Isolation of a Protein from the Plasma Membrane of Adrenal Medulla which Binds to Secretory Vesicles*

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Solubilized proteins of the plasma membrane of bovine adrenal medulla were fractionated on the basis of their affinity for secretory vesicles. The isolation procedure included preparation of a highly purified fraction of plasma membranes, its solubilization in detergent, and application to a column prepared from glutaraldehyde-fixed chromaffin granules. Using this technique, one major polypeptide (80% of the material bound) was isolated.

This protein has been shown to originate from the plasma membrane and has no affinity for fixed bovine adrenal medullary mitochondria or lysosomes. It is eluted most effectively by low pH (3.0) and can be rebound and re-eluted from fixed secretory granules. In sodium dodecyl sulfate and β-mercaptoethanol it has an apparent molecular weight of 51,000. In addition, two minor components, comprising about 20% of the material bound were detected having apparent molecular weights in sodium dodecyl sulfate of 14,000 and 62,000.

It is suggested that such a molecule could function as a plasma membrane-located receptor for chromaffin granules during the secretory process.

In order to isolate the putative plasma membrane-located ligand a novel approach was taken. The purification and partial characterization of a plasma membrane protein was carried out by affinity chromatography using the secretory granules themselves as the adsorbent.

EXPERIMENTAL PROCEDURES

Isolation of Chromaffin Granules—Bovine adrenal glands were obtained from the local slaughterhouse. The glands were placed in ice-cold physiological saline (0.9% NaCl solution) immediately upon removal from the animal. Within an hour the medullae were freed from the cortex and coarsely homogenized in 5 volumes of SME buffer using a Polytron homogenizer (Komet International Inc., Greenwich, Conn.) at lowest speed for 30 s. The homogenate was adjusted to 10% (w/v), further homogenized by one stroke in a motor-driven Durrall homogenizer (Kontes, Vineland, N.J.) and filtered through one layer of fine mesh cotton gauze. Following centrifugation at 1000 × g for 15 min in a Sorvall RC-2B centrifuge (GSA rotor), a crude granule pellet was prepared by centrifugation of the 1000 × g supernatant at 10,000 × g for 20 min, also in the GSA rotor. The pellet was resuspended to 10% of the starting volume of the homogenate and layered over step gradients of Percoll (Pharmacia Fine Chemicals AB, Uppsala, Sweden) as follows: In a 30-ml Corex tube, 5 ml of the resuspended crude granule fraction were layered over a three-step gradient consisting of 6 ml of 60% (w/v), 7 ml of 40%, and 6 ml of 20% Percoll. The gradients were centrifuged for 30 min at 10,000 × g in an SS-34 rotor (Sorvall). The material banded at the 40 to 60% interface was washed twice in SME buffer by centrifugation at 10,000 × g for 20 min to remove Percoll. The pink pellet obtained consisted of highly purified chromaffin granules (see "Results").

Fixation of Chromaffin Granules—The pellet obtained as described above was resuspended in SME buffer by two to four strokes in a Dounce homogenizer with a loose-fitting pestle (Kontes, Vineland, N.J.) in a concentration of 5 to 10 mg of protein/ml. One volume of 2-fold concentrated (4% w/v) glutaraldehyde was added dropwise to the granule suspension under gentle stirring at 4°C. Fixation was terminated after 3 to 4 h by the slow addition of a large excess of 0.1 M NH₄Cl. The granules were dialyzed overnight against 0.1 M NH₄Cl and then twice against NaCl/PO₄ for 4 h.

Isolation of Plasma Membranes—The same procedure was used as in the isolation of chromaffin granules, with the exception that the large granule fraction was prepared by centrifugation at 25,000 × g (Fig. 2). Then the 20 to 40% interface of the aforementioned Percoll gradient was collected and washed twice with SME buffer by centrifugation at 25,000 × g for 20 min. The pellets were resuspended in SME buffer and mixed with 2 volumes of 52% (w/w) sucrose (containing MOPS and EDTA, as in SME buffer), to give a final concentration of 40%. Twelve milliliters of this suspension was placed in the bottom of a cellulose nitrate centrifuge tube and overlaid with the pellet-breaking layers of sucrose: 14 ml of 36% (w/w), 8 ml of 32%, and 5 ml of 20%. The samples were then centrifuged for 120 min at 131,000 × g in an SW 27 rotor (Beckman Instruments, Palo Alto, Calif.). The 32 to 36% interface was used as the source of plasma membranes (see "Results").

