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Research Article

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QUANTITATIVE EFFECTS OF GLUCOSE, SULFONYLUREAS, SALICYLATE, AND INDOLE-3-ACETIC ACID ON THE SECRETION OF INSULIN ACTIVITY INTO PANCREATIC VENOUS BLOOD *

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It is now clear that the predominant metabolic effect of sulfonylurea compounds is to release insulin from pancreatic beta cells. In addition, assays by Yalow, Black, Villazon and Berson (1) of insulin concentrations in peripheral blood indicate that the secretory potency of sulfonylureas is substantially weaker than that of glucose. More quantitative comparison of insulin-releasing activities of individual substances, however, requires knowledge of both the concentration of insulin in pancreatic venous blood, and the latter's rate of flow. A suitable closed circuit enabling these measurements was devised by Metz (2), who determined total insulin outflow from the pancreaticoduodenal vein draining the right pancreatic limb in dogs during glucose infusion into the femoral vein. The latter technic was employed in the present study to determine total secretion of insulin activity from a segment of dog pancreas for 30 minutes after glucose or a hypoglycemia-inducing agent was injected into a peripheral vein. Although glucose and three sulfonylurea compounds all promptly initiated release of insulin, glucose was by far the most potent insulogenic substance. In contrast, neither salicylate nor indole-3-acetic acid altered the rate of insulin secretion.

METHODS

Mongrel dogs of either sex, weighing between 10 and 15 kg, were used in all experiments. During a quarantine period of 14 days, animals were metabolically stabilized on a daily ration of meat and Purina laboratory chow, which provided approximately 60 g protein, 20 g fat and 90 g carbohydrate. In early experiments, intact dogs were used after a 24-hour fasting period. However, it shortly became evident that the normal hyperglycemic response to anesthetic and surgical trauma caused premature discharge of insulin, which masked insulin-releasing activity of all agents except glucose itself (Table I). Accordingly, animals were starved and phlorhizinized for 3 days preoperatively, to deplete hepatic glycogen stores maximally and lower peripheral blood glucose to hypoglycemic levels. In dogs weighing 13 kg or less initially, 5 ml of a 40 per cent solution of phlorhizin in propylene glycol was injected subcutaneously approximately 64, 48, 40, 24, and 16 hours before operation; the total phlorhizin dosage was 10 g. Dogs weighing above 13 kg were given 6 ml per dose, and total dosage was 12 g. All animals also received two injections of 1:500 epinephrine-in-oil, 1 ml intramuscularly at 40 and 16 hours before surgery. Animals were anesthetized with pentobarbital sodium (Nembutal), 25 mg per kg intravenously, and laparotomized by midline incision. The posterior surface of the right pancreatic limb was exposed by delivering the free loop of the duodenum into the wound and reflecting it to the left. The cranial pancreaticoduodenal vein, draining all of the right limb of the pancreas except its distal tip, was usually visible and accessible with variable ease beneath the surface of the gland. The vessel was cannulated in retrograde direction (upstream against blood flow) about 2 cm distal to its confluence with the portal vein, using a plastic catheter of large bore (PE 240, OD 0.095 inch) to allow free flow of effluent. The vein was ligated proximal to the point of cannulation to prevent backflow of blood from the portal vein. Immediately before cannulation, the dog was heparinized with 1,500 U per kg of body weight. After cannulation, the entire venous outflow was collected uninterruptedly in a baseline (control) aliquot during the first 10 minutes; and then in three postinjection collections, each of 10 minutes' duration. Total volume and hematocrit were determined on all aliquots. In some animals an additional femoral venous aliquot was obtained before injection, and 10 minutes thereafter, for estimation of insulin activity in peripheral plasma. At the end of the baseline collection, the test substance was injected into a femoral vein. Eight dogs received 15 g of glucose (30 ml of 50 per cent solution) in 5 minutes. The sodium salts of tolbutamide (50 mg per kg to 6 dogs), chlorpropamide (50 mg per kg to 5 dogs), metahexamide (10 mg per kg to 5 dogs), salicylic acid (50 mg per kg to 4 dogs), and in-

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TABLE I

Responsiveness of intact versus phlorhizinized dogs to insulin-releasing stimuli

Substance	No. of dogs	Preinjection blood glucose	Pancreatic venous plasma insulin activity		p Value
			Preinjection −10 to 0 min	Postinjection 0 to 20 min	
		mg/100 ml	*μU/ml*	*μU/ml*	
		Intact dogs			
Glucose	4	105 ± 9*	100 ± 9	678 ± 161	<0.005
Tolbutamide	4	127 ± 9	94 ± 13	118 ± 10	<0.3
		Phlorhizinized dogs			
Glucose	8	48 ± 4	13 ± 8	615 ± 103	<0.001
Tolbutamide	6	53 ± 5	12 ± 5	73 ± 6	<0.001

* Mean ± SEM.

dole-3-acetic acid[1] (50 mg per kg to 4 dogs), were dissolved in 10 ml of distilled water and injected within 2 minutes. As control substances, 4 dogs were given 50 mg of sodium sulfadiazine per kg, and 3 others received 10 ml of 0.85 per cent sodium chloride solution. Oxalated samples for blood glucose were obtained from the femoral vein during the baseline period and every 15 minutes for 1 hour after administration of test material. The animal was then killed with pentobarbital, and the segment of pancreas which had been drained verified by retrograde injection of 20 ml of dilute methylene blue solution into the cannula.

