Processing of chromogranins in chromaffin cell culture: effects of reserpine and α -methyl-*p*-tyrosine

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Bovine chromaffin cell cultures were treated with either reserpine or α -methyl-*p*-tyrosine for up to 10 days. Afterwards the cells were harvested and the degree of proteolytic processing of secretogranin II, chromogranin A and chromogranin B was determined by immunoblotting and HPLC followed by RIA. There was a significant increase in the proteolysis of all three chromogranins after 4–6 days in the presence of reserpine. The small peptides formed in the presence of reserpine *in vitro* are also produced *in vivo*. A similar effect was observed with α -methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase, but the response took up to 10 days to develop. Both drugs decreased catecholamine levels but reserpine was more effective, reaching a

high degree of depletion after 4 days. In addition, experiments *in vitro* indicate that low millimolar amounts of either adrenaline (IC_{50} 5.2 mM) or noradrenaline (IC_{50} 2.4 mM) can significantly impair the proteolytic activity of recombinant murine prohormone convertase 1 when assayed with synthetic fluorogenic and/or peptidyl substrates. We conclude that a lowering of catecholamine levels in chromaffin granules leads to a concomitant increase in proteolytic processing of all secretory peptides. Apparently within chromaffin granules the endoproteases are inhibited by catecholamines and thus their removal leads to increased proteolysis.

INTRODUCTION

It is well established that treatment of cultured chromaffin cells with reserpine and tetrabenazine induces an increase in the endogenous proteolysis of proenkephalin [1–5] and chromogranin A [6]. There is also an increase in peptidyl-glycine amidation of a proenkephalin-derived peptide [3,7]. The adrenal secretory vesicles (the chromaffin granules) contain, in addition to proenkephalin and chromogranin A, other peptides, i.e. chromogranin B and secretogranin II, which are proteolytically processed by endogenous endoproteases (see [8,9]). It is not known whether reserpine also increases their proteolysis. The enzymes most likely to be involved in the processing of all these peptides are the prohormone-converting enzymes, PC1 and PC2 [10,11]. These proteins are present in chromaffin granules [12–14] and have been shown to be active on proenkephalin [15], chromogranin A [16] and secretogranin II [14].

The effect of reserpine on processing could in principle be caused by two effects: (i) an increase in the biosynthesis of these proteases, or (ii) an increase in the activities of the enzymes. This has not yet been investigated. The best characterized effect of reserpine and tetrabenazine in chromaffin cells is the specific blockage of the amine transporter localized in the membranes of chromaffin granules [17,18], leading as an end result to catecholamine depletion. Catecholamine depletion can also be induced by blocking their synthesis with α -methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase [19].

The present study has tried to answer three questions: (1) Does reserpine cause an increased proteolysis of all major peptides of chromaffin granules? (2) Does α -methyl-*p*-tyrosine induce an analogous effect? (3) Can catecholamines interfere directly with the proteolytic activity of convertases?

EXPERIMENTAL

Cell culture

Chromaffin cells were isolated from fresh bovine adrenal glands by collagenase (0.15 %, Boehringer Mannheim Biochemicals, Mannheim, Germany) digestion by a standard procedure [20] with some modifications. The use of differential centrifugation for purification of chromaffin cells after isolation was replaced by differential plating in culture medium overnight. On the next day cells were plated in collagen-coated 24-well plastic culture dishes (Costar, Cambridge, MA, U.S.A.) at a density of 10^6 cells per well. Culture medium consisted of Dulbecco's modified Eagle's medium/F12 Ham (1:1) containing 15 mM Hepes, 5 % (w/v) heat-inactivated fetal calf serum (Sebak, Austria), 2 mM L-glutamine, 100 i.u./ml penicillin, 100 μ g/ml streptomycin (all from Biological Ind., Israel) and 10 μ g/ml cytosine-arabinofuranoside (Sigma, Germany). Cells were kept at 5 % CO_2 in air and 37 °C. On the third day after isolation new medium containing reserpine (10 μ M, dissolved in DMSO with a final concentration of 0.1 %) or α -methyl-D,L-*p*-tyrosine methyl ester (5 mM) was added. Control cells for reserpine received an equivalent concentration of DMSO.

