V_{1a}- and V_2-type vasopressin receptors mediate vasopressin-induced Ca^{2+} responses in isolated rat supraoptic neurones

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1. The pharmacological profile of receptors activated by vasopressin (AVP) in freshly dissociated supraoptic magnocellular neurones was investigated using specific V_{1a}- and V_2-type AVP receptor agonists and antagonists.

2. In 97 % of AVP-responding neurones (1–3000 nM) V_{1a} or V_2 receptor type agonists (F-180 and dDAVP, respectively) elicited dose-dependent $[Ca^{2+}]_i$ transients that were suppressed by removal of external Ca^{2+}.

3. The $[Ca^{2+}]_i$ response induced by 1 μM F-180 or dDAVP was selectively blocked by 10 nM of V_{1a} and V_2 antagonists (SR 49059 and SR 121463A, respectively). The response to V_{1a} agonist was maintained in the presence of the V_2 antagonist, and the V_2 agonist-induced response persisted in the presence of the V_{1a} antagonist.

4. The $[Ca^{2+}]_i$ response induced by 1 μM AVP was partially (61 %) blocked by 10 nM SR 121463A. This blockade was increased by a further 31 % with the addition of 10 nM SR 49059. Similarly, the AVP-induced response was partially (47 %) decreased by SR 49059, and a further inhibition of 33 % was achieved in the presence of SR 121463A.

5. We demonstrate that AVP acts on the magnocellular neurones via two distinct types of AVP receptors that exhibit the pharmacological profiles of V_{1a} and V_2 types. However, since V_2 receptor mRNA is not expressed in the supraoptic nucleus (SON), and since V_{1b} receptor transcripts are observed in the SON, we propose that the V_2 receptor agonist and antagonist act on a 'V_2-like' receptor or a new type of AVP receptor that remains to be elucidated. The possibility that V_2 ligands act on the V_{1b} receptor cannot be excluded.

Vasopressin (AVP) magnocellular neurones in the hypothalamus exhibit phasic electrical activity that depends on intrinsic membrane properties and is controlled by extrinsic factors such as plasma osmolarity, blood volume and pressure (see review by Armstrong, 1995), and also by AVP itself. Indeed, a somato-dendritic release of AVP in the extracellular space of hypothalamic nuclei has been demonstrated by several studies (Leng & Mason, 1982; Di Scala-Guenot *et al.* 1987; Pow & Morris, 1989; Landgraf & Ludwig, 1991). Recently, we showed that AVP favours the expression of a specific phasic activity known to optimize the systemic release of AVP (Gouzènes *et al.* 1998*a*). This autocontrol was complex and depended on the initial state of activation of AVP neurones: AVP being excitatory for quasi-silent neurones or inhibitory for highly active neurones. These opposite effects may suggest the involvement of different types of AVP receptors in the control of the phasic pattern. Indeed, results from extracellular recordings performed *in vivo* or from rat hypothalamic slice preparation suggests that AVP-induced changes in firing rate of AVP neurones are mediated through the V_1 receptor (Inenaga & Yamashita, 1986; Dayanithi *et al.* 1995; Ludwig & Leng, 1997; Gouzènes *et al.* 1998*b*). On the other hand, in guinea-pig hypothalamic slices, AVP applied to neurones from the supraoptic nucleus (SON) inhibited spike generation by membrane depolarization (Abe *et al.* 1983). This inhibitory effect of AVP was mimicked by cAMP and enhanced by phosphodiesterase inhibitors (Abe *et al.* 1983), which suggests the involvement of V_2 receptors. Microspectrofluorimetric studies on dissociated supraoptic magnocellular neurones have shown that AVP induces an increase in $[Ca^{2+}]_i$ (Dayanithi *et al.* 1996) that requires an influx of external Ca^{2+} via voltage-dependent channels (Sabatier *et al.* 1997). The $[Ca^{2+}]_i$ response results from the activation of multiple intracellular transduction mechanisms involving the phospholipase C and adenylyl cyclase pathways (Sabatier *et al.* 1998), i.e. the classical

second messenger systems activated by V_1- and V_2-type receptors, respectively (Orloff & Handler, 1967; Stephens & Logan, 1986). The mediation of AVP-induced $[Ca^{2+}]_i$ response by V_{1a} receptors has been previously suggested by the use of a specific V_{1a} receptor antagonist, SR 49059 (Dayanithi *et al.* 1995, 1996). In addition, the expression of V_{1a} and V_{1b} but not V_2 mRNAs in magnocellular neurones has recently been reported (Hurbin *et al.* 1998).

In the present study, we have investigated the physiological significance of AVP receptors on freshly dissociated neurones from SON by evaluating the $[Ca^{2+}]_i$ responses evoked by specific V_{1a} and V_2 receptor agonists, F-180 and dDAVP, respectively. A preliminary report of this work has appeared in abstract form (Gouzènes *et al.* 1998*c*).