1 The abbreviations used are: SME buffer, 300 mM sucrose, 10 mM MOPS (morpholinoethanesulfonic acid), 5 mM EDTA; NaCl/PO₄ buffer, 0.137 M NaCl, 2.7 mM KCl, 0.4 mM NaH₂PO₄, 1.5 mM KH₂PO₄, 0.9 mM CaCl₂, 0.5 mM MgCl₂, WGA, wheat germ agglutinin; SDS, sodium dodecyl sulfate.

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Such a method did not increase the yield when compared to conventional techniques (Table I). It did, however, eliminate tonic conditions during isolation, and the relative hypotonicity which were as pure and as free from damage as possible, step by step, exceeded the necessity of the granules being exposed to extreme hyperosmotic conditions.

Radiolabeling of Plasma Membranes—The solubilized membranes were iodinated with \(^{125}\text{I}\) by the lactoperoxidase-catalyzed reaction. For each milliliter of membrane solution, 100 to 200 \(\mu\)l of \(^{125}\text{I}\) sodium iodide (Amersham, La Jolla, Cal.) were used. The reaction was initiated by the addition of 20 \(\mu\)l of membrane solution of 0.003% \(\text{H}_2\text{O}_2\), which was added in the same quantity again at 2 and 5 min. The iodination was carried out at 25°C for 10 min. The reaction was terminated by the addition of 100 \(\mu\)l of 0.5 M \(\text{KI}\). The solution was dialyzed three times against 100 to 200 volumes of 1% Triton X-100 in NaCl/PO\(_4\) for 6 h. The specific radioactivity of such a preparation ranged from 5.6 to 7.4 \(\times\) 10\(^6\) cpm/mg of protein.

Preparation of the Affinity Column—Columns for affinity chromatography were routinely made from 10-ml disposable plastic syringes (1.5 \(\times\) 6 cm). The first 1 ml of the column was filled with Bio-Gel P-30, 100 to 200 mesh (polyacrylamide beads, Bio-Rad Laboratories, Richmond, Cal.) which served as a support for the affinity adsorbent mixture. A 50% (v/v) slurry of fixed chromaffin granules in 1% Triton X-100 was mixed in a 1:1 ratio with a 50% (v/v) slurry of Bio-Gel P-30, 400 mesh, in the same buffer and poured onto the 100 mesh support. The fixed granule-bead mixture was overlaid with another 1 ml (packed volume) of 50 to 100 mesh beads. The column was extensively washed in 1% Triton X-100 in NaCl/PO\(_4\), then with the same quantity again at 2 and 5 min. The iodination was carried out at 25°C for 10 min. The reaction was terminated by the addition of 100 \(\mu\)l of 0.5 M KI. The solution was dialyzed three times against 100 to 200 volumes of 1% Triton X-100 in NaCl/PO\(_4\), then with the elution medium, and re-equilibrated with 1% Triton X-100 in NaCl/PO\(_4\).

Preparation of \(^{125}\text{I}\)-Labeled WGA—WGA was prepared by the method of Bloch and Burger (5). Lactoperoxidase labeling was carried out as described above for plasma membrane proteins, and the iodinated lectin purified by affinity chromatography on chick heart.

Electron Microscopy—Samples were fixed with an isotonic solution of glutaraldehyde (2%) in sodium cacodylate buffer, pH 7.0, for 2 to 3 h at 4°C, pelleted in an Eppendorf microfuge, postfixed with 1% \(\text{Os}_2\text{O}_3\), 400 mesh, in the same buffer and poured onto the 100 mesh screen. The fixed granule-bead mixture was overlaid with another 1 ml (packed volume) of 50 to 100 mesh beads. The column was extensively washed in 1% Triton X-100 in NaCl/PO\(_4\), then with the elution medium, and re-equilibrated with 1% Triton X-100 in NaCl/PO\(_4\).