Harvesting

Medium was removed and cells were washed with ice-cold PBS. After sonication in distilled water an aliquot was taken for analysis of catecholamines. The remaining extract was immediately boiled for 10 min. After centrifugation for 20 min at 13 500 *g* at 4 °C the supernatant was analysed for chromogranins A and B and secretogranin II peptides by RIA and immunoblotting.

Abbreviations used: AMC, 7-amino-4-methylcoumarin; hfurin, human furin; mPC1, murine prohormone convertase 1; PC1, PC2, prohormone-convertase 1, 2; SN, secretoneurin.

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Chromatography

Cell extracts were separated by gel-filtration HPLC on a Superose 12 HR 10/30 column (Pharmacia LKB, Sweden). Boiled extracts of rat liver (60 μ l, containing 300 μ g of protein) were added to each sample to prevent non-specific absorption of the small amounts of protein to the column. Chromatography was performed at a flow rate of 0.4 ml/min with 150 mM sodium phosphate/NaCl containing 0.02% NaN_3 . Fractions of 1 min were collected and analysed by RIA.

Radioimmunoassay

HPLC-purified peptides [GE-25 for chromogranin A, PE-11 for chromogranin B and secretoneurin (SN) for secretogranin II] as standards and ^{125}I -labelled peptides as tracers were used as described by Kirchmair et al. [21] with slight modifications. Standards and samples were incubated in RIA buffer with antisera (final dilutions: GE-25, 1:5400; PE-11, 1:9000; SN 1:13 500) for 24 h at 4 °C. Then tracer was added and samples were incubated for another 24 h at 4 °C. Bound/free separation was performed with dextran-coated charcoal. The antisera against GE-25 and secretoneurin recognize not only the free peptides but also the larger precursors including the prospective proproteins chromogranin A [22] and secretogranin II [21]. In RIA the antiserum against PE-11 (chromogranin B 524–534 [23]) reacts preferentially with the free C-terminal end of the peptide (B. Leitner and H. Winkler, unpublished work), but not with intermediate precursors or the propeptide chromogranin B. In immunoblotting the antibodies also recognize the precursor peptides.

Immunoblotting

Boiled cell extracts (or aliquots of cell suspensions) were separated by one-dimensional SDS/electrophoresis as described previously [24]. Afterwards proteins were transferred electrophoretically on to a nitrocellulose membrane and analysed by immunoblotting with peptide antisera against the respective peptide. For antisera a dilution of 1:250 was used. The antiserum against PC1 and PC2 [13] were supplied by R. Hogue-Angeletti (A. Einstein College of Medicine, New York, U.S.A.). For secretogranin II an antiserum against LF-19 (secretogranin II 133–151) was used [21]. Quantitation was done by FUJIX BAS 1000 PhosphorImager Analyzer.

Enzymic assays

The fluorogenic substrate pGlu-Arg-Thr-Lys-Arg-4-methylcoumaryl-amide (Peptide International, Louisville, KY, U.S.A.) was kept as a 2 mM stock solution in DMSO at –20 °C. Stock solutions of adrenaline and noradrenaline (both products were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.) were always prepared fresh in the assay buffer, their pH adjusted to 6.0 and used immediately; where ascorbic acid was used, it was added directly to the stock solutions of catecholamines at a molar ratio of 1:10.

Two endoproteases, human furin (hfurin) and murine prohormone convertase 1 (mPC1), were used in this study. The hfurin was obtained from the medium of somatomammotroph GH_4C_1 cells after infection with the corresponding recombinant vaccinia virus and partly purified as previously described [25]. The mPC1 was obtained from the medium of Sf9 insect cells after infection with corresponding recombinant baculovirus and was recovered through ammonium sulphate precipitation, as will be described elsewhere (A. Boudreault, N. Rondeau, N. G. Seidah, M. Chretien and C. Lazure, unpublished work).

For each assay, done in duplicate, the substrate was incubated with proteinase sample in a total volume of 100 μ l containing 50 mM sodium acetate, pH 6.0, and 10 mM CaCl_2 for mPC1 (3 units), and 50 mM sodium acetate, pH 7.0, and 1.0 mM CaCl_2 for hfurin (2 units); one unit of enzymic activity is defined as the amount of enzyme necessary to release 1 pmol of 7-amino-4-methylcoumarin (AMC) from the fluorogenic substrates (50 μ M) per minute. Incubations in the presence of catecholamines were performed in 96-well plates with different concentrations obtained by 2-fold serial dilution of the initial stock solution at 25 °C and in the presence of either 50 or 150 μ M fluorogenic substrate. All fluorescence measurements were made at various times on a Perkin-Elmer LS-50B spectrofluorimeter with an excitation wavelength of 370 nm and an emission wavelength of 460 nm. Measurements of initial rate were also made in the presence of ascorbic acid under identical conditions.

