

# Mechanisms of VIP-Induced Neuroprotection against Neonatal Excitotoxicity

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**ABSTRACT:** Two VIP receptors, shared with a similar affinity by pituitary adenylate cyclase-activating polypeptide (PACAP), have been cloned: VPAC1 and VPAC2. PHI binds to these receptors with a lower affinity. We previously showed that VIP protects against excitotoxic white matter damage in newborn mice. This article aimed to determine the receptor involved in VIP-induced neuroprotection. VIP effects were mimicked with a similar potency by VPAC2 agonists and PHI but not by VPAC1 agonists, PACAP 27 or PACAP 38. VIP neuroprotective effects were lost in mice lacking VPAC2 receptor. *In situ* hybridization confirmed the presence of VPAC2 mRNA. These data suggest that, in this model, VIP-induced neuroprotection is mediated by VPAC2 receptors. The pharmacology of this VPAC2 receptor seems unconventional as PACAP does not mimic VIP effects and PHI acts with a comparable potency.

**KEYWORDS:** VPAC2; PACAP; PKC; BDNF; PHI

## INTRODUCTION

Prepro-vasoactive intestinal peptide (VIP) mRNA codes for two neuropeptides: VIP and peptide histidine isoleucine (PHI) in rodents or VIP and peptide

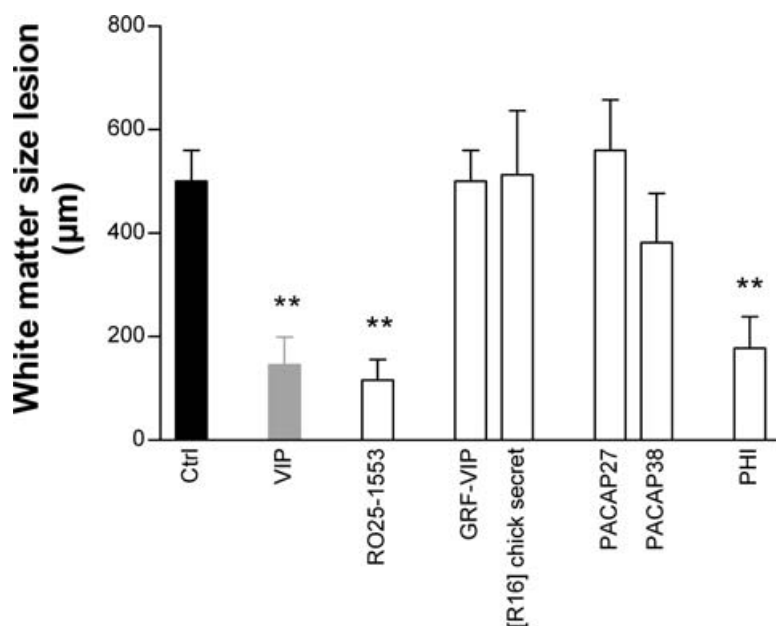
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histidine methionine (PHM) in humans. Two VIP receptors, shared with a similar affinity by pituitary adenylate cyclase-activating polypeptide (PACAP), have been cloned: VPAC1 and VPAC2 (for a review, see Ref. 1). PHI binds to these receptors with a lower affinity. Furthermore, PACAP-27 and PACAP-38, but not VIP, bind with high affinity to a specific PACAP receptor called the PAC1 receptor. VPAC receptors are preferentially coupled to  $G_{\alpha s}$  protein that stimulates adenylate cyclase activity and induces cAMP increase (for a review, see Ref. 1). VPAC receptors can also be coupled to  $G_{\alpha q}$  and  $G_{\alpha i}$  proteins that stimulate the inositol phosphate/calcium/protein kinase C (PKC) pathways.