Recovery—Protein 4.7 \(\pm\) 0.2; Catecholamines 14.7 \(\pm\) 3.9; Contamination—Mitochondria 2.8 \(\pm\) 0.3; Lyosomes 1.0 \(\pm\) 0.3; Stability—Protein (\(\mu\)g/mg granule protein) 22.3 \(\pm\) 5.0; Catecholamines (\(\mu\)g/mg granule protein) 13.3 \(\pm\) 0.6; Recovery—Protein 10.4 \(\pm\) 2.7; Catecholamines 32.6 \(\pm\) 9.0; Contamination—Mitochondria 3.8 \(\pm\) 0.5; Lyosomes 2.1 \(\pm\) 0.4; Stability—Protein (\(\mu\)g/mg granule protein) 231 \(\pm\) 36; Catecholamines (\(\mu\)g/mg granule protein) 209 \(\pm\) 45.

Values are expressed as a percentage of the total protein or catecholamines found in the whole homogenate.

Values represent the percentage of total activity of cytochrome oxidase (mitochondria) and acid phosphatase (lysosomes) found in the whole homogenate.

Pellets of chromaffin granules were incubated for 12 h at 4°C. They were resuspended in SME buffer and centrifuged once at 10,000 \(\times\) \(g\) for 20 min. The values represent the amount of protein and catecholamines released into the supernatant.

State brought about during their resuspension for further use. The data in Table I also show the granules isolated in this way to be significantly more stable (as determined by lysis resistance) than those isolated through barriers of sucrose. Furthermore, a good deal of time was saved in preparation since ultracentrifugation was eliminated altogether. Electron microcopy (Fig. 1) demonstrates the purity of Percoll-isolated granule fractions which are composed of adrenalin-containing (less dense) and noradrenalin-containing (dense core) vesicles. Morphometric measurements of granules isolated in this way revealed one mitochondrion per 90 to 100 chromaffin granules.

Fixation of the granules was determined to be adequate since they were resistant to lysis under hypotonic conditions monitored by release of protein and catecholamines.

**RESULTS**

**Isolation and Fixation of Chromaffin Granules.** In order to prepare chromaffin granules for affinity chromatography which were as pure and as free from damage as possible, step gradients of Percoll were used in the isolation procedure. Such a method increased the yield when compared to conventional techniques (Table I). It did, however, eliminate the necessity of the granules being exposed to extreme hypertonic conditions during isolation, and the relative hypotonicity which were as pure and as free from damage as possible, step by step, exceeded the necessity of the granules being exposed to extreme hypertonic conditions during isolation, and the relative hypotonic resistance of the granules was increased by the addition of 100 \(\mu\)l of 0.5 M KI. The solution was dialyzed three times against 100 to 200 volumes of 1% Triton X-100 in NaCl/PO\(_4\), then with the elution medium, and re-equilibrated with 1% Triton X-100 in NaCl/PO\(_4\).

**Table I**

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Protein</th>
<th>Catecholamines</th>
<th>Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.7 (\pm) 0.2</td>
<td>14.7 (\pm) 3.9</td>
<td>28.8 (\pm) 0.3</td>
</tr>
<tr>
<td>Stability</td>
<td>Protein ((\mu)g/mg granule protein)</td>
<td>22.3 (\pm) 5.0</td>
<td>231 (\pm) 36</td>
</tr>
<tr>
<td></td>
<td>Catecholamines ((\mu)g/mg granule protein)</td>
<td>13.3 (\pm) 0.6</td>
<td>209 (\pm) 45</td>
</tr>
</tbody>
</table>

Secretory Vesicle-binding Protein
FIG. 1. Electron micrograph of unwashed chromaffin granules derived from the 40 to 60% interface of a discontinuous gradient of Percoll (see "Experimental Procedures" for details). Magnification: a, × 10,000; b, × 45,000. (The small dense grains are Percoll particles.)

mental system, WGA, a protein known to bind to chromaffin granules in the presence of nonionic detergents (19) was poured over the column. This control was primarily undertaken to demonstrate that the granules were accessible to solubilized proteins and that the eluant was not merely coursing through the Bio-Gel support. The results, presented in Fig. 4, indicate that not only did WGA bind, but it could also be eluted from the column with low pH in a manner identical with previous observations using chitin as the affinity adsorbent for WGA (5). The small peak, representing WGA not bound, corresponds to a small amount of lectin inactivated during iodination and subsequent purification.

