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VIP Suppression in the Intestine and Cerebral Cortex Following Administration of VIP Antiserum to Newborn Rats

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The administration of vasoactive intestinal peptide (VIP) antiserum to newborn rats significantly reduced the VIP content, both in the cerebral cortex and in intestinal epithelial cells. The decrease was observed at postnatal days 14 and 21 and also in 90 day-old animals. The neonatal treatment produced a significant increase in the density of high- and low-affinity binding sites for VIP in the cerebral cortex at postnatal days 14 and 21 whereas in the intestinal epithelial cells only the low-affinity binding sites were up-regulated at the same time points. VIP suppression induced by neonatal administration of the corresponding antiserum may represent a useful approach to further characterize the physiological role of this neuropeptide.

Key words: Vasoactive intestinal peptide, VIP antiserum, VIP receptors, Intestinal epithelial cells, Cerebral cortex.

Vasoactive intestinal peptide (VIP) is a 28 amino acid peptide originally isolated from the duodenum and later found to be widely distributed both in the central and peripheral nervous system where it exhibits a neurotransmitter/neuromodulator role. In the brain, VIP is concentrated in the cerebral cortex, hypothalamus, amygdala and hippocampus, largely in cholinergic presynaptic neurons. In the peripheral nervous system it is found in peptidergic neurons innervating several tissues (21). VIP induces relaxation of smooth muscle and stimulates secretion of water and electrolytes from the gastrointestinal tract. VIP also stimulates glycogenolysis in the cerebral cortex. In autonomic ganglia, VIP is also found in acetylcholine-containing neurons and the signal

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induced by acetylcholine seems to be modulated by VIP (11, 23).

The different effects of VIP are mediated by its interaction with specific receptor sites, which have been demonstrated both in the brain and in the intestine. At least two receptor subtypes have been detected by radioligand binding analysis and studies with antagonists. These sites are positively coupled to adenylate cyclase so VIP stimulates the cellular accumulation of cyclic AMP. In addition, VIP has been reported to activate protein kinase C (PKC) (10, 12, 13).

VIP gene expression is developmentally determined and may be associated with neuronal growth and survival. VIP has been shown to be involved in neuronal growth, maturation and differentiation. Particularly, it has been suggested that VIP may have a regulatory role in the process of neuronal differentiation both in brain and in peripheral regions (11). The neurotrophic activity of VIP is perhaps related to an initial stimulating effect on glial cells with the subsequent release of glial-derived growth factors such as interleukin-1 or activity dependent neurotrophic factor (11) which have a direct trophic action on neurons. In addition, sustained activation of PKC is essential for subsequent responses such as cell proliferation and differentiation (2, 3).

Studies of VIP-gene expression show that VIP is almost undetectable in the brain before birth but is found in the intestine of the fetus and significant amounts of VIP-mRNA are detectable at birth. In contrast to the expression in the postnatal developing brain, in the intestine VIP-mRNA decreases during postnatal development and thereafter increases in the adult (9).

From the above developmental considerations on VIP-gene expression it could be expected that a deficiency of VIP during nervous system development may produce physiological changes in adult animals. Antibodies to other neuropeptides such as substance P or arg-vasopressin may be taken up by neurons (4, 5) and this may produce a peptide suppression which may lead in turn to lasting functional changes (1, 8, 16).

The present study was aimed at determining the biochemical consequences of the administration to neonatal rats of a specific VIP antiserum. Rats were treated with the antiserum on the second day of life and VIP levels and VIP receptors were measured at different times in the cerebral cortex and in the intestinal epithelial cells, that receive a dense VIP innervation. In the absence of selective VIP antagonists, VIP suppression would conceivably allow to get a further insight into the physiological role of this neuropeptide.

Materials and Methods

Animals and treatments.— Male Wistar rats were injected s.c. on the second day of life with VIP-antiserum (500 µg protein/rat). Control animals received the same amount of non-specific immunoglobulins (IgG). The volume injected was 100 µl. Animals were sacrificed by decapitation at postnatal days 14, 21 and 90.