### VIP BUT NOT PACAP PROTECTS THE NEWBORN BRAIN AGAINST EXCITOTOXIC LESIONS

In line with its previously reported neurotrophic properties,<sup>2</sup> VIP potently protects the developing brain against an excitotoxic insult in newborn mice.<sup>3</sup>



**FIGURE 1.** Effects of VIP, VPAC2 agonist (RO25-1553), VPAC1 agonists (GRF-VIP and [R16] chicken secretin), PACAP-27, PACAP-38, and PHI on white matter lesions induced by ibotenate injected to P5 mice. One microgram of each peptide was administered. Bar represents mean length of the lesions  $\pm$  SEM. Asterisks indicate difference from phosphate-buffered saline solution (PBS) control group (black bar); \*\* $P < 0.01$  in ANOVA with Dunnet's multiple comparison test.

In this *in vivo* model, VIP, coinjected with the glutamatergic agonist, ibotenate, in the brain of 5-day-old (P5) pups, reduces ibotenate-induced white matter lesions by up to 85 % when compared to controls. These ibotenate-induced white matter lesions mimic several aspects of periventricular leukomalacia, a white matter lesion often observed in human preterm infants. VIP effects are mimicked with a similar potency by VPAC2 agonists and PHI but not by VPAC1 agonists (FIG. 1).<sup>4,5</sup> Surprisingly, VIP-induced neuroprotection is not mimicked by a large range of doses of PACAP-27 or PACAP-38 (FIG. 1).<sup>3,5</sup>

### VPAC2 RECEPTORS MEDIATE VIP-INDUCED NEUROPROTECTION

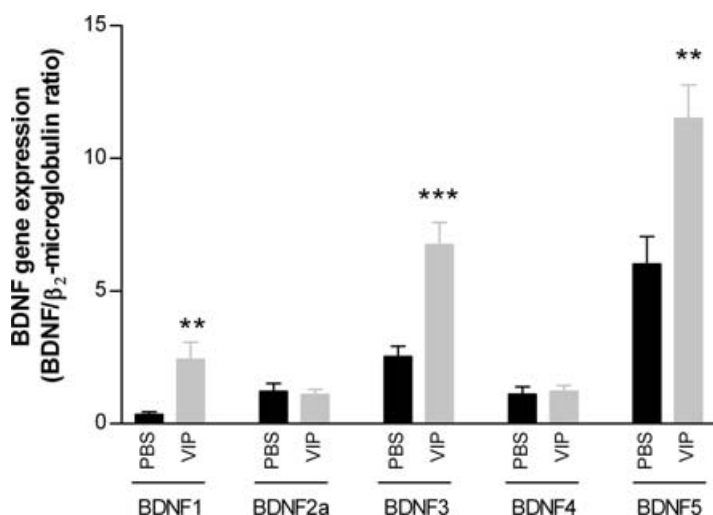
This atypical pharmacology of VIP-induced neuroprotection in newborn mice raised several hypotheses: (a) Activation of PAC1 receptors could have a toxic effect on the excitotoxic lesions while activation of VPAC receptors could be neuroprotective, leading to a lack of detectable effect for PACAP. (b) During some stages of brain development, the binding of VIP or PACAP to VPAC receptors leads to activation of separate transduction pathways. (c) VIP acts through a yet-to-be-identified specific VIP receptor which is not recognized by PACAP. Indeed, Ekblad *et al.*<sup>6</sup> characterized a PACAP-27 preferring receptor and a VIP-specific receptor, distinct from those that have been cloned (VPAC1, VPAC2, and PAC1 receptors), in intestine of rat and PAC1<sup>-/-</sup> mice.

The first stated hypothesis that activation of PAC1 receptors could have a toxic effect on the excitotoxic lesions while activation of VPAC receptors could be neuroprotective, leading to a lack of detectable effect for PACAP-38, can be ruled out by the lack of protective effects of PACAP-38 in PAC1<sup>-/-</sup> mice.<sup>5</sup> In contrast, VIP neuroprotective effects are completely abolished in mice lacking VPAC2 receptor.<sup>5</sup> *In situ* hybridization confirms the presence of VPAC2 mRNA in the postnatal day 5 white matter.<sup>5</sup> When analyzed between embryonic life and adulthood, VIP-specific binding site density peaks at postnatal day 5.<sup>5</sup> These data suggest that, in this model, VIP-induced neuroprotection is mediated by VPAC2 receptors. The pharmacology of this VPAC2 receptor seems unconventional as (a) PACAP does not mimic VIP effects, (b) PHI acts with a comparable potency, and (c) PACAP-27 modestly inhibits the VIP-specific binding while for PHI or VIP, inhibition is complete.

