Hyperglycemic and Glycogenolytic Effects of Vasoactive Intestinal Polypeptide[1] (37165)

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(Introduced by Andres Goth)

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Recently a polypeptide with broad biological activity was isolated from small intestine (1, 2). Both in chemical composition and in biological actions this polypeptide [vasoactive intestinal polypeptide (VIP)] bears certain similarities to pancreatic glucagon. Thus VIP contains 28 amino acid residues compared to glucagon's 29, and both polypeptides have the dipeptide histidylserine at the N-terminal end of the molecule (2). The effects of VIP in animals include peripheral vasodilation, increased cardiac output, smooth-muscle relaxation and hyperglycemia (2, 3). These similarities and the known presence in small intestine of glucagon-like biological and immunoreactivity, prompted this investigation. We set out to examine in some detail the hyperglycemic action of VIP, and to measure its glycogenolytic activity. In both aspects of the study the effects of VIP were compared to those of pancreatic glucagon.

Methods. In vivo *experiments.* In one series of 16 experiments on 8 dogs, we examined the effect on blood sugar of systemic infusions of partially purified VIP. The dose administered averaged 3.6 μg/kg. In a second series of 10 determinations on 6 dogs, we compared the systemic hyperglycemic effect of highly purified VIP to that of glucagon. The dogs were anesthetized with sodium pentobarbital (30 mg/kg body wt). Blood samples for the analysis of glucose concentration were collected from a cannula placed into the aorta via a femoral artery. Either glucagon (Eli Lilly & Co.) or highly purified VIP (Professor Viktor Mutt, Karolinska Institute) was then injected intravenously or into the root of the aorta, in a dose of 1 μg/kg. Fifteen minutes later, another arterial blood sample was collected. In each dog, both VIP and glucagon were infused, and the injections were separated by at least 1-hr intervals, to permit return of blood sugar to control values. Blood samples were heparinized and the plasma was saved for determination of glucose concentration by the enzymatic conversion of glucose to phosphogluconate.

In vitro *experiments.* Experiments on rabbit liver slices followed the procedure of Sutherland and DeDuve (4). The rabbits were killed by a blow to the head, and the liver was immediately removed and chilled on aluminum foil over ice. Slices were made approximately 1 cm^2 and 2–3 mm thick. These slices were incubated in a Dubnoff metabolic bath at 37°, in 25 ml beakers containing 3 ml of rabbit serum, and either glucagon or VIP, in a concentration of 0.2 μg/ml. The tissue slices were kept in constant motion and exposed to a slow flow of 95% O_2/5% CO_2. A control set of beakers were under the same conditions except for the absence of VIP or glucagon. Sugar concentrations were determined spectrophotometrically by a Technicon AutoAnalyzer, and results were expressed as milligrams of glucose per gram of

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liver. Comparisons were made after incubation for 30 and 45 min (7 expts each).

All results were analyzed for statistical significance by Student's t test, using paired comparisons.

Results. A. Systemic infusions. Systemic infusion of partially purified VIP (av dose 3.6 μg/kg) caused a 21.7% mean increase of blood glucose concentration over control blood levels ($p < 0.001$, Table I). The effects of smaller doses (1 μg/kg) of highly purified VIP and of glucagon were compared in six dogs. The VIP preparation caused a rise in mean blood glucose of 30.0 mg/100 ml, an increase of 27.3%. By comparison, a rise of 88.0 mg/100 ml (81.5% increase) was induced by the same dose of glucagon, given by the same route (Table II). The hyperglycemia induced by VIP was 33.5% that of glucagon. The magnitudes of the responses to the two polypeptides were significantly different ($p < 0.001$).

B. In vitro experiments with liver slices. A glycogenolytic effect of VIP on the liver was evident ($p < 0.01$) in the doses used, but was less pronounced than the glycogenolytic effect of glucagon under the same conditions (Table III). The increase in output of sugar with the addition of VIP was between 56.3% (at 45 min) and 63.2% (at 30 min) of that resulting from the addition of glucagon. The responses of isolated liver slices to either peptide were not different at the two incubation periods.

TABLE I. Hyperglycemic Effect of Partially Purified VIP (Av Dose, 3.6 μg/kg).

Dog no.	Glucose levels (mg/100 ml plasma)	
	Control	After Peptide
1	114	138
	134	146
2	130	146
	143	170
3	142	187
	106	132
4	77	83
5	112	176
	120	149
	118	120
6	111	140
	83	114
7	123	150
	117	164
8	95	114
	114	111
Mean	115	140[a]
SD ±	18.57	27.25

[a] Significantly different from control value ($p < .001$).

TABLE II. Systemic Hyperglycemic Effects of Glucagon and VIP (1 μg/kg).

Dog no.	Glucose levels (mg/100 ml plasma)			
	Control	Glucagon	Control	VIP
1	105	185	112	146
			108	137
2	118	178	122	137
	135	171	131	135
3	99	210	97	102
	96	226	110	206
4	111	196	112	135
5	100	207	96	102
			100	194
6	101	194	111	123
Mean	108	196[a]	110	140[b]
SD ±	14.13	15.41	10.24	35.79

[ab] Significantly different from control values ($p < .001$ and $< .025$, respectively) and from each other ($p < .001$).

