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Membrane-Delimited G Protein-Mediated Coupling between V_{1a} Vasopressin Receptor and Dihydropyridine Binding Sites in Rat Glomerulosa Cells

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SUMMARY

In rat glomerulosa cells, vasopressin stimulates intracellular calcium mobilization via at least two distinct mechanisms: the release of calcium from inositol-1,4,5-P_3-sensitive stores and the activation of transmembrane calcium influx. In this study, we focused on the second mechanism through three experimental approaches. By videomicroscopically examining Fura-2-loaded cells, we demonstrate that vasopressin induces a dose-dependent and receptor-mediated calcium influx fully inhibited by either 1 μM nifedipine or a pertussis toxin pretreatment and potentiated by 1 μM BAY K 8644. Patch-clamp experiments also indicate that vasopressin stimulates L-type calcium current by 87% and only weakly inhibits T-type calcium current. To further characterize the coupling between the vasopressin receptor and the dihydropyridine calcium channel, we performed binding studies using tritiated nitrendipine. With this technique, we showed that on intact cells, vasopressin is able to increase the specific binding of tritiated nitrendipine in a dose-dependent manner (K_{act} = 2 nM). Pharmacological studies using a series of vasopressin analogs revealed that this effect is mediated via a V_{1a} vasopressin receptor subtype. Furthermore, the vasopressin-stimulated nitrendipine binding was sensitive to pertussis toxin pretreatment, which affected only the maximum binding capacity of nitrendipine-binding sites. More interestingly, we demonstrate that vasopressin still increases nitrendipine binding to plasma membrane preparation and that GTP is absolutely necessary for such a hormonal effect. Altogether, these data confirm the existence of a tight and direct coupling between the V_{1a} vasopressin receptor and a dihydropyridine calcium channel via a pertussis toxin-sensitive G protein.

Steroid secretion by the ZG of the adrenal cortex is under multifactorial regulation. ACTH, Ang II, and K^+ ions represent the most important physiological secretagogues (1). However, other neuromodulators (e.g., serotonin, acetylcholine, epinephrine, dopamine) or peptidic hormones (e.g., endothelin, vasoactive intestinal peptide, AVP) also stimulate adrenal steroid secretions (2, 3). Both regulatory molecules modulate the basal secretion of ZG and/or fasciculata cells by interacting with specific receptors and producing multiple intracellular messengers. For example, ACTH stimulates adenylyl cyclase activity and, thus, PKA (4). In contrast, Ang II and AVP stimulate a phosphatidyl inositol-4,5-bisphosphate phospholipase C that is responsible for intracellular calcium mobilization from intracellular pools via the generation of Ins(1,4,5)P_3 (5) and activation of protein kinase C via the accumulation of diacylglycerol (6). In addition to these well-established second messenger cascades, the involvement of calcium influx represents a crucial parameter for adrenal steroid secretion. In the absence of external calcium, ACTH, Ang II, and AVP are unable to stimulate aldosterone secretion on human, rat, and bovine ZG cells (7–9) or glucocorticoid production on human or rat zona fasciculata cells (7, 8). Hormonal regulation of calcium channel activity is involved in these mechanisms: 1) ACTH, Ang II, and AVP stimulate calcium influx on human, bovine, and rat adrenocortical cells (7, 8, 10); 2) dihydropyridine antagonists such as nifedipine or nitrendipine partially inhibit the steroidogenic effect of ACTH and Ang II on rat and bovine adrenocortical cells (8–10); and 3) patch-clamp experiments performed on rat and bovine adrenocortical cells clearly demonstrate that both

ABBREVIATIONS: ACTH, adrenocorticotropin; AVP, arginine vasopressin; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; Ins(1,4,5)P_3, inositol-1,4,5-trisphosphate; PTX, pertussis toxin; PDBu, phorbol-12,13-dibutyrate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; AM, acetoxymethyl ester; BSA, bovine serum albumin; HBSS, Hanks' balanced salt solution; Ang II, angiotensin II; CVPA, β-mercapto-β,β-cyclopentamethylene-propionyl-Tyr-(Et)-Phe-Val-Asn-Cys-Pro-Arg-Gly-NH_2; LVPA, Phaa-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2; ZG, zona glomerulosa; MEM, minimal essential medium.

Ang II and ACTH receptors modulate the activity of L- and T-type calcium channels (10–13).

Nevertheless, the precise mechanisms by which steroidogenic hormones regulate calcium channel activities remained unclear. ACTH is known to primarily activate adenylyl cyclase activity, which in turn activates PKA. PKA then phosphorylates dihydropyridine-sensitive calcium channels, leading to the stimulation of a calcium influx (8, 11). However, this scheme is probably more complex because on both rat and human adrenocortical cells, the initial intracellular cAMP rise induced by ACTH is highly dependent on the presence of calcium in the extracellular medium (4, 14). It is also clear that the Ang II- and AVP-stimulated calcium influxes are abolished if rat or bovine adrenocortical cells are preincubated with PTX (15, 16). In contrast, Ang II- and AVP-stimulated phospholipase C activities are controlled by a PTX-insensitive G protein (15). Together, these data suggest that steroidogenic hormones may regulate calcium influx via distinct mechanisms.

In ZG cells, the nature of the coupling among the hormonal receptor, the G protein, and the calcium channel remained unclear. Many experimental findings favored indirect coupling mechanisms involving the second messengers generated on hormonal stimulation: 1) PKA, which was activated by cAMP, stimulates L-type calcium channels in rat adrenal cells and induces a sustained calcium influx (8, 11, 14); 2) the depletion of intracellular calcium stores by Ins(1,4,5)P_3 or thapsigargin induces calcium influx via diffusible unidentified messenger or messengers in adrenal cells and in vascular endothelial cells (17–19); and 3) PKC, which was secondarily activated by diacylglycerol, modulates dihydropyridine-sensitive calcium channel activity (20). We cannot exclude the possibility of a direct coupling between hormonal receptors and dihydropyridine-sensitive calcium channels as described by Yatani *et al.* (21) for β-adrenergic stimulation of cardiac L-type calcium channels and by Macrez-Leprêtre *et al.* for α_2-adrenergic stimulation of portal myocytes (22). Cohen and MacCarthy have shown that guanosine-5′-*O*-(3-thio)triphosphate stimulates T-type calcium channels on excised patch of bovine ZG cells (23). Patch-clamp experiments performed on intact rat sensory neurons and dorsal root ganglion also strongly suggest the presence of a direct coupling between $G_{\alpha i}/G_{\alpha o}$ and L-type calcium channels (24).

Our study aim was to investigate the coupling between the AVP receptor and dihydropyridine-sensitive calcium channels in rat adrenal ZG cells. Using complementary experimental approaches, we demonstrated the existence of a direct coupling between the AVP receptor/PTX-sensitive G protein and the L-type calcium channel.

Materials and Methods

Chemicals. AVP, the cyclopentamethylene vasopressin antagonist CVPA, and Ang II were obtained from Bachem (Voisins le Bretonneux, France). Collagenase and Eagle's MEM was from Gibco Life Technologies (Eragny, France). Deoxyribonuclease, Fura-2 AM, nifedipine, and PDBu were from Sigma Chemical (St. Quentin Fallavier, France). The linear AVP antagonist LVPA was a generous gift from Dr. M. Manning (Medical College of Ohio, Toledo, OH). It was radioiodinated according to the iodogen technique as described previously (7, 25) with a specific radioactivity of 2000 Ci/mmol and appropriately able to label both rat and human V_{1a} AVP receptors. Tritiated AVP (50 Ci/mmol) and tritiated nitrendipine (71 Ci/mmol) were from New England Nuclear (Les Ulis, France). PTX was from List Biochemicals (Campbell, CA), and staurosporine was purchased from Calbiochem (Meudon, France).

