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0014-4754/91/060623-03$1.50 + 0.20/0

Glycogenolytic effect of vasoactive intestinal peptide in the rat in vivo

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Received 16 May 1990; accepted 1 November 1990

Summary. The effects of VIP (300 pmol/kg), injected via the portal vein, on the glycogen content of the liver and on glycemia, were studied in the rat in vivo. VIP enhanced glycogenolysis and caused hyperglycemia in a time-dependent manner.
Key words. Glycogen; rat; VIP.

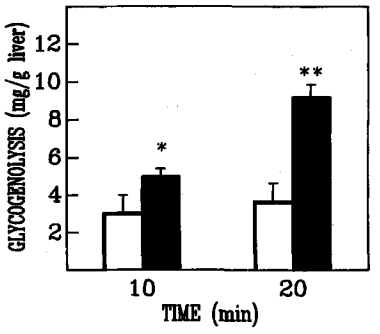
Vasoactive intestinal peptide (VIP), a highly basic octacosapeptide originally isolated from porcine small intestine [1], is widely distributed in neurons throughout the body [2]. There is experimental evidence indicating a metabolic role for VIP. In vitro studies have shown that VIP stimulates glycogenolysis in rabbit [3] and rat [4] liver slices, and isolated rat hepatocytes [5-7]. In vivo studies have shown that VIP causes hyperglycemia when administered i.v. to dogs [3, 8] and to man [9]. However, although most metabolic effects of VIP have been studied in rat hepatocytes (in vitro), little is known about its role in the rat in vivo. Therefore, the aim of this paper was to study the glycogenolytic effect of VIP in the rat in vivo. The peptide was injected directly into the superior mesenteric vein of anaesthetized rats to study its effect on hepatic glycogen content, blood glucose levels, and basal plasma insulin and glucagon levels.

Materials and methods

VIP was obtained from Peninsula Laboratories Europe (Merseyside, UK); bovine serum albumin (BSA) from Sigma Chemical (St Louis, Mo, USA). The peptide was dissolved in 0.9% NaCl-1% BSA. Male Wistar rats weighing 250–350 g were used. The animals were fed a standard diet ad libitum. The experiments were performed on anesthetized rats (pentobarbital sodium, 50 mg/kg i.p.) after a short-term fast (4–6 h) in the postabsorptive state. Anesthetized rats were injected with 1 ml VIP solution (300 pmol/kg). Control rats were injected with 0.9% NaCl-1% BSA. As described before [10], pieces of hepatic lobes from the same rat, weighing approximately 0.3 g, were tied off and rapidly excised before injection and at 10 and 20 min after injection, and immediately processed to obtain the glycogen by alcoholic precipitation. Blood samples (0.9 ml) were taken from the jugular vein before and at 5, 10 and 20 min after the injection. The blood obtained was heparinized and 20 µl were taken to measure blood glucose levels, the remaining blood was immediately centrifuged and plasma separated and stored at −20 °C. An ELISA kit (Boehringer Mannheim GmbH, W. Germany) was employed to measure plasma insulin. Radioimmunoassay (kit from Medgenix, Brussels, Belgium) was employed to measure plasma glucagon. Glycogen was determined by enzymatic conversion (amyloglycosidase from Boehringer Mannheim GmbH, W. Germany) to glucose which was determined by the glucose oxidase method. Blood glucose levels were also determined by this method. Student's t-test was used to test the degree of significance.

Results and discussion

For the first time the glycogenolytic effect of VIP in the rat in vivo has been investigated. The injection into the superior mesenteric vein of VIP (300 pmol/kg) produced a decrease in glycogen content of liver. This dose is similar to that employed by other authors for in vitro studies to obtain a maximal glycogenolytic effect [6, 7]. The basal content of hepatic glycogen after 4-6 h fasting was 51.3 ± 5 mg/g liver. The results of glycogenolysis are ex-



Glycogenolysis after the injection of VIP (300 pmol/kg; closed bars) or saline-1 %BSA (open bars) into the superior mesenteric vein. Means ± SEM are shown. *p < 0.05, **p < 0.001 (n = 8), probability of random differences between the group injected with VIP and the control group.

