

Role of protein kinase C and carboxyl-terminal region in acute desensitization of vasopressin V1a receptor

Nicolas Ancellin^a, Laurence Preisser^a, Bruno Corman^a, Alain Morel^{a,b,*}

^aService de Biologie Cellulaire, CEA, Centre d'Etudes de Saclay, 91191 Gif-sur-Yvette Cedex, France

^bUniversité d'Angers, 2 rue Lavoisier, 49000 Angers, France

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Abstract The role of protein kinase C activation and carboxyl-terminal region in rapid desensitization of the vasopressin V1a receptor was investigated in *Xenopus* oocytes. Preincubation of the oocytes with vasopressin or with the diacylglycerol analog 1-oleoyl-2-acetyl-sn-glycerol (OAG), or direct injection of active protein kinase C, all blunted the calcium response of the V1a receptor. Truncation of the 51 terminal amino acids (S374STOP) modified neither the intracellular calcium response to vasopressin nor its desensitization by vasopressin or OAG. These data suggest that desensitization of the V1a receptor is mediated by PKC activation and that its carboxyl-terminal domain is not required for signal transduction and rapid desensitization.

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Key words: Vasopressin V1a receptor; Protein kinase C; Desensitization; Intracellular calcium; *Xenopus* oocyte

1. Introduction

Vasopressin V1a receptor is widely distributed in several tissues such as blood vessels, kidney, choroid plexus, liver or central nervous system [1]. It belongs to the superfamily of the G-protein-coupled receptors with seven transmembrane domains. Binding of vasopressin (AVP) to the V1a receptor activates phospholipase C and produces inositol tri-phosphate and diacylglycerol, which respectively increase intracellular calcium and activate protein kinase C (PKC). Like most G-protein-coupled receptors already studied [2], the V1a receptor undergoes desensitization when stimulated by its agonist. This homologous desensitization is characterized by the inability of the receptor to induce a further intracellular response when it is successively challenged by vasopressin. Such rapid desensitization is probably linked to uncoupling of the receptor from G proteins within seconds, whereas internalization would occur within minutes. Both rapid desensitization and internalization have been demonstrated for the V1a receptor in native tissue and in cells expressing the cloned receptor [3–6].

The most frequently invoked mechanism for rapid desensitization refers to phosphorylation of threonine and serine residues by protein kinase A or C and/or by G receptor kinase [7]. This phosphorylation may be consecutive to binding of the ligand to the receptor or to activation of protein kinase by second messengers [7–11]. The role of protein kinase A activation by cAMP on desensitization has been well demonstrated for adenylyl cyclase coupled receptor. On the other hand, the effect of protein kinase C activation by diacylglycerol on the desensitization process is less documented.

The V1a receptor, like other transmembrane G protein receptors, has a number of threonine and serine residues which are putative sites for phosphorylation and may be involved in desensitization. These residues are located either on the intracellular loops or on the intracellular C-terminal tail of the receptor. Current experiments on adenylyl cyclase coupled receptors with a C-terminal tail which has been truncated or mutated indicate that phosphorylation of carboxyl-terminal residues is implicated in rapid desensitization [7]. Data on the role of C-terminal tail in phospholipase C coupled receptors are more conflicting. Carboxyl terminus truncation of the alpha-1b adrenergic receptor, for example, impairs agonist-dependent desensitization [12]. In contrast, experiments with angiotensin AT1a receptor brought evidence for a role of the carboxyl-terminal residues in the mechanisms of rapid desensitization [9], whereas other experiments did not [10]. It is in this respect that the roles of C-terminal tail and protein kinase C activation in desensitization of the V1a receptor was further investigated in this study.