Preparation of intestinal epithelial cells and cerebral cortex membranes.— Immediately after sacrifice, the jejuno-ileal segment was removed and washed with ice-cold 0.25 M NaCl. Cells were isolated according to the method of PRIETO *et al.* (19). The gut portion was shaken for 20 min in 100 ml of a dispersing solution containing 2.5 mM EDTA and 0.25 M NaCl (pH=7.5). Cells were collected by centrifugation at 2000 g for 5 min, washed in 100 ml of 0.15 M NaCl and centrifuged again. This procedure was repeated three times. After isolation, cells were resuspended in incubation buffer at appropriate dilution and used in the experiments.

For cerebral cortex membranes preparation, the brain was immediately removed

from the skull and cerebral cortex was dissected. Fresh cortex was homogenized on ice in 0.32 M sucrose using a glass-teflon homogenizer. The homogenate was centrifuged at 1000 g for 3 min. The supernatant was subjected to further centrifugation at 10000 g for 40 min. The resultant pellet was resuspended in the incubation buffer for binding assay.

Protein concentration was determined by the LOWRY method (15).

VIP radioimmunoassay.— VIP was assayed by a double antibody radioimmunoassay as previously described (14, 25), using a polyclonal VIP antiserum prepared by CACICEDO and SANCHEZ-FRANCO (14).

Radioligand binding assay.— Experimental conditions for VIP binding were essentially as described (18). Briefly, cell homogenates were suspended in an incubation buffer containing 35 mM Tris-HCl (pH=7.5), 50 mM NaCl, 1.5 % bovine serum albumin and 1 mg/ml bacitracin at a protein concentration of 100-200 µg/ml. Incubation was carried out for 90 min at 15 °C with 45 pM porcine [^{125}I]-VIP. Bound peptide was separated by centrifugation and the radioactivity determined by gamma spectrometry. Nonspecific binding was determined in the presence of the unlabelled VIP (10^{-10}-10^{-7} M). The biphasic Scatchard plot for Kd and Bmax values, was obtained from the displacement curves, using a fixed concentration of the labelled ligand and different concentrations of the cold ligand (7).

Drugs and chemicals.— Bovine serum albumin (BSA; Sigma, UK), synthetic porcine VIP (Peninsula, USA), [^{125}I]-VIP (2000 Ci/mmol) (Amersham, UK). Other reagents were obtained from Merck.

Results

VIP radioimmunoassay.— VIP content in rat intestinal epithelial cells increased during postnatal development in control animals. This increase was not observed in the cerebral cortex (table I).

Neonatal treatment with VIP-antiserum injected s. c. produced a significant decrease in VIP content at all periods tested, both in the cerebral cortex and in the intestinal epithelial cells. The decrease was more marked in intestinal cells than in cerebral cortex (table I). A maximal reduction of about 90 and 60 % respectively, was observed.

Radioligand binding assay.— Figure 1 shows an example of the displacement of binding of [^{125}I]VIP to intestinal epithelial cells by different concentrations of unlabelled VIP. The Scatchard plot obtained from these binding data was curvilinear and resulted in a best fit for a model of two VIP binding sites of high and low affinity. The values of the dissociation constant as well as the binding capacity for the high- and low-affinity sites in intestinal epithelial cells from IgG and VIP-antiserum treated animals are depicted in table II. [^{125}I]VIP binding to rat cerebral

Table I. *Effect of neonatal administration of VIP antiserum or nonspecific immunoglobulins (IgG) on VIP content (pmol/mg protein in rat intestinal epithelial cells) and in cerebral cortex in rat.*
Rats were injected on the second day of life with 500 µg protein/rat (s.c.) of IgG or VIP antiserum. Values are means ± S.E.M. (n=6). * P< 0.05 vs. control rats.

