Translocation of cytosolic annexin 2 to a Triton-insoluble membrane subdomain upon nicotine stimulation of chromaffin cultured cells

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Received 24 February 1997

Abstract To gain a better understanding of the function of annexin 2, we have investigated the subcellular distribution of the monomeric and heterotetrameric forms of annexin 2 and their relationship to the cytoskeleton upon stimulation of chromaffin cells. Quantitative immunoblotting has revealed that in resting cells a large amount of annexin 2 is monomeric and cytosolic. Upon nicotine stimulation 80% of total annexin 2 becomes associated with a Triton-X100-insoluble fraction where the monomeric and the heterotetrameric forms are found. The translocation of monomeric annexin 2 is Ca^{2+}-dependent and complete at 1 μM free Ca^{2+}. We have shown that about 66% of the annexin 2 associated with the Triton-X100-insoluble fraction is soluble in octylglucoside while the remnants are insoluble in the detergent and remain likely associated with actin filaments and associated cytoskeleton proteins. The octylglucoside-soluble fraction contains integral proteins from the plasma membrane and from granule membrane, but does not contain caveolin. Moreover, upon nicotine stimulation, a redistribution of proteins was detected in this fraction. These dynamic processes appear concomitantly with the phosphorylation of annexin 2 in this compartment and with catecholamine release. It is suggested that the soluble octylglucoside fraction may represent a special lipidic membrane compartment where the NSF attachment proteins and the cytosolic proteins like annexin 2 and rab3a may become concentrated upon stimulation of the cell. The presence of annexin 2 is consistent with its proposed function on granule and target membrane proteins required for the close apposition of two distinct membranes and supports its functional role in the regulated exocytosis/endocytosis process.

Key words: Annexin; Chromaffin cell; Exocytosis

1. Introduction

Annexin 2 differs from other members of the annexin family in that it forms a tetrameric complex of two heavy chains of 36 kDa with two p11 polypeptides. p11 itself forms a tight dimer which is believed to bridge the two annexin 2 heavy chains, thus mediating the formation of the heterotetramer annexin 2, $p36_2p11_2$. While both monomeric p36 and $p36_2p11_2$ complex exist within the cell, p11 has so far only been found complexed to p36. p11 is a protein of the S-100 family which has lost its ability to bind calcium and shows a calcium-independent interaction with p36.

Immunofluorescence microscopy reveals a submembranous localization of p36 and p11 in a variety of cultured cells [1]. Both p36 and p11 are associated with the cytoplasmic face of the membrane. Since p11 itself is not able to interact with phospholipids or cytoskeletal structures, its presence in the cortical cytoskeleton suggests an interaction with p36 in vivo [2].

Recently [3], it has been shown that in resting chromaffin cells the p36 heavy chain of annexin 2 was localized in the cytosol while the p11 light chain was concentrated in the cortical subplasmalemma. Under nicotine stimulation, monomeric annexin 2 (p36 heavy chain) translocated to the cell periphery where p11 was localized. The data obtained were based on confocal microscopy of resting and stimulated cells and on immunoreactivity of antibodies but not on protein quantification. Thus, it was suggested that following stimulation p11 may be the docking ligand for p36 in the Triton-X100-insoluble fraction where the heterotetramer was likely formed.

If the presence of the monomeric p36 heavy chain in the cytosol of the resting cells was supported by previous studies [4], it was the first time that p11 was immunolocalized in the subplasmalemma in a form that did not seem associated with p36. It was also the first time that the heterotetramer appeared as a dynamic form susceptible to dissociation and reassociation according to the resting or stimulated state of the cells.

In order to confirm these data, in the present study we quantified the p36 heavy chains and the p11 light chains and characterized their respective localization in the cell.

2. Materials and methods

2.1. Culture of chromaffin cells

Bovine chromaffin cells were isolated from fresh adrenal glands by retrograde perfusion with collagenase A from *Clostridium histolyticum* (Boehringer-Mannheim, Mannheim, Germany) and purified as described by Bader et al. [5]. They were suspended in Dulbecco's modified Eagle's medium supplemented as described by Delouche et al. [15]. Cells were usually cultured as monolayers on 60-mm-diameter cell culture dishes (Costar, Cambridge, MA, USA) at 37°C in a water-saturated 5% CO_2 atmosphere at a density of $8{\cdot}10^6$ cells/well. Cells were used 2 days after plating.