Cell preparation. The rat ZG cells were obtained from adrenal glands of female Sprague-Dawley rats and prepared as described previously (15). Briefly, adrenal capsules were digested with 1.2 mg/ml collagenase and 25 μg/ml DNase dissolved in Eagle's MEM followed by mechanical dispersion of the tissue. Dispersed cells were then filtered through nylon gauze, centrifuged for 10 min at 100 × *g*, resuspended in MEM supplemented with 2% fetal calf serum, and incubated for 4 hr at 37° in a humid incubator before use (freshly dispersed ZG cells).

In some experiments, freshly dispersed ZG cells were cultured as described previously (15) in MEM containing 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells plated at a density of 10^5 cells/dish were grown at 37° in a humidified atmosphere (95% air/5% CO_2). The culture medium was changed 24 hr after plating, and the cells were used after 2 or 3 days of culture.

PTX treatment. Freshly dispersed ZG cells were incubated for 4 hr at room temperature in MEM supplemented with 2% fetal calf serum and 1 μg/ml PTX. ZG cell primary cultures were infected in the final 18 hr with 0.1 μg/ml PTX. Under both of these two conditions, back-ADP-ribosylations performed on infected ZG cells indicated that all PTX-sensitive G protein α subunits were completely ADP-ribosylated (data not shown and Ref. 26).

[^{3}H]Nitrendipine binding to freshly dispersed ZG cells. Binding studies were performed according to Aguilera *et al.* (10). Briefly, freshly dispersed ZG cells were centrifuged for 10 min at 100 × g and resuspended in an HBSS incubation medium containing 130 mM NaCl, 3.5 mM KCl, 1.8 mM $CaCl_2$, 2.5 mM $MgCl_2$, 3.5 mM $NaHCO_3$, 0.5 mM HEPES, pH 7.4, 1 g/liter glucose, and 1 mg/ml BSA. Thereafter, ZG cells (~200,000/assay) were incubated for 60 min at 25° in 500 μl of HBSS incubation medium with or without the drugs or hormones to be tested and supplemented with 0.2 nM [^{3}H]nitrendipine (total binding). Nonspecific binding was determined by the addition of 1 μM unlabeled nifedipine in the incubation medium, as described by Aguilera *et al.* (10). The reaction was stopped by the addition of 3 ml of ice-cold HBSS and rapid filtration through GF/C Whatman glass-fiber filters that had been presoaked for 2 hr with BSA solution (10 mg/ml). The filters were then washed three times with 3 ml of ice-cold HBSS, and radioactivity was counted for 15 min in a Packard 1600TR liquid scintillation analyzer. Table 1 illustrates typical total and nonspecific binding values measured with intact cells. Nonspecific values represented 67 ± 3% and 81 ± 6% of total binding for ZG cells incubated with or without maximum doses of AVP in the presence of 0.2 nM [^{3}H]nitrendipine (seven separate experiments), respectively. Despite these high nonspecific binding values, measurement of specific nitrendipine-binding sites remained very reproducible from one experiment to another (data mean ± standard error of three separate experiments) and very reliable (maximum difference observed for a quadruplicate determination never exceeded 6%).

[^{3}H]Nitrendipine binding to ZG cell membrane preparation. Three-day-cultured ZG cells were washed with HBSS deprived of $CaCl_2$ and $MgCl_2$; scraped with a rubber policeman; resuspended in an ice-cold medium containing 0.1 mM ATP, 10 mM Tris·HCl, pH 7.4, 3 mM $MgCl_2$, 0.5 mM EGTA, 0.05 mg/ml soybean trypsin inhibitor, 0.5 mg/ml bacitracin, and 0.1 mM phenylmethylsulfonyl fluoride; and homogenized in a Dounce homogenizer (Kontes, Vineland, NJ). The cell extract was centrifuged at 4° (15 min at 25,000 × *g*). The pellet resuspended in the same medium was used immediately to measure nitrendipine-binding sites. Crude plasma membranes (18–25 μg of protein/assay) were incubated for 60 min at 25° in 100 μl of an incubation medium containing 0.2 nM [^{3}H]nitrendipine, 5 mM Tris·HCl, pH 7.4, 1 mM $MgCl_2$, 0.05 mg/ml soybean trypsin inhibitor, 0.2 mg/ml bacitracin, 1 mg/ml BSA, and the drugs or hormones to be tested (total binding). Nonspecific binding was determined in each condition by the addition of 1 μM unlabeled nifed-

TABLE 1

Influence of pertussis toxin treatment on specific nitrendipine binding to rat glomerulosa cells

Freshly dispersed ZG cells (0.18 ± 0.02–10^6 cells/assay) were incubated 4 hr in MEM culture medium supplemented with 2% fetal calf serum without (control cells) or with 1 μg/ml PTX. Cells were then centrifuged and resuspended in an HBSS incubation medium containing 0.2 nM [^{3}H]-nitrendipine (total binding) or 0.2 nM [^{3}H]-nitrendipine plus 1 μM unlabeled nifedipine (nonspecific binding). AVP 30 nM or vehicle were also added in the incubation medium. Specific bindings were calculated. Results (expressed as dpm specifically bound per cell ± standard error) were the mean of three distinct experiments each performed in quadruplicate.

Effectors	[^{3}H]Nitrendipine binding					
	Control cell			PTX-treated cell		
	Total binding	Nonspecific binding	Specific binding	Total binding	Nonspecific binding	Specific binding
	dpm/assay					
None	197 ± 4	146 ± 4	51 ± 4	197 ± 5	148 ± 5	49 ± 4
+AVP (30 nM)	277 ± 20*	146 ± 5	131 ± 16**	220 ± 5	147 ± 5	73 ± 5*

*, $p < 0.05$ difference compared with corresponding control values.
**, $p < 0.001$, difference compared with corresponding control values.

ipine in the incubation medium. The reaction was stopped, and the radioactivity remaining on the filters were determined, as described above. Table 2 illustrated the typical total and nonspecific binding values that were measured. For membranes, nonspecific values were relatively high (61 ± 2% and 74 ± 2% of total binding for membranes incubated with or without maximum doses of AVP in the presence of 0.2 [^{3}H]nitrendipine (seven experiments), respectively). However, the specific nitrendipine binding determinations remained very reproducible from one experiment to another (data in this table are mean ± standard error of triplicate determinations).

Specific AVP binding to freshly dispersed ZG cells. AVP binding studies were performed using the same experimental procedure as that described for [^{3}H]nitrendipine (see above). Freshly dispersed ZG cells were incubated for 45 min at 30° in HBSS incubation medium supplemented with either [^{3}H]AVP or ^{125}I-LVPA (total binding) or the same amount of labeled hormones plus 1 μM unlabeled AVP (nonspecific binding). Radioactivity associated with ZG cells was determined as described above, and specific binding was calculated as the difference between total and nonspecific binding.

Measurement of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was measured on ZG cells grown for 2–3 days on glass coverslip as described previously (7). Before the experiments, the cells were preincubated for 40 min at 37° in a serum-free culture medium supplemented with 20 mM HEPES, pH 7.4, and 3.3 μM concentration of fluorescent calcium chelator Fura-2 AM. The cells were washed three times with 3 ml of HBSS buffer. The coverslips were placed in a perfusion chamber and mounted on an inverted Zeiss Axiovert 110 microscope (Zeiss, New York). The cells were then incubated at room temperature in HBSS buffer with or without the hormones to be tested. In some experiments, the free calcium concentration of HBSS was reduced to 100 nM by the addition of 0.5 mM EGTA (low-calcium medium). The cells were alternately excited with a 150-W xenon lamp light at 340 or 380 nm, and the emission was measured at 500 nm. Filter changes were managed with a MSP 21 microprocessor (Zeiss). Height video frames were taken every 2 sec with a low-light-level SIT LH 4036 camera (Lhésa, Paris) digitalized on-line with a Quantel Crystal imaging system and averaged. The camera dark noise was substracted for each averaged image, and calcium concentrations were calculated according to the procedure described previously with the appropriate software. $[Ca^{2+}]_i$ values were determined individually on the final image of each cell soma using the Crystal particle analysis package with custom-made computer software.