Discussion. The data on the relative hyperglycemic potency of the two polypeptides are subject to two possible sources of imprecision. First, the VIP preparation used was nearly but not completely pure, lacking the final purification step (2). Secondly, although multiple injections in the same animals were separated by at least 1 hr, and the control blood sugars were close (108 and 110 mg/100 ml, Table II), reduction in glycogen stores of the liver after one injection could have decreased the hyperglycemic response to the second injection. Nevertheless, the results offer strong evidence that VIP induces hyperglycemia in anesthetized dogs, and glycogenolysis in isolated rabbit liver, and that in both actions VIP is relatively weaker than glucagon.

The demonstration of the glucagon-like property of this newly isolated polypeptide could provide another clue to the role of the small intestine in the regulation of carbohydrate metabolism. As early as 1948, Sutherland and DeDuve (4) demonstrated that ex-

TABLE III. Glycogenolytic Effect of Glucagon and VIP on Isolated Rabbit Liver Slices.

	Glucose (mg/g liver)		
	Control	Glucagon	VIP
A. 30 min			
	5.41	7.63	7.76
	13.44	22.38	18.22
	6.21	10.43	8.94
	4.13	20.77	15.09
	3.68	7.40	5.09
	6.76	8.13	8.06
	5.96	8.22	7.47
Mean	6.51	12.14[a]	10.09[a]
SD ±	3.25	6.54	4.71
B. 45 min	7.00	10.31	8.18
	13.42	26.72	22.06
	3.21	8.20	4.32
	4.13	20.13	15.13
	3.69	6.51	5.59
	7.90	11.18	9.28
	7.27	8.17	7.19
Mean	6.66	13.03[b]	10.25[b]
SD ±	3.53	7.51	6.26

[a] Significantly different from control values ($p < .01$) and from each other ($p < .05$).

[b] Significantly different from control values ($p < .01$) and from each other ($p < .01$).

tracts of gastrointestinal mucosa contained a glycogenolytic factor like that which they found in the pancreas. It has now been established that insulin titers in the blood rise to higher levels after the ingestion of a given load of glucose than after the intravenous infusion of the same load of glucose (5). The ingestion of glucose also is accompanied by an increase in plasma "enteroglucagon" or "glucagon-like immunoreactivity" (GLI) (6, 7). These and similar data have led some workers to propose models of a "hormonal entero-insular axis" to describe the intestinal influence on glucose metabolism (7).

The identity of intestinal factor or factors influencing sugar metabolism remains ill-defined. Valverde *et al.* found that enteroglucagon (or GLI) is contained in two peptide fractions obtained on gel chromatography of jejunal extracts (8). Using labeled insulin and glucagon as tracers, these authors concluded that the first peptide peak was larger in molecular weight than insulin (at least 7000), and the second was roughly the size of glucagon. The larger compound(s) has been found to have no hyperglycemic activity, while the smaller one(s) possesses glycogenolytic activity, which is qualitatively similar but quantitatively less than that of pancreatic glucagon (8). Further fractionation of these peptide peaks has been accomplished by Moody *et al.*, using ion-exchange chromatography (9).

In view of the findings reported here it seems possible that VIP may be a component of the factors that have been labeled "enteroglucagon" (GLI). Confirmation of this possibility must await further data, such as the determination of the insulin-releasing activity of VIP, and the development of a specific radioimmunoassay for this polypeptide.

Besides the ability to stimulate glycogenolysis, VIP shares other properties with pancreatic glucagon. Other studies have shown VIP to have a positive inotropic effect on the myocardium in intact, anesthetized dogs and in isolated cat papillary muscle (10). Further, VIP stimulates lipolysis in isolated fat cells of rats (E. K. Frandsen, G. S. Agerbak and A. J. Moody, personal communication). These similarities, along with other data (H. Estep and S. I. Said, unpublished data), suggest that the two polypeptides may have a common mode of action, *i.e.*, stimulation of adenyl cyclase. The possible role of VIP in the regulation of sugar metabolism or other metabolic functions remains to be determined.

Summary. The hyperglycemic and glycogenolytic actions of a recently isolated vasoactive intestinal polypeptide (VIP), were compared with those of pancreatic glucagon. In intact, anesthetized dogs, 1 μg of nearly pure VIP raised plasma glucose concentration 33.5% as much as the same dose of glucagon did. The addition of VIP (0.2 μg/ml) to slices of rabbit liver incubated *in vitro*, stimulated glycogenolysis 56–63% as much as glucagon did. VIP may be one of the components of "enteroglucagon" or "glucagon-like immunoreactivity" known to occur in intestinal extracts. Because of its vasodilator and glycogenolytic activity, VIP may play a part in the regulation of intestinal blood flow and

of sugar metabolism.

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