Blood glucose was determined in duplicate by the Somogyi-Nelson method (3). Aliquots of pancreaticoduodenal and femoral venous blood were centrifuged immediately, and the separated plasma was refrigerated at 7° C. All samples were assayed within a week of procurement, and all specimens from an animal were assayed simultaneously. Plasma insulin activity was assayed by determining the glucose uptake from 2 ml of undiluted plasma by isolated rat hemidiaphragm, adhering strictly to the technic described by Vallance-Owen and Hurlock (4). Male Wistar rats, weighing between 90 and 130 g, were used after food was withheld for 18 to 24 hours. Glucose concentration of plasma or buffered salt solution (GBSS) was 300 mg per 100 ml. When glucose was the test substance, and its concentration in postinjection plasmas exceeded 300 mg per 100 ml, the glucose level of GBSS and all plasma samples was brought up to that in the most hyperglycemic plasma. Samples of plasma, GBSS alone, or GBSS containing 10, 100, or 1,000 μU of insulin[2] per ml GBSS, were incubated in a Warburg apparatus for 90 minutes at 37.2° C under an atmosphere of 95 per cent O_2 and 5 per cent CO_2. Residual glucose was determined by the iodometric method of King (5). Diaphragms were dried for 2 hours at 110° C, cooled, and weighed. Glucose uptake was expressed as micrograms per 10 mg of dried diaphragm. "Net glucose uptake" signified total glucose uptake from a sample minus baseline glucose uptake by diaphragm incubated in GBSS alone. Technical limitations prevented simultaneous performance of both a dose-response curve and all samples from a single experiment. Accordingly, at intervals of 2 to 4 weeks a dose-response curve was done, by determining net glucose uptake from triplicate 2-ml aliquots of GBSS containing 10, 100, and 1,000 μU of insulin per ml, respectively. The mean of 24 individual dose-response curves yielded the standard dose-response curve (Figure 1) from which experimental values for net glucose uptake were converted into microunits of "effective insulin concentration" per milliliter of plasma. Conversion values between 1 and 10 μU per ml of plasma were estimated by extrapolation. The few glucose uptakes exceeding the equivalent for an insulin concentration of 1,000 μU per ml of GBSS (Table II) were included in calculations as the latter value.

Pancreaticoduodenal venous plasma flow (PP flow) was derived by multiplying the rate of pancreaticoduodenal venous blood flow (PB flow) by the "plasmacrit":

$$\text{PB flow (ml/min)} \times \frac{(100 - \text{hematocrit})}{100} = \text{PP flow (ml/min)} \quad [1]$$

The concentration of insulin activity in pancreaticoduodenal venous plasma (PIA), multiplied by pancreaticoduodenal venous plasma flow, yielded outflow rate of plasma insulin activity (PIA flow):

$$\text{PIA (μU/ml)} \times \text{PP flow (ml/min)} = \text{PIA flow (μU/min)} \quad [2]$$

[1] Indole-3-acetic acid was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. The sodium salt was prepared by dissolving the acid in 5 ml of warm 0.5 N NaOH, and neutralizing with dilute HCl to pH 7 on pHydrion paper.

[2] Five times recrystallized zinc insulin was kindly supplied by Dr. W. R. Kirtley, Eli Lilly and Company, Indianapolis, Ind.

TABLE II

Concentration and secretory rate of insulin activity in pancreatic venous plasma during 30 minutes after injection of test substance

Dog	Mean net glucose uptake				Mean plasma insulin activity				Rate of plasma flow				Flow rate of insulin activity				Mean increase of insulin outflow rate	
	Control	0–10 min	10–20 min	20–30 min	Control	0–10 min	10–20 min	20–30 min	Control	0–10 min	10–20 min	20–30 min	Control	0–10 min	10–20 min	20–30 min	0–30 min	
	μg/10 mg diaphragm				*μU/ml*		*μU/ml*		*ml/min*		*ml/min*		*μU/min*		*μU/min*		*μU/min*	%
									Glucose									
1	54	234	357		7	407	1,000		4.18	5.84	5.56		29	2,377	5,560		3,940	
2	33	275	259	302	2	670	561	898	4.02	5.01	4.28	3.36	8	3,357	2,401	3,017	2,917	
3	31	138	178	258	2	89	170	551	6.53	7.64	4.16	2.92	13	604	707	1,609	960	
4	35	455	268	155	2	1,000	623	110	2.00	2.69	2.61	2.60	4	2,690	1,626	286	1,530	
5	70	175	242	310	12	162	452	971	4.05	6.87	5.98	4.14	49	1,113	2,703	4,020	2,563	
6	129	285	207	350	66	753	274	1,000	3.68	5.68	3.89	1.99	243	4,277	1,066	1,990	2,201	
7	61	356	277	279	9	1,000	685	703	3.72	5.34	4.72	2.95	33	5,340	3,233	2,074	3,516	
8	25	471	500	373	1	1,000	1,000	1,000	6.98	8.73	6.57	3.07	7	8,730	6,570	3,070	6,116	
Mean	55	299	286	290	13	634	596	748	4.40	5.98	4.72	3.00	48	3,561	2,983	2,295	2,968	100.0
SEM	12	43	36	26	8	134	92	124	0.59	0.65	0.45	0.25	29	813	686	346	567	19.1
p		<0.001	<0.001	<0.001		<0.001	<0.001	<0.001		<0.001	<0.5	<0.1		<0.001	<0.001	<0.001	<0.001	
									Tolbutamide									
9	21	135	155	117	1	74	110	50	3.16	3.43	3.47	2.66	3	254	382	133	253	
10	30	139	121	126	2	80	55	62	3.64	3.42	3.20	2.83	7	274	176	175	201	
11	86	177	184	166	22	166	190	136	3.46	5.48	4.99	2.07	76	910	948	282	637	
12	99	158	167	85	32	117	140	21	3.34	3.70	3.95	3.05	107	433	553	64	243	
13	47	90	169	101	5	25	144	33	2.75	2.81	2.98	2.14	14	70	429	71	176	
14	70	164	111	184	12	132	43	190	2.50	2.51	2.48	2.13	30	331	107	405	251	
Mean	59	144	151	130	12	99	114	82	3.14	3.56	3.51	2.48	40	379	433	188	294	9.9
SEM	13	11	12	12	5	20	23	27	0.18	0.44	0.36	0.17	17	116	124	54	70	2.4
p		<0.001	<0.001	<0.005		<0.001	<0.001	<0.05		<0.25	<0.3	<0.005		<0.01	<0.01	<0.05	<0.005	
									Chlorpropamide									
15	14	120	183	170	1	54	189	148	6.67	6.70	5.64	6.80	67	382	1,066	1,006	751	
16	60	146	122	196	9	91	57	232	5.67	4.61	2.97	2.21	51	420	169	513	316	
17	64	202	87	99	10	265	24	32	2.68	2.69	2.47	2.65	27	689	59	85	251	
18	82	134	192	157	19	73	217	114	2.10	1.80	1.81	2.84	40	131	393	324	243	
19	48	250	160	258	5	505	141	551	2.88	3.17	3.22	2.91	14	1,601	454	1,603	1,205	
Mean	54	170	149	176	9	196	126	215	4.00	3.79	3.22	3.48	40	645	428	706	553	18.6
SEM	11	24	20	26	3	85	37	90	0.91	0.86	0.65	0.84	9	254	175	270	188	6.4
p		<0.005	<0.005	<0.005		<0.1	<0.02	<0.1		<0.4	<0.2	<0.6		<0.05	<0.1	<0.05	<0.02	
									Metahexamide									
20	44	145	111		4	91	44		4.71	3.56	2.07		19	396	91		225	
21	70	165	200	125	12	133	246	60	2.38	2.02	2.06	1.95	29	269	507	117	269	
22	98	173	61	92	31	156	10	26	2.49	4.48	3.52	2.67	77	699	35	69	191	
23	20	193	169	165	1	221	144	133	4.70	5.72	3.28	2.22	5	1,264	472	295	672	
24	84	132	162	235	20	70	128	412	2.38	2.38	2.26	2.06	48	167	269	849	380	
Mean	63	162	141	154	14	134	114	158	3.33	3.63	2.64	2.23	36	559	275	333	347	11.7
SEM	14	11	25	31	5	26	41	88	0.56	0.68	0.22	0.14	12	208	94	110	87	2.9
p		<0.005	<0.05	<0.1		<0.005	<0.05	<0.2		<0.6	<0.3	<0.4		<0.05	<0.1	<0.2	<0.005	