In addition, the effect of added catecholamines in the presence of ascorbic acid was examined by monitoring the cleavage of synthetic peptidyl substrates, such as the pro-parathyroid hormone tridecapeptide, representing positions –6 to +7 of the human pro-parathyroid hormone, by reverse-phase HPLC as previously described [26].

Catecholamine assay

Catecholamines were quantified by HPLC [27].

RESULTS

The proteolytic processing of the three investigated chromogranins in the presence of reserpine or α -methyl-*p*-tyrosine was evaluated by HPLC and by immunoblotting. In the presence of these compounds the catecholamine content of the cultures decreased compared with the controls. As shown in Figure 1, reserpine led to a faster decline. After 4 days only 15% of the catecholamines remained, whereas with α -methyl-*p*-tyrosine this level was reached only after 8 days.

Secretogranin II

The immunoblotting results are shown in Figure 2. For these experiments an antibody was used that is directed against a

Figure 1 Catecholamine levels in chromaffin cells

The catecholamine levels of cell cultures in the presence of reserpine or α -methyl-*p*-tyrosine (α -m-*p*-tyrosine) are given as percentages of controls cultured for the same times ($n = 5$ for reserpine and $n = 8$ to 17 for α -methyl-*p*-tyrosine). The S.E.M. is given only for values where it was not too small to be drawn.

Figure 2 Immunoblotting of extracts from chromaffin cells

Boiled extracts of chromaffin cell cultures were subjected to immunoblotting. An antiserum against PE-11 (chromogranin B; CgB) was used. The cells were treated with reserpine (R) for 1 day (1d) or 6 days (6d), or with α -methyl-*p*-tyrosine (Tyr) for 10 days (10d). C, controls; SgII, an antiserum against LF-19 (secretogranin II 133–151) was used. The cells were treated for 6 days with reserpine.

sequence close to the N-terminal end (LF-19; secretogranin II 133–151) of secretogranin II [13]. After 4 days, and even more after 6 days, in the presence of reserpine the band representing secretogranin II had declined whereas the band representing the N-terminal piece (secretogranin II 1–153 [14]) had increased. In controls secretogranin II represented 36.3 % of the total counts and this declined to 9 % after 6 days, whereas for the N-terminus the values were 51.4 % and 75.5 % respectively (see Table 1).

The results obtained by HPLC chromatography followed by RIA are shown in Figure 3. For these experiments an antibody against secretoneurin [21] was used. After 6 days in the presence of reserpine the high-molecular-mass immunoreactive peaks had declined whereas the peak representing the free peptide secretoneurin (see [21]) had significantly increased (from 17.9 % to 43.7 % of total immunoreactivity; see Table 1).

An analogous result was obtained in the presence of α -methyl-*p*-tyrosine for 10 days, but the breakdown of secretogranin II was less marked: the free peptide secretoneurin increased from 43.3 % to 54 % (see Table 1). It can be seen that after 10 days the control values (43.3 %) were also higher than those after 6 days (17.9 %).

Figure 3 Processing of secretogranin II

Cell extracts were subjected to HPLC–molecular sieve chromatography followed by RIA for secretoneurin. The elution time is given on the abscissa; the ordinate gives the immunoreactivity in fmol per fraction. The elution positions of secretogranin II (SgII) and free secretoneurin (SN) are indicated. (A) Cells treated with reserpine for 6 days; (B) cells treated with α -methyl-*p*-tyrosine for 10 days.