Measurement of calcium influx. Unidirectional calcium influx was measured in ZG cell primary culture according to Schilling *et al.* (19). Briefly, ZG cells grown for 3 days on glass coverslips were loaded with Fura-2 AM as described above. Cells were then incubated in a low-calcium medium (100 nM free calcium) and stimulated with 100 nM Ang II. After treatment, neither a calcium influx nor an intracellular calcium mobilization occurred because both the intracellular and extracellular free calcium concentrations were similar, and the Ins(1,4,5)P_3-sensitive intracellular calcium stores were emptied. Then, the incubation medium was aspirated and replaced with HBSS medium containing 1.8 mM free $CaCl_2$ with or without the drug to be tested. $[Ca^{2+}]_i$ values were then measured every 8 sec. Control experiments demonstrated that under these conditions, the $[Ca^{2+}]_i$ rapidly rose linearly with time within a few minutes and reached a plateau. Because the calcium detected inside the cell by the Fura-2 probe originated from the external medium, the slope of the intracellular calcium accumulation kinetics curve gave a good estimation of the calcium influx. Data obtained by this technique were similar to those previously obtained for the same cells with the ^{45}Ca technique: 100 nM AVP stimulated calcium influx by 2–3-fold regardless of the technique that was used (15, 16).

TABLE 2

Modulation of specific nitrendipine binding by vasopressin on rat glomerulosa cell membrane preparations.

Effectors	[^{3}H]nitrendipine binding					
	Control cell			PTX-treated cell		
	Total binding	Nonspecific binding	Specific binding	Total binding	Nonspecific binding	Specific binding
	dpm/assay					
Control	258 ± 8	206 ± 5	52 ± 3	260 ± 10	207 ± 6	53 ± 6
GTP (0.5 μM)	254 ± 7	208 ± 6	46 ± 2	256 ± 8	207 ± 8	49 ± 4
AVP (0.1 μM)	258 ± 9	206 ± 6	52 ± 5	255 ± 9	209 ± 5	46 ± 3
AVP (0.1 μM) +GTP (0.5 μM)	303 ± 8*	206 ± 5	97 ± 5**	258 ± 7	206 ± 5	52 ± 3
GTPγs (10 μM)	304 ± 8*	207 ± 5	97 ± 6**	259 ± 10	205 ± 6	54 ± 6

ZG cells were cultured for 3 days in standard culture medium and treated for the last 18 hr with 0.1 μg/ml PTX (PTX-treated cell) or vehicle (control cell). Crude plasma-membranes (17 μg of protein per assay) were prepared from control and PTX-treated cells and incubated for 60 min at 25°C with 0.2 nM [^{3}H]nitrendipine (total binding) or 0.2 nM [^{3}H]nitrendipine plus 1 μM nifedipine (nonspecific binding) in the presence of various effectors. Results (expressed as dpm specifically bound per assay) are the mean ± standard error of three distinct experiments each performed in quadruplicate.
** $p < 0.05$, difference compared with corresponding control values

Electrophysiological studies. The measurements of calcium currents in rat ZG cells was performed according to Durroux *et al.* (27). The physiological solutions used in the experiments had the following compositions. For calcium current measurement, the extracellular solution contained 100 mM NaCl, 10 mM $CaCl_2$, 35 mM tetraethylammonium chloride, 1 mM $MgCl_2$, 5.4 mM CsCl, 5 mM HEPES, and 1 g/liter glucose, pH 7.4. The pipette solution contained 126 mM CsCl, 18 mM NaCl, 1 mM $CaCl_2$, 11 mM EGTA, 2 mM $MgCl_2$, 5 mM HEPES, 3 mM ATP, and 0.4 mM GTP, pH 7.3. For membrane potential measurements, the extracellular solution contained 140 mM NaCl, 5.4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 1 g/liter glucose, pH 7.3. The pipette solution contained 125 mM KCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM HEPES, 11 mM EGTA, 3 mM ATP, and 0.4 mM GTP, pH 7.2. Solutions containing AVP were made daily from stock solutions. Before use, the solutions were filtered through 0.2-μm Millipore filters. All the experiments were performed at room temperature.

The culture dish (1-ml volume solution) was mounted on the stage of an inverted microscope, and the cells were observed at a magnification of ×300. Ionic currents and membrane potentials were obtained using the patch-clamp method in the voltage-clamp and current-clamp configurations, respectively (11, 27). Patch electrodes with a resistance of 3–5 MΩ were pulled from Pyrex glass (Corning 7740, Corning Glass Works, NY) capillaries. An Axopatch IB (Axon Instruments, Burlingame, CA) was used for recordings. Pulse stimulation and data acquisition were performed with an A/D interface DAS 16F (Metrabyte, Taunton, MA) and an IBM-compatible computer under the control of a custom-made program. Linear leak and capacitive currents were subtracted. Currents were filtered at 2 kHz and sampled at 5 kHz. For recordings in the continuous mode, the membrane potential was recorded on an analog tape (D/C 2.5 kHz band-pass) and digitalized afterward at 200 Hz (cutoff frequency, 100 Hz). Analysis was performed with a custom-made program. Each value is representative of several experiments performed on a number of (n) as indicated in the text.

Data analysis. Data are mean ± standard error of a varying number of separate experiments. Half-maximum effective concentrations (ED_{50}) were obtained directly from dose-response curves. Statistical analysis of the data was performed using the one-way analysis of variance test. Homogeneity of variance was assessed by Barlett's test, and p values were obtained from Dunnett's tables.

Results

Regulation of $[Ca^{2+}]_i$ by AVP in rat ZG cells: involvement of dihydropyridine-sensitive calcium channels and PTX-sensitive G proteins. As illustrated in Fig. 1A, AVP strongly stimulated the $[Ca^{2+}]_i$. On the addition of 100 nM AVP, $[Ca^{2+}]_i$ increased in 30 sec from 90 to 250 ± 18 nM (four experiments) (transient phase). Thereafter, the $[Ca^{2+}]_i$ weakly decreased and reached an equilibrium which remained stable for 3 to 4 min at a value around 170 ± 15 nM (four experiments) (plateau phase). When a similar experiment was performed in a low-calcium medium, the transient phase was weakly affected but the plateau phase was completely suppressed. This suggested that the transient phase corresponded to a calcium mobilization from intracellular pool and the plateau phase corresponded to a stimulation of calcium influx. Fig. 1B shows that the hormonal stimulation of intracellular calcium mobilization was dose dependent. Maximum effects were observed for AVP concentrations around 100 nM, and the concentration of hormone leading to a half-maximum effect was 2.0 ± 0.8 nM (four experiments). These effects were receptor mediated because AVP-induced calcium mobilization was abolished by a 5-min preincubation