2.2. Cell stimulation

After 2 days of plating, cultured chromaffin cells were incubated in 2 ml Locke's solution: 154 mM NaCl, 5.6 mM KCl, 2.5 mM $CaCl_2$, 3.6 mM $HNaCO_3$, 1.2 mM $MgSO_4$, 5.6 mM glucose, 0.5 mM ascorbic acid and 10 mM HEPES, pH 7.5, containing 5% fetal bovine serum. The cells were then incubated at 37°C in the tissue culture incubator for 1 h. In phosphorylation studies, 300 μCi carrier-free ^{32}P-orthophosphate (Amersham) was added. The cells were incubated at 37°C for 4 h. Half of the cells were stimulated by introducing 20 μM nicotine in Locke's solution. Incubation was conducted at 25°C for 1 min. To terminate stimulation, EGTA was added to the medium in order to adjust the free calcium concentration to 10^{-5} M. Then cells were rinsed with a 10^{-5} M calcium Locke's solution and frozen in liquid nitrogen. To the other half of the cells which were used as control, EGTA was added so that free calcium was 10^{-8} M and rinsed with a Locke's solution which was adjusted at 10^{-8} M calcium. In the

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PII S0014-5793(97)00594-2

experiment where the effect of calcium is studied on non-stimulated cells, EGTA was added to the medium so that the free calcium concentration was 10^{-8}, 10^{-7}, 10^{-6} M. The cells were then rinsed with a Locke's solution adjusted at the respective calcium concentrations and frozen in liquid nitrogen.

2.3. Subcellular fractionation

Frozen culture cells were rapidly thawed, and 35 μl of 0.1 mM sodium orthovanadate, 0.1 mM PMSF, 20 μg/ml leupeptin, benzonuclease, 50 μg/ml pepstatin, aprotinin, trypsin inhibitor and 0.1 μM okadaic acid were added. The cell culture dishes were scraped with a plastic blade, then the mixture was incubated for 10 min on ice and centrifuged for 45 min at 200 000×*g*. The supernatant was saved (cytosol) and the pellet was homogenized with a Hamilton microsyringe in 150 μl of 10 mM imidazole, pH 7.3, 75 mM KCl, 2 mM $MgCl_2$, 1 mM NaN_3 and 0.5% Triton X100 containing the inhibitors mentioned above. The suspension was then incubated for 10 min at 0°C and centrifuged for 45 min at 200 000×*g*. The supernatant was saved (Triton-X100-soluble fraction) and the pellet was suspended in 150 μl of 150 mM NaCl, 60 mM octylglucoside, 25 mM MES, pH 6.5, containing the same inhibitors, sonicated, incubated for 30 min at 0°C and centrifuged for 15 min at 14 000×*g*. The supernatant was saved (octylglucoside-soluble fraction) and the pellet was dissolved in 100 μl of 2 mM EDTA, 0.5% SDS, pH 8.5, with the same inhibitors (octylglucoside-insoluble fraction). The proteins of the supernatant (octylglucoside soluble fraction) were precipitated by adding 80% acetone (v/v), the solution was stored at −20°C during 12 h, and then centrifuged for 20 min at 10 000×*g*. The pellet was dissolved in 100 μl of 2 mM EDTA, 0.5% SDS, pH 8.5, with inhibitors (octylglucoside-soluble fraction). For the immunoprecipitation experiments, the octylglucoside supernatant was added directly to the antibody-conjugated protein A beads, the octylglucoside-insoluble fraction was homogen-