TABLE II—(*Continued*)

Dog	Mean net glucose uptake				Mean plasma insulin activity				Rate of plasma flow				Flow rate of insulin activity				Mean increase of insulin outflow rate	
	Control	0–10 min	10–20 min	20–30 min	Control	0–10 min	10–20 min	20–30 min	Control	0–10 min	10–20 min	20–30 min	Control	0–10 min	10–20 min	20–30 min	0–30 min	
	μg/10 mg diaphragm				*μU/ml*		*μU/ml*		*ml/min*		*ml/min*		*μU/min*		*μU/min*		*μU/min*	%
									Salicylate									
25	16	130	50	111	1	67	6	43	4.23	5.61	3.97	3.65	4	376	24	157	182	
26	46	66	0	78	4	11	0	17	3.72	4.47	2.70	3.50	15	55	0	60	23	
27	10	26	7		1	1	1		7.52	7.85	3.96		8	9	4		0	
28	79	76	93	109	18	16	27	42	2.62	2.61	2.60	2.37	47	42	70	100	24	
Mean	38	75	38	99	6	24	9	34	4.52	5.14	3.31	3.17	19	121	25	106	57	1.9
SEM	16	21	22	11	4	15	6	8	1.06	1.10	0.38	0.40	10	86	16	28	42	1.4
p		<0.25		<0.1		<0.4	<0.8	<0.2		<0.1	<0.2	<0.4		<0.4	<0.8	<0.3	<0.25	
									Indole-3-acetic acid									
29	95	6	8	−20	35	1	1	0	5.28	4.37	4.15	4.17	185	4	4	0	0	
30	74	76	126	144	15	16	62	89	5.70	7.39	5.27	3.40	86	118	327	303	163	
31	24	72	52	78	1	14	6	17	5.05	4.45	3.50	3.92	5	62	21	67	45	
32	62	52	32	42	9	6	1	4	2.87	4.93	4.30	3.86	26	30	4	15	0	
Mean	64	52	55	61	15	9	18	28	4.73	5.27	4.31	3.84	76	54	89	96	52	1.8
SEM	15	16	26	34	7	7	15	21	0.63	0.69	0.40	0.48	40	21	80	71	38	1.3
p		<0.6	<0.8	<0.9		<0.6	<0.9	<0.6		<0.6	<0.6	<0.3		<0.7	<0.9	<0.8		
									Sulfadiazine									
33	47	28	−2	−9	5	1	0	0	2.88	3.08	3.40	2.79	14	3	0	0	0	
34	72	80	53	67	14	18	7	11	4.64	3.37	3.93	3.49	65	61	28	38	0	
35	80	65	−8	−26	18	11	0	0	3.33	5.62	5.12	4.33	60	62	0	0	0	
36	92	112	100	106	26	44	33	38	2.69	5.30	3.13	3.98	70	233	103	151	92	
Mean	73	71	36	34	16	19	10	12	3.39	4.34	3.90	3.65	52	90	33	47	23	0.8
SEM	10	17	20	31	9	18	16	9	0.44	0.66	0.46	0.44	13	39	24	36	23	0.8
p		<0.9	<0.2	<0.2		<0.9	<0.8	<0.8		<0.3	>0.5	<0.7		<0.4	<0.6	<0.9		
									Sodium chloride									
37	45	25	43	9	5	1	4	1	3.58	4.07	2.97	3.11	18	4	12	3	0	
38	64	14	72	104	10	1	14	36	6.83	5.28	5.21	6.87	68	5	73	247	40	
39	40	54	8	32	3	7	1	1	6.00	6.40	4.94	5.99	18	45	5	6	1	
Mean	50	31	41	48	6	3	6	13	5.47	5.25	4.37	5.32	35	18	30	85	14	0.5
SEM	7	12	18	29	2	2	4	12	0.98	0.67	0.70	1.13	17	14	22	81	23	0.8
p		<0.4	<0.5	<0.9		<0.6		<0.7		<0.8	<0.02	<0.5		<0.6	<0.5	<0.5	<0.8	