2. Materials and methods

2.1. Construction of the mutant receptor cDNAs and expression vectors

Using the cloned rat V1a receptor [13,14], three different mutant stops were constructed by the Altered Site II in vitro mutagenesis System (Promega) as described by the manufacturer. Mutant oligonucleotides were as follows: S374STOP, 5'-CCATGCTGCACT-GAATGG CGCAGAAATTCGCC-3', Q367STOP, 5'-GCAAGACT-GCGTTCTAAAGTTTCCCATG CTGC-3', H360STOP, 5'-TAC-ATGTTTTTCAG TGGCTAACTCCTGCAAGAC-3'. The first mutant was truncated at serine-374 (Fig. 1) of the carboxyl-terminal region of the receptor (S374STOP). This truncation removed the 51 last amino acids of the C-terminal tail. The second one was truncated at glutamine-367 (Q367STOP) and lacked the 58 C-terminal amino acids. The third one was truncated at histidine-360 (H360STOP) and lacked the 65 C-terminal amino acids.

In a second series, a stretch of nucleotides coding for a 9-amino acid epitope (TagHA) derived from the influenza virus hemagglutinin protein (YPYDVPDYA) was introduced in the three resulting cDNA and the wild type at their N-terminus *CspI* restriction site [15]. This modification in the structure of the receptors did not alter the calcium response induced by vasopressin when expressed in oocytes (data not shown). All cDNA were sequenced before expression.

2.2. Oocyte expression and intracellular calcium detection by photoprotein aequorin

Female albino oocyte-positive *Xenopus laevis* were purchased from Xenopus One (Ann Arbor, MI, USA). Synthetic messenger RNA (cRNA) corresponding to the rat vasopressin V1a receptor (wild type or mutant) [13,14] was produced by in vitro transcription [16] using the mCAP mRNA Capping Kit from Stratagene. Two days after injection of the synthesized messenger cRNA (30 nl at 0.1 mg/ml) in defolliculated oocytes, the photoprotein aequorin (Friday Harbour Laboratory, USA) was injected (20 nl at 1 mg/ml). Changes in the intracellular calcium concentration following addition of vasopressin were detected by light emission [17] using a luminometer (Mini-Lumat LB9506, Berthold). Results were expressed as relative units of

*Corresponding author at address a. Fax: 33 1 69-08-35-70.
E-mail: amorel2@cea.fr

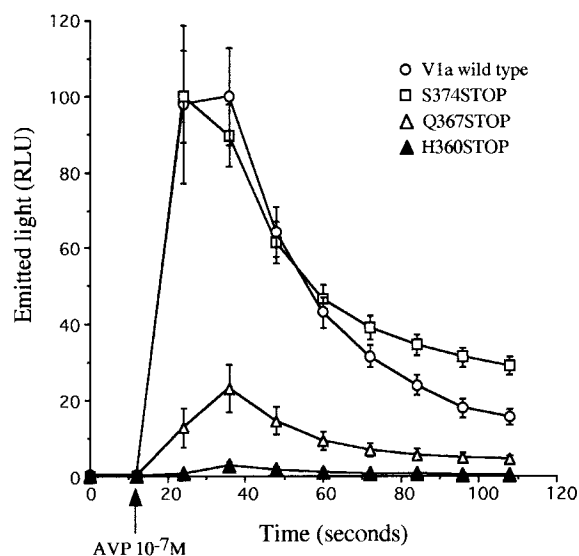


Fig. 3. AVP-dependent intracellular calcium signalling of full length and truncated V1a receptors. Oocytes were injected with cRNA of full length V1a receptor (○), or receptor which C-terminal tail was truncated at serine-374 (□) or glutamine-367 (△) or histidine-360 (▲). Three days later they were stimulated with AVP, and intracellular calcium was detected by aequorin. Mean of 3 different experiments.

desensitization of the V1a receptor was subsequently addressed by direct injection within the oocyte of the catalytic fragment of the PKC. Increasing intracellular PKC activity through such a procedure blunted the response to vasopressin in V1a-expressing oocytes (Fig. 2). These experiments indicate that the activation of PKC by diacylglycerol following occupation of the V1a receptor by vasopressin contributes to its rapid desensitization.