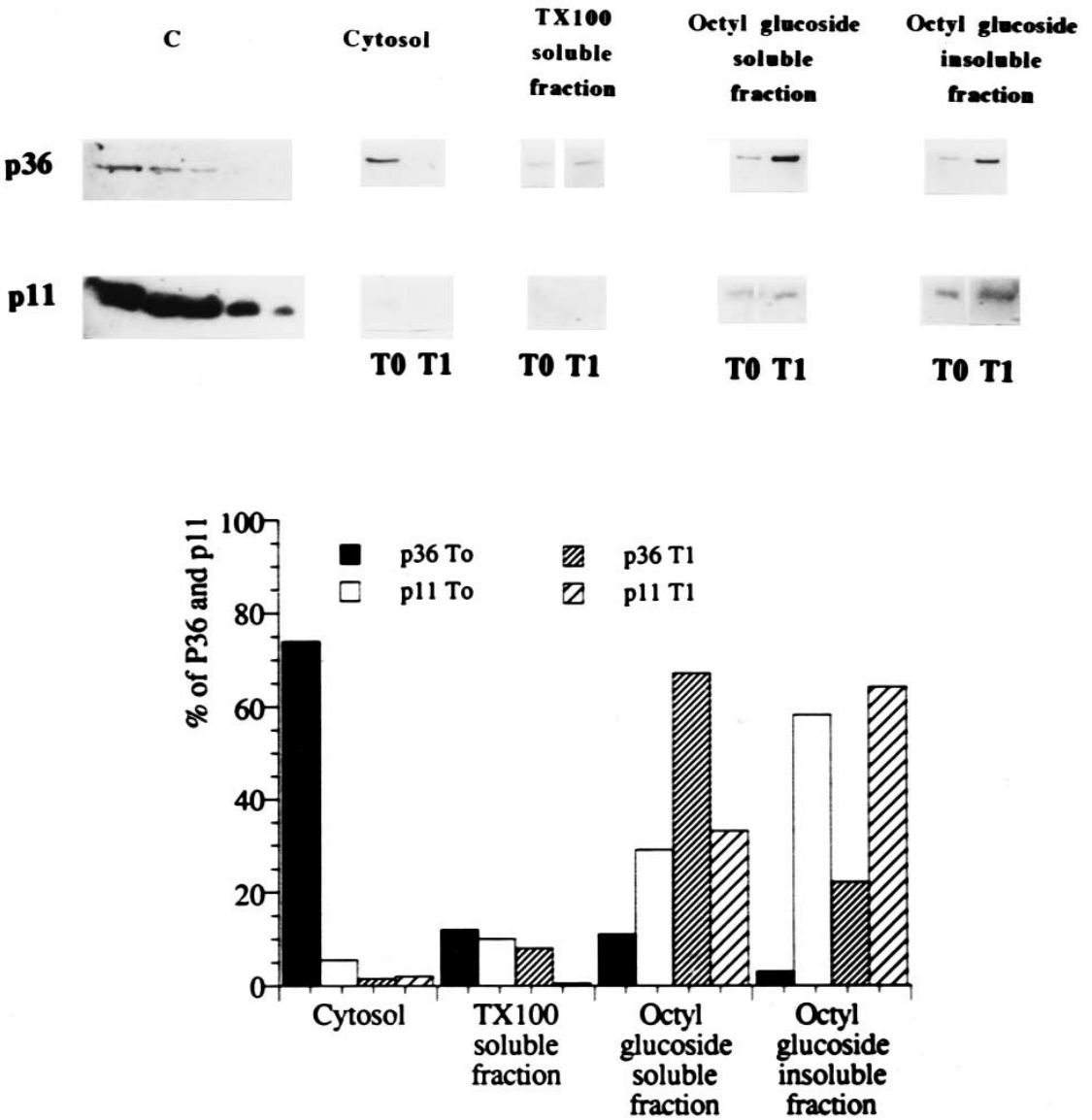

Fig. 1. Intracellular distribution of p36 and p11 in resting and stimulated chromaffin cells. Top panel: Each lane is an immunoblot of one subcellular fraction in resting cells (T0) or stimulated cells (T1). 100 μg of cytosolic proteins as well as 30 μg of Triton-X100-soluble, octylglucoside-soluble and -insoluble proteins were loaded on a 15% SDS polyacrylamide gel. After transfer to nitrocellulose sheets were incubated with primary antibodies: a monoclonal raised against p11 and a polyclonal raised against p36. Bound antibodies were visualized using alkaline-phosphatase-coupled secondary antibody reagents. Bottom panel: Percentage of p36 and p11 in each subcellular fraction. p36 and p11 bands in the different fractions in resting and stimulated cells were quantified on a Phosphor-Imager after incubation of immunoblots with ^{125}I-protein A. The percent of each band, p36 or p11, was determined in relation to the total p36 or p11 protein in the cell. This graph represents 3 experiments.