The effectiveness of the affinity column in binding solubilized membrane proteins is shown in Fig. 5A. Following sample application, the column was washed with the equilibration buffer until the base-line had stabilized. Elution was carried out, in preliminary experiments, with 0.05 N HCl containing 1% Triton X-100 (pH 1.5). pH measurements of the eluted fractions indicated that at pH 2.5 to 3.0 the majority (>95%) of the bound material could be eluted from the column. It was observed that a further reduction in pH was unnecessary, as no additional activity appeared in the effluent. In subsequent experiments, such as those shown in Fig. 5, bound material was eluted with 0.05 N acetic acid containing 1% Triton X-100 (pH 2.6). In terms of recovery, the peak eluted at low pH comprised 8.6% of the total radioactivity recovered from the column.

In addition, it was determined that neither high pH (up to 9.5) high salt (up to 0.5 M NaCl) nor EDTA (up to 5 mM) was competent in eluting bound protein from the column. In order to demonstrate that the protein bound to and eluted from the column was derived from the plasma membrane, a fraction possessing lower marker enzyme specific activities was solubilized, radioactivity labeled, and passed over the column. The less enriched fraction used was the 36 to 40% interface of the sucrose gradient used to purify plasma membranes (see Fig. 2). As is shown in Table II, the 32 to 36% fraction had about 1.6 to 2.5 times the activity of acetylcholinesterase and (Na⁺-K⁺)-ATPase compared to the 36 to 40% interface. A comparison of Fig. 5A with Fig. 5B shows that the ratio of labeled protein bound and eluted from the affinity
TABLE II
Specific activities of marker enzymes from sucrose gradients during plasma membrane isolation

The principal value given is the purification factor arrived at by dividing the specific activity of the fraction by the specific activity of the whole homogenate. All specific activities (indicated in parentheses) are given in micromoles of substrate converted per h per mg protein except for dopamine-β-hydroxylase which is given in nanomoles per h per mg of protein and monoamine oxidase which is given in arbitrary units per h per mg of protein. Values represent the average of three separate determinations ± S.D.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Acetylcholinesterase</th>
<th>(Na⁺ - K⁺)-ATPase</th>
<th>5'-Nucleotidase</th>
<th>Mg²⁺-ATPase</th>
<th>Monoamine oxidase</th>
<th>Glucose-6-phosphatase</th>
<th>Dopamine-β-hydroxylase</th>
<th>Cytosol c oxidase</th>
<th>Acid phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>(13.6 ± 0.75)</td>
<td>(93.9 ± 8.6)</td>
<td>(3.03 ± 0.3)</td>
<td>(31.4 ± 3.5)</td>
<td>(2.0 ± 0.02)</td>
<td>(1.24 ± 0.10)</td>
<td>(8.8 ± 0.7)</td>
<td>(56.2 ± 4.4)</td>
<td>(0.44 ± 0.08)</td>
</tr>
<tr>
<td>32-36% Interface</td>
<td>16.9</td>
<td>6.3</td>
<td>4.2</td>
<td></td>
<td>2.0</td>
<td>2.5</td>
<td>0.3</td>
<td>1.6</td>
<td>0.9</td>
</tr>
<tr>
<td>36-40% Interface</td>
<td>6.3</td>
<td>6.7</td>
<td>6.1</td>
<td>3.9</td>
<td>3.1</td>
<td>5.5</td>
<td>0.2</td>
<td>4.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Pellet</td>
<td>(8.4 ± 0.6)</td>
<td>(37.2 ± 3.6)</td>
<td>(2.94 ± 0.2)</td>
<td>(28.5 ± 2.5)</td>
<td>(3.03 ± 0.04)</td>
<td>(2.73 ± 0.20)</td>
<td>(4.9 ± 0.5)</td>
<td>(147.7 ± 6.1)</td>
<td>(1.03 ± 0.1)</td>
</tr>
<tr>
<td>Homogenate</td>
<td>(5.4 ± 0.2)</td>
<td>(13.3 ± 1.1)</td>
<td>(1.83 ± 0.1)</td>
<td>(28.5 ± 3.5)</td>
<td>(0.627 ± 0.04)</td>
<td>(4.51 ± 0.4)</td>
<td>(2.2 ± 0.2)</td>
<td>(8.2 ± 3.5)</td>
<td>(6.1 ± 1.4)</td>
</tr>
</tbody>
</table>

Fig. 3. A typical field from a plasma membrane preparation as seen in thin section. The sample was prepared as described under “Experimental Procedures.” Magnification: × 60,000.