METHODS

Preparation of dissociated magnocellular neurones

For each experiment, two adult male Wistar rats (Iffa-Credo, France; 150–300 g) were killed by decapitation with a guillotine following the guidelines laid down by the French/European ethical committee (licence no. 005039/04-08-1992/G. Dayanithi), and supraoptic neurones were isolated as previously described (Lambert *et al.* 1994) with modifications (Sabatier *et al.* 1997). Briefly, the isolated SONs were incubated for 45 min at 25 °C in oxygenated Locke buffer supplemented with deoxyribonuclease I (0·5 mg ml^{-1}), proteases X and XIV (1 mg ml^{-1} each). All enzymes and other standard chemicals were purchased from Sigma (USA). The Locke buffer contained (mM): 140 NaCl, 5 KCl, 1·2 KH_2PO_4, 1·2 $MgSO_4$, 1·8 $CaCl_2$, 10 glucose and 10 Hepes; pH 7·2, and the osmolarity was 295–300 mosmol l^{-1}. Tissue pieces were then rinsed with Locke buffer and submitted to mechanical trituration. Dissociated cells were plated onto glass coverslips and incubated for 1 h at 37 °C with Locke buffer supplemented with 1·5 μM fura-2 AM (acetoxymethyl ester form of fura-2) plus 0·1% (w/v) Pluronic F-127 (Molecular Probes Inc., Eugene, OR, USA). $[Ca^{2+}]_i$ measurements were performed with the fast fluorescence photometer system (FFP; Zeiss, Oberkochen, Germany; for details, see Dayanithi *et al.* 1996). Only cells with dendritic processes and a soma diameter more than 12 μm were used, since these large neurones have been previously demonstrated to contain either AVP or OT (Oliet & Bourque, 1992; Lambert *et al.* 1994). Control and test solutions were applied in the vicinity of the recorded neurone (1 mm) using a gravity-driven perfusion system with a flow rate of 100 μl min^{-1}. Complete solution change around the neurone was achieved within 2 s. The free Ca^{2+}/EGTA buffer contained (mM): 0·1 EGTA, 140 NaCl, 5 KCl, 1·2 KH_2PO_4, 1·2 $MgSO_4$, 10 glucose and 10 Hepes; pH 7·2, and the osmolarity was 295–300 mosmol l^{-1}. The Ca^{2+} concentration in the Ca^{2+}-free EGTA buffer was approximately 100 nM, which corresponds to the resting $[Ca^{2+}]_i$ typically observed in neurones.

Test compounds

The V_2 receptor agonist dDAVP (1-deamino-8-D-AVP), the V_{1a} receptor agonist [Phe^2,Orn^8]vasotocin and the V_2 receptor antagonist desGlyNH_2-d(CH_2)$_5$-[D-Ile^2,Ile^4]AVP were generously given by Dr C. Barberis (INSERM U-469, Centre de Pharmacologie-Endocrinologie, Montpellier, France). The V_{1a} receptor antagonist, SR 49059 ((2*S*)1[(2*R*,3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulphonyl))-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide), and V_2 receptor antagonist SR 121463A (1-[4-(*N*-tert-butylcarbamoyl)-2-methoxybenzene sulphonyl]-5-ethoxy-3-spiro-[4-(2-morpholino ethoxy)cyclohexane]-indol-2-one, fumarate; equatorial isomer) were gifts from Sanofi Recherche (Centre de Toulouse, Toulouse, France). The V_{1a} agonist F-180 (Hmp-Phe-Ile-Hgn-Asn-Cys-Pro-Dab(Abu)-Gly-NH_2) was kindly supplied by Dr J. L. Junien (Ferring France, Gentilly, France). AVP and OT were purchased from Boehringer Mannheim (Meylan, France). Concentrated stock solutions of substances were prepared in DMSO (SR 49059 and SR 121463A) or distilled water (dDAVP) and stored at −20 °C.

Data analysis

In the figures, the $[Ca^{2+}]_i$ traces represent the ratio values between two fluorescence wavelengths (A: 340 nm/B: 380 nm). The dose–response relationship (Fig. 3) was constructed with peak amplitude values all normalized to that of the response induced by 100 nM agonist. The dDAVP dose–response curve was fitted with a single Hill equation:

$$Y = Y_{max}/(1 + (EC_{50}/X)^{n_H}).$$

The dose–response curve for F-180 was fitted with a double Hill equation:

$$Y = Y_{max_1}/(1 + (EC_{50(1)}/X)^{n_{H(1)}}) + Y_{max_2}/(1 + (EC_{50(2)}/X)^{n_{H(2)}}),$$

where Y is the normalized peak amplitude (%), Y_{max} is the maximum normalized peak amplitude (%), EC_{50} is the concentration required to obtained the half-maximum peak amplitude (nM), X is the concentration of the agonist (nM) and n_H is the Hill coefficient.

Fits were made using Origin software (Microcal, Northampton, USA). All values are expressed as means ± S.E.M., and the results were analysed using Student's paired t test.