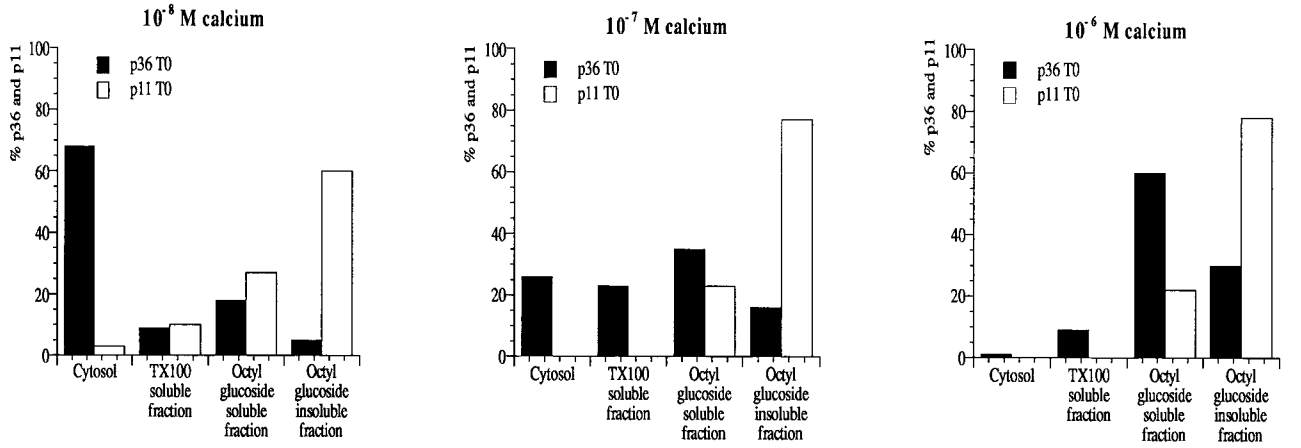

Fig. 2. Effect of the concentration of free calcium on the intracellular distribution of p36 and p11 in resting chromaffin cells. Resting cells were rinsed in Locke's solutions, adjusted respectively to 10^{-8}, 10^{-7}, 10^{-6} M free calcium. p36 and p11 bands in the different fractions (cytosol, Triton-X100-soluble, octylglucoside-soluble and -insoluble) were quantified on a Phosphor-Imager after incubation of immunoblots with ^{125}I-protein A. The percent of each band was determined in relation to the total p36 or p11 protein in the cell.

ized in 10 mM Imidazole, pH 7.4, 150 mM NaCl, 10 mM EGTA, 2 mM EDTA, 1 mM DTT, centrifuged for 40 min at 200 000×*g*, and the supernatant was added to the antibody-conjugated protein A beads.

2.4. Immunoprecipitation of phosphorylated annexin 2

Anti-annexin 2 serum was obtained and affinity-purified as previously described [15]; it was then filtered on a column of purified native annexin 1 coupled to Affigel 10; 80 μg of the affinity-purified antibody, which had been incubated with 35 mg of protein A Sepharose 4B beads for 7 h at 4°C, was added to each subcellular fraction (see Section 2.3). The mixtures were then incubated for one night at 4°C with rocking and centrifuged at 20 000 rpm (microfuge) for 5 min. The protein A Sepharose 4B antibody pellet was washed with 50 mM Tris buffer, pH 7.4, + 0.05% Triton X100, 50 mM Tris, pH 7.4, 0.5 M LiCl, 50 mM LiCl, 50 mM Tris buffer, pH 7.4. Each washing was centrifuged at 20 000 rpm for 5 min. The final pellet was dissolved in 40 μl of the loading buffer used for SDS-PAGE.

2.5. Quantification of proteins

Protein samples were separated on 15% SDS-PAGE and either blue-stained or transferred to nitrocellulose or Immobilon filters. For quantification of phosphorylated annexin 2 the sheets were dried and submitted to a Phosphor-Imager. For quantification of p36 and p11 proteins, on the same gel, standard curves were obtained from known quantities of purified heterotetrameric annexin 2 (0.05–1 pmol). Sheets of nitrocellulose were incubated with polyclonal antibody against lung annexin 2 1/500 which was prepared in our laboratory [6] and with mouse monoclonal anti p11 1/500 (Affinity). Blots incubated with primary antibodies were probed using appropriate secondary antibodies: phosphatase alkaline anti-mouse IgG, 1/7500, phosphatase alkaline anti-rabbit IgG, 1/10000 (Promega). Antibody–antigen complexes were detected by the enzymatic reaction. The sheets were also incubated with rabbit anti-mouse IgG, 1/5000 (Interchim), for the monoclonal antibody, and all the sheets were incubated overnight at 4°C with ^{125}I-labeled protein A (100 μCi/ml). The other proteins were characterized using a variety of primary antibodies at the following concentrations: monoclonal antibodies anti-actin 1/2000 (Amersham), anti-vimentin 1/8000 (Boehringer Mannheim), anti-VAMP2 1/10000 (clone 69,I) and anti-αSNAP 1/10000 (generous gift of Dr. R. Jahn, Yale University, New Haven, CT), anti-syntaxin 1/20000 (Sigma, clone HPC-1), anti-SNAP-25 1/40000 (Valbiothec, SMI 81), anti-caveolin 1/3000 (Affinity), and polyclonal antibodies anti-brain-spectrin 1/500, anti-brain-annexin 1 1/700 (prepared in our laboratory [6,7]), anti-smooth-muscle myosin 1/500 (generous gift of Dr. R. Cassoly), anti-DBH 1/5000 from our laboratory, anti-cytochrome *b*561 1/2000 (obtained from Dr. D. Apps) and anti-rab3a 1/1000 (generous gift of Dr. F. Darchen). Blots were incubated with primary antibodies and were probed using appropriate secondary antibodies: horseradish peroxidase anti-mouse IgG, 1/10000, and anti-rabbit IgG, 1/10000 (Promega). Antibody–antigen complexes were detected by enhanced chemiluminescence (NEN) and quantified by densitometry.

