Mechanism of Biosynthesis of Soluble and Membrane-bound Forms of Dopamine \( \beta \)-Hydroxylase in PC12 Pheochromocytoma Cells*

Esther I. Sabban\textsuperscript{¶}, Lloyd A. Greene\textsuperscript{¶**}, and Menek Goldstein\textsuperscript{¶}

*From the Departments of \textsuperscript{¶}Psychiatry, \textsuperscript{¶}Cell Biology, and \textsuperscript{¶}Pharmacology, New York University Medical Center, New York, New York 10016

Dopamine \( \beta \)-hydroxylase was present as 2 subunit forms (apparent \( M_r = 77,000 \) and 73,000) in the PC12 pheochromocytoma cell line as detected by immunoprecipitation from \([35S]\)methionine-labeled cultures, and analyzed by sodium dodecyl sulfate gel electrophoresis and fluorography. The \( M_r = 77,000 \) form was present in a crude membrane fraction, while the \( M_r = 73,000 \) form was soluble. Both forms appeared to be present in approximately equal amounts, and both were glycosylated. Treatment of PC12 cells with tunicamycin, a potent inhibitor of core glycosylation in the endoplasmic reticulum, completely inhibited the appearance of the \( M_r = 77,000 \) and \( M_r = 73,000 \) forms, and 2 new immunoreactive polypeptides were obtained (apparent \( M_r = 67,000 \) and 63,000).

Pulse-chase experiments suggested that the \( M_r = 77,000 \) form is initially synthesized (by 5 min) and a portion is converted in 15–90 min to the \( M_r = 73,000 \) form. Thereafter, the ratio between forms remains relatively constant, at least for several hours. Translation of mRNA from bovine and rat adrenals, and immunoprecipitation, indicated that dopamine \( \beta \)-hydroxylase is initially synthesized as a single polypeptide (apparent \( M_r = 67,000 \)). The subcellular site of biosynthesis of dopamine \( \beta \)-hydroxylase was determined by isolation of mRNA from free and membrane-bound polysomes from bovine adrenal medulla. Translation in a cell free system and immunoprecipitation localized the synthesis of dopamine \( \beta \)-hydroxylase on membrane-bound polysomes.

These experiments suggest that both soluble and membrane-bound forms of dopamine \( \beta \)-hydroxylase are synthesized and core glycosylated in the endoplasmic reticulum, and that there probably is a precursor-product relationship between the \( M_r = 77,000 \) and the \( M_r = 73,000 \) subunit forms of dopamine \( \beta \)-hydroxylase.

Dopamine \( \beta \)-hydroxylase (EC 1.14.17.1) is the enzyme which catalyzes the formation of norepinephrine from dopamine (Kaufman and Friedman, 1965) and consequently it is the marker enzyme for the noradrenergic neuronal system (Geffen et al., 1969; Goldstein et al., 1972). In nerve terminals and in adrenal chromaffin cells, dopamine \( \beta \)-hydroxylase is present in both the membrane and soluble content of the noradrenergic vesicles or chromaffin granules, respectively (Smith and Kirshner, 1967; Lagercrantz, 1976; Winkler, 1976). The soluble form of dopamine \( \beta \)-hydroxylase can be secreted with the catecholamines (De Potter et al., 1969; Weinshilboum et al., 1971; Viveros et al., 1968). The soluble and membrane forms in the adrenal have been found to be similar immunohchemically (Slater et al., 1981) and are reported to consist of four glycosylated subunits each with molecular weight of about 75,000 (Park et al., 1976; Fong et al., 1980; Rush and Geffen, 1980).

