Divergent changes of chromogranin A/secretogranin II levels in differentiating human neuroblastoma cells

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Human neuroblastoma cells were cultured either in the absence or presence of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) known to induce neuronal differentiation. This treatment led to a marked increase in the concentration of secretogranin II but to a decrease of chromogranin A. Analogous changes were observed for the respective mRNAs. Thus during differentiation of these cells the biosynthesis of two vesicle constituents of large dense core vesicles is differentially regulated as determined both at the mRNA and the protein level. Levels of both synaptin/synaptophysin and SV2 were also elevated but to a smaller degree than that of secretogranin II.

Neuroblastoma; Chromogranin A; Secretogranin II; Synaptin/synaptophysin; SV2

1. INTRODUCTION

The catecholamine-storing organelles of the adrenal medulla, the chromaffin granules, and the large dense core vesicles of sympathetic nerve contain a complex 'secretory cocktail' [1]. Apart from the hormones these vesicles comprise neuropeptides and the so-called chromogranins [2,3]. Previous studies have already established that the biosynthesis of these components is not regulated en bloc allowing a significant blending of the secretory cocktail (adrenal medulla [4,5]; PC12-cells [6]; see also [7]).

In the present study the levels of various components of secretory vesicles and their mRNAs were studied in human neuroblastoma cells (SH-SY5Y). These cells differentiate morphologically, biochemically, and functionally, and are growth inhibited when treated with nanomolar concentrations of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [8–11]. Notably, the noradrenaline concentration increases 200–300-fold, dense core granules become more frequent, and the resting membrane potential increases [8–10]. Furthermore, the SH-SY5Y cells express muscarinic acetylcholine receptors [11,12], and when the TPA-treated cells are exposed to acetylcholine, the stored noradrenaline is released via a Ca^{2+}-dependent process [11]. In the present paper we demonstrate that TPA treatment of these cells downregulates the biosynthesis of chromogranin A whereas that of secretogranin II (chromogranin C; for nomenclature see [13]) is markedly elevated. Levels of SV2 [14] and synaptin/synaptophysin [15] are moderately increased.

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2. MATERIAL AND METHODS

2.1. *Cell culture*

The SH-SY5Y cells [16], kindly provided by June Biedler (Sloan Kettering Institute for Cancer Research, New York, NY), were cultured and differentiated by 16 nM TPA as described [9]. Essentially, 10^6 cells were seeded per 8.5 cm dish with or without differentiation inducer, and the cells were harvested at the indicated time points. Once the cells were harvested, they were stored at −70°C as a pellet, until they were lyophilized and analysed.

2.2. *Immunoblotting*

The harvested cells were subjected to quantitative immunoblotting as already described in detail [17]. The following rabbit antisera were used: anti-human chromogranin A, anti-rat secretogranin II [18], anti-synaptin/synaptophysin (kindly provided by R. Jahn, Munich). For SV2 a monoclonal antibody, 10 H3 [14], was employed. Protein was determined according to Lowry [19].

2.3. *RNA-extraction*

Cells were cultured in 6 well dishes for 5 days at a density of 2×10^5 per well. RNA was extracted with a guanidinium thiocyanate method [20]. Total RNA from two wells was combined, size-fractionated on denaturing agarose gels transferred electrophoretically to nylon membranes and hybridized for chromogranin A with a 57-mer oligonucleotide against human chromogranin A (bases 224–280) [21] labelled with T4 polynucleotide kinase. For secretogranin II a 756 bp *BglII* fragment of an adrenal medullary bovine secretogranin II clone [22] labelled by random priming was used.

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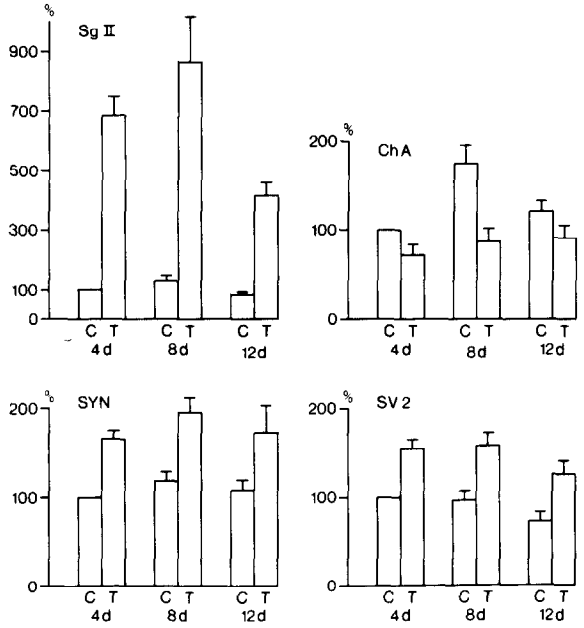