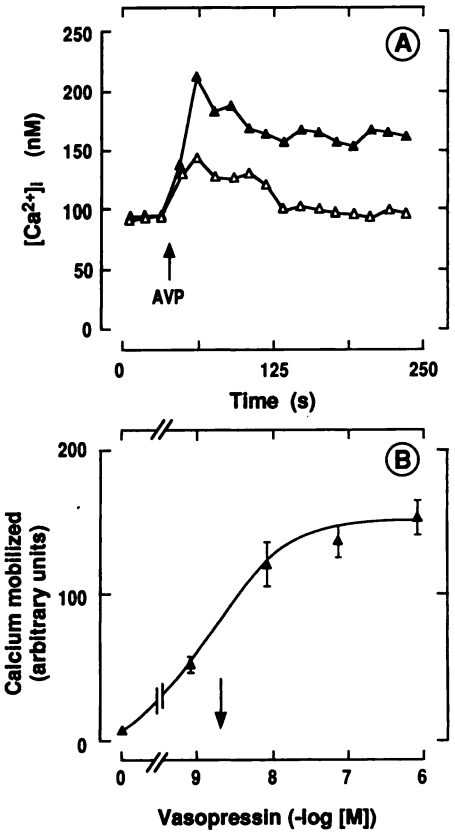

Fig. 1. Mobilization of intracellular calcium by AVP in rat ZG cells. A, ZG cell primary cultures were grown for 3 days onto glass coverslips. Cells were then loaded with Fura-2 AM, placed in a perfusion chamber, and mounted on an inverted microscope. $[Ca^{2+}]_i$ values were measured every 15 sec before and after the addition of 100 nM AVP as described in Materials and Methods. Two incubation media differing in free $CaCl_2$ concentration were used: (▲), 1.8 mM; (△), 100 nM. Results are the mean of $[Ca^{2+}]_i$ originating from 20–40 AVP-sensitive individual cells. Data are representative of four distinct experiments. Standard errors did not exceed 10% of each value. B, $[Ca^{2+}]_i$ values of 3-day ZG primary cultures were measured every 15 sec before and after the addition of increasing amounts of AVP as described in A. Mobilized intracellular calcium was measured by integrating the kinetic curve of $[Ca^{2+}]_i$ from 0 to 4 min and plotted as a function of the concentration of the hormone used. Results are mean ± standard error of $[Ca^{2+}]_i$ originating from 20–40 AVP-sensitive individual cells from three representative experiments.

with 100 nM of CVPA, a well-known V_{1a} AVP antagonist compound (data not shown) (25).

To verify whether dihydropyridine-sensitive calcium channels were involved in the hormone-stimulated calcium influx, we tested the effects of BAY K 8644 or nifedipine. As seen in Fig. 2A, 1 μM BAY K 8644 alone did not modify the basal $[Ca^{2+}]_i$ level. However, in the presence of 100 nM AVP, it potentiated both the transient and the plateau phases of the calcium response. This probably reflected a stimulation of a calcium influx because when experiments were performed in a calcium-free medium, BAY K 8644 was unable to potentiate the hormonal response (Fig. 2B).

In an experimental condition allowing the measurement of a calcium influx (preincubation of the cells with Ang II in a low-calcium medium; see Materials and Methods), we demonstrated that 100 nM AVP stimulated the basal calcium influx by 2.6-fold (Fig. 3A). Nifedipine (1 μM) did not affect

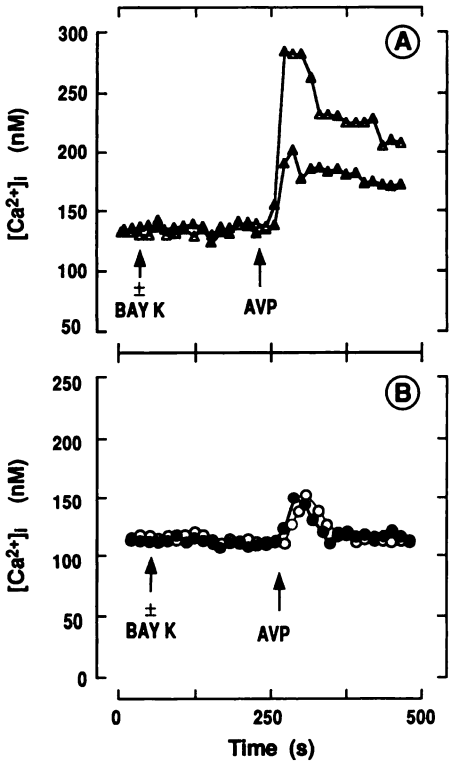

Fig. 2. BAY K 8644 potentiates AVP-stimulated intracellular calcium mobilization in rat ZG cells. Three-day ZG cell primary cultures were first incubated at room temperature with (△, ○) or without (▲, ●) 1 μM BAY K 8644 in an incubation medium containing either 1.8 mM (A) or 100 nM (B) free $CaCl_2$. After 4 min of incubation, the media were removed and replaced with equivalent fresh media supplemented with 100 nM AVP. $[Ca^{2+}]_i$ values were measured every 15 sec. Results are mean of $[Ca^{2+}]_i$ values originating from 20–40 AVP-sensitive individual cells from a single experiment that was representative of three experiments. In this typical experiment, the standard error did not exceed 10% of each value.

the basal calcium influx (Fig. 3B) but completely prevented its stimulation by 100 nM AVP (Fig. 3, B and A).

When ZG cells were preincubated for 18 hr before the experiments with 0.1 μg/ml PTX, AVP was no longer able to potentiate calcium influx (Fig. 3C). Furthermore, PTX pretreatment did not affect the basal level of calcium influx (Fig. 3, B and C).

Characterization of tritiated dihydropyridine-binding sites of rat ZG cells: effect of AVP. As described by Aguilera *et al.* (10) and further illustrated in Fig. 4A, rat ZG cells exhibited specific nitrendipine-binding sites. Specific binding was saturable and dose dependent. The Scatchard representation of the dose-response curve (Fig. 4B) was linear, suggesting the existence of a single class of nitrendipine-binding sites exhibiting a K_d value of 0.21 ± 0.02 nM (three experiments) and a maximum binding site of 3500 ± 600 sites/cell (three experiments). More interestingly, the addition of 100 nM AVP in the incubation medium increased the specific binding of nitrendipine to 7000 ± 580 sites/cell (three experiments) without significantly affecting the affinity of tritiated nitrendipine for its specific receptor K_d value of 0.25 ± 0.06 nM (three experiments). This AVP effect was saturable and dose dependent (ED_{50} = 5.0 ± 0.1 nM, three experiments) (Fig. 4C). The maximum increase in specific nitrendipine-binding sites (200 ± 15%, four experiments)