Differences in the soluble and bound forms of dopamine \( \beta \)-hydroxylase do appear, however, to exist. For instance, subtle differences in these forms were detected through peptide mapping (Slater et al., 1981). Charge-shift crossed immunoelectrophoresis has differentiated between an amphiphilic membrane-bound and a more hydrophilic soluble form (Bjerrum et al., 1979). These results have suggested that a small hydrophobic tail may anchor the enzyme in the membrane, and have raised the possibility of a biosynthetic relationship between the two forms. Recently, immature adrenomedullary vesicles in microsomal and Golgi fractions were shown to contain a high proportion of the amphiphilic form of dopamine \( \beta \)-hydroxylase (Helle and Serck-Hanssen, 1981). Pulse-chase studies with perfused adrenals suggested that the kinetics of incorporation of soluble and membrane forms of dopamine \( \beta \)-hydroxylase into vesicles were similar, but different from that of newly synthesized chromogranin (Ledbetter et al., 1978). On the other hand, Winkler et al. (1972) showed in labeling experiments with radioactive amino acids that the membrane proteins of adrenal-medullary granules were labeled considerably later than proteins in the soluble content. The membrane and releasable form of dopamine \( \beta \)-hydroxylase appeared to turn over at different rates, and it was suggested that the two forms are synthesized as different molecules and that there is no appreciable exchange between the membrane-bound and soluble pools of dopamine \( \beta \)-hydroxylase (Gagnon et al., 1976; Winkler, 1977).

We have utilized the PC12 pheochromocytoma cell line to investigate the mechanisms of biosynthesis of the soluble and membrane-bound forms of dopamine \( \beta \)-hydroxylase. Established from a transplantable rat pheochromocytoma, PC12 cells proliferate in serum-containing medium and possess the differentiated properties of chromaffin cells, including the presence of chromaffin granules, and the synthesis, storage, and release of dopamine and noradrenaline (Greene and Tischler, 1976, 1982; Greene and Rein, 1977). These cells have the advantage of providing large amounts of homogeneous material for biochemical analysis. In this paper, we report that in PC12 cells, subunits of dopamine \( \beta \)-hydroxylase isolated by immunoprecipitation, are present in soluble and membrane forms (apparent \( M_r = 73,000 \).
and 77,000, respectively). Both subunit forms are glycosylated by the tunicamycin-sensitive pathway. Evidence is presented to support a precursor-product relationship between these forms.

**EXPERIMENTAL PROCEDURES**

**Materials**—The following materials were purchased from commercial sources: \(^{[35}S\)methionine (800 Ci/ml), carrier-free \(^{[32}P\)orthophosphate, t-\(^{[5,6}H\)fucose (56 Ci/mmol), and liquiflor (New England Nuclear); \(^{[35}S\)methionine (amersham); \(^{[3}H\)-labeled molecular weight markers (Bethesda Research Laboratories); bovine liver catalase (17,000 units/mg), and N-glycine-HCl (N-Methyl-N-Benzyl-2-propynylamine) (Sigma); protein A-Sepharose (Pharmacia); Trasylol (MoBay Chemicals, New York, NY); tunicamycin (Calbiochem); Triton X-100 (Eastman); RPMI 1640, methionine-free RPMI 1640 (select amine kit), dialyzed horse serum, and dialyzed fetal calf serum (for tissue culture dishes, were labeled with \(^{[35}S\)methionine (20-150 Ci/ml) either in complete medium (labeling time 8 h) or methionine-free medium supplemented with 10% dialyzed horse serum, 5% dialyzed fetal calf serum, and 0.5% complete horse serum (labeling for less than 8 h). The experimental additives were also present in the medium used for labeling. Before addition to the medium, the labeled methionine was concentrated on ice under a stream of N, to remove the mercaptoethanol used as a preservative.

**Cell Growth and Labeling**—PC12 pheochromocytoma cells were grown as previously described in a humidified atmosphere containing 7.5% CO\(_2\) at 37 °C (Greene and Tischler, 1976; Greene and Rein, 1977). The cells, grown in monolayer cultures on collagen-coated tissue culture dishes, were labeled with \(^{[35}S\)methionine (20-150 Ci/ml) either in complete medium (labeling time 8 h) or methionine-free medium supplemented with 10% dialyzed horse serum, 5% dialyzed fetal calf serum, and 0.5% complete horse serum (labeling for less than 8 h). The experimental additives were also present in the medium used for labeling. Before addition to the medium, the labeled methionine was concentrated on ice under a stream of N, to remove the mercaptoethanol used as a preservative. For pulse-chase experiments, the cells were preincubated with the methionine-free media for 30 min, before addition of \(^{[35}S\)methionine. The pulse labeling was terminated by addition of excess methionine (final concentration 1 mM). For labeling with \(^{[3}H\)mannose (33-200 Ci/ml), \(^{[3}H\)fucose (50 Ci/ml), or \(^{[3}H\)glucosamine (50 Ci/ml), complete medium was used.