Postnatal day	Intestinal epithelial cells		Cerebral cortex	
	IgG (Controls)	VIP antiserum	IgG (Controls)	VIP antiserum
14	4.14 ± 0.25	1.22 ± 0.18*	0.58 ± 0.20	0.23 ± 0.10*
21	6.36 ± 0.63	0.72 ± 0.07*	0.34 ± 0.07	0.15 ± 0.03*
90	7.30 ± 0.80	0.57 ± 0.10*	0.82 ± 0.20	0.33 ± 0.10*

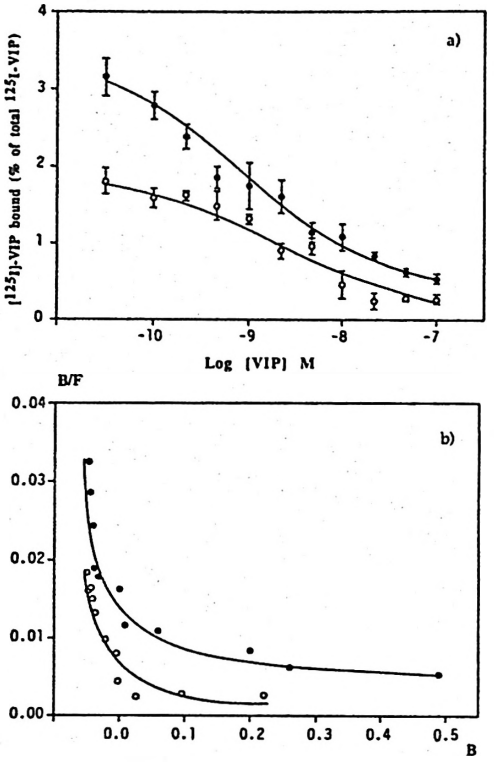

Fig. 1. *Displacement of [125I]-VIP binding to homogenates of intestinal ephitheliasl cells from 14-day-old rats by unlabelled VIP (a) and Scatchard plot of the same binding data (b).*
Animals treated on the 2nd day of life with VIP antiserum (o) or with non-specific IgG(•). Data indicate the mean ± S.E.M. from six duplicate experiments.

cortex homogenates was also indicative of a high- and a low-affinity site (table III).

In intestinal epithelial cells, there was no significant change either in the Kd or Bmax for high-affinity binding sites at any postnatal days after VIP antiserum treatment. However, neonatal treatment with the antiserum resulted in a significant increase of the low affinity binding sites in animals aged 14 and 21 days as compared to the matched IgG-treated group, without any modification in Kd values (table II).

In rat cerebral cortex (table III), Bmax values for high- and low- affinity binding sites were significantly increased in animals of 14 and 21 days of age after the neonatal treatment with VIP antiserum. The Kd values were not significantly affected. Again, no effect was detected after VIP-antiserum administration in 90-day-old animals.

Discussion

The results of the present study show that neonatal administration of VIP antiserum induces a reduction in VIP content both in the intestinal epithelial cells and in the cerebral cortex of the rat. VIP-antiserum could be internalized by VIP neu-

Table II. *Effect of neonatal IgG (control) or VIP antiserum (VIP-A) administration on equilibrium parameters of [125I]-VIP binding to homogenates of rat intestinal epithelial cells.*
Rats were injected on the second day of life with 500 µg protein/rat of IgG or VIP-A, Data are means ± S.E.M. of 6 Scatchard plots, each plot constructed with 10 points determined in duplicate. Kd and Bmax are expressed in nM and fmol/mg of protein respectively. * P< 0.05 vs. control rats.

Postnatal day		High-affinity binding sites		Low-affinity binding sites	
		Kd	Bmax	Kd	Bmax
14	control	0.66 ± 0.09	95 ± 8	56.5 ± 7.4	1530 ± 123
	VIP-A	0.48 ± 0.10	130 ± 18	59.3 ± 9.6	3500 ± 204*
21	control	1.24 ± 0.15	31 ± 4	47.8 ± 5.3	1359 ± 138
	VIP-A	1.27 ± 0.08	42 ± 6	32.3 ± 8.5	1875 ± 126*
90	control	3.03 ± 0.21	141 ±17	129.1 ± 6.9	1675 ± 187
	VIP-A	3.07 ± 0.19	140 ± 5	134.6 ± 10.3	1928 ± 213

Table III. *Effect of neonatal IgG (control) or VIP antiserum (VIP-A) administration on equilibrium parameters of [^{125}I]-VIP binding to homogenates of rat cerebral cortex.*
Rats were injected on the second day of life with 500 µg protein/rat of IgG or VIP-A. Data are means ± S.E.M. of 6 Scatchard plots, each plot constructed with 10 points determined in duplicate. Kd and Bmax are expressed in nM and fmol/mg of protein respectively. * $P < 0.05$ vs. control rats.