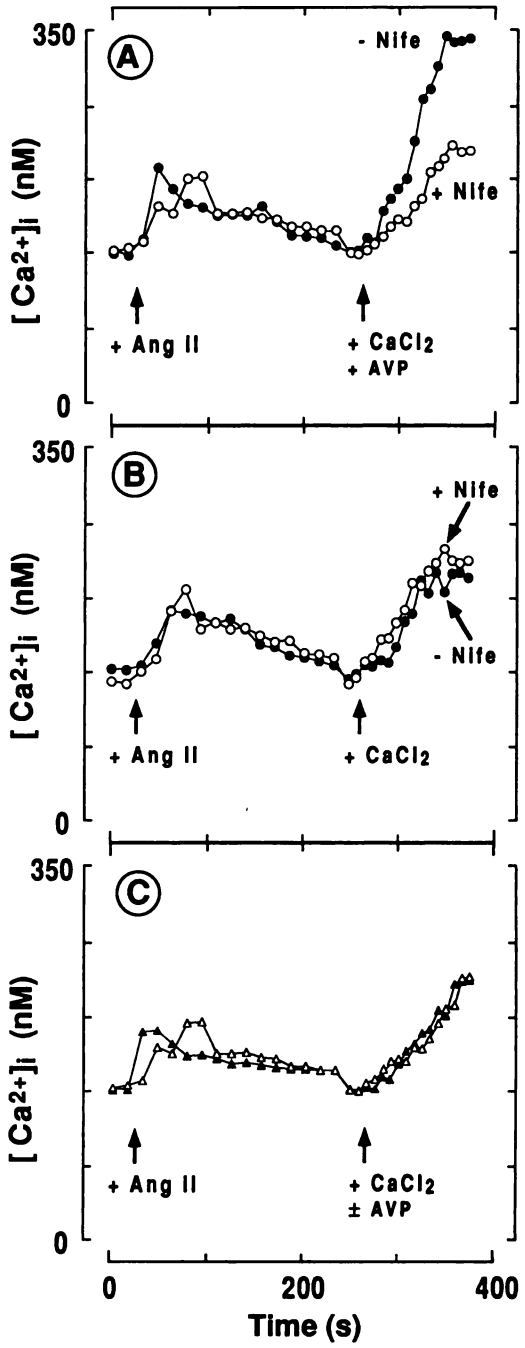

Fig. 3. Nifedipine (*Nife*) and PTX pretreatments inhibit AVP-stimulated calcium influx in rat ZG cells. A and B, Three-day ZG cell primary cultures were loaded with Fura-2 AM, and $[Ca^{2+}]_i$ was measured in individual cells as described in the legend to Fig. 1. ZG cells were stimulated with 100 nM Ang II in a low-$CaCl_2$ medium (100 nM), and $[Ca^{2+}]_i$ values were measured every 15 sec. Then, the incubation media were removed and replaced with a similar one supplemented with (○) or without (●) 1 μM nifedipine. After a 5-min incubation period, these media were aspirated and replaced by similar media supplemented with 1.8 mM $CaCl_2$ (B) or with 1.8 mM $CaCl_2$ plus 100 nM AVP (A). $[Ca^{2+}]_i$ values were then measured every 8 sec. C, Three-day ZG cell primary cultures were preincubated with PTX as described in Materials and Methods. ZG cells were then first stimulated with 100 nM Ang II in a low-$CaCl_2$ medium and then with (△) or without (▲) 100 nM AVP in a medium containing 1.8 mM $CaCl_2$. $[Ca^{2+}]_i$ values were measured every 8 sec. Results are mean of $[Ca^{2+}]_i$ originating from 20–40 AVP-sensitive individual cells from a single experiment that was representative of three experiments. In these typical experiments, the standard error did not exceed 10% of each value.

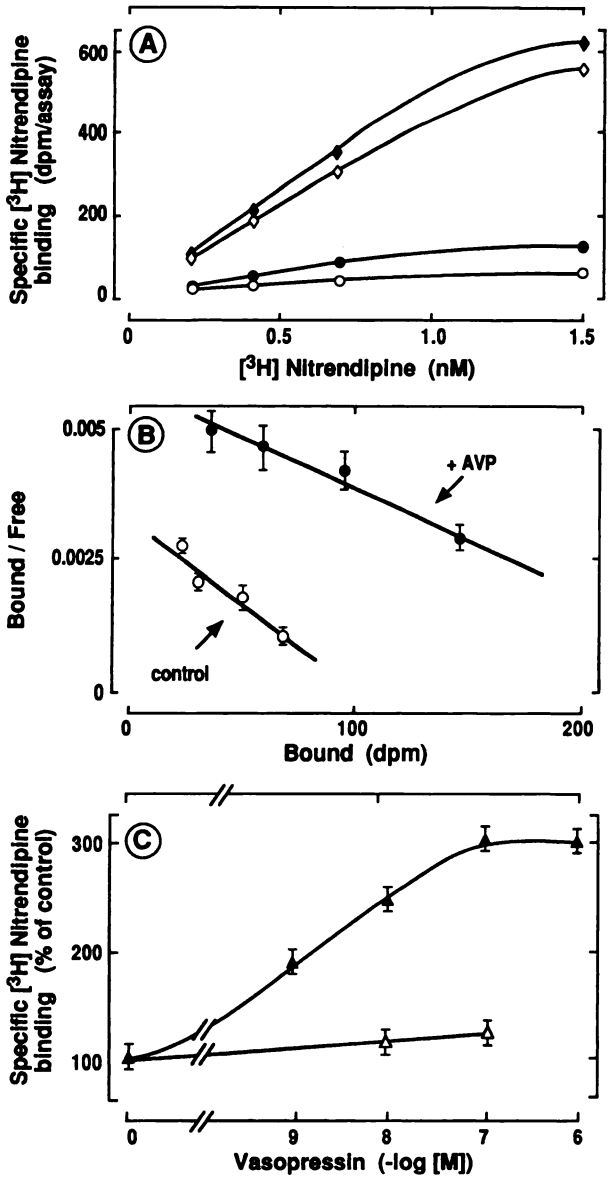

Fig. 4. Influence of AVP and an AVP antagonist on specific nitrendipine binding to rat ZG cells. A and B, Freshly dispersed ZG cells (0.16 ± 0.02–10^6) were prepared as described in Materials and Methods and incubated for 60 min at 25° with increasing amounts of [^{3}H]nitrendipine in the absence (total binding, (◇, ◆) or the presence of 1 μM unlabeled nifedipine (nonspecific binding). AVP (100 nM) (◆, ●) or vehicle (◇, ○) were also added to the incubation media. Specific bindings were calculated (○, ●). Results are mean ± standard error of triplicate determinations originating from a single experiment that was representative of three experiments. A, Dose-response binding curve. B, Corresponding Scatchard plot. C, Freshly dispersed ZG cells (0.19 ± 0.02 10^6 cells/assay) were incubated in the presence of 0.2 nM [^{3}H]nitrendipine without (control) or with increasing amounts of AVP. CVAP (1 μM) (Δ) or vehicle (▲) was also added to the incubation media. Specific binding was calculated as described above and expressed as a percentage of control specific binding (100% = 46 ± 5 dpm/assay). Results are mean ± standard error of triplicate determinations originating from a single experiment that was representative of three experiments. In this experiment, nonspecific binding was unaffected by the addition of hormones and represented 83% of basal total binding.

was observed for 100 nM AVP (Fig. 4C). Such an effect was receptor mediated because 1 μM CVPA, a AVP antagonist, completely prevented the effect of 10 and 100 nM AVP (Fig. 4C).

As seen in Table 1, PTX treatment prevented the stimulatory effect of AVP on nitrendipine specific binding. This effect was partial (50%) and could not be accounted for by a partial PTX ADP-ribosylation of G protein αi subunits (see Materials and Methods).

Additional experiments presented in Table 3 also confirmed the link between AVP receptor and dihydropyridine-binding sites. The specific [^{3}H]AVP binding was increased by 160% when 0.1 μM BAY K 8644, a calcium channel agonist, was added to the incubation medium. In contrast, when 1 μM nifedipine, a calcium channel antagonist, was added, no potentiation of specific [^{3}H]AVP binding was observed. When ^{125}I-LVPA, a AVP antagonist (25), was used to label AVP receptor, 0.1 μM BAY K 8644 had no effect.

Effects of guanyl nucleotides and AVP on the specific binding of [^{3}H]nitrendipine to rat ZG membrane preparations. As observed in intact cells, tritiated nitrendipine bound specifically to crude plasma membrane preparations derived from ZG primary cultures. Maximum binding capacity was 40 ± 4 fmol/mg of protein and K_d = 0.18 ± 0.1 nM (three experiments) (data not shown).

AVP was also able to potentiate the specific nitrendipine binding, but as illustrated in Table 2, this hormonal stimulation required GTP. When added separately, 0.5 μM GTP and 100 nM AVP did not significantly affect specific nitrendipine binding. However, when added together, specific nitrendipine binding was increased. A similar effect was observed with 10 μM guanosine-5′-*O*-(3-thio)triphosphate (Table 2) and was completely suppressed when membranes were prepared from PTX-treated rat ZG cells. For intact cells, AVP stimulated specific nitrendipine binding in a dose-dependent manner (Fig. 5A). Maximum stimulation (1.8 ± 0.12-fold, three experiments) was observed for 100 nM AVP (ED_{50} for AVP = ~10 nM). dDAVP, a specific V_2 AVP agonist, was unable to affect specific nitrendipine binding, even at 1 μM. In contrast, oxytocin weakly but significantly ($p < 0.05$) increased specific nitrendipine binding (Fig. 5A). As illustrated in Fig. 5B, the potentiation of nitrendipine binding induced by 30 nM AVP could be completely antagonized using increasing doses of the specific V_{1a} AVP antagonist CVPA. The inhibition constant determined for CVPA was calculated as described previously (25) (K_i = 3.0 ± 0.15 nM; three experiments).

Effects of modulators of protein kinase C activity on the specific binding of [^{3}H]nitrendipine to rat ZG membrane preparations. Because AVP stimulated protein kinase C in rat ZG cells (6), we studied the influence of drugs that modulate the activity of this enzyme on AVP-stimulated nitrendipine binding. As illustrated in Fig. 6, neither staurosporine (a PKC inhibitor) nor PDBu (a PKC activator) was able to affect the specific binding of [^{3}H]nitrendipine to rat ZG plasma membranes, even at 100 nM. Similarly, the presence of these two drugs did not modify the potentiating effect of AVP.