**Immunoprecipitation**—The cell lysates were washed twice with 0.15 M NaCl, pH 7.2 (PBS), then scraped and centrifuged 10 min at 5,000 g. The supernatant was collected and the pellet was washed twice and the lysate was centrifuged again at 5,000 g. The supernatant was used for immunoprecipitation.

**Assays**—The activity of dopamine \(\beta\)-hydroxylase was assayed according to Nagatsu and Udenfriend (1972), except that bovine serum albumin (0.1% Tris was added to each of the substrates). Freshly prepared cell lysates or soluble and membrane fractions of cell lysates were used. Protein was determined according to Bradford (1976) with the Bio-Rad assay kit. The catecholamines were extracted with alumina and analyzed by high pressure liquid chromatography as previously described (Sabban et al., 1981).

**Preparation of mRNA**—The RNA from rat adrenals or bovine adrenal medulla was prepared according to Liu et al. (1979). Free and membrane-bound polysomes were prepared from bovine adrenal medulla according to the procedure developed by Ramsey and Steele (1976, 1977, 1979) for rat liver polysomes. The RNA in the polysome pellet was extracted with phenol and glycine hydrochloride by a modification of the procedure of Cox (1964), as previously described (Sabban et al., 1981). The poly(A) mRNA was purified by oligo(dT)-cellulose chromatography (Aviv and Leder, 1972) and used for translation experiments.

**Cell Free Translations and Immunoprecipitation**—In vitro translation was carried out in a wheat germ lysate at 25 °C (Roman et al., 1976, 1977, 1979) with \(^{[35}S\)methionine. For immunoprecipitation, we used the equal 

**RESULTS**

**Subunit Forms of Dopamine \(\beta\)-Hydroxylase**—In PC12 cells—In order to characterize the subunit forms of dopamine \(\beta\)-hydroxylase, PC12 cells were labeled with \(^{[35}S\)methionine for several hours. The cell lysate was immunoprecipitated with antibodies prepared against rat dopamine \(\beta\)-hydroxylase, the immunoprecipitates were analyzed on 6-12% linear gradient polyacrylamide slab gels in SDS, and the radioactive proteins were detected by fluorography. Two specific subunit forms of immunoprecipitated dopamine \(\beta\)-hydroxylase were consistently detected with apparent molecular weights of 73,000 and 77,000 (Fig. 1). These forms are present in approximately equal amounts as indicated by their approximately equal intensity of \(^{[35}S\)methionine label. When cells were labeled for longer periods of time (24 h), these two bands were still present although they were somewhat more diffuse.

The presence of ascorbic acid, which has been found to stabilize dopamine \(\beta\)-hydroxylase against proteolysis in vitro (Wong et al., 1981), had no effect on the distribution of the subunit forms of dopamine \(\beta\)-hydroxylase. Thus, when 1 and 5 mM ascorbic acid was freshly added to PC12 cells during a 2-h preincubation and a 2-h labeling period, the two forms were still present in near equal amounts.

**Subunit Forms in Soluble and Membrane Fractions**—Dopamine \(\beta\)-hydroxylase in the adrenal is known to exist in membrane and soluble fractions of chromaffin granules. We carried out a crude subcellular fractionation of \(^{[35}S\)methionine-labeled PC12 cells to find the localization of the subunit forms of dopamine \(\beta\)-hydroxylase. Cells were lysed by brief sonication in hypotonic solution in the presence of protease inhibitors. A crude membrane fraction was prepared. The soluble and membrane fractions were brought to 2% sodium.
storage sites were lysed. Cholamines indicated that the norepinephrine and dopamine were soluble and hence, that under these conditions, the membrane fraction, while the 78,000-M, subunit form was greatly enriched in the soluble fraction (Fig. 2). Analysis of the fractions for catecholamines indicated that the norepinephrine and dopamine were soluble and hence, that under these conditions, the storage sites were lysed. 