The column is proportional to the content of plasma membrane in the fraction being studied. Based on trichloroacetic acid-precipitable radioactivity, exactly twice as much material (8.6% compared to 4.3% of total counts per min) could be eluted from the column using 32 to 36% interface membranes as compared to ones from the 36 to 40% interface. Such a value agrees well with the 1.6- to 2.5-fold purification factor based on marker enzyme activities. A further control was undertaken to ascertain the specificity of the protein derived from solubilized plasma membranes for chromaffin granules per see. When columns were prepared from fixed bovine adrenal medullary mitochondria and lysosomes, instead of chromaffin granules, labeled protein was not bound and all of the radioactivity was recovered in the void volume (Fig. 5C). Lastly, the plasma membrane-derived material which was bound and subsequently eluted from fixed granules (as in Fig. 5A), retained, to a large extent, its capacity for being bound and eluted following pH neutralization (Fig. 5D).

Polyacrylamide Gel Electrophoresis of Eluted Materials—Gel electrophoresis (Fig. 6, a to c) of the plasma membrane protein bound to the affinity column showed it to be composed

Fig. 4. Affinity chromatography of [125I]-WGA from a column of fixed chromaffin granules. One milligram of [125I]-labeled WGA (specific activity: 300,000 cpm/mg) was applied to a 10-ml column of fixed granules as indicated under “Experimental Procedures.” Elution was carried out using 0.05 N HCl in 1% Triton X-100 (pH 1.5). The pH was measured in each fraction and is shown below the abscissa.

Fig. 5. Affinity chromatography of solubilized plasma membranes. A, 10 mg of [125I]-labeled plasma membrane protein (specific activity: 6 × 10⁸ cpm/mg of protein) was chromatographed on a 10-ml column of fixed chromaffin granules. The arrow indicates here, as well as in B, C, and D, the point at which the pH of the effluent reached 3.0. Sample application and elution is described under “Experimental Procedures.” B, 10 mg of solubilized membranes from the 36 to 40% interface were passed over a column identical with that used in A. C, chromatography of solubilized plasma membranes on a 10-ml column of glutaraldehyde-fixed mitochondria. D, the material eluted at low pH from A was reapplied to the affinity column of fixed chromaffin granules. All the data shown here are representative of at least three experiments performed identically.
to the purification of one major and two minor polypeptides referred to collectively in this discussion as the "binding protein."

A similar study showed tubulin migrating more slowly (data not shown). The autoradiogram (Fig. 6d) indicated that this was also a minor component of about 51,000 in the presence of SDS and β-mercaptoethanol. The autoradiogram (Fig. 6d) indicated that this was also the major source of the labeled material observed in the elution patterns of Fig. 5, and that there is a lesser component of about 14,000 which either stains poorly with Coomassie blue, or is highly iodinated by lactoperoxidase. In addition, a minor component can be seen in the stained gel at about 62,000 which is not significantly iodinated. When the bands were sliced out of the gel and assayed for radioactivity, the 62,000, 51,000, and 14,000 molecular weight species possessed 5%, 80%, and 15% of the total, respectively.

Two prominent proteins in secretory cells are actin ($M_j = 42,000$) and tubulin ($M_i = 53,000$ to 55,000). Isolated plasma membranes have also been shown to contain actin and this protein has been found to be associated (22) or capable of reassociating (23, 24) with chromaffin granules. To rule out the possibility that either of these two ubiquitous cellular proteins represents the major component possessing an affinity for chromaffin granules, iodinated G-actin and tubulin were electrophoresed together with the granule-bound material on polyacrylamide gels. Fig. 6e indicates that G-actin clearly migrates ahead of the granule-binding protein while a similar study showed tubulin migrating more slowly (data not shown).

**DISCUSSION**

The use of cellular organelles as affinity adsorbents has led to the purification of one major and two minor polypeptides whose further characterization and possible role in exocytosis can now be evaluated. The maintenance of isotonic conditions throughout the subcellular fractionation procedure yielded highly purified and stable chromaffin granules. This lessened the likelihood that proteins from the granule matrix, released upon lysis, could be covalently linked to the surface of the vesicles (during glutaraldehyde fixation) which could either result in the blocking of existing affinity sites, or in the creation of new ones.