Modulation of calcium current and membrane potential by AVP in rat ZG cells. To study the modulation of the inward calcium current, the K^+ outward current was blocked by replacing K^+ with Cs^+ in both the bathing and pipette solutions. Tetraethylammonium (35 mM) was also added to the bathing medium. Two types of Ca^{2+} currents were recorded: the T- and L-type currents. As previously shown, they differ in activation threshold and kinetics (27).

TABLE 3

Effects of BAY K 8644 and nifedipine on specific vasopressin binding sites from rat glomerulosa cells

Freshly dispersed ZG cells (0.21 ± 0.02 − 10^6 cells/assay) were incubated as described in Materials and Methods for 45 min at 30° with either 1 nM [^{3}H]AVP or 30 pM ^{125}I-LVPA (concentrations corresponding to the K_d value for each labeled hormone used), in the presence (Total binding) or the absence of 1 μM unlabeled AVP (nonspecific binding). When specified, BAY K 8644 and nifedipine were added in the incubation media. Specific bindings were calculated in each condition as the difference between total and nonspecific binding and expressed as dpm per assay. Results are the mean ± standard error of three distinct experiments each performed in triplicate.

Effectors	Vasopressin binding sites					
	[^{3}H]AVP			[^{125}I]LVPA		
	Total binding	Nonspecific binding	Specific binding	Total binding	Nonspecific binding	Specific binding
	dpm per assay					
None	342 ± 9	250 ± 14	92 ± 5	3270 ± 220	1460 ± 300	1810 ± 140
nifedipine (1 μM)	335 ± 18	250 ± 12	85 ± 8	3200 ± 180	1460 ± 290	1740 ± 150
BAYK (0.1 μM)	397 ± 16*	252 ± 13	145 ± 6**	3300 ± 190	1510 ± 280	1790 ± 150

* $p < 0.05$, difference compared with control conditions.
** $p < 0.01$, difference compared with control conditions.

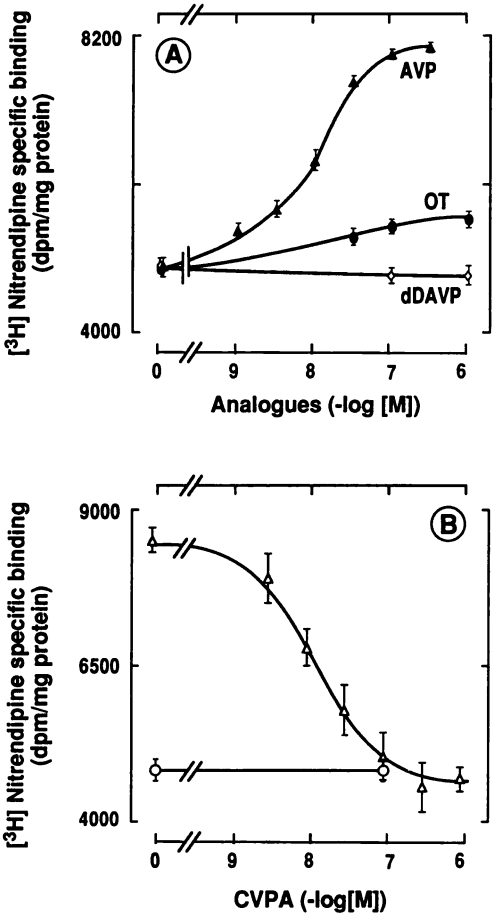

Fig. 5. Influence of AVP analogs on the specific nitrendipine binding to ZG cell membrane preparations. A, ZG cell crude plasma membranes (20 μg of protein/assay) were prepared as described in Materials and Methods and incubated for 60 min at 25° in a medium containing 0.2 nM [^{3}H]nitrendipine (total binding) or 0.2 nM [^{3}H]nitrendipine plus 1 μM nifedipine (nonspecific binding) with or without (control) increasing amounts of unlabeled AVP (▲) oxytocin (●) or [1-deamino,8-D-arginine]vasopressin (●). B, ZG cell crude plasma membranes (16 μg of protein/assay) were incubated with (△) or without (○) 30 nM AVP and increasing amounts of CVAP as described in A. Under each experimental condition, specific bindings were calculated. Results (dpm specifically bound/mg of protein) are mean ± standard error of triplicate determinations originating from a single experiment that was representative of three experiments. In this experiment, nonspecific binding was found to be unaffected by the addition of hormones and represented 75% of control total binding.

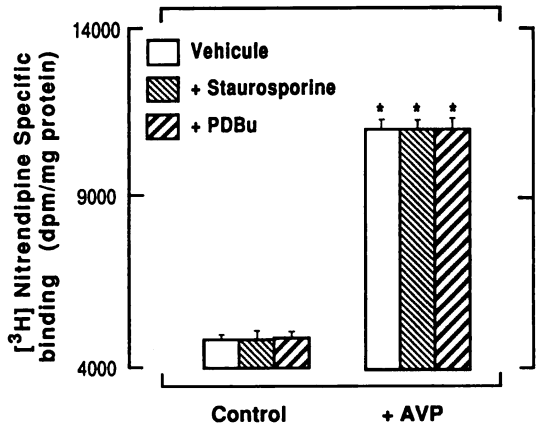

Fig. 6. Influence of PKC activity modulators on nitrendipine specific binding to ZG cell membrane preparation. ZG cell crude plasma membranes (21 μg of protein/assay) were prepared as described in Materials and Methods and incubated for 60 min at 37° in a medium containing 0.2 nM [^{3}H]nitrendipine (total binding) or 0.2 nM [^{3}H]nitrendipine plus 1 μM unlabeled nifedipine (nonspecific binding) with or without (control) 100 nM AVP. PDBu (100 nM), staurosporine (100 nM), or vehicle was also added to the assay medium. Specific binding was calculated under each condition. Results (dpm specifically bound/mg of protein) are mean ± standard error of triplicate determination originating from a single experiment. In this experiment, nonspecific binding was unaffected by the addition of drugs or hormones and represented 72% of control total binding.

Fig. 7, A and B, shows the current recorded from a holding potential of −80 mV for a step depolarization to −20 mV in two different cells. In control conditions, the current inactivated rapidly, indicating that the T-type current was predominantly activated. A sustained calcium current corresponding to the L-type current was also observed at the end of the pulse, but its amplitude varied from one cell to the other (Fig. 7, A and B).

The addition of AVP (1 nM) to the bath increased the amplitude of the L-type current. On seven different cells originating from four distinct primary cultures, the mean increase was found to be 87 ± 22% of the control value (Fig. 7A). The figures also showed that the peak current amplitude decreased. This was confirmed by experiments in cells in which only the T-type current was recorded (data not shown and Fig. 7B). The decrease in the peak current observed after application of 1 nM AVP indicated that the T-type current was reduced by 23 ± 4% (seven experiments). Such effects

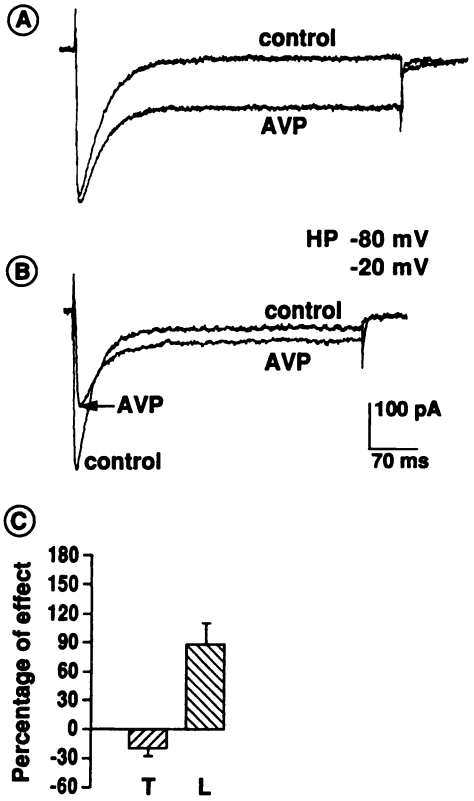

Fig. 7. Effect of AVP on calcium currents. Calcium currents were recorded from a holding potential of −80 mV by a step depolarization to −20 mV. A and B, Current traces obtained in control and after the addition of 1 nM AVP. C, Modulation of the T- and L-type currents by AVP (1 nM). Amplitudes were measured at the peak and steady state current, respectively. The T-type current amplitude was decreased by 23 ± 4% (seven experiments), whereas the L-type current was increased by 87 ± 22% (seven experiments).

were receptor mediated because no modification of L- or T-type calcium currents were observed when the cells were preincubated 15 min before the addition of AVP with 100 nM CVPA, a V_{1a} AVP antagonist (four experiments; data not shown).