The assay of dopamine β-hydroxylase activity was carried out on the membrane and soluble fractions (Table 1). Dopamine β-hydroxylase activity was recovered in both fractions, supporting the view that both subunit forms are indeed active dopamine β-hydroxylase. However, under the conditions used, the membrane form has an almost 10-fold higher specific activity when expressed per mg of total protein. Since the similar labeling of the 78,000- and 77,000-M, subunit forms with [35S]methionine suggests that they are present in near equal molar amounts, and since both fractions contained similar amounts of protein (Table 1), the variation in activity may indicate that the 77,000-M, membrane form is intrinsically more active. Alternatively, it may be less susceptible to inactivation during the fractionation. 

Since phosphorylation is known to modulate the activity of many enzymes including tyrosine hydroxylase (Raese et al., 1977; Latendre et al., 1977; Joh et al., 1978; Markey et al., 1980; Yamauchi and Fujisawa, 1979; Lazar et al., 1982), we examined whether the two subunit forms might be differentially phosphorylated. PC12 cells were labeled with [35S]methionine for 4 h and aliquots of the total protein (lane A) were immunoprecipitated with antisera specific for dopamine β-hydroxylase (lane B) or with preimmune sera (lane C), analyzed on a 6–12% polyacrylamide slab gel in the presence of SDS and processed for fluorography as described under "Experimental Procedures." The soluble fraction contained 52% and the membrane had 48% of the trichloroacetic acid-precipitable [35S]Met label. The solutions were adjusted to contain 2% sodium dodecyl sulfate and aliquots (5 μl) were taken to analyze the total profiles of proteins in the soluble (lane A), and membrane fractions (lane C), and to compare these to the postnuclear supernatant (lane B). The remainder of the material in each fraction (145 μl) was immunoprecipitated with specific antibodies against dopamine β-hydroxylase. The immunoprecipitates from soluble, membrane-bound, and total postnuclear supernatant are shown in lanes D, F, and E, respectively. This experiment was repeated twice and comparable results were obtained.

**TABLE I**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Dopamine β-hydroxylase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/dish</td>
<td>% pmol octopamine/mg/min</td>
</tr>
<tr>
<td>Postnuclear supernatant</td>
<td>2.34 ± 0.81 100.0 143.7 ± 16.7</td>
</tr>
<tr>
<td>Soluble</td>
<td>1.43 ± 0.66 55.7 ± 4 25.2 ± 3.9</td>
</tr>
<tr>
<td>Membrane</td>
<td>1.10 ± 0.34 44.3 ± 4 249.0 ± 37.0</td>
</tr>
</tbody>
</table>
Mechanism of Biosynthesis of Dopamine β-Hydroxylase

A B C

FIG. 3. [3H]mannose-labeling of dopamine β-hydroxylase.
The fluorograph of an SDS gel shows the total [3H]mannose-labeled protein profile obtained from cells treated in the absence (A and C) and presence of 15 µg/ml of tunicamycin (B). The cells were treated with D-[2,6-3H]mannose for 4 h (33 &i/ml (A and B) or for 24 h (200 &i/ml (C). The former (cells shown in A and B corresponding to 80,000 and 25,000 cpm of trichloroacetic acid-precipitable material respectively) were immunoprecipitated with antibodies to dopamine β-hydroxylase (D and E) and the entire immunoprecipitates were compared to immunoprecipitates from [35S]Met-labeled cells (F). Arrows indicate positions of the two dopamine β-hydroxylase bands.