Effect of VIP (300 pmol/kg) injected into the superior mesenteric vein on blood glucose, plasma insulin and glucagon levels. V (group injected with VIP), C (group injected with saline-1 %BSA). Means ± SEM are shown. *p < 0.05, **p < 0.001 (n = 6) probability level of random difference between V and C group.

		0 min	5 min	10 min	20 min
Blood glucose	C	4.0 ± 0.2	4.1 ± 0.2	4.1 ± 0.2	4.1 ± 0.2
(mM)	V	4.1 ± 0.1	4.5 ± 0.1	4.9 ± 0.2	5.5 ± 0.2**
Plasma insulin	C	13.5 ± 1.0	13.8 ± 1.5	13.2 ± 1.2	14.0 ± 0.8
(μU/ml)	V	14.6 ± 0.3	19.0 ± 1.4*	19.1 ± 1.1*	21.5 ± 0.7**
Plasma glucagon	C	82 ± 10	75 ± 10	78 ± 12	80 ± 10
(pg/ml)	V	87 ± 10	140 ± 7**	94 ± 20	100 ± 25

pressed as the difference of glycogen concentration between the basal conditions and after the injection of VIP or saline-1 % BSA (figure). The glycogenolysis was significantly enhanced by VIP at 10 min compared with controls ($p < 0.05$), and much further increased at 20 min ($p < 0.001$). VIP caused slightly increased blood glucose levels, but the differences from controls were only significant at 20 min after the injection ($p < 0.05$) (table).

Baseline levels of plasma insulin were slightly, but significantly, increased from 5 min after the injection ($p < 0.05$) (table). Basal glucagon levels were enhanced with a peak at 5 min after the injection ($p < 0.05$), and there were no significant differences at 10 and 20 min. Presumably, these effects could be explained by the well-established fact that VIP stimulates glucagon release much more than insulin secretion [11, 12]. This effect is the likely cause of the hyperglycemia observed, since blood glucose levels were only significantly increased at 20 min after the injection of VIP. However, glycogenolysis was observed as early as 10 min after the injection of VIP. So our results show that VIP has a glycogenolytic effect in the rat in vivo, but not a direct hyperglycemic action, though the secondary increase of glucagon, probably reinforced by the remaining VIP, could cause the hyperglycemia observed.

VIP-containing fibers are abundant in the gut [2] and the fibers are mainly from intrinsic neurons [13]. Different experimental approaches have shown that the cell bodies reside in Meissner's plexus from which they extend fibers to Auerbach's plexus and the rest of the gut wall [14]. However, VIP neurons have never been described in the liver. VIP release from the gastrointestinal tract after stimulation of the vagus nerve [15] and electrical field stimulation of the ileum [16] has been reported. VIP is known to increase during fasting as well as during prolonged physical exercise, but not during short-term physical exercise [17, 18], and this suggests that VIP has an energy-mobilizing function during exercise. Our results on the hepatic glycogenolysis caused by VIP in the rat in vivo are in accordance with previous reports indicating that VIP could have a role as a polypeptide of substrate need [17, 18]. Furthermore, in response to feeding of a protein meal in dogs, VIP was shown to be released into the splanchnic portal circulation [19]. So VIP as well as glucagon could prevent insulin-induced hypoglycemia after a meal that is rich in protein, in part directly, but to a great extent secondarily stimulating glucagon secretion, since the foregoing results show that after short-term fasting (in the postabsorptive state), VIP causes hepatic glycogenolysis but a belated hyperglycemia, after the peak in plasma glucagon levels.

Acknowledgments. This work was supported by a grant from the Junta de Andalucía (07/CLM/MDM). Víctor Sánchez is a doctoral fellow of the University of Sevilla, Spain, sponsored by Monte de Piedad y Caja de Ahorros de Sevilla.

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