The activation of PKC by PDBu, however, has no effect on the L-type current amplitude (four experiments), whereas the T-type current amplitude was slightly decreased (25%) in two cases (data not shown).

Current clamp experiments were also performed to determine whether AVP could affect the membrane potential of ZG cells. Fig. 8 shows that the application of 100 nM AVP induced a membrane depolarization from −50 to −20 mV. In five different cells, the mean AVP-induced depolarization was found to be 31 ± 4 mV.

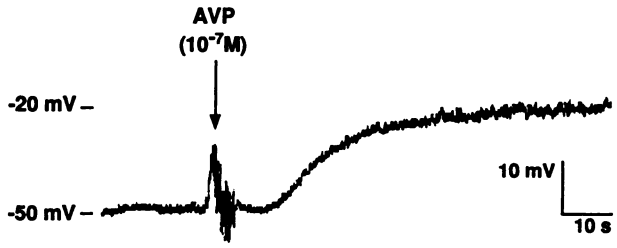

Fig. 8. Modulation of the membrane potential by AVP. The membrane potential was recorded in the current clamp mode. The application of 100 nM AVP induced a slow depolarization.

Influence of potassium on specific nitrendipine binding and on the intracellular calcium mobilization. As illustrated in Fig. 9A, KCl increased the specific binding of [^{3}H]nitrendipine to freshly dispersed ZG cells. This effect was dose dependent (ED_{50} = 8 ± 0.8 mM, three experiments) and maximum for KCl concentration at ~20 mM. The maximum increase in [^{3}H]nitrendipine specific binding (180 ± 8% of control values, three experiments) was similar to those obtained with maximum doses of AVP (Fig. 4). PTX pretreatment did not modify the stimulatory effect of KCl on [^{3}H]nitrendipine specific binding.

KCl also mobilized calcium in rat ZG cells. As shown in Fig. 9B, this effect was dose dependent (ED_{50} = 10 ± 1 mM, three experiments). The maximum stimulation obtained for 20 mM KCl was similar to that obtained with AVP (compared Figs. 1B and 9B). Calcium mobilized on KCl stimulation arose only from the extracellular medium because no effect of KCl was observed when experiments were performed in a low-calcium medium (data not shown). The addition of 0.1 or 1 μM nifedipine to the incubation medium also completely blocked the KCl-induced calcium mobilization (Fig. 9B). In contrast, 1 μM BAY K 8644 potentiated such a response (Fig. 9B). The KCl-stimulated calcium influx was not altered in PTX-treated cells.

Discussion

Previous studies on rat ZG cells have shown that V_{1a} receptor is coupled to a phosphatidyl inositol-4,5-P_2 phospholipase C via a G protein that is insensitive to PTX (15). However, in the same cells, AVP also stimulates a calcium influx via another unidentified pathway (15, 16). We discuss the nature of the coupling between the hormonal receptor and the calcium channel or channels.

Characterization of the AVP-stimulated calcium influx in rat ZG cells. As previously demonstrated in many cell types, including rat, human, and bovine ZG cells (7, 9, 28), intracellular calcium mobilization induced by AVP could be resolved into two steps: a rapid and transient peak followed by a sustained plateau phase. Calcium mobilized during the peak phase mainly originates from Ins(1,4,5)P_3-sensitive pools and thus is mainly controlled by the hormonal activation of phospholipase C. This phase is insensitive to PTX because PTX treatment does not affect AVP-stimulated inositol phosphate accumulations (15). In contrast, the sustained plateau phase is mainly due to calcium influx because it was completely suppressed when external calcium was omitted from the incubation medium (Fig. 1) and was sensitive to PTX treatment. Such results confirm previous studies performed on similar cells using the ^{45}Ca uptake technique (15, 16). These mechanisms are not specific to the action of AVP on rat ZG cells because similar results have been described for Ang II on the same cells (16) and with various other calcium-mobilizing hormones on many cellular systems, such as rat hepatocytes (29, 30). Altogether, these data confirm the existence of a coupling between calcium-mobilizing receptors and calcium channel or channels via a PTX-sensitive G protein.

Nature of the calcium channel coupled to AVP receptor. Results presented in this study suggest a coupling between dihydropyridine-sensitive calcium channels and AVP receptors because 1) nifedipine, a specific L-type calcium

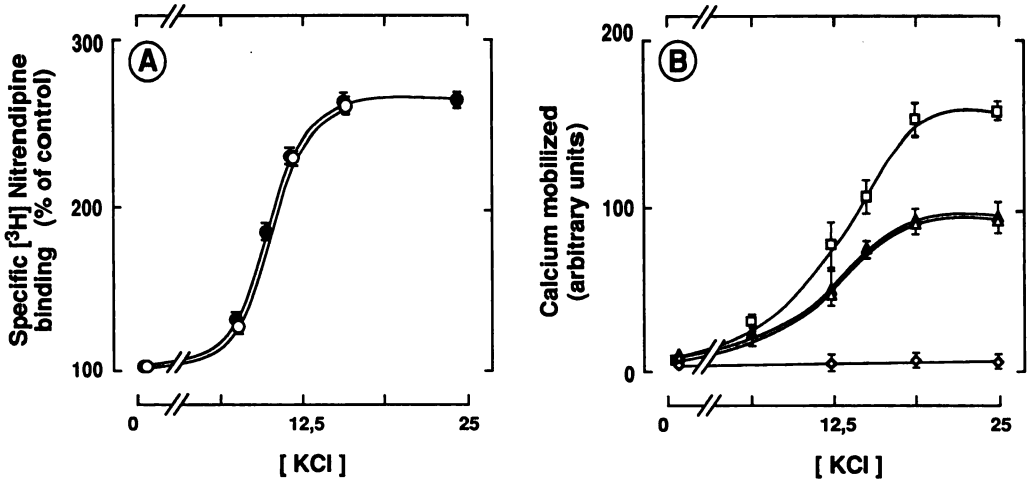

Fig. 9. Influence of KCl on nitrendipine binding and intracellular calcium mobilization. A, Freshly dispersed ZG cells (0.20 ± 0.02 10^6 cells/assay) preincubated for 4 hr at 37° with (○) or without (●) PTX were further incubated for 60 min at 25° with 0.2 nM [^{3}H]nitrendipine (total binding) or 0.2 nM [^{3}H]nitrendipine plus 1 μM nifedipine (nonspecific binding) in the presence of increasing concentrations of KCl. Specific binding was calculated and expressed as a percentage of specific binding measured in the absence of KCl (100% = 80 ± 7 dpm specifically bound/assay). Results are the mean of triplicate determinations from a single experiment that was representative of three. Nonspecific binding was found to be unaffected by KCl and represented 69% of basal total binding. B, Three-day ZG cell primary cultures preincubated for 18 hr at 37° with (△) or without (□, ◇, ▲) 0.1 μg/ml PTX were loaded with Fura-2 AM, and $[Ca^{2+}]_i$ values were measured on individual cells as described in the legend to Fig. 1. Increasing amounts of KCl were added to the incubation medium, and $[Ca^{2+}]_i$ values were measured every 15 sec for 4 min without effector (△, ▲) with 0.1 μM nifedipine (◇) or with 1 μM Bay K (□). The intracellular calcium mobilized was measured by integrating the kinetic curve of $[Ca^{2+}]_i$ from 0 to 4 min and plotted as a function of the KCl concentration used. Results are the mean of $[Ca^{2+}]_i$ originating from 20 to 40 AVP-sensitive individual cells from a single experiment representative of 3. In these typical experiments the standard error did not exceed 10% of each value.