FIG. 4. Effect of tunicamycin treatment on dopamine β-hydroxylase. PC12 cells were preincubated for 2 h in complete medium with tunicamycin (at the concentrations specified below), and then labeled in methionine-free medium with [35S]Met in the presence of the same concentrations of tunicamycin. The total profile of labeled proteins, as resolved by SDS gel electrophoresis, is shown from a comparable amount of lysate (from about 2 x 10⁵ cells) of control cells (lane A), and cells treated with 6 µg/ml (lane B) or 10 µg/ml (lane C) of tunicamycin. Lanes D–L show fluorographs of immunoprecipitates run on a separate gel in which electrophoresis was continued for several hours after the marker dye ran off in order to maximize the separation of the subunit forms. The immunoprecipitates from control cells (D–F) are compared to those treated with 6 µg/ml (G–I) or 10 µg/ml (J–L) of tunicamycin and were obtained with preimmune serum (D, G, and J) or antiserum to dopamine β-hydroxylase (E, F, H, I, K, L). Approximately equal amounts of cells (4 x 10⁶ cells) corresponding to 2 x 10⁶ cpm (control), 1.7 x 10⁶ cpm (6 µg/ml of tunicamycin), or 1.6 x 10⁶ cpm (10 µg/ml of tunicamycin) of [35S]Met-labeled protein was used for the immunoprecipitations. Arrows indicate positions of bands that were specifically immunoprecipitated by the antiserum to dopamine β-hydroxylase.

sugars and then processed for immunoprecipitation to detect whether both subunit forms are glycosylated. After treatment for 4 h with [3H]mannose, both subunit forms of dopamine β-hydroxylase were labeled, suggesting that both are glycosylated (Fig. 3). In longer labeling (several days), the immunoprecipitated material was extremely diffuse with electrophoretic mobility corresponding to M, = 73,000–77,000. Labeling with [3H]fucose also revealed two subunit forms which incorporate similar amounts of [3H]fucose (not shown).

To further delineate the glycosylation of the multiple forms of dopamine β-hydroxylase, PC12 cells were treated with tunicamycin. The latter drug blocks glycosylation by interfering with the formation of dolichol-bound N-acetyl glucosamine derivatives (Struck and Lennarz, 1977; Tkacz and Lampen, 1975). Tunicamycin (6 µg/ml) inhibited incorporation of [3H]glucosamine into protein in PC12 cells by over 80%, while reducing protein synthesis by only 13%, as monitored by incorporation of [35S]methionine into protein. As shown in Fig. 4, the synthesis of the 73,000- and 77,000-M, subunit forms was almost completely inhibited in the tunicamycin-treated cultures and two new specific immunoreactive polypeptides (apparent M, = 63,000 and 67,000) were obtained. It should be noted that, while similar numbers of trichloroacetic acid-precipitable counts were used for each immunoprecipitation shown in Fig. 4, the immunoreactive forms in the tunicamycin-treated cells were considerably reduced, suggesting that the non-glycosylated forms may be more rapidly degraded. Quantification of densitometer scans of the results, shown in Fig. 4, showed that in tunicamycin-treated cells the relative amount of immunoreactive dopamine β-hydroxylase in the two new specific polypeptides is 16% of the original forms. These results also showed that the antibodies recognized the non-glycosylated form of dopamine β-hydroxylase. Addition of up to a 5-fold concentration of antiserum did not increase the amount of the M, = 67,000 and 63,000 polypep-
ties immunoprecipitated, thus ruling out the possibility that the nonglycosylated material was not as efficiently immunoprecipitated under our experimental conditions.

**Relationship between the Subunit Forms**—Pulse-chase experiments were carried out to determine if there is a biosynthetic relationship between the multiple forms. When PC12 cells were labeled with [³⁵S]methionine for 5 min, only the 77,000-M₀ subunit form was detected (Fig. 5). A chase with unlabeled methionine subsequently showed the appearance of the 73,000-M₀ subunit form in near equal amounts to the 77,000-M₀ subunit form by 90 min. This proportion between the 73,000- and 77,000-M₀ subunit forms remained constant for at least several hours (Fig. 5). This is consistent with results in nerve growth factor-treated PC12 cells (Sabban et al., 1983), in which the 73,000-M₀ subunit predominates, and in which pulse-chase experiments show that the 77,000-M₀ subunit form is synthesized first. Due to the large reduction in the amount of dopamine β-hydroxylase in tunicamycin-treated cells, it was not feasible to carry out pulse-chase experiments with them, and to directly determine whether the 67,000-M₀ form is a precursor for the 63,000-M₀ form observed in tunicamycin-treated cells.

