Interaction of vasoactive intestinal peptide (VIP) with cholinergic stimulation of glucagon secretion

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Summary. VIP-containing nerve fibers as well as cholinergic nerve fibers have a ubiquitous distribution in the body and both types of nerves have been demonstrated to innervate the pancreatic islets. The present study shows, in the intact, conscious mouse, that VIP and the cholinergic agonist carbachol stimulate glucagon secretion in a dose-dependent manner. Furthermore VIP and carbachol were found to exert potentiating interactions on glucagon secretion. These results suggest the existence of an interactive neural regulation of glucagon secretion, exerted by acetylcholine and VIP.

Vasoactive intestinal peptide (VIP) is a 28 amino acid polypeptide with structural similarities to secretin, glucagon, and gastric inhibitory polypeptide. It induces vasodilatation, stimulation of glycogenolysis and lipolysis, secretion from small intestine and exocrine pancreas and inhibition of gastric acid secretion, and affects hormonal release from the endocrine pancreas[2]. VIP has been found to be localized in granules in certain nerve terminals and is presumed to be a neurotransmitter[2-8]. In the pancreas, VIP-containing nerve fibers were recently described to run along acini and, furthermore, to form a network surrounding the pancreatic islets[9]. Thus VIP-containing nerve fibers may have the potential ability to influence glucagon secretion. Cholinergic stimulation is known to enhance glucagon secretion[10,11] and we present here functional evidence that VIP and the cholinergic agonist carbachol potentiate each other's effects on glucagon secretion.

Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark) were used. They were fed standard pellets (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum before and throughout the experiments. Pure porcine VIP or the cholinergic agonist carbachol (Brit Drug Houses Ltd, Poole, England) was injected i.v. in a tail vein; 10 µl/g b.wt was given. Blood samples from conscious mice were taken by orbital puncture at different time intervals after the injection. Controls were injected with saline; to all solutions 1 g/l gelatine was added to avoid adsorption to tubes. In another series of experiments a half-maximal dose of VIP (4.25 nmoles/kg) or saline, was injected i.v. 15 sec prior to a rapid i.v. injection of a half-maximal dose (0.16 µmoles/kg) of carbachol; controls received a double injection of saline also spaced by 15 sec. 5 µl/g b.wt was given in each injection. Blood was taken 2 min later (peak level of plasma glucagon after VIP and carbachol). Concentrations of plasma immunoreactive glucagon (IRG) were analyzed with radioimmunoassay[12] using K 964 (Novo Res Institute, Copenhagen, Denmark). This antiserum is raised in rabbits and directed against the C-terminal residue of porcine pancreatic glucagon. The assay was performed on small (25 µl) samples of unextracted plasma. Plasma glucose concentrations were determined with a glucose oxidase method[13].

Both VIP and carbachol were found to stimulate glucagon secretion in a dose-dependent manner. Peak levels of plasma IRG were achieved 2 min after the injection, both for VIP and carbachol. Figure 1 shows the dose-response curves for VIP and carbachol, respectively. Plasma glucose was 8.3 ± 0.4 mmoles/l in the saline-injected control group; neither VIP nor carbachol induced any change in plasma glucose during the 1st 2 min after injection. Figure 2 shows the combined effect of carbachol and VIP on glucagon secretion. Half-maximal doses of the 2 secretagogues were used. Plasma IRG was enhanced 0.36 ± 0.03 ng/ml by VIP, and 0.46 ± 0.08 ng/ml by carbachol; thus the expected value of their combined effects can be calculated to 0.82 ± 0.09 ng/ml if they exerted additive effects. However, the combined action of VIP and carbachol enhanced plasma IRG by 1.5 ± 0.15 ng/ml ($p < 0.01$ vs the expected value). Thus the 2 secretagogues potentiated each other's effects by about 85%.