RESULTS

This study was performed on a total of 206 AVP-responding neurones (from 40 preparations) that displayed stable resting $[Ca^{2+}]_i$ levels. Those neurones initially displaying spontaneous $[Ca^{2+}]_i$ oscillations were discarded. Consistent with our previous study (Dayanithi *et al.* 1996), we also observed that neurones responding to OT ($n = 13$) were insensitive to AVP (1 μM), F-180 (V_{1a} receptor agonist, 1 μM) and dDAVP (V_2 receptor agonist, 1 μM).

Characteristics of the agonist-induced $[Ca^{2+}]_i$ increase

The $[Ca^{2+}]_i$ responses to F-180 and dDAVP (1 μM) were compared with those induced by AVP (1 μM) in 58 neurones (Fig. 1). Most of these AVP-responding neurones (72%) exhibited $[Ca^{2+}]_i$ responses to both F-180 and dDAVP, 16% responded to F-180 only, 9% responded to dDAVP only, and 3% responded neither to F-180 nor to dDAVP. Responses to F-180 and AVP displayed a short latency (8·4 ± 0·4 s and 7·4 ± 0·5 s, $n = 51$), a comparable amplitude (peak $[Ca^{2+}]_i$ rise evoked by F-180 represented 119 ± 17% of that induced by AVP; $n = 51$), and similar profiles, although individual variations were noted. Conversely, the response to dDAVP markedly differed from that observed in response to AVP (see Fig. 1), with a significantly longer latency (29·3 ± 7·3 *vs.* 7·3 ± 0·5 s, $P < 0·005$, $n = 47$), a smaller peak amplitude (48 ± 6% of the AVP response; $n = 47$) and more varied profiles (see examples in Fig. 4).

Twelve AVP-responsive neurones were subjected to four successive applications of F-180 (1 μM given every 2–3 min; Fig. 2*A*). In all neurones tested, F-180 induced reproducible

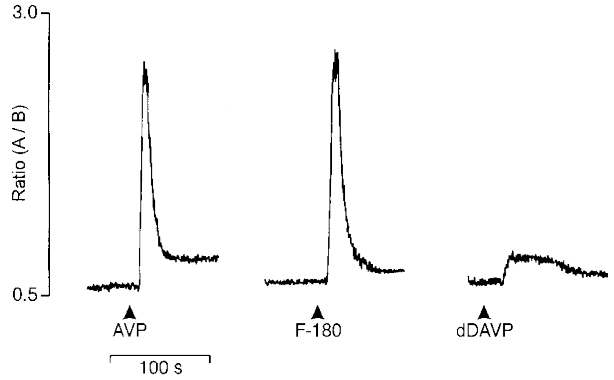

Figure 1. The traces represent the $[Ca^{2+}]_i$ responses induced by F-180 (V_{1a} agonist) and dDAVP (V_2 agonist) in a selected neurone responding to AVP

The three substances were tested at 1 μM. Traces are expressed as the ratio between two fluorescence wavelengths: 340 nm (A)/380 nm (B).

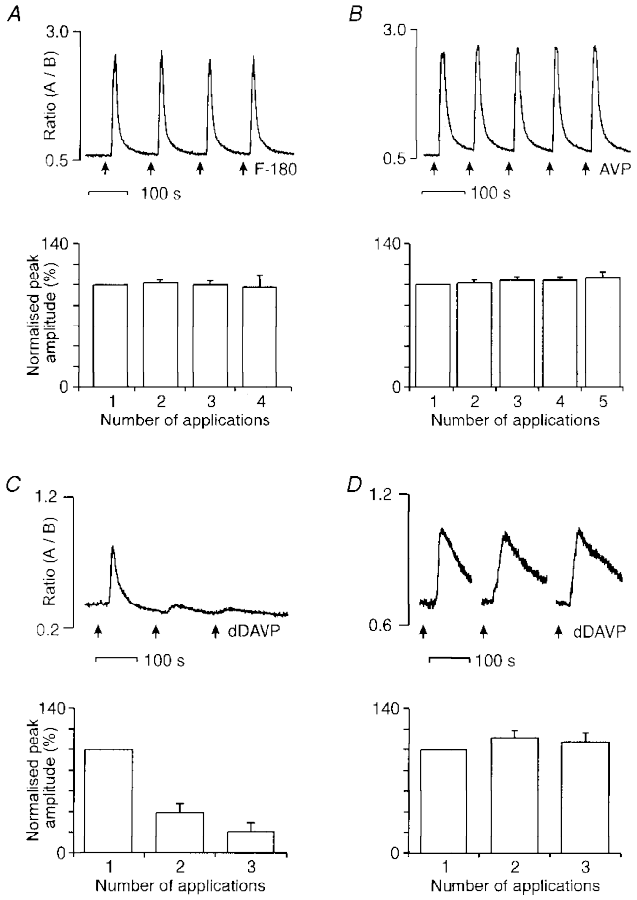

Figure 2. The $[Ca^{2+}]_i$ responses to repeated applications of 1 μM F-180 (*A*), AVP (*B*) and dDAVP (*C* and *D*)

In each case, the upper Ca^{2+} traces show individual examples of responses. The lower histograms correspond to the mean values of the responses obtained in several neurones. The peak amplitude of the successive responses were expressed as a percentage of the first peak amplitude (% normalized peak amplitude). *A* and *B*, reproducibility of the responses to successive application of F-180 ($n = 15$ neurones) and AVP ($n = 5$ neurones). *C*, tachyphylaxis obtained with dDAVP applications every 2 min ($n = 10$ neurones). *D*, a longer interval (8–10 min) between successive applications of dDAVP prevented tachyphylaxis ($n = 6$ and 3 for the second and the third applications, respectively).