**Translation Product for Dopamine β-Hydroxylase**—In order to confirm that only one form of dopamine β-hydroxylase is initially synthesized, we examined the translation product for dopamine β-hydroxylase in a cell-free system. Isolation of newly synthesized dopamine β-hydroxylase, using mRNA from PC12 cells, gave a high nonspecific background, probably due to low molecular weight mRNA’s are more efficiently translated. Therefore, we examined the translation product for dopamine β-hydroxylase using mRNA from total rat adrenals, or from bovine adrenal medullae, both of which contain higher concentrations of dopamine β-hydroxylase (and presumably the corresponding mRNA) than the PC12 cells.

The mRNA was translated in a cell-free system and the newly synthesized polypeptide of dopamine β-hydroxylase isolated by immunoprecipitation. With rat adrenal mRNA, only one specific polypeptide (apparent M₀ = 67,000) was observed in the higher molecular weight region (not shown). Its electrophoretic mobility was identical to that of the larger form of dopamine β-hydroxylase in tunicamycin-treated PC12 cells. In translation of mRNA from bovine adrenal medulla (Fig. 6), a single polypeptide (apparent M₀ = 67,000) is obtained, along with an additional band of apparent M₀ = 32,000; the latter had an identical electrophoretic mobility to newly synthesized phenylethanolamine N-methyltransferase (Sabban et al., 1982). These results, although obtained in adrenals, support the findings in PC12 cells that dopamine β-hydroxylase is initially synthesized as a single polypeptide (apparent M₀ = 67,000).

**Site of Synthesis of Dopamine β-Hydroxylase**—The previously mentioned results with tunicamycin suggested that dopamine β-hydroxylase may be synthesized on membrane-bound polysomes since tunicamycin interferes with core glycosylation which takes place in the endoplasmic reticulum. The oligosaccharide-lipid donor for glycosylation of asparagine residues has been found localized on the luminal side of microsomes (Snider and Robbins, 1982).

In order to ascertain the subcellular site of synthesis of dopamine β-hydroxylase, free and membrane-bound polysomes were prepared from bovine adrenal medulla. The mRNA was extracted, and used in cell-free translations in a wheat germ extract system. This procedure yielded 2.6 times more free than bound mRNA. Both were active in directing protein synthesis. Immunoprecipitation of equal amounts of trichloroacetic acid-precipitable [³⁵S]Met-labeled protein, were immunoprecipitated with antibodies to dopamine β-hydroxylase and analyzed by gel electrophoresis and fluorography.

**Fig. 6. Site of synthesis of dopamine β-hydroxylase on membrane-bound polysomes.** The translation products in a wheat germ system obtained with mRNA from total bovine adrenal medulla (T) and from free (F) and membrane-bound polysomes (B) from the same source is shown. The translation products of mRNA from free (F) and bound (B) polysomes, corresponding to 2 × 10⁶ cpm of trichloroacetic acid-precipitable [³⁵S]Met-labeled protein, were immunoprecipitated with antibodies to dopamine β-hydroxylase and analyzed by gel electrophoresis and fluorography.

**Subunit Forms of Dopamine β-Hydroxylase**—The PC12 cells have been shown to synthesize two subunit forms of dopamine β-hydroxylase with apparent M₀ = 73,000 and 77,000. These forms were separated electrophoretically on gradient polyacrylamide-SDS slabs of immunoprecipitated...
dopamine \( \beta \)-hydroxylase. Two forms of dopamine \( \beta \)-hydroxylase were detected previously by charge shift immunoelectrophoresis of \( \beta \)-hydroxylase from adrenal medulla (Bjerrum et al., 1979). However, separation of two subunit forms, to our knowledge, has not previously been described on polyacrylamide gels, although dopamine \( \beta \)-hydroxylase often is represented as a rather diffuse band. The differentiation between these two forms probably reflects the enhanced sensitivity of long gradient polyacrylamide slab gels over the commonly used 7.5% or 10% polyacrylamide gels as well as the greater resolution of fluorography over sliced gels and scintillation counting. It should, however, be noted that in longer labeling times these bands appeared more diffuse and the distinction was somewhat less clear-cut. This may reflect alterations in the carbohydrate moiety during the lifetime of the molecule.