Chromogranin A

The chromogranin A proteolysis was analysed by HPLC only. As shown in Figure 4A, in the presence of reserpine there was an

Table 1 Quantitative analysis of chromogranin processing

The immunoblot results indicate how much of the total radioactivity (see Figure 2) is found in the fastest-moving band. The HPLC results indicate for chromogranin A (CgA) how much of the total immunoreactivity was eluted after 33 min (see Figure 4A). For chromogranin B (CgB) the immunoreactive peak in controls was taken as 100 % (see Figure 5). For secretogranin II (SgII) the percentage of total immunoreactivity in the peak given by free secretoneurin (see Figure 3) is presented. Where variation is shown, results are means $\pm$ S.E.M.; the number of experiments is given in parentheses. Significance of results compared with control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	CgA		CgB		SgII	
	Control	Treated	Control	Treated	Control	Treated
Reserpine, 6 days:						
Immunoblotting	—	—	9.5 $\pm$ 1(4)	26.0 $\pm$ 1.5*** (4)	51.4 $\pm$ 2.8(5)	75.5 $\pm$ 6.1** (5)
HPLC	14.7(2)	57.6(2)	100.0 $\pm$ 0(3)	402.0 $\pm$ 31.7*** (3)	17.9 $\pm$ 4.3(3)	43.7 $\pm$ 0.9*** (4)
α -Methyl- <i>p</i> -tyrosine, 10 days:						
Immunoblotting	—	—	9.7 $\pm$ 0.3(3)	18.0 $\pm$ 0.6*** (3)	—	—
HPLC	30 $\pm$ 2.9(3)	41.4 $\pm$ 0.1* (3)	100.0 $\pm$ 0(3)	268.5 $\pm$ 34.9** (3)	43.3 $\pm$ 2.5(3)	54.0 $\pm$ 2.2* (3)

Figure 4 Processing of chromogranin A

See Figure 3 for details. GE-25 immunoreactivity (chromogranin A; CgA) was determined. The elution positions of chromogranin A and of free GE-25 are indicated. (A) Cells treated with reserpine for 6 days; (B) cells treated with α -methyl-*p*-tyrosine for 10 days.

increased breakdown of chromogranin A. Although in controls there was no immunoreactive peak representing the free peptide GE-25 (see [22]), after 6 days in the presence of reserpine this peak was significant. In controls the immunoreactive material eluting after 33 min represented 14.7% of the total (mean value of two closely agreeing experiments); in the presence of reserpine this was 57.6 (see Table 1). After 10 days of culture, breakdown in controls was higher (30% eluting after 33 min), but even after that long time free GE-25 could not be detected (see Figure 4B). However, in the presence of α -methyl-*p*-tyrosine this breakdown was further increased (to 41.4% eluting after 33 min; see Table 1) and free GE-25 became apparent (see Figure 4B).

Chromogranin B

The immunoblotting results are shown in Figure 2. After 6 days in the presence of reserpine the large chromogranin B immunoreactive proteins have declined whereas smaller peptides have increased. Thus the fastest moving band represents in controls 9.5% of the total radioactivity and after reserpine 26% (see Table 1). For α -methyl-*p*-tyrosine the corresponding values were 1% and 18%.

The results obtained by HPLC are shown in Figure 5. It should be noted that in the RIA the antiserum hardly reacted with the large PE-11 precursors (chromogranin B) and so only a

Figure 5 Processing of chromogranin B

See Figure 3 for details. PE-11 immunoreactivity was determined. This antiserum does not give a significant reaction with larger precursors in the RIA. The elution position of the free peptide PE-11 is indicated. (A) Cells treated with reserpine for 6 days; (B) cells treated with α -methyl-*p*-tyrosine for 10 days.

Figure 6 Immunoblotting with antisera against PC1 and PC2

Aliquots of the cells were subjected to immunoblotting. C, controls; R, after 6 days of reserpine.

relatively small peptide was recognized but this does not represent free PE-11. Because the antibody in the RIA reacts with the free C-terminal end of PE-11 this peptide must represent PE-11 elongated at its N-terminal end (see Figure 5A). In the presence of reserpine the major immunoreactive peptide eluted at 41 min

Figure 7 Inhibition of recombinant mPC1 by catecholamines

The fluorescence of released AMC by recombinant mPC1 on incubation in the presence of 150 μ M pGlu-Arg-Thr-Lys-Arg-4-methylcoumaryl-amide was measured after 120 min at 25 $^{\circ}$ C in the presence of increasing amounts of adrenaline ($\blacktriangle$) or noradrenaline ($\circ$). The rate of AMC release per hour is plotted against the concentration of the catecholamines; each point represents the mean of two closely agreeing measurements.

increased to 402% compared with controls, whereas after α -methyl-*p*-tyrosine treatment a value of 269% is found.