VIP-containing nerve fibers seem to have a ubiquitous distribution in the body[2-8], and VIP is known to modulate endocrine and exocrine secretion[2-9]. Since VIP-containing nerve fibers are localized in close connection to pancreatic islets and since VIP has been shown to stimulate insulin, glucagon, and somatostatin secretion[9,14,15] it is conceivable that VIP might be of importance for the regulation of islet

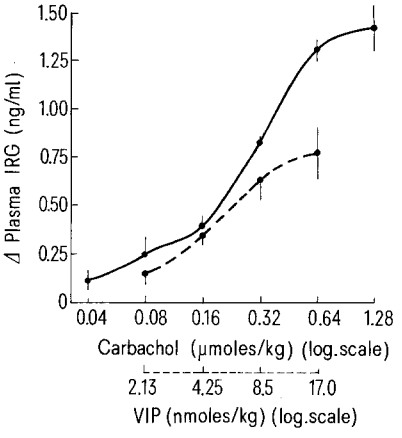

Figure 1. Dose-response relationships between dose of carbachol (µmole/kg) (●——●), or VIP (nmole/kg) (●– – –●), and glucagon secretion. Plasma concentrations of immunoreactive glucagon (IRG) were measured in samples taken 2 min after an i.v. injection of the secretagogue, respectively. Means and SEMs are shown. There were 8-12 animals in each group. Abscissa: dose of secretagogue (log scale). Ordinate: increase in plasma concentrations of glucagon above basal (ng/ml).

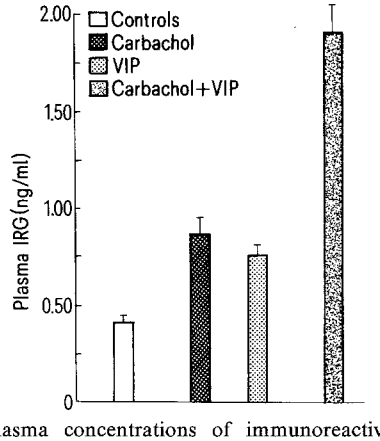

Figure 2. Plasma concentrations of immunoreactive glucagon (IRG) 2 min after an i.v. injection of saline, carbachol (0.16 µmoles/kg), VIP (4.25 nmoles/kg) or carbachol plus VIP (0.16 µmoles/kg and 4.25 nmoles/kg, respectively). There were 30-40 animals in each group. Bars indicate SEMs.

hormone secretion. The in vivo interaction between VIP-containing nerve fibers and other factors, neural as well as nutritional, which influence hormone secretion, is not known. The present study suggests that a potentiating synergism might be exerted through an interaction between cholinergic nerve fibers and VIP-containing nerve fibers. VIP and carbachol both displayed a marked glucagon secretory effect. The importance of these actions on the glucagon cell might well be expressed through an interactive neural regulation of glucagon secretion exerted by acetylcholine and VIP.

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2 S.I. Said, in: Gastrointestinal Hormones, p.245. Ed. G.B.J. Glass. Raven Press, New York 1980.
3 J. Fahrenkrug, Digestion *19*, 149 (1979).
4 L.I. Larsson, J. Fahrenkrug, O. Schaffalitzky de Muckadell, F. Sundler, R. Håkanson and J. Rehfeld, Proc. natl Acad. Sci. USA *73*, 3197 (1976).
5 S.I. Said and R.N. Rosenberg, Science *192*, 907 (1976).
6 L.I. Larsson, Histochemistry *54*, 173 (1977).
7 B. Ahrén, J. Alumets, M. Ericsson, J. Fahrenkrug, L. Fahrenkrug, R. Håkanson, P. Hedner, I. Lorén, A. Melander, C. Rerup and F. Sundler, Nature *287*, 343 (1980).
8 F. Sundler, J. Alumets, R. Håkanson, J. Fahrenkrug and O. Schaffalitzky de Muckadell, Histochemistry *55*, 173 (1978).
9 A.E. Bishop, J.M. Polak, I.C. Green, M.S. Bryant and S.R. Bloom, Diabetologia *18*, 73 (1980).
10 J.E. Gerich and M. Lorenzi, in: Frontiers in Neuroendocrinology, vol.5, p.265. Eds W.F. Ganong and L. Martini. Raven Press, New York 1978.
11 I. Lundquist, B. Ahrén, R. Håkanson and F. Sundler, in: Diabetes 1979, p.57. Ed. W.K. Waldhäusl. Excerpta Medica, Amsterdam, Oxford, Princeton 1980.
12 L.G. Heding, Diabetologia *7*, 10 (1971).
13 M.L. Bruss and A.L. Black, Analyt. Biochem. *84*, 309 (1978).
14 J. Szecówka, E. Sandberg and S. Efendić, Diabetologia *19*, 137 (1980).
15 B. Ahrén and I. Lundquist, Diabetologia *20*, 54 (1981).