2.6. Other methods

Protein concentration was determined by the methods of Bradford [8] or Lowry [9]. SDS-PAGE were carried out according to Laemmli [10] on 25 or 10 cm plates.

Free Ca^{2+} concentrations were buffered using balanced Ca^{2+}/EGTA solutions [11].

3. Results and discussion

The Triton-X100-insoluble fraction was generally assumed to be cytoskeleton. However, in a number of recent papers [12–14], it has been shown that the Triton-X100-insoluble fraction might be heterogeneous and contained caveolin, some integral and signal transducing molecules as well as particular sphingoglycolipids that were solubilized in some detergents like octylglucoside or CHAPS.

In our hands, the Triton-X100-insoluble fraction was in part solubilized in octylglucoside giving two subfractions, one octylglucoside-soluble (OGS), which represents 12±4%

Table 1
Quantification of p36 and p11 in Triton-X100-insoluble fraction and octylglucoside fractions, in resting and stimulated cultured cells

	Resting cells			Stimulated cells		
	p36 (pmol)	p11 (pmol)	p36/p11	p36 (pmol)	p11 (pmol)	p36/p11
Triton-X100-insoluble fraction	1.85±0.15	2.05±0.05	0.9±0.1 (n=2)	14 ±4	1.85±0.4	8.2±4 (n=3)
Octylglucoside-soluble fraction	2.3 ±0.2	0.9 ±0.7	2.6±0.3 (n=3)	10.5±0.3	1.1 ±0.25	9.6±2.2 (n=3)
Octylglucoside-insoluble fraction	0.7 ±0.2	1.8 ±0.5	0.4±0.1 (n=3)	3.5±0.2	2.1 ±0.5	1.7±0.45 (n=3)

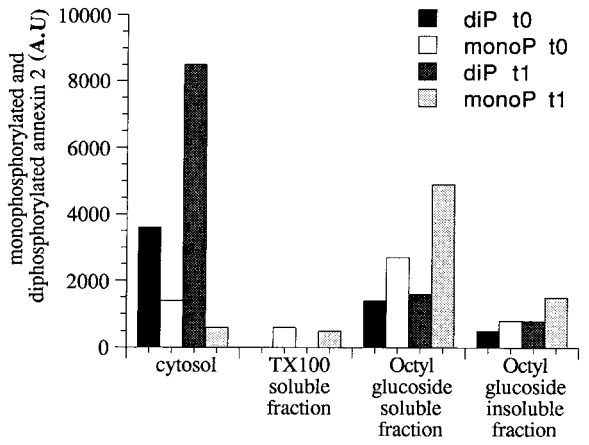

Fig. 3. Intracellular distribution of phosphorylated annexin 2 in resting and nicotine-stimulated chromaffin cells. The cells were pre-incubated in the culture medium containing ^{32}P-orthophosphate for 4 h, then stimulated and washed as described in Section 2. The cells were divided into 4 fractions: cytosol, Triton-X100-soluble fraction, octylglucoside-soluble and -insoluble fractions. Each fraction was immunoprecipitated with an antibody directed against native hetero-tetrameric annexin 2 and immunopurified as described in Section 2. Immunoprecipitates were eluted by boiling in SDS sample buffer, electrophoresed on 10% SDS-polyacrylamide gels, transferred to nitrocellulose, and immunoblotted. ^{32}P-labelled bands were visualized on a Phosphor-Imager and expressed as arbitrary units (A.U.).

of total proteins and one octylglucoside-insoluble (OGI), which represents 14 ± 5% of total proteins. We quantified the two subunits of annexin 2, p36 and p11, in the two fractions. As shown in Table 1, in resting cells, a ratio of 2 p36/1 p11 and 1 p36/2 p11 was determined respectively in the OGS and the OGI fractions. These data suggested that in the OGI fraction some p11 was free from p36 and that in each fraction p36 was in part present under the heterotetrameric form $p36_2p11_2$.