PC1 and PC2

The protein levels for these enzymes were determined by immunoblotting (see Figure 6). For PC1 several immunoreactive bands were found. The presence of reserpine did not alter this pattern and there was no change in amounts: after 6 days with reserpine the level was $94.3 \pm 10.9\%$ (mean $\pm$ S.E.M.; $n = 5$) compared with 6-day controls. PC2 cells treated with reserpine for 6 days had an increased level when compared with controls ($179.4 \pm 21.6\%$, $n = 11$), whereas after 10 days with α -methyl-*p*-tyrosine there was only an insignificant increase to $125 \pm 26\%$ ($n = 5$). In contrast, cells treated for 6 days with reserpine also had an increased level of total chromogranin A immunoreactivity as measured by RIA ($201.6 \pm 44.6\%$, $n = 10$).

Effects of catecholamines on proteolytic activity of recombinant mPC1 *in vitro*

As shown in Figure 7, addition of either adrenaline or noradrenaline to the incubation buffer led to a marked decrease in the release of AMC from the fluorogenic substrate pGlu-Arg-Thr-Lys-Arg-4-methylcoumaryl-amide. In the case of recombinant mPC1, one can estimate an IC_{50} of 2.4 mM for noradrenaline and 5.2 mM for adrenaline. Similar IC_{50} values of 4.6 and 7.1 mM respectively were obtained with another prohormone convertase, recombinant hfurin. Considering that the mechanism of inhibition is still under investigation, this result might be indicative of a general effect of catecholamines on this class of endoproteases. Addition of ascorbic acid to the solution of either adrenaline or noradrenaline in a 1:10 molar ratio seems in the case of recombinant mPC1 to increase slightly the inhibition by noradrenaline but to decrease that by adrenaline; however, a more thorough study of this effect will need to be performed to confirm these results. Nevertheless it is clear that addition of ascorbic acid, by diminishing the oxidation of catecholamines, allows the measurement of fluorescence over a longer period. Indeed it is likely, considering the spectrometric properties of adrenochrome, a possible oxidized catabolite of noradrenaline,

that it could interfere with the measurement of the released AMC moiety.

To document further the effect of catecholamine on the enzymic activity of convertases, preliminary studies made by measuring the initial rate of cleavage with a fluorogenic substrate or by monitoring the cleavage of peptidyl substrates by reverse-phase HPLC agree with the above-mentioned results. Indeed, the initial rate of cleavage in the presence of 5.0 mM noradrenaline or 8.6 mM adrenaline was diminished by 40–60% compared with the initial rate measured in the absence of these compounds; this was seen with either enzyme, and in each case noradrenaline appeared more efficient than adrenaline (results not shown). Finally, incubation in the presence of the same amounts of noradrenaline or adrenaline of synthetic peptidyl substrates known to represent more efficiently cleaved substrates [25] also led to a marked decrease in the cleavage of these peptides; again, noradrenaline seemed more efficient than adrenaline (results not shown). These results taken together indicate that either recombinant hfurin or mPC1 can be inhibited by low millimolar amounts of the catecholamines.

DISCUSSION

In a previous study [6] it was shown that in cultured chromaffin cells the processing of chromogranin A to pancreastatin is increased by reserpine after 2–4 days. We have confirmed this finding by measuring another peptide formed from chromogranin A, i.e. GE-25. Both pancreastatin and GE-25 are formed *in vivo* [22,28,29], although in chromaffin cells processing of chromogranin A to these small peptides is rather limited [22,28,30]. Reserpine apparently induces an increase of the proteolytic processing of chromogranin A to peptides that are found in other tissues, e.g. endocrine pancreas or brain, in high concentrations [22]. Thus reserpine seems to accelerate a physiological process.