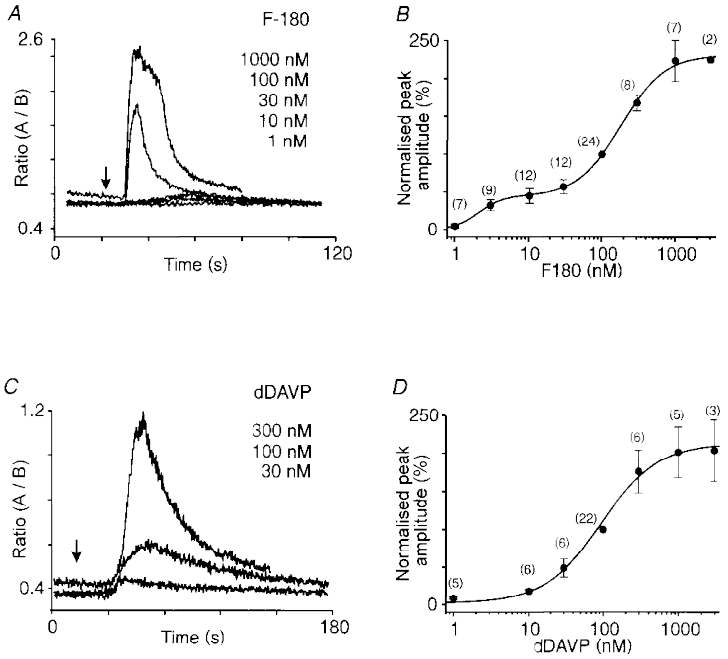

Figure 3. Dose-dependent effect of V_{1a} and V_2 agonists

The $[Ca^{2+}]_i$ traces in *A* and *B* illustrate the responses to increasing concentrations of F-180 and dDAVP, respectively. The graphs in *C* and *D* show the dose–response relationships of the mean $[Ca^{2+}]_i$ transients induced by F-180 ($n = 24$) and dDAVP ($n = 22$), respectively. For each cell tested, values of peak amplitude responses were expressed as a percentage of that induced by 100 nM agonist (normalized peak amplitude, %).

$[Ca^{2+}]_i$ responses. Similarly, the $[Ca^{2+}]_i$ responses to successive applications of AVP (1 μM given every 2–3 min) were generally stable in each of the five neurones tested (Fig. 2*B*). In contrast, the responses to successive applications of dDAVP (1 μM, given every 2–3 min) showed a strong tachyphylaxis, as the third application of dDAVP induced a response with a peak amplitude reaching only $21 \pm 9\%$ of that to the first test ($n = 10$; Fig. 2*C*). However, tachyphylaxis could be prevented by increasing the delay between applications to 10 min ($n = 6$; see Fig. 2*D*). Consequently, all subsequent experiments with dDAVP were performed with one application every 10 min.

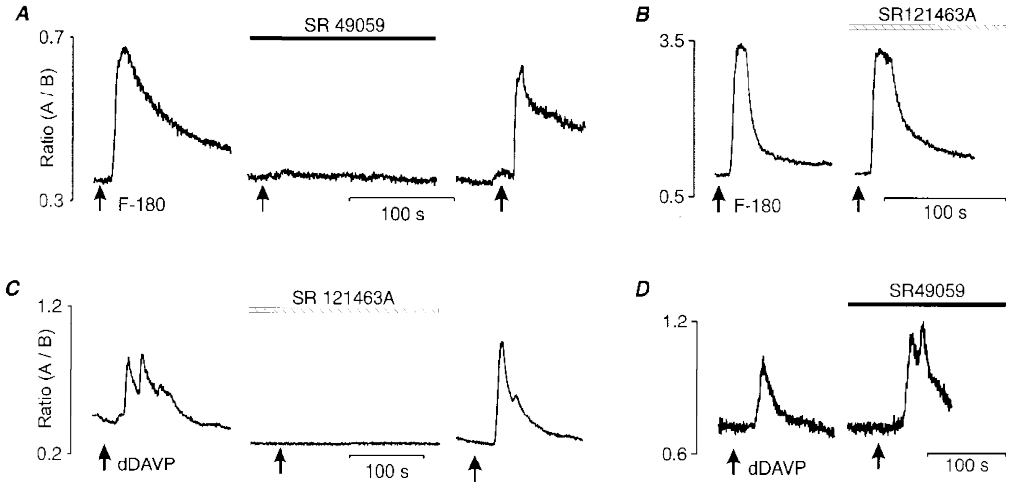

Figure 4. Specificity of the responses to V_{1a} and V_2 receptor agonists

The $[Ca^{2+}]_i$ transient elicited by F-180 (1 μM) was totally blocked by 10 nM of the specific V_{1a} receptor antagonist (SR 49059, *A*), but was maintained in the presence of 10 nM V_2 receptor antagonist (SR 121463A, *B*). Similarly, response to dDAVP (1 μM) was abolished after pre-incubation with 10 nM SR 121463A (*C*) but persisted in the presence of 10 nM SR 49059 (*D*). *A* and *B* show the reversibility of the blockade induced by each antagonist.

