin and glucagon concentrations before and after arginine. Finally, 5-HTP did not alter fasting serum cortisol levels (18 ± 1.7 μg/dl before 5-HTP; 18 ± 2.0 μg/dl after 5-HTP).

Side effects, although mild and transient, were frequently observed during the period of 5-HTP administration. They were similar to those described in the literature: 7 subjects complained of nausea and some abdominal discomfort; 6 subjects experienced increased bowel activity (mild diarrhea); irritability and mild headache were recorded in 4 cases; and 3 volunteers complained of unusual tiredness and mild depression. Side effects disappeared spontaneously after 1–3 days in all the cases without requiring any therapy or modification of schedule doses. Screening laboratory tests outlined in Materials and Methods remained unaffected after 5-HTP treatment.

Effects of p-CPA

A significant reduction in urinary 5-HIAA followed the administration of this inhibitor of serotonin synthesis (Table 2), although the effect was relatively small when compared with the dramatic change in 5-HIAA observed after the administration of 5-HTP. After p-CPA therapy, the glucose disposal rate constant and plasma glucose concentrations during IVGTT remained unchanged; nevertheless, both postglucose insulin and postglucose glucagon levels were higher after p-CPA (at 5, 40, and 50 min for insulin; at 5, 10, and 20 min for

Table 8. Intravenous Glucose Tolerance Test: Before and After p-CPA (*N* = 12)

		−10*	−5	+5	+10	+15	+20	+30	+40	+50	+60
Plasma glucose (mg/dl)											
Before	Mean	82	84	300	382	350	307	245	196	167	143
	SEM	1.1	1.4	17.7	15.9	12.7	11.7	10.8	11.3	11.1	11.7
After	Mean	84	86	305	384	350	322	259	206	171	148
	SEM	1.0	1.0	28.5	14.5	12.1	9.7	10.5	8.9	9.4	11.7
	t	1.34	1.02	0.15	0.09	0	0.96	0.90	0.99	0.29	0.02
Plasma insulin (μU/ml)											
Before	Mean	10	10	79	67	73	74	56	46	47	41
	SEM	1.4	1.0	12.8	13.7	13.7	14.8	8.8	7.0	7.6	6.7
After	Mean	11	12	95	75	87	77	67	59	57	48
	SEM	1.1	1.4	14.4	12.6	17.8	13.2	8.7	6.7	7.1	4.5
	t	0.56	1.16	2.55	1.70	1.91	0.62	1.63	2.94	2.59	1.47
	p			<0.05		<0.1			<0.02	<0.05	
Plasma glucagon (pg/ml)											
Before	Mean	193	204	152	123	122	120	127	129	126	128
	SEM	23.0	20.0	20.5	16.8	16.9	16.7	16.9	17.1	16.3	19.3
After	Mean	214	209	183	148	132	136	135	136	139	131
	SEM	25.5	25.2	22.8	18.0	15.9	16.8	15.3	16.3	17.8	17.2
	t	1.72	1.40	2.62	2.72	1.13	2.77	1.81	1.14	1.61	0.43
	p			<0.05	<0.02		<0.02	<0.1			

*Minutes before (−) or after (+) p-CPA administration.

Table 9. Intravenous Arginine Test: Before and After p-CPA ($N = 12$)

		−15*	−5	+15	+30	+45	+60	+90	+120
Plasma glucose (mg/dl)									
Before	Mean	81	83	99	100	85	71	78	86
	SEM	1.1	1.0	3.2	2.5	3.5	3.3	1.8	1.1
After	Mean	82	84	102	103	87	73	78	87
	SEM	1.1	1.2	2.1	2.6	3.2	2.6	1.8	1.1
	t	0.56	0.64	0.88	0.83	0.42	0.48	0	0.56
Blasma insulin (μU/ml)									
Before	Mean	12	11	59	77	49	16	8	7
	SEM	1.7	1.0	9.8	13.6	7.3	2.5	1.1	1.0
After	Mean	13	12	78	110	60	19	9	9
	SEM	1.3	1.4	20.0	20.5	10.9	2.8	1.1	1.1
	t	0.47	0.58	1.04	1.55	1.19	1.07	0.56	0.67
Plasma glucagon (pg/ml)									
Before	Mean	185	185	624	786	625	424	254	219
	SEM	22.4	22.5	67.2	61.2	55.7	35.2	23.9	21.1
After	Mean	215	211	670	841	692	481	244	221
	SEM	33.1	26.0	74.7	67.8	68.9	40.8	24.6	22.9
	t	1.96	2.18	0.78	1.13	1.22	2.32	0.29	0.06
	p	<0.1					<0.05		

*Minutes before (−) or after (+) p-CPA administration.

glucagon) (Table 8), although the area of insulin and glucagon secretion did not differ significantly during IVGTT before/after p-CPA (Table 5 and Fig. 1).

p-CPA treatment was responsible for a mild elevation of arginine-stimulated plasma glucagon levels, which was significant only at 60 min (Table 9), without affecting fasting or arginine-stimulated plasma insulin (ΔI), glucose (ΔG), or glucagon (Δ glucagon) before/after arginine infusion (Tables 7 and 9, Fig. 2). Finally, fasting serum cortisol levels remained unaffected by p-CPA therapy (14.7 ± 1.4 μg/dl before p-CPA; 14.4 ± 1.1 μg/dl after p-CPA).

All the subjects showed excellent tolerance to the administered p-CPA doses. Only one subject complained of mild and transient abdominal discomfort, which disappeared spontaneously after 2 days. No abnormalities of the screening laboratory tests were observed in any case.

DISCUSSION

The present results show that marked serotoninergic activation, similar to that recorded in the carcinoid syndrome,[10] produces a mild deterioration of carbohydrate tolerance, documented by the lowered glucose disposal rate constant and by potentiation of the hyperglycemic effect shown after arginine infusion in healthy human subjects. These adverse effects on glucose tolerance were not associated with relative hypoinsulinemia or relative hyperglucagonemia.

A possible direct interference of indoleamine in glucose production or utilization may be responsible for the described adverse effect on glucose tolerance; in this respect Levine et al. have shown a glycogenolytic effect after the endoportal administration of serotonin in isolated rat liver that was reversed by methysergide.[28] Indirect mechanisms cannot be excluded either; interactions with

adrenergic mechanisms[2,29] or a stimulatory effect on circulating growth hormone levels[30,31] after oral administration of 5-HTP may represent meaningful examples.

Serotoninergic inhibition by p-CPA was not followed by an increase of the glucose disposal rate constant in normal subjects; the observed potentiation of glucose-mediated insulin release was counterbalanced by decreased suppressibility of glucagon release. We could not reproduce the observation by Marco et al.[32] of an inhibitory effect of p-CPA on arginine-stimulated insulin secretion, but we confirmed mild potentiation of arginine-induced glucagon release by p-CPA.

The well-known carbohydrate intolerance in patients suffering from carcinoid syndrome with elevated serum serotonin and the decreased carbohydrate tolerance now reported in normal human subjects under the effects of marked serotoninergic activation suggest that excessive indoleamine production rather than the neoplastic syndrome is responsible for the carbohydrate intolerance in carcinoid tumors.

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