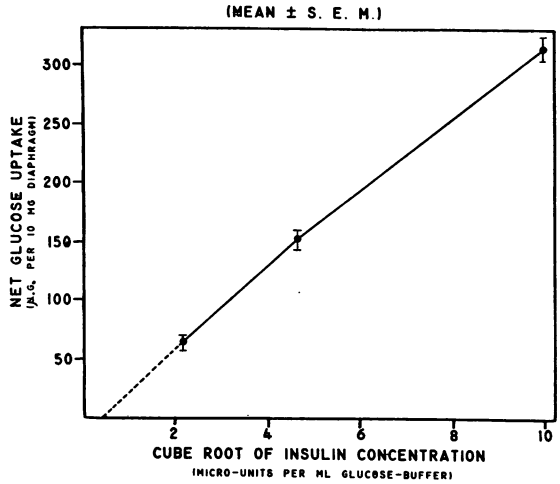

FIG. 1. STANDARD DOSE-RESPONSE CURVE. Experimental values for net glucose uptake from plasma were converted into "microunits of effective insulin concentration per milliliter of plasma" from this curve. Points on the abscissa are cube roots of 10, 100, and 1,000 μU insulin per ml of glucose-buffered salt solution (GBSS), respectively. Conversion values below 10 μU per ml were estimated from the extrapolated segment of the curve.

RESULTS

A. Dose-response curve (Figure 1)

All 24 individual dose-response curves, and hence the mean curve derived from them, were curvilinear when plotted on the usual semilogarithmic coordinates. However, as Vallance-Owen, Hurlock and Please (6) originally demonstrated and validated statistically, and as Wright (7) and Gundersen (8) subsequently confirmed, the dose-response curve formed virtually a straight line when net glucose uptake was plotted linearly on the ordinate, and insulin concentration as cube root on the abscissa. Respective mean net glucose uptakes for insulin concentrations of 10, 100, and 1,000 μU per ml of GBSS were 63 ± 6 (SEM) μg per 10 mg diaphragm; 151 ± 8 μg per 10 mg diaphragm; and 313 ± 10 μg per 10 mg diaphragm. On the extrapolated portion of the curve, net glucose uptake of 20 μg per 10 mg diaphragm was equivalent to 1 μU per ml GBSS. The slope of this dose-response curve (31.9 μg of glucose uptake per cube root of insulin level in microunits per milliliter) was considerably steeper than those of Vallance-Owen and co-workers and Wright (23.1 and 23.7 μg of glucose uptake per cube root of insulin concentration, respectively), and essentially the same as recent ones obtained by Gundersen (8).

B. Secretory rates of insulin activity in pancreaticoduodenal venous plasma after administration of test substances (Table II, Figures 2 and 3)

1. After glucose. At the end of the 5-minute injection of 15 g of glucose, peripheral blood glucose level (mean ± SEM) was 417 ± 21 mg per 100 ml, and 25 minutes later it had fallen to 236 ± 12 mg per 100 ml. Glucose evoked a prompt and prodigious rise in pancreatic outflow of insulin activity, with only a slight decrease during the 30 minutes of observation. The increased secretion was due to greatly enhanced titers of insulin activity in pancreatic venous blood, since neither pancreatic blood flow nor hematocrit changed significantly. Respective *concentrations* of effective insulin activity were 13 ± 8 μU per ml of plasma, baseline; 634 ± 134 μU per ml between 0 and 10 minutes; 596 ± 92 μU per ml between 10 and 20 minutes; and 748 ± 124 μU per ml between 20 and 30 minutes. Derived *secretory rates* of insulin activity (Figure 2) were 48 ± 29 μU per minute, baseline; 3,561 ± 813 μU per minute between 0 and 10 minutes; 2,983 ± 686 μU per minute between 10 and 20 minutes; and 2,295 ± 346 μU per minute between 20 and 30 minutes.

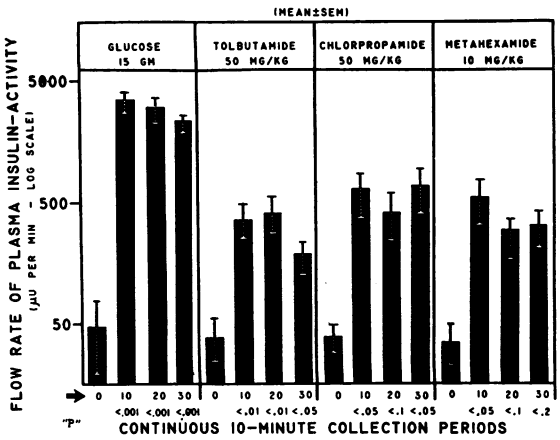

FIG. 2. INSULIN ACTIVITY IN PANCREATIC VENOUS BLOOD AFTER GLUCOSE AND SULFONYLUREAS. Glucose and sulfonylurea compounds caused prompt release of insulin activity into pancreatic venous blood. Beta-cell stimulation by glucose, however, was greater and more sustained than by any sulfonylurea.