Postnatal day		High-affinity binding sites		Low-affinity binding sites	
		Kd	Bmax	Kd	Bmax
14	control	0.65 ± 0.15	64 ± 3	26.3 ± 3.8	1180 ± 300
	VIP-A	0.56 ± 0.08	100 ± 20*	21.7 ± 1.6	2140 ± 126*
21	control	1.17 ± 0.30	130 ± 20	38.6 ± 3.5	2860 ± 300
	VIP-A	1.32 ± 0.25	260 ± 30*	25.0 ± 2.8	5000 ± 600*
90	control	3.09 ± 0.75	315 ±17	27.5 ± 8.2	1571 ± 212
	VIP-A	2.43 ± 0.68	384 ± 36	43.9 ± 9.9	2019 ± 268

rons inducing a decrease in VIP levels and the subsequent changes in receptor density. This possibility would be in keeping with previous studies showing that specific antibodies against peptide neurotransmitters, such as substance P or vasopressin were able to penetrate into neurons in vivo and were transported anterograde and retrogradely (4, 5). In vivo administration of these antibodies to adult animals does not produce neuronal damage whereas in the more vulnerable developing nervous system may produce an immunolesion (4-6). Moreover, the results indicate that the antiserum is able to penetrate a still inmature blood-brain barrier (20).

In agreement with previous studies (19, 22), our results indicate the existence of two apparent classes of VIP binding sites both in rat intestinal epithelial cells and in cerebral cortex. Although the nature of the high- and low- affinity receptors and their possible interrelationship have not been determined, it has been suggested that guanine nucleotides may modulate the interconversion of both states or that receptor occupancy by VIP may induce a transition of a low- to a high affinity state (17).

In addition to the decrease of VIP content in rat intestinal epithelial cells, VIP-antiserum induces a concomitant increase in the number of low-affinity binding sites, which was only significant on postnatal days 14 and 21 and was compensated in adult animals. In the cerebral cortex there was an increase in the density of both low- and high-affinity sites. These changes may be a consequence of the decrease in VIP content induced by the administration of the antiserum. It is possible to suppose that a prolonged inhibition of the tissue exposure to VIP, bound to the antiserum at a critical stage of neuronal development, causes a marked compensatory increase in receptor number. This interpretation would represent a parallelism between the administration of the antiserum and the typical effect of treatment with receptor antagonists that may up-regulate receptor number.

The compensatory effect observed in adult animals may be related to an increase in the VIP-gene expression at this period of development. This interpretation is consistent with previous studies on the ontogeny of the VIP-gene expression that shows a local and age-dependent regulation of the gene expression so the maximal expression level is observed in the intestine at this period. In the cerebral cortex, although VIP-mRNA content decreases as compared to total mRNA after

reaching a maximal level at postnatal day 14, the peptide content might still be increasing and it has been suggested that this is related to an additional post-translational control (9).

In summary, administration of a VIP-antiserum to newborn rats induces long-term changes in VIP content both in intestinal epithelial cells and in cerebral cortex which are accompanied by an increase in the number of binding sites for this peptide. These changes suggest that neonatal administration of specific antibodies may be a useful approach to elucidate the role of neuropeptides in the control of different physiological functions.

J. L. DÍAZ-JUÁREZ, D. FRECHILLA, G. ROMERO, F. SÁNCHEZ-FRANCO y J. DEL RÍO. *Reducción de VIP en intestino y corteza cerebral tras la administración de antisuero frente a VIP en ratas neonatas.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 5 (2), 109-116, 1994.

La administración de un anticuerpo anti-VIP a ratas recién nacidas reduce significativamente los niveles de VIP en corteza cerebral y en células epiteliales de intestino. Esta disminución se observa tanto a los 14 y 21 días de edad como a los 90 días. El tratamiento neonatal produce un aumento significativo en la densidad de los sitios de unión de alta y de baja afinidad en corteza cerebral de rata medidos a los 14 y 21 días de edad, mientras que en células epiteliales de intestino sólo se observa este aumento en los sitios de baja afinidad. La supresión de los niveles de VIP por la administración del correspondiente antisuero puede representar una aproximación de interés para profundizar en la caracterización del papel fisiológico de este neuropéptido.