Plasma membrane-enriched fractions were purified to a level which was in good agreement with previously published results. There was a slight improvement using our technique over the procedures described by Zinder et al. (25), Wilson and Kirschner (26), or Nijar and Hawthorne (27), although the procedures and the enrichment in marker enzyme specific activity differed in each case.

The use of fixed chromaffin granules and solubilized plasma membranes is not entirely free from the criticism that additional proteins possessing affinity for chromaffin granules might exist. First, solubilization in detergents, although it does allow the isolation of basically one active species, may be too selective and a similarly active, but poorly solubilized species would be overlooked. In addition, other proteins with an affinity for the granules may lose their activity upon solubilization of the membrane. Second, fixation of the granule may result in the inability to isolate a protein (possibly multivalent) whose affinity would be enhanced by the presence of freely mobile receptor sites within the granule membrane capable of forming patches or clusters. Despite these criticisms, the approach presented here and the results obtained comprise an essential first step in the examination of exocytosis at the molecular level. On the basis of its affinity for the granule surface, the binding protein can now be used as an affinity adsorbent in the isolation of its receptor from granule membranes, and piece by piece, an exocytotic system can be reconstituted.

The exact mechanism of binding of the protein to the granules is unclear. We have previously demonstrated, using plant lectins and neuraminidase, that there is carbohydrate-containing material on the cytoplasmic surface of chromaffin granules (19). For the sake of simplicity, it was hoped that such structures would serve, based on the specificity conferred by their oligosaccharides, as the receptors for the binding protein on the chromaffin granule. We have not yet been able to demonstrate such a specificity. The protein, when bound to fixed granules, could neither be eluted by a variety of glycopeptides derived from a pronase digest of whole chromaffin granules.

Creutz et al. (28) have demonstrated the existence of another protein, synexin, in the soluble phase of fractionated adrenal medulla. In our hands, this protein, capable of aggregating chromaffin granules in the presence of Ca++, binds very weakly, if at all, to columns of fixed granules. This is consistent with previous studies in which it was shown that aggregation by synexin was inhibited by prior fixation of the granules in glutaraldehyde (28). Furthermore, synexin has a lower apparent molecular weight in SDS than the protein described here, 47,000 as compared to 51,000 (28).

We would like to propose that the protein isolated in this study serves to bring about the anchoring of the chromaffin granule to the plasma membrane during exocytosis. Morphological studies on a variety of secretory systems have shown the granules to be in extremely close contact with the cell membrane immediately prior to fusion (1–3). In certain protozoans, intramembranous particles have been seen to be aggregated into rosettes to which secretory granules are bound.
prior to and during release of their contents (29, 30). In the last step prior to the breakdown of the two membranes during exocytosis the fusion site is devoid of intramembranous particles on faces belonging to either granules or plasma membranes (2, 3). One model, consistent with these morphological data, postulates that upon stimulation of the secretory cell the binding protein becomes available to the granule surface. The granules, arriving at the membrane by either a passive (e.g. Brownian movement) or an active (e.g. contractile elements) mechanism, are bound to the cell membrane by the formation of a binding protein·receptor complex. The next step would involve the clearance of the fusion site of the elements corresponding to intramembranous particles, in this case, the binding protein·receptor complex. Lastly, fusion occurs in particle-free areas perhaps as a result of the high lysophosphatidylcholine content of granule membranes (19, 31).

Preliminary experiments on functional aspects of this protein have been carried out in a system where chromaffin granules can be observed to fuse with one another in a fashion similar to that observed in the phenomenon of compound exocytosis. The extent of fusion could be determined morphometrically from electron micrographs of freeze-fractured preparations (32). Phospholipid vesicles containing the binding protein showed increased binding to secretory granules when compared to control vesicles. Chromaffin granule fusion was inhibited in the presence of such liposomes, implying the involvement of additional factors in fusion between granules and plasma membranes.

The validity of such a model ultimately depends upon proving a defined functional role for the binding protein in a secreting system in vivo. Two necessary correlates are the localization of a part or all of this protein on the inner bound receptor. Both investigations are currently underway.

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D I Meyer and M M Burger


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