Furthermore, supporting an atypical pharmacological profile of this VPAC2 receptor, stearyl norleucine VIP, a specific VIP agonist that does not activate adenylate cyclase, mimics VIP effects and treatment with forskolin, an adenylate cyclase activator, fails to provide a VIP-like protection.<sup>3</sup> In contrast, VIP protective effects are abolished by a PKC inhibitor and a mitogen-associated protein kinase (MAPK) inhibitor in a dose-dependent manner.<sup>3,7</sup>

## POTENTIAL MECHANISMS UNDERLYING THE ATYPICAL PHARMACOLOGY OF VIP EFFECTS

In order to explain the observed characteristics of VPAC2 receptors in this model of neuroprotection, some hypotheses can be formulated: (a) During some stages of brain development, the binding of VIP or PACAP to VPAC2 receptors leads to activation of separate transduction pathways. This differential coupling could be secondary to VPAC2 receptors dimerization (homo- or heterodimers) or to their interaction with larger oligomeric complexes, as demonstrated for other types of G protein-coupled receptors (GPCRs) (for a review, see Ref. 8). A variant of this hypothesis would be a developmental change in the G proteins available for the receptor to couple to in the relevant cells. (b) An alternative hypothesis has been suggested by recent studies. A first study identified a deletion variant of the mouse VPAC2 receptor in immune cells.<sup>9</sup> This natural deletion abrogates VIP-induced cAMP production without apparent alterations of expression or ligand binding. Second, Langer and Robberecht<sup>10</sup> showed that mutations in the proximal domain of the third intracellular loop of the VPAC1 receptor reduced the capability of VIP to increase adenylate cyclase activity without any change in the calcium response, whereas mutations in the distal part of the loop markedly reduced the calcium



**FIGURE 2.** Effects of VIP on BDNF mRNA expression. Real-time PCR quantification of BDNF-1 to -5 mRNA variants in brains treated with ibotenate + 1  $\mu$ g VIP or with ibotenate + PBS. Intracerebral injections were performed on P5 and tissues were collected at the site of injection 4 h later. Data are presented as mean BDNF variant/ $\beta_2$ -microglobulin ratios  $\pm$  SEM. Asterisks indicate statistically significant differences between PBS-treated controls and pups treated with VIP (\*\* $P$  < 0.01 in a  $t$ -test; \*\*\* $P$  < 0.001 in a  $t$ -test).

increase and G $\alpha$ i coupling but only weakly reduced the adenylate cyclase activity. Based on these studies, we can hypothesize that a yet-to-be-identified substitution or deletion in the newborn mouse VPAC2 receptor transcript, through RNA editing for instance, might be able to induce VIP specificity and modulate the coupling with different G proteins.

### **BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) MEDIATES VIP-INDUCED NEUROPROTECTION**

Interestingly, VIP neuroprotective effects against neonatal excitotoxic damage have recently been shown to be mediated by BDNF production (as demonstrated by BDNF-5 primers which recognize all BDNF mRNA variants) which induces a secondary axonal sprouting and white matter repair through MAPK pathway activation.<sup>11</sup> In particular, VIP induces through activation of this atypical VPAC2 receptor an increased expression of BDNF-1 and -3 mRNA variants (FIG. 2). These BDNF-1 and -3 mRNA transcripts have been recently shown to be decreased in brains of Alzheimer patients.<sup>12</sup>

### **CONCLUSION**

Altogether, these data strongly support the hypothesis that, in newborn mice, VIP neuroprotective effects against an excitotoxic insult are mediated by VPAC2 receptors showing atypical pharmacological properties.

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