Dose dependence of the responses

To evaluate the dose–response relationships for the two agonists (F-180 and dDAVP), the neurones were systematically tested with each compound at 100 nM. Two or three other concentrations (from 1 nM to 3 μM) were applied to the same neurone in a random order. Threshold concentration was 3 nM for F-180 and 30 nM for dDAVP. The dose–response curve obtained with F-180 (Fig. 3*B*) was best fitted with the sum of two Hill equations ($EC_{50} = 2{\cdot}1 \pm 0{\cdot}5$ and 184 ± 18 nM, $n_H = 2{\cdot}64 \pm 1{\cdot}24$ and $1{\cdot}49 \pm 0{\cdot}21$, respectively), whereas the dose–response curve with dDAVP (Fig. 3*D*) presented a single slope only ($EC_{50} = 95 \pm 13$ nM and $n_H = 1{\cdot}15 \pm 0{\cdot}15$).

Specificity of the $[Ca^{2+}]_i$ responses

The specificity of the two agonists was studied using specific V_{1a} and V_2 receptor antagonists (Fig. 4). The $[Ca^{2+}]_i$ response to F-180 was inhibited by $97 \pm 2\%$ by 10 nM SR 49059, a specific V_{1a} receptor antagonist ($n = 6$; Fig. 4*A*). SR 49059 did not affect the resting $[Ca^{2+}]_i$ level and inhibition of the response to F-180 recovered after wash-out of the antagonist. Similarly, the responses to 100 nM [Phe^2,Orn^8]vasotocin, another V_{1a} receptor agonist, were suppressed by 10 nM SR 49059 (by $97 \pm 1\%$; $n = 6$; figure not shown). In contrast, the mean amplitude of the response induced by F-180 was unaffected by the presence of 10 nM SR 121463A, a specific V_2 receptor antagonist, ($105 \pm 5\%$ of respective control response; $n = 10$; Fig. 4*B*; $P = 0{\cdot}59$). The same concentration of SR 121463A, reversibly inhibited (by $91 \pm 5\%$) the response to dDAVP ($n = 9$; Fig. 4*C*; $P < 0{\cdot}001$). The mean amplitude of the $[Ca^{2+}]_i$ rise induced by dDAVP was unchanged in the presence of the V_{1a} antagonist, SR 49059 ($127 \pm 15\%$ of the respective control response, $n = 5$; Fig. 4*D*; $P = 0{\cdot}09$).

The effects of the V_{1a} and V_2 receptor antagonists were also tested on the $[Ca^{2+}]_i$ rise induced by 1 μM AVP (Fig. 5). The V_2 receptor antagonist SR 121463A (10 nM) and desGlyNH$_2$-d(CH$_2$)$_5$-[D-Ile^2,Ile^4]AVP (100 nM) reduced the response to AVP by $61 \pm 6\%$ ($n = 9$; see example in Fig. 5*A*; $P < 0{\cdot}001$) and by $53 \pm 15\%$ ($n = 4$; figure not shown), respectively. A near complete inhibition ($92 \pm 3\%$; $n = 9$; $P < 0{\cdot}001$) of the AVP response was obtained by combined application of SR 49059 and SR 121463A (see example in Fig. 5*A*). Similarly, the response to AVP was reduced by $46 \pm 7\%$ ($n = 5$) with SR 49059 given alone, and further decreased up to $79 \pm 4\%$ ($n = 5$) by subsequent addition of SR 121463A (see example in Fig. 5*B*).

Origin of the Ca^{2+} rise induced by the agonists

The responses induced by V_{1a} or V_2 receptor agonists were also tested in Ca^{2+}-free EGTA buffer to investigate the dependence upon external Ca^{2+} (Fig. 6*A* and *B*). The $[Ca^{2+}]_i$ responses induced by 1 μM F-180 or dDAVP were completely abolished in a Ca^{2+}-free EGTA buffer ($n = 10$ and 4, respectively), suggesting that extracellular Ca^{2+} is necessary for the action of either agonist.