Sertoli cells of adult rats in vitro. III. Purification of androgen-binding protein from the culture medium[1]

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Summary. A method giving a high yield for the isolation and purification of the androgen-binding protein (ABP) from the nutritional medium of cultured Sertoli cells from adult rats is described.

Androgen-binding protein (ABP) is a secretory product of Sertoli cells which has been purified and characterized from the testis and the epididymus of several mammals[3-10]. Moreover, it is known that Sertoli cells in vitro are still able to synthesize ABP and release it into the medium[11,12]. This capacity makes them a useful source for the purification of this protein.

This paper describes a method for isolating ABP from the medium of cultured rat Sertoli cells and confirms previous findings[13] on the possibility of making cultures of Sertoli cells from adult rats.

Materials and methods. Sertoli cells of 4-month-old Long Evans rats were isolated and cultured as previously described[13]. The cells were subcultured into 10 roller-bottles, each containing 150 ml of Eagle's medium (Flow Lab., Rockville) supplemented with 10% foetal calf serum (Eurobio Lab., Paris). When monolayers became confluent, the nutritional medium was removed and replaced by an equal volume of fresh medium. After 10 days of culture the pooled media (1500 ml) were used for ABP purification. Protein concentration was evaluated according to Lowry et al.[14]. Proteins were precipitated with 40% saturated ammonium sulphate at 0 °C. The sediment, recovered by centrifugation, was dissolved in 5 ml of 0.1 M tris-HCl buffer, pH 7.4, with 1 M NaCl and dialyzed against the same buffer. The chromatography was performed by a 2089 Uvicord III, 280 nm filter, 11300 Ultrograd (LKB, Sweden). The gel filtration was performed at 0 °C on a column (2.5 × 100 cm) of superfine Sephadex G-200 eluted with 0.1 M tris-HCl buffer pH 7.4 containing 1 M NaCl. The flow rate was 10 ml/h and fractions of 2 ml were collected, starting from 100 ml of eluent. The ion exchange chromatography was performed according to Cervone et al.[15]. Immunoelectrophoresis, using anti-whole rat and anti-bovine sera (Behring) and normal rat and bovine sera as controls was carried out according to Sega et al.[16]. Isoelectric focusing on polyacrylamide gel was performed by BIO-RAD equipment according to Wringley[17]. The pH gradient was measured by a BIO-RAD profiler. Gels were scanned at 590 nm with PMQ3 Zeiss spectrophotometer. ABP activity was determined on 0.3 ml (40 mg/ml) of fraction A from DEAE cellulose chromatography with ^{3}H-testosterone (2×10^{-9} M, average sp. act. 44Ci/mmole) purchased from New England Nuclear Co.[11].

Results. The fractions between the 2 peaks[7,10] were collected, concentrated and refiltered on Sephadex G-200 (fig. 1).

Purification procedure for ABP from Sertoli cell culture medium

Step	Total protein (mg)	Total pmoles bound	Sp. act. pmoles (mgprotein)$^{-1}$	Yield %	Purification (fold)
Whole medium	8000	31,818	3.9		
$(NH_4)_2SO_4$	50	30,860	617	97	158
1st Sephadex G200	10	22,274	2227	70	571
2nd Sephadex G200	3	20,683	6894	65	1767
DEAE-cellulose	1.5	17,500	11,667	55	2991

The specific activity of ABP after each step was determined according to Fritz et al.[11].