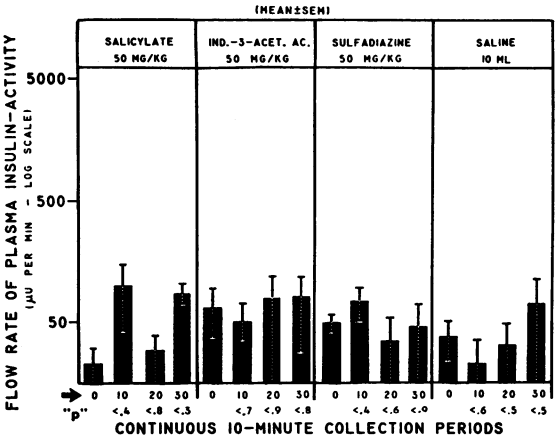

FIG. 3. INSULIN ACTIVITY IN PANCREATIC VENOUS BLOOD AFTER HYPOGLYCEMIA-INDUCING AND CONTROL SUBSTANCES. The two nonsulfonylurea, hypoglycemia-inducing substances, salicylate and indole-3-acetic acid, were as devoid of insulin-releasing activity as the control substances, sulfadiazine and saline.

All of these increases above baseline exceeded $p < 0.001$ level of significance.

2. After sulfonylurea compounds. The effects of tolbutamide, chlorpropamide, and metahexamide on release of insulin activity resembled that of glucose qualitatively, but not quantitatively. As a group, they sharply augmented insulin activity in pancreatic venous blood during the first 10 minutes, although maximal secretory rates in individual experiments occurred in any of the three collection intervals. The magnitude of response to these agents, however, was only a fraction of that following glucose (Figure 2). Secretion rates of insulin activity in animals given tolbutamide were 40 ± 17 μU per minute, baseline; rising to 379 ± 116 μU per minute ($p < 0.01$) in the first 10 minutes; reaching a maximum of 433 ± 124 μU per minute ($p < 0.01$) between 10 and 20 minutes; and dropping to 188 ± 54 μU per minute ($p < 0.05$) between 20 and 30 minutes. Dogs injected with chlorpropamide had corresponding values of 40 ± 9 μU per minute, baseline; 645 ± 254 μU per minute ($p < 0.05$) between 0 and 10 minutes; dropping slightly to 428 ± 175 μU per minute ($p < 0.1$) between 10 and 20 minutes; and rising again to 706 ± 270 μU per minute ($p < 0.05$) during the last period. Insulin output after metahexamide rose from baseline of 36 ± 12 μU per minute to a maximal value of 559 ± 208 μU per minute ($p < 0.05$) within 10 minutes, then subsided to 275 ± 94 μU per minute ($p < 0.1$) in the 10 to 20 minute interval, and remained at 333 ± 110 μU per minute ($p < 0.2$) during the last period. All increases of insulin flow rates above baseline levels were due to enhanced concentrations of insulin activity in pancreaticoduodenal venous plasma, and not to increased plasma flow rates.

3. After salicylate and indole-3-acetic acid. Neither sodium salicylate nor sodium indole-3-acetate stimulated release of insulin activity (Figure 3). Interval flow rates in animals given salicylate were 19 ± 10 μU per minute, baseline; 121 ± 86 μU per minute between 0 and 10 minutes; 25 ± 16 μU per minute between 10 and 20 minutes; and 106 ± 28 μU per minute between 20 and 30 minutes. Mean plasma salicylate concentration 15 minutes after injection was 28 ± 3 mg per 100 ml. For indole-3-acetic acid, baseline flow rate was 76 ± 40 μU per minute; and the three postinjection values were 54 ± 21, 89 ± 80, and 96 ± 71 μU per minute, respectively.

4. After sulfadiazine and saline. Similarly, administration of sodium sulfadiazine or sodium chloride as control substances elicited no change in preinjection outflow rates of insulin activity (Figure 3).

C. Comparison of insulogenic potencies

The last column in Table II shows the mean increase of insulin outflow rate above baseline value during the entire 30-minute interval. After glucose administration, the average increase in pancreatic venous insulin activity was $2,968 \pm 567$ μU per minute (100.0 ± 19.1 per cent). The average increase after tolbutamide was 294 ± 70 μU per minute (9.9 ± 2.4 per cent). After chlorpropamide the mean increase was 553 ± 188 μU per minute (18.6 ± 6.4 per cent), and after metahexamide the corresponding mean enhancement was 347 ± 87 μU per minute (11.7 ± 2.9 per cent). All of these increments were significant at $p < 0.02$ or better. In contrast, after salicylate the increase above baseline value was only 57 ± 42 μU per minute (1.9 ± 1.4 per cent), and after indole-3-acetic acid it was 52 ± 38 μU per minute (1.8 ± 1.3 per cent).

TABLE III

Insulin activity in peripheral versus pancreatic venous plasma of phlorhizinized dogs (μg net glucose uptake/10 mg diaphragm)

Substance	Femoral vein			Pancreaticoduodenal vein		
	Baseline	10 min	p Value	Baseline	0–20 min	p Value
Glucose	5 ± 16*	22 ± 13	<0.3	55 ± 12	293 ± 40	<0.001
Tolbutamide	8 ± 28	22 ± 15	<0.7	59 ± 13	147 ± 8	<0.001
Salicylate	−12 ± 6	8 ± 18	<0.4	38 ± 16	57 ± 18	<0.6
Indole-3-acetic acid	24 ± 6	−8 ± 10	<0.2	64 ± 15	52 ± 21	<0.6
Saline	15 ± 3	27 ± 21	<0.5	50 ± 7	36 ± 4	<0.8

* Mean ± SEM.