In nicotine-stimulated cells, 10 p36/1 p11 were found in the OGS fraction and 3 p36/2 p11 in the OGI fraction. Thus, in the two fractions there was an excess of p36, a large excess in OGS in comparison with OGI. Finally, following nicotine stimulation, most of the monomeric cytosolic annexin 2 was translocated to the Triton-X100-insoluble fraction: 75% in OGS and 25% in OGI, respectively (Fig. 1). This translocation was essentially Ca^{2+}-dependent and complete at 1 μM calcium (Fig. 2).

Upon nicotine stimulation, annexin 2 was phosphorylated by protein kinase C concomitantly with catecholamine secretion [15]. Phosphorylation of annexin 2 was evaluated in the different fractions (Fig. 3). It is interesting to note that the two forms of annexin 2 phosphorylated in vitro by protein kinase C [16] were present: the diphosphorylated form (2 PO_4/36 kDa chain), molecular mass 40 kDa, and the monomeric form (1 PO_4/36 kDa chain), molecular mass 38 kDa; the diphosphorylated form was predominant in cytosol, the monophosphorylated form only present in OGS and OGI but predominant in OGS. The two forms increased upon nicotine stimulation. From these results it was suggested that upon stimulation the unphosphorylated p36 which disappeared from cytosol was chiefly phosphorylated in OGS under a monophosphorylated form, the subsequent diphosphorylation of the heavy chain being followed by its dissociation from the membrane and its increase in cytosol.

In order to characterize some specific proteins in the OGS and OGI subfractions, we tested in each fraction antibodies directed either against specific plasma membrane proteins (syntaxin, SNAP 25), specific granule proteins (DBH, cytochrome *b*561, VAMP2), cytosolic proteins (annexin 1, rab3a) or cytoskeletal proteins (actin, vimentin, myosin, spectrin).

As shown in Table 2 and Fig. 4, in the resting cells, the granule proteins and plasma membrane proteins were present in the two soluble detergent fractions; the proteins known as cytoskeletal (spectrin, myosin, vimentin) were concentrated in the OGI. αSNAP partitioned in all the fractions; strikingly, 24% was found in the OGI. 74% F-actin was localized in the OGS and OGI (Triton-X100-insoluble fraction). In the cytosol, we found the totality of annexin 1, 17% of G-actin and 9% αSNAP. This is consistent with the data already known for the localization of annexin 1 [17]. 50% of αSNAP leaked from digitonin-permeabilized cells [18] in a free-calcium medium (5 mM EGTA); this high level might represent the amount we found in the cytosol and associated with the cytoskeleton.

As shown in Table 2 and Fig. 4, in the nicotine-stimulated cells, the integral protein of the plasma membrane, syntaxin,

Table 2
Identification of specific proteins in chromaffin cells, subcellular fractions

	Resting cells				Stimulated cells			
	Cytosol	TX100-soluble fraction	OG-soluble fraction	OG-insoluble fraction	Cytosol	TX100-soluble fraction	OG-soluble fraction	OG-insoluble fraction
VAMP2[a]	–	72	28	–	–	63	37	–
DBH[a]	8	43	45	4	7	28	56	9
Cyt. *b*561[a]	–	62	38	–	–	36	63	–
Rab3a[a]	–	56	44	–	–	32	68	–
Syntaxin[b]	–	39	61	–	–	38	60	2
SNAP25[b]	–	–	100	–	–	–	100	–
αSNAP[c]	9	23.5	44	23.5	4	24	44	28
AnnexinI[c]	92	8	–	–	–	30	67	3
Actin[d]	17	9	33	41	32.5	14	20	34
Spectrin[d]	–	–	10	90	–	–	18	82
Myosin[d]	–	–	–	100	–	–	–	100
Vimentin[d]	–	–	–	100	–	–	–	100

[a]Proteins of the chromaffin granules.
[b]Proteins of the plasma membrane.
[c]Proteins of the cytosol.
[d]Proteins of the cytoskeleton.