DISCUSSION

In this study, the use of specific agonists and antagonists allowed us to define the pharmacological properties of AVP receptors expressed by AVP-responding neurones of the SON (i.e. vasopressinergic neurones, see Dayanithi *et al.* 1996). In most of these neurones, both the V_{1a} and V_2 receptor agonists, F-180 and dDAVP, induced dose-dependent rises in $[Ca^{2+}]_i$ that were totally abolished in a Ca^{2+}-free EGTA buffer and inhibited by their respective selective antagonist. The response to AVP was decreased by

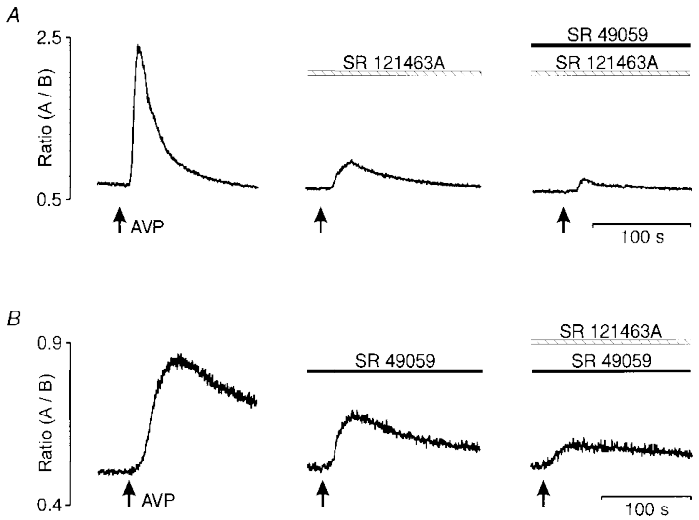

Figure 5. Simultaneous activation of V_{1a}- and V_2-type receptors by AVP

Pre-incubation with 10 nM SR 49059 or SR 121463A (middle panels in *A* and *B*) partially inhibited the $[Ca^{2+}]_i$ response normally induced by AVP (left panels in *A* and *B*). At the same dose, each antagonist was shown to prevent the $[Ca^{2+}]_i$ transients elicited by the respective agonist (see Fig. 4). Blockade of AVP response was nearly complete when the two antagonists were pre-incubated simultaneously (right panels in *A* and *B*).

both V_{1a} and V_2 receptor antagonists, suggesting the simultaneous activation of V_{1a}- and V_2-type receptors by AVP. Because AVP was previously shown to induce an increase in $[Ca^{2+}]_i$ in AVP-sensitive neurones (Dayanithi *et al.* 1996), and because F-180 and dDAVP elicited responses only in AVP-sensitive neurones, these agonists are most likely to act on AVP neurones.

Characteristics of the responses to V_{1a} and V_2 receptor agonists

The $[Ca^{2+}]_i$ responses to the V_{1a} receptor agonist, F-180, and to AVP were similar, i.e. the responses occurred with a short latency and did not desensitize. The reproducibility of the AVP responses could be due to the use of a rapid and short duration of application of the peptide rather than static incubation over long periods, which might have caused desensitization as previously reported (Dayanithi *et al.* 1996). The dose–response relationship to F-180 was best described by a double Hill function, which suggests the activation of two receptor types with different affinities. Such a hypothesis is plausible since the existence of low- and high-affinity states for the V_{1a} receptor has been demonstrated in smooth muscle cells (Stassen *et al.* 1987; Gopalakrishnan *et al.* 1991) and in the liver (Gopalakrishnan *et al.* 1988). The biphasic dose–response to F-180 could result from complex interactions between different second messenger pathways (see Sabatier *et al.* 1998), which could modulate the activation of Ca^{2+} channels. Indeed, it has been shown that the rise in $[Ca^{2+}]_i$ activates the neurospecific type I calmodulin-sensitive adenylyl cyclase, which in turn exerts a positive feedback regulation of Ca^{2+} channels by cAMP-dependent protein kinase (Choi *et al.* 1993). In the present work, $[Ca^{2+}]_i$ responses induced by F-180 were also abolished in a free Ca^{2+} buffer, suggesting the involvement of a Ca^{2+} influx but not excluding an additional participation of Ca^{2+} from internal stores, as has been previously demonstrated for AVP (Dayanithi *et al.* 1996; Sabatier *et al.* 1997). In cultured hippocampal (Brinton *et al.* 1994) and cortical (Son & Brinton, 1998) neurones, the absence of Ca^{2+} in the extracellular medium has been shown to abolish the rise in $[Ca^{2+}]_i$ induced by a V_1 vasopressin receptor agonist, but the inositol-1-phosphate formation persisted. These studies indicate that the absence of extracellular Ca^{2+} does not affect the binding characteristics of the V_1 vasopressin receptor. They further showed that the activation of phospholipase C, and thus the production of the phosphatidylinositols, was involved in the response to the V_1 receptor agonist, but was not sufficient to increase the $[Ca^{2+}]_i$.