D. Changes in pancreaticoduodenal venous plasma flow (Table II)

Except in the glucose group, in which the larger volume of injected solution caused significant rise in pancreatic venous blood flow during the first collection period, no important increases in blood flow were observed. Toward the end of the 40 minutes of continuous hemorrhage, blood flow declined progressively and was accompanied by slight hemodilution. Only in the tolbutamide-treated animals was the change significant (baseline of 3.14 ± 0.18 ml per minute fell to 2.48 ± 0.17 ml per minute, $p < 0.005$).

E. Insulin activity in femoral venous plasma after administration of test substances (Table III)

Baseline concentrations of insulin activity in peripheral plasma were consistently lower than respective baseline titers in pancreaticoduodenal venous plasma. Ten minutes after administration of glucose, tolbutamide, salicylate, indole-3-acetic acid or saline, no enhancement of insulin activity was detectable in femoral venous blood from these phlorhizinized dogs.

F. In vitro effects on glucose uptake of test substances added to plasma (Table IV)

Sulfonylureas, added to baseline plasma in clinically effective concentrations and incubated *in vitro,* had no effect on net glucose uptake by rat diaphragm. In contrast, when salicylate was added to baseline plasma, net glucose uptake did increase from the control value of 69 ± 11 to 119 ± 13 μg per 10 mg diaphragm ($p < 0.005$).

G. Changes in peripheral blood glucose (Table V)

None of the test substances caused depression of femoral venous blood glucose during 60 minutes after injection. In particular, mean baseline blood glucose of 55 ± 3 mg per 100 ml for all three sulfonylurea compounds actually rose slightly to 57 ± 3 mg per 100 ml at 30 minutes, and 69 ± 5 mg per 100 ml at 60 minutes.

DISCUSSION

Vallance-Owen correctly emphasizes that his bioassay technic does not measure total insulin content of plasma, but estimates instead, "plasma-

TABLE IV

Effect of hypoglycemia-inducing substances, added to plasma in vitro, on glucose uptake by rat hemidiaphragm

Substance*	Conc.	Glucose uptake		p Value
		Baseline plasma	Plasma +substance	
	mg/100 ml		*μg/10 mg diaphragm*	
Tolbutamide	25–50	55 ± 22†	60 ± 11	<0.9
Chlorpropamide	50	15 ± 15	8 ± 11	<0.8
Metahexamide	50	15 ± 20	−2 ± 13	<0.4
Salicylate	30–50	69 ± 11	119 ± 13	<0.005
Indole-3-acetic acid	50	59 ± 14	57 ± 22	<0.9
Sulfadiazine	50	70 ± 23	83 ± 23	<0.7

* As sodium salt.
† Mean ± SEM.

TABLE V

Femoral venous blood glucose concentrations after administration of hypoglycemic agents

Substance	No. of dogs	Preinjection	Minutes after injection 15	30	45	60
			mg per 100 ml			
Tolbutamide	6	53 ± 5*	50 ± 7	54 ± 6	63 ± 6	73 ± 6
Chlorpropamide	5	54 ± 5	51 ± 4	51 ± 5	52 ± 5	56 ± 8
Metahexamide	5	61 ± 3	65 ± 5	67 ± 3	72 ± 3	76 ± 3
Salicylate	4	43 ± 4	42 ± 4	50 ± 6	52 ± 8	57 ± 7
Indole-3-acetic acid	4	45 ± 3	46 ± 3	42 ± 3	44 ± 4	49 ± 5
Sulfadiazine	4	56 ± 6	62 ± 5	65 ± 8	66 ± 7	67 ± 9
Saline	3	43 ± 6	40 ± 6	40 ± 7	38 ± 6	41 ± 6

* Mean ± SEM.

insulin activity, or the effective plasma-insulin concentration, i.e., the sum of insulin and its synergists, if any, on the one hand and its antagonists on the other" (9). Nevertheless, in the present study, the conclusion seems reasonable that the flow rates of insulin activity in pancreaticoduodenal venous effluent truly reflected the rate of insulin secretion by beta cells. The isolated rat diaphragm is sufficiently specific for assaying insulin activity that it does not detect "insulin activity" in blood from totally depancreatized animals (10, 11), in contrast to the more sensitive but apparently less specific rat epididymal fat pad (12, 13). Similarly, by Vallance-Owen's method, two totally pancreatectomized patients exhibited no circulating insulin activity either before or after ingesting 100 g of glucose (Table VI). Further indications that augmented outflow of insulin activity from a pancreatic segment actually signified increased secretion of insulin were the absence of concomitant enhancement of insulin activity in peripheral blood, and the demonstration that *in vitro* addition of sulfonylureas did not cause non-

TABLE VI

Lack of insulin activity in plasma of totally depancreatized patients

Patient	Days without insulin	Total glucose uptake: GBSS alone	Fasting plasma	Plasma after glucose*	Plasma insulin activity: Fasting	After glucose
		μg/10 mg diaphragm			*μU/ml plasma*	
L.F.	5	287† ±6	292 ±16	290 ±22	<1‡	<1
J.B.	2	289 ±20	284 ±14	309 ±20	0	1

* 100 g by mouth.
† Mean ± SEM.
‡ 20 μg glucose uptake/10 mg diaphragm = 1 μU insulin activity/ml.

specific increase of glucose uptake by rat diaphragm.