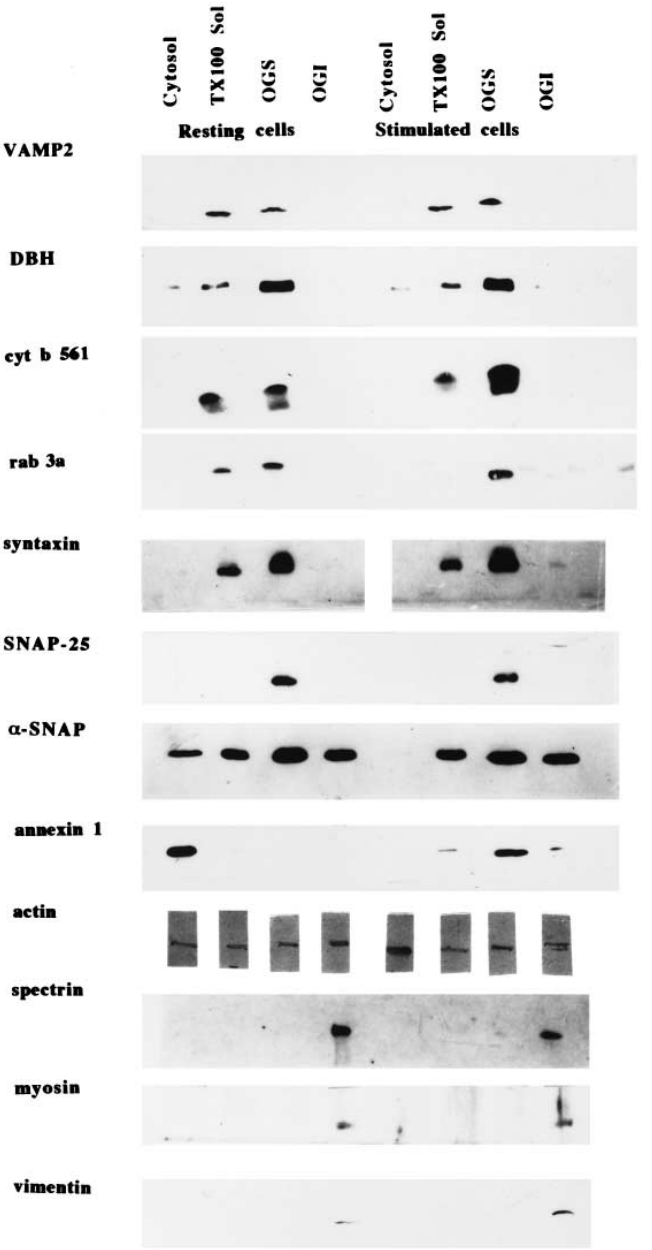

Fig. 4. Identification of proteins in the subcellular fractions of chromaffin cells in a resting and a stimulated state. Following subcellular fractionation, 100 μg of cytosolic proteins and 30 μg of Triton-X100-soluble, octylglucoside-soluble and -insoluble proteins were loaded on a 15% SDS-polyacrylamide gel and transferred on Immobilon-P. Immunoblots were probed with antibodies raised against VAMP2, DBH, cyt. *b*561, rab3a, syntaxin, SNAP-25, α-SNAP, annexin 1, actin, spectrin, myosin and vimentin. Proteins were visualized by the ECL system (Amersham).

was constant in relation to the resting cells. Interestingly, whereas annexin 2 was translocated to OGS and OGI and was phosphorylated (Fig. 3), a fraction of the proteins of the granules membrane DBH, cytochrome *b*561, VAMP2 and also rab3a increased in OGS whereas they decreased in the Triton X100 fraction. Concomitantly, annexin 1 was translocated from the cytosol to OGS and αSNAP appeared to translocate from the cytosol to the OGI fraction. It has been shown that under calcium stimulation in permeabilized chromaffin cells, αSNAP did not leak and was retained in the cell [18]; our results suggest that during the calcium-dependent step, αSNAP would associate with the cytoskeleton. Actin increased in the cytosol and decreased in the OGS and OGI fractions upon stimulation; this was in accord with previous studies that provided evidence of depolymerization of F-actin at the exocytosis sites [19–21].