Conversely, $[Ca^{2+}]_i$ responses induced by the V_2 receptor agonist, dDAVP, were smaller and delayed compared with those elicited by either AVP or F-180, and the dose–response curve showed a single slope. Figure 3 clearly illustrates the fact that the latency of the responses to dDAVP and F-180 does not depend on the concentrations that induced a significant response (i.e. over 30 nM). Indeed, the latency to dDAVP response is similar from 30 nM (i.e. below the half-maximal dose) to 300 or 1000 nM (illustration not shown), and is longer than the latency to F-180 responses from 100

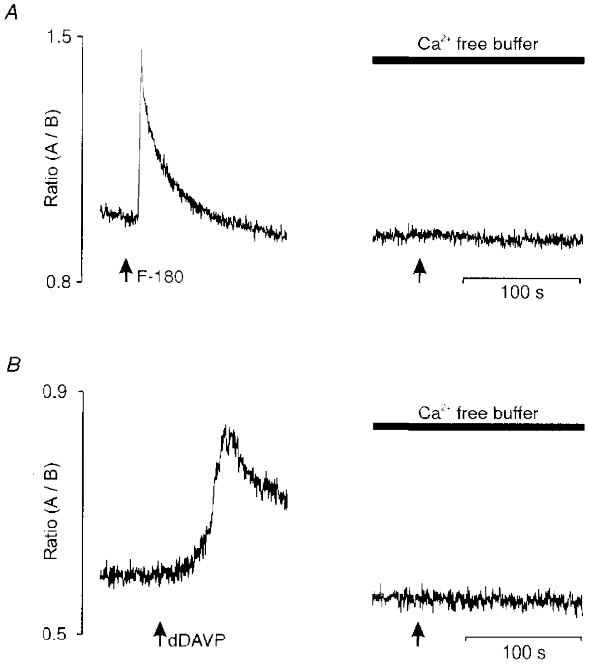

Figure 6. Block of $[Ca^{2+}]_i$ responses to V_{1a} and V_2 receptor agonists by suppression of extracellular Ca^{2+}

In *A* and *B*, the left panel represents the $[Ca^{2+}]_i$ response induced by 1 μM F-180 and dDAVP, respectively, in the presence of normal Locke buffer containing 1·8 mM external Ca^{2+}. Pre-incubation in a Ca^{2+}-free EGTA buffer (see Methods) for 10 min abolished the response to each agonist (right panels).

to 1000 nM. The threshold of the dDAVP response was 30 nM, i.e. much higher than the concentration (0·7 nM) required to elicit $[Ca^{2+}]_i$ changes in rat medullary collecting tubules (Champigneulle *et al.* 1993). The tachyphylaxis obtained with a short interval between applications of dDAVP seems to be directly linked to the agonist characteristics rather than the receptor itself, since AVP showed reproducible responses. In the present study, dDAVP triggered a rise in $[Ca^{2+}]_i$ that was abolished in a Ca^{2+}-free buffer, as has been demonstrated in rat inner medullary collecting tubes (Ishikawa & Saito, 1990; Naruse, 1992). This result supports the activation of Ca^{2+} influx by V_2 receptors, although the possibility of an additional mobilization of Ca^{2+} from internal stores is not excluded, as is the case for AVP (Dayanithi *et al.* 1996). Indeed, mobilization of Ca^{2+} from intracellular stores by V_2-like receptors has been observed in a subpopulation of neurones from circumventricular organs (Jursak *et al.* 1995). Whatever the origin of the Ca^{2+}, the transduction pathways responsible for the $[Ca^{2+}]_i$ rise induced by dDAVP in the present work are still unknown. The involvement of the cAMP pathway is an interesting issue, since typically the V_2 receptor has been reported to be linked to activation of adenylyl cyclase (see Zingg, 1996).

Which AVP receptors subtypes are present on magnocellular neurones?

Based on their pharmacological profile and transduction signals, three types of vasopressin receptors have been described in the periphery: V_{1a}- and V_{1b}-types, both coupled to the phospholipase C, and V_2-type, coupled to the adenylate cyclase (see Zingg, 1996). The V_{1a} receptor agonist, F-180, is a selective vasoconstrictor and appears to be the most specific ligand of the V_{1a} receptor available to date (Aurell *et al.* 1991; Bernadich *et al.* 1998). Similarly, SR 49059 is a potent, non-peptidic antagonist of rat and human vasopressin V_{1a} receptors (Serradeil-Le-Gal *et al.* 1993; Guillon *et al.* 1995). The dDAVP and SR 121463A are respectively considered as a specific agonist (Manning *et al.* 1976) and a selective antagonist of the V_2 receptor (Serradeil-Le-Gal *et al.* 1996).

The affinities of AVP for peripheral vasopressin receptors in the rat are: $K_d = 1{\cdot}7$ nM for the V_{1a} receptor and $K_d = 0{\cdot}4$ nM for the V_2 receptor. The affinities of the V_2 agonist dDAVP are: $K_d = 250$ nM for the V_{1a} receptor and $K_d = 0{\cdot}3$ nM for the V_2 receptor. Concerning the V_{1a} agonist, F-180, the relative affinities studied in COS cells are: $K_d = 5{\cdot}8$ nM for the V_{1a} receptor and $K_d > 10\,000$ nM for the V_2 receptor. Therefore, the V_{1a} agonist presents a very good selectivity between V_{1a} and V_2 receptors (see Barberis & Tribollet, 1996).