At the same time, experimental values for concentrations and flow rates of insulin activity denoted only *relative* quantitation of insulin secretion. Since the cranial pancreaticoduodenal vein in the dog drains a segment of gland containing about 30 per cent of total islet cell volume (14), only a fraction of total insulin secretion was measured. A more critical dissuader from misinterpreting relative values as absolute, however, is the fact that reported "normal" values for insulin content of blood differ widely (15). This discrepancy even exists between workers using undiluted plasma and rat diaphragm as assay tissue, but varying otherwise in method. Metz (2) found baseline insulin activity in pancreaticoduodenal venous blood of dogs to vary from 10 to 31 mU per 100 ml of plasma (100 to 310 μU per ml) at blood glucose levels between 37 and 86 mg per 100 ml, with enhancement to values of 330 and 570 mU per 100 ml plasma (3,300 and 5,700 μU per ml) when blood glucose was 300 mg per 100 ml. These absolute values, about ten times greater than those recorded under similar experimental conditions in the present study, are largely due to his ten times less sensitive dose-response curve, since actual net glucose uptakes from plasma were essentially the same in both laboratories. In contrast, the specific immunoassay of Yalow and Berson (16) yielded values for actual insulin concentrations in human plasma very similar to those for biologic "insulin activity" found by Vallance-Owen and co-workers (4, 6), Wright (7), and Seltzer and Smith (17). These comparable results by two entirely different pro-

cedures suggest that absolute concentrations of circulating insulin are, in fact, low rather than high.

Even in relative terms, however, the parameter of insulin-releasing activity enabled sharp qualitative and quantitative classification of test substances. Glucose and all three sulfonylureas stimulated prompt discharge of insulin, whereas salicylate and indole-3-acetic acid had no effect. The insulogenic potency of glucose far exceeded that of sulfonylureas. Rapidly induced hyperglycemia evoked a tremendous outpouring of insulin, whose secretory rate promptly reached a maximal level which was essentially sustained for 30 minutes. The slight drop during the last period may have reflected the waning glycemic stimulus as blood glucose level fell rapidly, since Metz (18) found that glucose infused at mounting rates for 45 minutes continued to elicit proportional increase of insulin secretion. In contrast to the massive response to glucose, the insulin-releasing potency of all three sulfonylurea compounds was much smaller, both in magnitude and duration. Mean outflow rates for the nine periods of observation never exceeded 20 per cent of the maximal rate for glucose (Table II and Figure 2). Moreover, in Figure 4 are compared respective mean increases of insulin secretion during the entire 30 minutes after injection of glucose and sulfonylureas. Considering enhancement by glucose as 100 per cent, corresponding insulogenic potencies were 9.9 per cent for tolbutamide, 18.6 per cent for chlorpropamide, and 11.7 per cent for metahexamide. Although blood levels of sulfonylurea were not measured, maintenance of effective concentrations was assured by the known half-life disappearance rates for tolbutamide, chlorpropamide, and metahexamide of 4.6, 35, and 19 hours, respectively (19, 20).

The insulogenic omnipotence of glucose has previously been intimated in published clinical observations. One hour after giving 50 g of glucose orally, Vallance-Owen and associates found an increment in plasma insulin activity of 333 μU per ml in normal subjects (4), and 219 μU per ml in maturity-onset diabetics (6). Two and one-half hours after similar individuals took 2 g of tolbutamide by mouth, the average increase of plasma insulin activity was 114 μU per ml in normal subjects, and 130 μU per ml in adult diabetics (21). Similarly, both in normal subjects and in untreated mild diabetics, Yalow and coworkers (1) found greater enhancement of plasma insulin levels after glucose than after tolbutamide. In nondiabetics the average increase after glucose was 119 μU per ml, compared to only 28 μU per ml after tolbutamide; in diabetic patients corresponding mean increases were 132 μU per ml after glucose and 52 μU per ml after tolbutamide. In these clinical studies, the full insulogenic superiority of glucose over sulfonylurea compounds was probably masked for several reasons. In addition to significant hepatic binding of intra-portal-venous insulin during its first traverse of the liver (22), and dilution of the remainder upon entering the post-hepatic circulation, isolated values of insulin concentration tend to obscure the sustained nature of the glycemic stimulus compared to the transient one invoked by sulfonylureas.

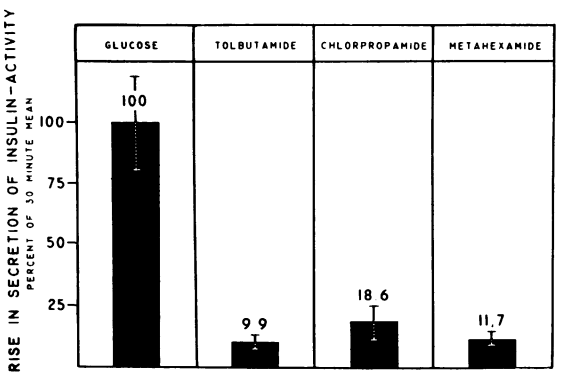

FIG. 4. COMPARATIVE INSULOGENIC POTENCIES OF GLUCOSE AND SULFONYLUREAS. Respective vertical bars indicate the mean increase above baseline value in secretory rate of insulin activity during the entire 30 minutes after injection of test substance. The insulogenic omnipotence of hyperglycemia is obvious.

An opposite interpretation might be that the observed insulogenic superiority of glucose was an artifactual sequela of the chronic hypoglycemia induced in experimental animals. The hypoglycemic state is combated by the counterregulatory release of epinephrine (23), adrenocorticosteroids (24), and glucagon (25). Epinephrine and cortisol strongly inhibit the enhancing effect of insulin on glucose uptake by rat diaphragm (26, 27), wherefore their presence in excess in test plasmas could have blunted the full betacytotropic effects of sulfonylurea agents. However, even in animals whose hypoglycemia was obliterated by glu-

cose administration, it is unlikely that preinjection plasma levels of epinephrine or cortisol changed significantly during the next 30 minutes, since epinephrine-in-oil had been used in preparing all animals, and circulating cortisol titers are known to be markedly elevated during surgical trauma per se (28).