In conclusion, the soluble octylglucoside fraction in chromaffin cells does not seem to represent caveolae-enriched plasma membrane domains since caveolin was not immunocharacterized with the antibody used. However, heterotetrameric annexin 2 has already been found in the caveolae and a role for calcium and annexin 2 in the formation of caveolae was suggested recently by Parkin et al. [22]. Our results support the calcium dependence of the translocation of annexin 2 to OGS. It is worth noting that in this domain the annexin 2 heterotetramer like the integral membrane protein, syntaxin,

remained constant. This suggests that in the resting state the heterotetramer might be already bound to the plasma membrane and might crosslink some chromaffin granules. In the stimulated state, the major monomeric form of annexin 2 translocated to this domain with a second set of granules, the associated protein rab3 and likely protein kinase C [15,23], annexin 2 was then phosphorylated. Hence, OGS might represent a membrane microdomain, an active zone of signal transduction, containing the sites of exocytosis.

In the cytoskeletal OGI compartment, upon stimulation and p36 translocation, heterotetrameric annexin 2 increased and was likely bound to actin filaments or spectrin according to previous observations [4]. Annexin 2 was also phosphorylated and this phosphorylation might affect the organization of actin filaments. Finally, in the course of stimulation of chromaffin cells, annexin 2 might function in two compartments of the cell, at the level of the plasma membrane and at the level of the cortical actin filament network.

Acknowledgements: This work was supported by the Centre National de la Recherche Scientifique. We thank Dr. J. Ayala for a critical reading of the manuscript.

References

[1] Osborn, M., Johnsson, N., Wehland, J. and Weber, K. (1988) Exp. Cell Res. 175, 81–96.
[2] Gerke, V. (1989) Cell Motil. Cytoskeleton 14, 449–454.
[3] Chasserot-Golaz, S., Vitale, N., Sagot, I., Delouche, B., Dirrig, S., Pradel, L.A., Henry, J.P., Aunis, D. and Bader, M.F. (1996) J. Cell Biol. 133, 1217–1236.
[4] Zokas, L. and Glenney, J.R. (1987) J. Cell Biol. 105, 2111–2121.
[5] Bader, M.F., Thierse, D., Aunis, D., Ahnert-Hilger, G. and Gratzl, M. (1986) J. Biol. Chem. 261, 5777–5783.
[6] Regnouf, F., Nguyen, E., Cassoly, R. and Pradel, L.A. (1985) Eur. J. Biochem. 153, 313–319.
[7] Regnouf, F., Rendon, A. and Pradel, L.A. (1991) J. Neurochem. 56, 1985–1996.
[8] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–272.
[10] Laemmli, U.K. (1970) Nature 227, 680–685.
[11] Fabiato, A. and Fabiato, F. (1979) J. Physiol. (Paris) 75, 463–505.
[12] Siargiacomo, M., Sudol, M., Lan, T.Z. and Lisanti, M.P. (1993) J. Cell Biol. 122, 789–807.
[13] Chun, M., Liyanage, U.K., Lisanti, M.P. and Lodish, H.F. (1994) Proc. Natl. Acad. Sci. USA 91, 11728–11732.
[14] Brown, D.A. and Rose, J.K. (1992) Cell 68, 533–544.
[15] Delouche, B., Pradel, L.A. and Henry, J.P. (1997) J. Neurochem. 68, 1720–1727.
[16] Regnouf, F., Sagot, I., Devilliers, G., Cartaud, J., Henry, J.P. and Pradel, L.A. (1995) J. Biol. Chem. 270, 27143–27150.
[17] Drust, D.S. and Creutz, C.E. (1991) J. Neurochem. 56, 469–478.
[18] Morgan, A. and Burgoyne, R.D. (1995) EMBO J. 14, 232–239.
[19] Cheek, T.R. and Burgoyne, R.D. (1987) J. Biol. Chem. 262, 11663–11666.
[20] Neng, W.Y., Chung, Y.Y. and Wagner, P.D. (1992) J. Biol. Chem. 267, 8396–8403.
[21] Nakata, T. and Hirokawa, N. (1992) J. Neurosci. 12, 2186–2197.
[22] Parkin, E.T., Turner, A.J. and Hooper, N.M. (1996) Biochem. Soc. Trans. 24, 444S.
[23] Sarafian, T., Pradel, L.A., Henry, J.P., Aunis, D. and Bader, M.F. (1991) J. Cell. Biol. 114, 1135–1147.