The consequence of protein kinase C activation would be an increased phosphorylation of intracellular proteins. Among proteins which are putative targets for phosphorylation is the receptor itself, as evidenced for the vasopressin V2 receptor [8]. Although not yet demonstrated, it could be hypothesized that the parent V1a receptor is also a target for phosphorylation. In this respect, one may question which phosphorylated site of the receptor should be responsible for desensitization. A predominant role for phosphorylation of the intracellular C-terminal tail has been frequently invoked in these mechanisms of desensitization [8–12]. The strongest experimental evidence for such a role of the C-terminal tail is the lack of rapid desensitization of receptors which C-terminal tail is truncated [7,9,12,20]. The predominant role of the C-terminal region is also supported by the absence of rapid desensitization of receptors lacking the intracytoplasmic C-terminal domain, such as the gonadotropin-releasing hormone receptor [19]. However, other experiments indicated that the carboxyl-

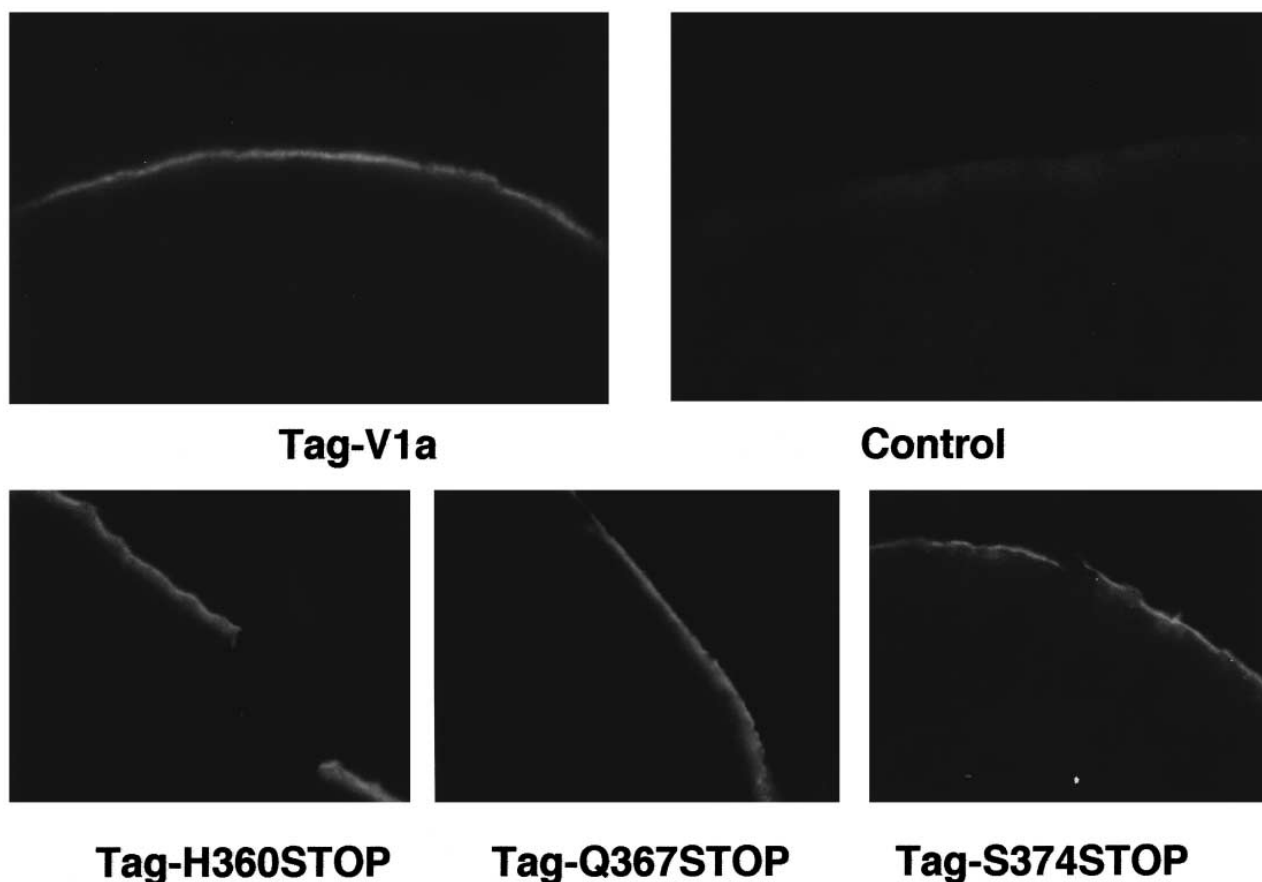


Fig. 4. Immunofluorescent localization of wild-type and carboxy-terminal truncated V1a receptor. Oocytes expressing epitope-tagged wild-type V1a receptor (Tag-V1a) or receptor which C-terminal tail was truncated at serine-374 (Tag-S374STOP), glutamine-367 (Tag-Q367STOP) or histidine-360 (Tag-H360STOP) was treated as described in Section 2. Controls was oocytes expressing non-tagged V1a receptor treated in same condition.

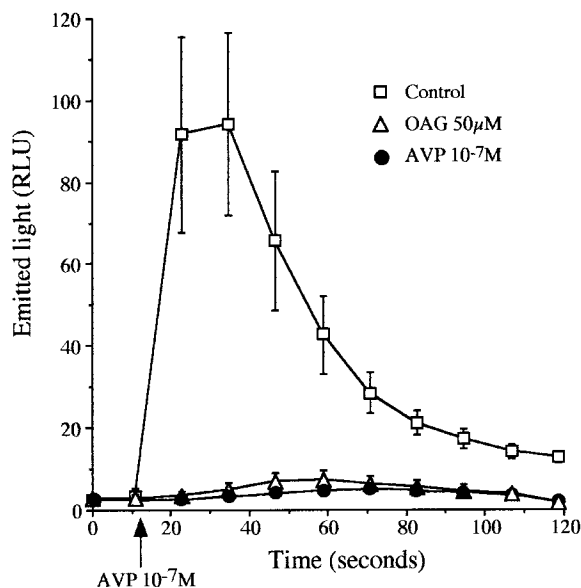


Fig. 5. Desensitization of the V1a receptor truncated on serine-374 (S374STOP). Oocytes were injected with cRNA of the mutated S374STOP vasopressin receptor. Three days later they were stimulated with AVP, and intracellular calcium was detected by aequorin. (□) Control. (△) Oocytes were preincubated in 50 μ M of the cell permeable diacylglycerol analog OAG for 10 min. (●) Oocytes were incubated for 2 min in 10^{-7} M AVP and then extensively washed before new stimulation. Mean of 3 different experiments.