channel antagonist, completely prevents AVP-stimulated calcium influx (Fig. 3); 2) BAY K 8644, a specific dihydropyridine calcium channel agonist, strongly potentiates AVP-stimulated calcium mobilization (Fig. 2); 3) patch-clamp experiments reveal that even at a low concentration (1 nM), AVP stimulates L-type calcium current (Fig. 7) and that 0.1 μM nifedipine blocks this current (27); and 4) a close correlation exists between the presence of specific dihydropyridine-binding sites and the ability of AVP to stimulate calcium influx. WRK_1 cells, which like rat ZG cells possess V_{1a} AVP-binding sites tightly coupled to PLC (31) but not dihydropyridine-binding site, exhibited only a rapid and transient calcium mobilization phase on AVP stimulation (data not shown).

Nature of the AVP receptor subtype coupled to L-type calcium channel. Data presented in this study suggest that a V_{1a} AVP receptor subtype is coupled to dihydropyridine-sensitive calcium channel via a PTX-sensitive G protein. Thus, dDAVP and oxytocin, which are specific for the V_2 AVP receptor and oxytocin receptor, respectively, are inactive or weakly active (Fig. 5A). In contrast, CVPA, a specific V_{1a} antagonist, abolishes the AVP effect on nitrendipine binding with a K_i value in the nanomolar range (Fig. 5B). Whether it is exactly the same hormonal receptor subtype that couples the PLC and L-type calcium channel remains unknown.

Nature of the coupling between AVP receptor and dihydropyridine-sensitive calcium channel. To further study the coupling between the AVP receptor and the L-type calcium channel, we decided to characterize the dihydropyridine-sensitive calcium channel using a specific tritiated probe. Such an experimental approach was used by Macrez-Leprêtre *et al.* (22) and Mironneau *et al.* (32) to outline the interaction between L-type calcium channels and α-adrenergic receptor from the rat portal vein. As shown by Aguilera *et al.* (10) and further illustrated in this study, rat ZG cells exhibited specific nitrendipine-binding sites of high affinity and low capacity. More interestingly, we demonstrated that on AVP stimulation, the maximum number of specific binding sites increases (Fig. 4). Such data could be explained by the depolarization induced by AVP activation. Under basal conditions, only the inactivated L-type calcium channels exhibiting a high affinity for tritiated nitrendipine (K_d value ~0.5 nM) are detectable (33). On depolarization induced by hormonal stimulation, the resting L-type calcium channels, which are not detectable by the binding technique due to their low affinities for the radiolabeled ligand used (K_d value ~0.1-1.0 μM) activate and further inactivate. They become thus measurable because their affinities increase (33). Data presented in Fig. 9 favor such an explanation: KCl, which is known to depolarize rat adrenal ZG cells (34), stimulates both nitrendipine binding and calcium influx with the same ED_{50} value. However, calcium influx and specific [^{3}H]nitrendipine binding induced by KCl and AVP differ in PTX sensitivity. This may indicate that the AVP effect cannot be accounted for by only a depolarization of the membrane. Indeed, it has been shown that in smooth muscle, AVP increases the L-type channel openness (N_{Po}, where N is the number of channels and Po is the probability of finding the channel in the open state), mainly by increasing the transition to the gating mode 2, a mode also favored by BAY K 8644 (35). By using the patch-clamp method in whole-cell or single-channel configurations, it is difficult to determine whether a hormone increases the current amplitude by increasing N or Po. In our study, we showed that AVP increases the number of dihydropyridine-binding sites, and it may be postulated that N has been increased. The direct involvement of G protein in the signaling cascade between the AVP receptor and the calcium channels is strengthened by the

absence of an inhibitory effect of PDBu on calcium currents. Similar results were found in other cell types (36).

In membrane preparations in which no variation of membrane potential could be observed, AVP still increased the specific binding of tritiated nitrendipine (Fig. 5 and Table 2). Like on intact cells, this effect was saturable, dose dependent (K_d = 1 nM), and receptor mediated because it was blocked by an AVP antagonist. More interestingly, without GTP, AVP was unable to increase the specific binding of nitrendipine (Table 2). Similarly, on membrane preparations derived from adrenal cells preincubated with PTX, AVP had no effect (Table 2). Such data strongly suggest that AVP receptor interacts with L-type calcium channel via a PTX-sensitive G protein. Results presented in Table 3 corroborate this hypothesis; we demonstrate that the occupation of the L-type calcium channel by an agonist and not by an antagonist also modifies the binding of tritiated AVP to its specific receptor. It can be suggested that the AVP/receptor/G protein ternary complex modifies the three-dimensional structure of the L-type calcium channel and thus the interaction of its distinct subunits. Such a modification may regulate the binding of tritiated nitrendipine because as recently reviewed, the dihydropyridine-binding site of the L-type calcium channel is an allosteric domain (37).

The tight interaction among AVP receptor, G protein α subunit, and dihydropyridine-sensitive calcium channels probably plays an important role in modulating calcium influx on rat adrenal ZG cells. We thus propose that on intact cells, different mechanisms regulate hormonal calcium influx: those that are PTX insensitive and probably arise from cell depolarization induced by the hormonal inhibition of a potassium channel via a G protein activation, as described previously for Ang II-stimulated cell depolarization (38), and those that are PTX sensitive and involve coupling among AVP receptor, G protein α subunit, and dihydropyridine-sensitive calcium channels. Whether this last coupling is direct or second messenger mediated remains to be determined. However, the mechanism by which AVP receptor activates dihydropyridine-sensitive calcium channels does not imply, as observed for ACTH (8, 14), the involvement of PKA because in adrenal ZG cells, AVP does not increase the intracellular cAMP (26). Similarly, PKC is not involved in these activation processes because 1) phorbol ester does not stimulate, like AVP, the specific nitrendipine binding to ZG plasma membrane preparations, and 2) staurosporine does not prevent the potentiating effect of AVP on the specific nitrendipine binding (Fig. 6). Data from Boland and Bean (39), who proposed that G protein α subunit may directly interact with the α1 subunit of N-type calcium channel, and from our experiments performed on membrane preparation favor a direct coupling, but we cannot exclude that second messengers locally synthesized or $\beta\gamma$ G protein subunits released during hormonal receptor activation indirectly modulate the L-type calcium channel as recently described by Ikeda (40) and Herlitze *et al.* (41) for various neuronal calcium channels. Thus, we prefer the term "membrane-delimited G protein gating" to describe such coupling processes, as recently suggested by Clapham (42).

The involvement of G protein in activating calcium channel was reviewed by Clapham (42). G proteins $\alpha i/\alpha o$ subunits were shown to be negatively coupled to voltage-dependent calcium channels in tissues such as neuronal cerebellar granule cells or dorsal root ganglia or cell lines (42, 43). To our knowledge, only a few studies have described a positive coupling between hormonal receptors and L-type calcium channel triggered by a PTX-sensitive G protein (Ref. 22 and this study). Such a positive regulation of dihydropyridine-sensitive calcium channels seems to be a characteristic of peripheral tissues such as the portal vein (22) or adrenal ZG cells because negative regulation has generally been found only on neuronal tissues (39, 42, 43).

In conclusion, our studies demonstrate that V_{1a} AVP receptor is positively coupled to dihydropyridine-sensitive calcium channels via a membrane-delimited G protein mechanism in rat adrenal ZG cells.

Acknowledgments

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