Palabras clave: Péptido intestinal vasoactivo, Anticuerpo anti-VIP, Receptores VIP, Células epiteliales intestinales, Corteza cerebral.

References

1. Achaval, M., Martínez-Murillo, R., Rodrigo, J., Aguado, F., de Felipe, M. C. and del Río, J. (1991): *Neurosci. Lett..*, 126, 75-78.
2. Brenneman, D. E., Neale, E. A., Foster, G. A., d'Autremont, S.W. and Westbrook, G. L. (1987): *J. Cell Biol.*, 104, 1603-1610.
3. Brenneman, D. E., Nicol, T., Warren, D. and Bowers, L. M. (1990): *J. Neurosci. Res.*, 25, 386-394.
4. Burlet, A. J., Leon-Henri, B. P., Robert, F. R., Arahmani, A., Fernette, B. M. L. and Burlet, C. R. (1987): *Exp. Brain Res.*, 65, 629-638.
5. Chan-Palay, V. (1979): *Anat. Embryol.*, 156, 241-254.
6. Costa, M., Creffen, L. B., Rush, R. A., Brigges, D., Blessing, W. W. and Heath, J. W. (1979): *Brain Res.*, 173, 65-78.
7. de Blasi, A., O'Reilly, K. and Motulsky, H. J. (1989): *TIPS*, 10, 227-229.
8. de Felipe, M. C., Molinero, M. T. and del Río, J. (1989): *Brain Res.*, 485, 301-308.
9. Gozes, I., Shani, Y and Rostène, W. H. (1987): *Mol. Brain Res.*, 2, 137-148.
10. Gozes, I., McCune, S. K., Jacobson, L., Warren, D., Moody, T. W., Fridkin, M. and Brenneman, D. E. (1991): *J. Pharmacol. Exp. Ther.*, 257, 959-966.
11. Gozes, I. and Brenneman, D. E. (1993): *J. Mol. Neurosci.*, 4, 1-9.
12. Hedlund, B., Abens, J. and Bartfay, T. (1983): *Science*, 220, 519-521.
13. Labourthe, M., Prieto, J. C., Amiranoff, B., Dupont, C., Hui Bon Hoa, D. and Rosselin, G. (1979): *Eur. J. Biochem.*, 96, 239-248.
14. Lorenzo, M. J., Sánchez-Franco, F., De Los Frailes, M. T., Reichlin, S., Fernández, G. and Cacicedo, L. (1989): *Endocrinology*, 125, 1983-1990.
15. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951): *J. Biol. Chem.*, 193, 265-275.
16. Moratalla, R., Borrell, J., Sánchez-Franco, F. and del Río, J. (1987): *Behav. Brain Res.*, 23, 231-237.
17. O'Dorisio, M. S. (1987): *Fed. Proc.*, 46, 192-195.
18. Prieto, J. C., Labourthe, M. and Rosselin, G. (1979): *Eur. J. Biochem.*, 96, 229-237.
19. Prieto, J. C., Labourthe, M., Hui Bon Hoa, D. and Rosselin, G. (1981): *Acta Endocrinol.*, 96, 100-106.
20. Risau, W. and Wolburg, H. (1990): *Trends Neurosci.*, 13, 174-178.
21. Said, S. and Mutt, V. (1970): *Science*, 169, 1217-1218.
22. Said, S. (1984): *Peptides*, 5, 143-150.
23. Said, S. (1986): *J. Endocrinol. Invest.*, 9, 191-200.

24. Sánchez, F., Carretero, J., Sánchez-Franco, F., Riesco, J. M., Blanco, E., Juanez, J. A. and Vázquez, R. (1990): *Neuropeptides,* 17, 127-134.

25. Sánchez, F., Lara, J. I., Cacicedo, L., Sánchez-Franco, F., Carretero, J. and Vázquez, R. (1991): *Biomed. Res.,* 12, 71-75.