terminal region is not the only site of phosphorylation responsible for acute desensitization. Truncation of the terminal domain of the endothelin A receptor [20], for example, did not affect its rapid desensitization nor the agonist-dependent intracellular increase in calcium concentration.

In the present experiment, the contribution of the C-terminal domain of the V1a receptor to the diacyl glycerol-induced desensitization was investigated with different mutants. The first mutant (H360STOP) was truncated at histidine-360, which removed the last 65 C-terminal amino acids (Fig. 1). When expressed in oocytes, this H360STOP mutant did not elicit calcium response to vasopressin (Fig. 3). Oocytes expressing Q367STOP which lack the 58 C-terminal amino acids showed a small but significant intracellular calcium response after agonist stimulation. In contrast, stimulation of the S374STOP expressing oocyte with AVP elicited a calcium response which was comparable to that observed with the full-length V1a receptor (Fig. 3).

The differences in calcium response of the three mutants may be due to change in signalling capacity of the truncated receptors or to their expression at the membrane of the cell. The presence of the mutant receptors on the surface of the oocytes was evidenced by immunocytochemistry using epitope-tagged HA receptor. Comparable fluorescent signals at the surface of the oocytes were found for the epitope-tagged wild type and the three truncated receptors (Fig. 4). It indicates that all the tested receptors were well expressed at the cell surface. The difference in their calcium response to AVP is therefore likely attributable to the truncated 6 and 11 amino acids including the two adjoining cysteines in positions 372 and 371, and the single cysteine in position 365.