The antagonists we used are very potent and selective. The affinities of the V_{1a} antagonist for peripheral receptors in the rat are: $K_d = 2$ nM for the V_{1a} receptor and $K_d = 275$ nM for the V_2 receptor. The affinities of V_2 antagonist SR 121463A are: $K_d = 10\,600$ nM and $K_d = 1{\cdot}42$ nM for V_{1a} and V_2 receptors, respectively (see Serradeil-Le-Gal *et al.* 1996). At the concentrations we used, the V_2 agonist (1 μM) is able to bind to V_{1a} receptors, but in our experiments the response to V_2 agonist is not affected by the V_{1a} antagonist (10 nM), while it is totally blocked by the V_2 antagonist (10 nM). However, we also have to consider that the relative affinities and even the nature of vasopressin receptors expressed in the central nervous system are not well known and remain to be clearly elucidated.

In the present work, 10 nM SR 49059 abolished the $[Ca^{2+}]_i$ response to F-180, but not that to dDAVP. Similarly, 10 nM SR 121463A completely suppressed the $[Ca^{2+}]_i$ response to dDAVP but not that to F-180. These results argue for the presence of two distinct types of AVP receptors on the cell body of the large majority of AVP-responding neurones. These two AVP receptor types appear to be simultaneously activated by AVP, since the selective V_{1a} and V_2 receptor antagonists show additivity in the blockade of the AVP-induced response. In agreement with these results, we recently demonstrated that the $[Ca^{2+}]_i$ rise induced by AVP in AVP magnocellular neurones involved both the phospholipase C and adenylyl cyclase intracellular pathways (Sabatier *et al.* 1998), the established transduction pathways of V_{1a} and V_2 receptors, respectively. Furthermore, AVP has been reported to induce a rise in cAMP levels in neurones from the SON, and cAMP to mimic the electrophysiological effect of AVP on magnocellular neurones (Abe *et al.* 1983).

Taken together, these results clearly suggest a possible expression of V_{1a} and V_2 receptors on AVP magnocellular neurones. However, a different picture arose from a recent study from our laboratory using RT-PCR, which showed the presence of V_{1a} and V_{1b} receptor mRNAs, but not the presence of the V_2 transcript, in the SON (Hurbin *et al.* 1998). By *in situ* hybridization, these authors further visualized V_{1a} and V_{1b} mRNAs in AVP magnocellular neurones. It is of interest to note that a similar observation was made by Vaccari and collaborators (Vaccari *et al.* 1998) that the supraoptic nucleus expresses V_{1b} transcripts. With regard to the V_{1a} receptor, these results fit with the present study. For the V_{1b} receptor, the lack of specific ligands limits its pharmacological characterization (Barberis & Tribollet, 1996). Nevertheless, since a residual AVP response persisted in the presence of both V_{1a} and V_2 receptor antagonists, we suggest the presence of another receptor type on AVP magnocellular neurones, which could be the V_{1b} receptor type. With regard to V_2 receptors, the results obtained with RT-nested PCR (Hurbin *et al.* 1998) are in apparent contradiction with the present pharmacological data. Similarly, the V_2-type receptor has not been identified in the brain by either autoradiography or immunocytochemistry (see Burbach *et al.* 1995), whereas several pharmacological studies attest for its central involvement in different functions such as the proconvulsive effect of AVP (Croiset & De Wied, 1997), analgesia (You *et al.* 1995), cardiovascular regulation (Lowes *et al.* 1993) and Ca^{2+} responses in circum-

ventricular organs (Jursak *et al.* 1995). Thus the nature of the AVP receptor mimicking the V_2-type pharmacology or activating the cAMP pathway remains to be elucidated.

In conclusion, our results demonstrate that AVP-responding neurones express at least two distinct AVP receptors that have the pharmacological profile of V_{1a} and V_2 receptors. The existence of functional V_{1a}-type receptors is corroborated by the presence of V_{1a} receptor mRNA in AVP neurones, but this is not the case for the receptor exhibiting the pharmacological profile of the V_2 type. The nature of this V_2-like receptor has yet to be defined. There is no direct solution offered in this paper for the discrepancies between the data (absence of V_2 receptor mRNA *vs.* V_2 agonist-induced $[Ca^{2+}]_i$ response) obtained within our research group. However, we can clearly hypothesize that the V_2-agonist could act on a different type (V_2-like?) or a new type of receptor that may have a similar pharmacology to 'classical' V_2-type receptors. Nevertheless, it appears that AVP magnocellular neurones express multiple AVP receptor types that could account for the complex effects of AVP on the electrical activity of AVP neurones observed *in vivo* (Gouzènes *et al.* 1998*a*). The inhibitory effect of AVP seems to be mediated via the V_{1a} receptor, as revealed by the excitatory effect of the V_{1a} receptor antagonist on the phasic pattern of AVP neurones (Dayanithi *et al.* 1995; Ludwig & Leng, 1997; Gouzènes *et al.* 1998*b*). In contrast, Inenaga & Yamashita (1986) have suggested that the activation of V_1 receptors results mostly in neuronal excitation. However, our preliminary results suggest that the excitatory effect could be mediated by V_2-type receptors (Gouzènes *et al.* 1998*b*). A pharmacological characterization of all receptors activated by AVP expressed on AVP magnocellular neurones, as well as intracellular pathways activated by the different agonists, is under investigation. This will help in defining the exact nature of the receptor types activated by AVP on AVP magnocellular neurones.

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