The implication that the physiological stimulus represented by a rising blood sugar level can stimulate normal beta cells maximally and indefinitely, suggests that glucose and sulfonylureas impinge differently upon the beta cell. Glucose appears to be truly "insulogenic" in that it not only triggers release of stored insulin granules, but also engenders formation and secretion of new insulin in proportion to the concentration of glucose in pancreatic arterial blood. In contrast, sulfonylurea compounds seem merely capable of "solubilizing" preformed, stored insulin and enabling it to be rapidly discharged. Thereafter, so long as effective sulfonylurea blood levels persist, spontaneously synthesized insulin is promptly secreted regardless of arterial glucose concentration. Volk and Lazarus (29) demonstrated totally degranulated beta cells during chronic tolbutamide-induced hypoglycemia in rabbits and dogs. Moreover, after 9 months of daily tolbutamide administration to rats, Baender (30) found that several days after stopping the drug, normal beta cell granulation had been restored. Of similar significance may be the repeated clinical observation that, although sulfonylureas normalize glucose levels in optimally responsive ketoacidosis-resistant diabetics, the oral glucose tolerance test itself frequently shows little improvement (31, 32). This apparent paradox is actually a predictable sequela, if pancreatic islets continuously bathed in a sulfonylurea solution cannot conserve a depot of insulin to release upon hyperglycemic demand.

Recent clinical trials with salicylate in diabetic patients have shown that its ability to lower blood sugar is equal to that of sulfonylurea and phenethylbiguanide compounds. Unfortunately, normalization of fasting blood glucose in maturity-onset diabetics was achieved by Reid and MacDougall (33) at blood levels of salicylate (above 40 mg per 100 ml) too toxic to encourage its therapeutic use. However, when this agent was selectively used only in mild adult diabetics whose blood glucose was optimally responsive to tolbutamide, fasting normoglycemia was indefinitely maintained at well tolerated blood levels between 25 and 35 mg per 100 ml (34). In the present study, failure of the latter concentrations to manifest betacytotropic activity confirms the exclusively extrapancreatic action of salicylate. Manchester, Randle and Smith (35) showed significant augmentation of glucose uptake by rat diaphragm when salicylate was added *in vitro,* a finding verified in Table IV; and Falcone (36) subsequently localized a salicylate-induced block in oxidative phosphorylation, which presumably results in accelerated disposal of glucose via anaerobic glycolysis. The metabolic action of salicylate thus closely stimulates the peripheral mechanism whereby phenethylbiguanide substances also cause disappearance of circulating glucose (37).

The hypoglycemia-inducing property of indole-3-acetic acid was discovered by Mirsky, Diengott and Perisutti (38) during studies elucidating its ability to inhibit insulinase activity. Clinical titration in diabetic patients revealed this physiological end-product of tryptophan metabolism to be fully as effective as tolbutamide in reducing fasting hyperglycemia (39). Because indole-3-acetic acid is also a powerful plant growth hormone, it might have possessed an "insulotropic" action similar to that by which animal growth hormone stimulates anatomical and functional hypertrophy of beta cells (40). However, not only was the agent devoid of betacytotropic activity, but its addition to the *in vitro* assay system did not increase glucose uptake by rat diaphragm. In addition, even profound hypoglycemic responses of elderly diabetics to the substance were not accompanied by increased plasma insulin activity (39). In a negative sense, therefore, these findings support the conclusion of Mirsky and coworkers that indole-3-acetic acid preponderantly blocks destruction of insulin by insulinase.

Finally, the apparent failure of proved hypoglycemia-inducing agents to cause further lowering of blood sugar was an expected sequela of the experimental procedure. Preliminary efforts in intact dogs had consistently shown that the necessarily extensive surgical manipulations caused peripheral *hyper*glycemia, which not only evoked premature insulin secretion but also canceled the glucose-depressing activity of the agent under

scrutiny. When the animal was depleted of glycogen by starvation, phlorhizin, and epinephrine to prevent otherwise uncontrollable hyperglycemic interference, the normal hypoglycemic response to the test substance was also blunted.

SUMMARY

Respective betacytotropic activities of glucose, sulfonylurea compounds, salicylate, and indole-3-acetic acid were semiquantitatively compared by measuring the effect of each agent on the outflow rate of insulin activity in pancreaticoduodenal venous blood of phlorhizinized dogs.

Glucose and three sulfonylureas (tolbutamide, chlorpropamide, and metahexamide) all promptly enhanced concentration of insulin activity in the pancreatic venous effluent. However, whereas the response to glucose was both intense in magnitude and sustained, insulin release by sulfonylureas was only moderate in degree and transient in duration. Mean enhancement of insulin secretion by glucose was five to ten times the output after sulfonylurea compounds. Neither salicylate nor indole-3-acetic acid stimulated pancreatic release of insulin activity.

The data suggest that: *1*) both glucose and sulfonylurea compounds cause prompt release of insulin from pancreatic beta cells; *2*) the betacytotropic activity of glucose is far more potent than that of sulfonylureas; *3*) glucose is truly insulogenic; i.e., capable of engendering formation of new insulin, whereas sulfonylureas seem only able to release preformed stores of insulin; and *4*) salicylate and indole-3-acetic acid cause peripheral hypoglycemia via extrapancreatic mechanisms.

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CORRECTION

On page 1798 of the article "The Influence of Barbiturates on Coumarin Plasma Levels and Prothrombin Response," by Dayton *et al.* (J. clin. Invest. 1961, **40**) the legend to Figure 1 should have the following addendum: "which was preceded by the same dose of barbital daily for 3 prior days."