Desensitization of the functional S374STOP mutant deleted with the last 51 terminal amino acids was compared to that of

the V1a wild type. This receptor retained the two cysteines residues in positions 371 and 372 which are suspected to be involved in the palmitoylation of the receptor and its anchorage to the membrane [21]. The AVP induced homologous desensitization performed on this truncated mutant was not different from that of the wild-type receptor (Fig. 5). Preincubation with OAG of the oocytes expressing the S374STOP mutant nearly abolished the calcium response to vasopressin, similarly to the full-length V1a receptor (Fig. 5). It indicates that the last 51 terminal amino acids are not necessary for acute desensitization of the V1a receptor.

In conclusion, V1a receptor undergoes rapid desensitization which can be mimicked by diacylglycerol analog and increased PKC activity. This desensitization is observed with truncated receptor which lacks most of its carboxyl-terminal domain, but which intracellular signalling was comparable to that of the wild-type receptor. It indicates that the C-terminal tail is not required for signal transduction and rapid desensitization of V1a receptor.

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References

- [1] Howl, J. and Wheatley, M. (1995) *Gen. Pharmacol.* 26, 1143–1152.
- [2] Haudorff, W.P., Caron, M.G. and Lefkowitz, R.J. (1990) *FASEB J.* 4, 2881–2889.
- [3] Cantau, B., Guillon, G., Alaoui, M., Chicot, D., Balestre, M. and Devilliers, G. (1988) *J. Biol. Chem.* 263, 10443–10450.
- [4] Caramelo, C., Tsai, P., Okada, K., Briner, V. and Schrier, R. (1991) *Am. J. Physiol.* 260, F46–F52.
- [5] Grier, C., Nambi, P., Aiyar, N. and Crooke, S.T. (1989) *J. Biol. Chem.* 264, 5384–5391.
- [6] Nathanson, M., Burgstahler, A., Orloff, J., Mani, A. and Moyer, S. (1994) *Am. J. Physiol.* 267, C94–C103.
- [7] Lohse, M.L. (1993) *Biochim. Biophys. Acta* 1179, 171–188.
- [8] Innamorati, G., Sadeghi, H., Eberle, A.N. and Birnbaumer, M. (1997) *J. Biol. Chem.* 272, 2486–2492.
- [9] Thomas, W.G., Thekkumkara, T.J., Motel, T.J. and Baker, K.M. (1995) *J. Biol. Chem.* 270, 207–213.
- [10] Balmforth, A.J., Lee, A.J., Bajaj, B.P.S., Dickinson, C.J., Warburton, P. and Ball, S.G. (1995) *Eur. J. Pharmacol.* 291, 135–141.
- [11] Ozelebi, F., Holtmann, M.H., Rentsch, R.U., Rao, R. and Miller, L.J. (1995) *Mol. Pharmacol.* 48, 818–824.
- [12] Lattion, A.L., Diviani, D. and Cotecchia, S. (1994) *J. Biol. Chem.* 269, 22887–22893.
- [13] Morel, A., O'carroll, A.M., Bronstein, M.J. and Lolait, S.J. (1992) *Nature (London)* 356, 523–526.
- [14] Innamorati, G., Lolait, S.J. and Birnbaumer, M. (1996) *Biochem. J.* 314, 710–711.
- [15] Kolodziej, P. and Young, R.A. (1991) *Methods Enzymol.* 194, 508–519.
- [16] Nielsen, D.A. and Shapiro, D.J. (1991) *Nucleic Acids Res.* 14, 5936.
- [17] Sandberg, K., Markwick, A.J., Trinh, D.P. and Catt, K.J. (1988) *FEBS Lett.* 241, 177–180.
- [18] De Chaffoy De Courcelles, D., Roevens, P. and Van Belle, H. (1984) *Biochem. Biophys. Res. Commun.* 123, 589–595.
- [19] McArdle, C., Willars, G., Fowkes, R., Nahorski, S., Davidson, J. and Forrest-Owen, W. (1996) *J. Biol. Chem.* 271, 23711–23717.
- [20] Cyr, C., Rudy, R. and Kris, R. (1993) *J. Biol. Chem.* 268, 26071–26074.
- [21] Morello, J.P. and Bouvier, M. (1996) *Biochem. Cell Biol.* 74, 449–457.