The membrane form appeared to have a 10-fold higher specific activity than soluble dopamine \( \beta \)-hydroxylase in this system. However, the possibility that the soluble form may be inactivated more rapidly cannot be ruled out. In studies on dopamine \( \beta \)-hydroxylase in the rat adrenal, Giarratana et al. (1975) also reported a much higher (6-fold) specific activity of dopamine \( \beta \)-hydroxylase in a particulate than a soluble fraction obtained from a total homogenate. They showed by immunotitration that the differences were not due to altered amounts of enzyme, but rather that the particulate fraction has a higher homospecific activity (activity/amount of the specific protein measured immunologically (Rush et al., 1974)). In contrast, others (Rush et al., 1974; Helle and Serck-Hanssen, 1981) who studied the specific activity of dopamine \( \beta \)-hydroxylase in isolated granules found homospecific activity of the soluble fraction about 5-fold higher than membrane-bound forms of dopamine \( \beta \)-hydroxylase.

While immunological data are subject to the possibility of detecting a contaminating protein, this is unlikely in the present study. The presence of enzyme activities in the soluble and membrane-bound fractions would indicate that both subunit forms (\( M_r = 73,000 \) and 77,000) indeed represent dopamine \( \beta \)-hydroxylase. However, the order of magnitude difference in specific activity between them makes the argument ambiguous—since small contamination of the soluble form with the membrane form could alter the results. More conclusive evidence that both forms probably are indeed bona fide dopamine \( \beta \)-hydroxylase comes from the pulse-chase experiments, particularly in nerve growth factor-treated cells in which the 77,000-\( M_r \) form is almost completely converted to the 73,000-\( M_r \) form (Sabban et al., 1983).

The pulse-chase experiment on untreated PC12 cells showed that initially the 77,000-\( M_r \) subunit form is synthesized and apparently processed to the 73,000-\( M_r \) form in 15-90 min. The similarity of the labeling pattern between 90 min to 4 h indicates that equilibrium has been achieved and that the label does represent total protein, and not just rate of synthesis. It should, however, be noted that during the chase (Fig. 5), there is an increase in the amount of both subunits. We did not detect any immunoreactive higher molecular weight precursor. The increase in radioactivity probably reflects variation in the “actual” labeling time during the chase, assuming a lag for entry of \(^{35}\)Smethionine into the cellular pool and a similar lag for entry of the unlabeled chase methionine. Moreover, any nascent chains whose biosynthesis began during the pulse, and which are completed during the chase, would show up in the immunoprecipitate. In support of these possibilities, a comparable experiment employing antis serum to tyrosine hydroxylase also showed increased labeling of tyrosine hydroxylase during the chase period, although in this case there is no processing.

Site of Synthesis of Dopamine \( \beta \)-Hydroxylase—We have shown directly that dopamine \( \beta \)-hydroxylase is synthesized exclusively on membrane-bound polysomes of bovine adrenals. While parallel experiments could not be carried out with PC12 cells, it appears likely that a similar mode of synthesis pertains in this system as well. Such a mechanism is consistent with the site of synthesis of a protein destined for secretion (as occur in a variety of systems) and of most plasma membrane proteins (Palade, 1975; Blobel, 1978; Sabatini and Kreibich, 1976; Sabatini et al., 1982). Thus, dopamine \( \beta \)-hydroxylase would be expected to contain an NH\(_2\)-terminal signal sequence which directs vectorial discharge into the endoplasmic reticulum and which is subsequently removed (Blobel and Sabatini, 1971; Blobel and Dobberstein, 1975). However, surprisingly the electrophoretic mobility of the translation products is identical to one of the forms in tunicamycin-treated cells. Perhaps, dopamine \( \beta \)-hydroxylase may contain an internal insertion signal similar to ovalbumin (Lingappa et al., 1979) or the major transmembrane erythrocyte glycoprotein, band 3 (Sabban et al., 1981; Braell and Lodish, 1982). The mechanism by which dopamine \( \beta \)-hydroxylase is inserted into membranes should be investigated further.

The exclusive localization of mRNA for dopamine \( \beta \)-hydroxylase on membrane-bound polysomes would indicate, as previously suggested (Gagnon et al., 1976), that the portion of the dopamine \( \beta \)-hydroxylase which is detected in the supernatant during subcellular fractionation is due to leakage from vesicles during the preparation and that dopamine \( \beta \)-hydroxylase is not present in a free form in the cytosol.