We have now shown that this increase in proteolysis not only, as previously shown, affects proenkephalin (see the Introduction section) and chromogranin A but also the other major secretory proteins [8] of chromaffin granules, chromogranin B and secretogranin II. This latter precursor is processed to secretoneurin, which *in vivo* is present in a low concentration in adrenal medulla but at much higher levels in brain and other organs where processing of secretogranin II is nearly complete [21]. The processing of chromogranin B to PE-11 has not been investigated before. Apparently in bovine tissue the free peptide is not produced; only a slightly larger peptide (about 23 amino acids) elongated at the N-terminal end of PE-11 is formed, whereas in rat tissues the free peptide PE-11 also arises (A. Laslop, B. Leitner, G. Rathmair, R. Fischer-Colbrie and H. Winkler, unpublished work). Thus we conclude that under normal conditions breakdown of the major secretory components of chromaffin granules is limited, whereas processing of all components is increased in the presence of reserpine. We have already demonstrated previously that such a concomitant proteolysis can also be observed when various tissues are compared. Thus in posterior pituitary and endocrine pancreas the hormone precursors for vasopressin and insulin respectively, and also chromogranin A and secretogranin II, are processed to a high degree [16,22,30,31] whereas in anterior pituitary and in chromaffin cells chromogranin A, B and secretogranin II are processed in a very limited way [21,22]. This would indicate that the processing of all these secretory peptides might depend on the same enzymes and on their levels. In fact it has been established that the most likely enzymes to be involved in prohormone and proneuropeptide processing are the endoproteases PC1 and PC2.

These enzymes are apparently also responsible for the proces-

sing of chromogranin A (PC2 [16]), chromogranin B (PC1 and PC2; A. Laslop, C. Weiss, H. Winkler and N. Seidah, unpublished work) and secretogranin II (PC1 and PC2 [14,32]).

By what mechanism can reserpine increase the activity of these enzymes, leading to a faster proteolysis of the secretory proteins? The most obvious action of reserpine is its binding to the catecholamine transporter of chromaffin granules [17,18]. This blocks catecholamine uptake and finally leads to catecholamine depletion. We have sought to mimic this effect by another drug achieving catecholamine depletion by a different mechanism. For this purpose the rather specific inhibitor of tyrosine hydroxylase, α -methyl-*p*-tyrosine, was useful. It effectively depleted catecholamines but more slowly than reserpine. We have now established that this compound also induces an increase in proteolytic processing of the secretory peptides but rather more slowly and to a smaller degree, which apparently matches its action on the catecholamine levels when compared with reserpine. Thus we can reach the firm conclusion that depletion of catecholamines from chromaffin granules induces increased proteolysis. The final question, of the mechanism by which this is brought about, is more difficult to answer. The protein levels of the endoprotease PC1 did not change after reserpine treatment, whereas that of PC2 after 6 days of treatment with reserpine was approx. 80% higher than in the 6-day controls. However, because chromogranin A, as the major secretory component of chromaffin granules, was elevated to a similar degree, the ratio of PC2 to the major substrate chromogranin A did not change. It is rather difficult to find an explanation for these increased levels, but reserpine becomes bound irreversibly to the membranes of chromaffin granules and might thus interfere with their biosynthesis, which includes recycling of membranes [33], in a complex way. Furthermore reserpine treatment also induces higher protein levels of the enzyme tyrosine hydroxylase by an unknown mechanism [34].

In any case the effect of reserpine on protein levels of chromaffin granules seems unlikely to be the explanation for the proteolysis increase, because the more specific agent α -methyl-*p*-tyrosine did not lead to a significant increase in PC2 levels. One is therefore left with the suggestion that proteolysis is changed simply by the decrease in catecholamine levels within chromaffin granules. An inhibition by catecholamines has already been demonstrated for another proteolytic enzyme thought to be present in those organelles [35]. This enzyme is a metalloprotease cleaving proenkephalin fragments at a single arginine residue. Thus catecholamines at a concentration of 0.6 M [36] within chromaffin granules are good candidates for inhibiting proteolysis in these organelles. Indeed, our experiments *in vitro* with either recombinant hFurin or mPC1 appear to indicate that even low millimolar amounts of adrenaline or noradrenaline can impair the proteolytic activities of either enzyme. These inhibitory effects of catecholamines are therefore in agreement with the above documented effects of catecholamine depletion on proteolysis in chromaffin granules. In addition it does provide an explanation of why processing of the chromogranins in chromaffin granules is slow under physiological conditions despite the presence of significant amounts of the endoproteases PC1 and PC2 and a half-life of these organelles of 1 week [37].

We can therefore conclude that both reserpine and α -methyl-*p*-tyrosine induce an increase in the proteolytic processing of the major secretory peptides of chromaffin granules, i.e. chromogranins A and B and secretogranin II. Catecholamines inhibit *in vitro* the endoprotease PC1 involved in this proteolysis.

Apparently within chromaffin granules the endoproteases are inhibited by catecholamines and thus their depletion as caused by reserpine or α -methyl-*p*-tyrosine induces a higher rate of proteolysis.

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