There appears to be only one translation product for dopamine \( \beta \)-hydroxylase, with apparent \( M_r = 67,000 \). This value may seem somewhat low since subunits of bovine dopamine \( \beta \)-hydroxylase, with electrophoretic mobility corresponding to apparent molecular weight of 73,000-75,000, is reported to contain about 5% carbohydrate (Wallace et al., 1973; Geissler et al., 1977; Fischer-Colbrie et al., 1982). However, it is not unusual for glycoproteins, and particularly sialoglycoproteins to have somewhat anomalous electrophoretic mobilities.

It should be noted that immunoprecipitation from the translation with anti-dopamine \( \beta \)-hydroxylase antiserum also isolates a polypeptide with identical electrophoretic mobility to newly synthesized phenylethanolamine-N-methyltransferase (apparent \( M_r = 32,000 \)). Moreover, this polypeptide is much more prominent in immunoprecipitates from free polysomes, and thus, is unlikely to represent a degradation product of dopamine \( \beta \)-hydroxylase. These results are interesting in light of recent suggestions by Joh and co-workers of possible similarities in domains between the enzymes involved in the synthesis of catecholamines (Joh et al., 1981).

The results presented here, as well as those by Helle and Serck-Hanssen (1981), suggest post-translational processing of dopamine \( \beta \)-hydroxylase from the membrane to soluble form. We cannot, however, rule out the possibility of formation of the 73,000-\( M_r \) and 77,000-\( M_r \) forms at different rates from a higher molecular weight precursor which is unrecognized by antiserum to native dopamine \( \beta \)-hydroxylase. However, the processing of the subunit form to the 73,000-\( M_r \) form seems the more likely interpretation of the data. This event occurs relatively quickly (within about 15-90 min), and it appears from the pulse-chase data that the processing does not occur initially, the distribution of the two forms remains relatively intact. Glycosylation does not appear to be necessary for this process, since in tunicamycin-treated cells, two subunit forms (albeit of lower apparent molecular weight) are obtained. These results indicate that the nonglycosylated forms may be less stable since they are reduced by about 6-fold in the tunicamycin-treated cells. Indeed, it has been
Mechanism of Biosynthesis of Dopamine $\beta$-Hydroxylase

suggested for a number of proteins that the function of the carbohydrate moiety is to prevent degradation, and there is evidence that the nonglycosylated forms of several proteins, such as fibronectin or the acetylcholine receptor, are more susceptible to proteolysis (Olden et al., 1982).

In summation, the experiments presented here lead to the following model for the biosynthesis of dopamine $\beta$-hydroxylase. The enzyme is synthesized in the endoplasmic reticulum on membrane-bound polyomes ($M \approx 67,000$) and rapidly glycosylated to a $77,000-M_r$ form. This membrane-bound form can be processed to a $73,000-M_r$ soluble form rapidly within $15-90$ min. If not converted then, the distribution remains relatively constant, at least for several hours.

These findings on the mechanism of biosynthesis of dopamine $\beta$-hydroxylase should be helpful in designing further experiments on the biogenesis of chromaffin granules and neuronal vesicles. In particular, it would be of considerable interest to elucidate the factors which regulate whether do-
Additions and Corrections

Vol. 258 (1983) 7812–7818

Mechanism of biosynthesis of soluble and membrane-bound forms of dopamine β-hydroxylase in PC12 Pheochromocytoma Cells.

Esther L. Sabban, Lloyd A. Greene, and Menek Goldstein

Page 7818, left column, the last line of text was omitted during printing. The last sentence should read:

In particular, it would be of considerable interest to elucidate the factors which regulate whether dopamine β-hydroxylase will be soluble or membrane-bound.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.
Mechanism of biosynthesis of soluble and membrane-bound forms of dopamine beta-hydroxylase in PC12 pheochromocytoma cells.

E L Sabban, L A Greene and M Goldstein


Access the most updated version of this article at http://www.jbc.org/content/258/12/7812

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/12/7812.full.html#ref-list-1