Fig. 1. Levels of constituents of storage vesicles in human neuroblastoma cells. Cells were cultured for up to 12 days in the absence or presence of 16 nM TPA (T) and were then analysed by quantitative immunoblotting. The results are expressed per mg protein as percentages of the levels in controls at day 4 which were taken as 100%. Mean values (several determinations for 3 different cultures) ± SE are shown ($n=9$ for Ch A; $n=10$ for Sg II; $n=12$–13 for SYN: $n=10$ for SV2). The differences between controls and TPA-treated cultures were statistically significant for all components and at each time point ($P<0.05$ to $P<0.001$). Ch A, chromogranin A; Sg II, secretogranin II (chromogranin C); SYN, synaptin/synaptophysin.

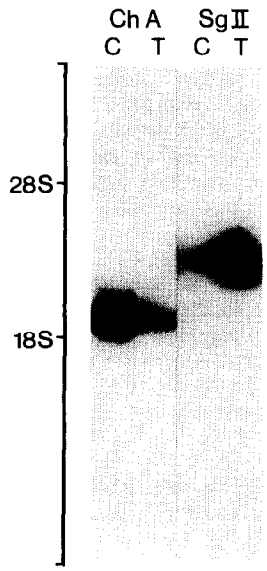

Fig. 2. Northern blots for the mRNA of chromogranin A and secretogranin II. Total RNA was extracted from control (C) and TPA (T)-treated cultures (after 5 days). The blots were either hybridized with chromogranin A (Ch A) or secretogranin II (Sg II) probes. The positions of the 28 S and 18 S ribosomal RNAs are indicated at the left side of the figure.

3. RESULTS

Several constituents of sympathetic large dense core vesicles and synaptic vesicles were determined in human neuroblastoma cell cultures in the absence or presence of TPA. Fig. 1 compiles the results. In control cultures over 12 days the levels of secretogranin II, synaptin/synaptophysin and SV2 did not change significantly whereas that of chromogranin A reached a higher level after 8 days. TPA treatment induced significant changes. A marked increase was observed for secretogranin II already after 4 days. At 8 days the level was even higher and then declined, which in part might be due to an increase in the population of non-responding cells [9]. On the other hand, chromogranin A levels were reduced when compared to controls. For the two membrane components, SV2 and synaptin/synaptophysin, increased levels from days 4 to 12 were observed.

Fig. 2 demonstrates the changes in the levels of the mRNAs for chromogranin A and secretogranin II induced by TPA. The chromogranin A message was significantly reduced by this agent (38.7 ± 4.2%, SE, of controls; $n=6$, $P<0.001$). On the other hand the secretogranin II message was markedly increased (1001.6 × 129.0%, SE, $n=6$, $P<0.001$).

4. DISCUSSION

The subcellular localization of the 4 components measured in this study is not identical. Chromogranin A and secretogranin II are constituents of chromaffin granules [2] and of large dense core vesicles in nerve [23]. SV2 and synaptin/synaptophysin are also present in these organelles [24–26] but are also found in translucent synaptic vesicles in apparently higher concentration [27]. Let us first discuss the differential changes in the biosynthesis of chromogranin A/secretogranin II which are relevant for the secretory content of large dense core vesicles. Previous studies have established that TPA treatment of human neuroblastoma cells leads to a differentiation which is accompanied by profound changes, including axonal growth and increased expression of the GAP43 gene coding for the axonal growth-area associated protein 43 [8–11,28]. In analogy, IMR-32 human neuroblastoma cells acquire regulated secretory properties after drug-induced differentiation [29]. These differentiated cells contain typical secretory granules comprising chromogranins/secretogranin [29]. We have now demonstrated that differentiation does not simply turn on the biosynthesis of chromogranin A/secretogranin II together. Quite to the contrary, a marked increase of secretogranin II is paralleled by a fall of chromogranin A and these changes are apparently based on a different regulation of their respective mRNAs. Previous studies have established that chromogranin A, B and secretogranin

II are not always regulated together. Thus, only the biosynthesis of chromogranin A depends on corticosteroids [4–6]. The present result provides a further clear-cut example of a differential regulation of these peptides costored within the same organelle [30]. An analogous behaviour to secretogranin II has recently been found for another protein apparently stored in large dense core vesicles of PC12 cells [31].

Our results suggest that TPA-induced differentiation of sympathetic cells to a more neuronal phenotype is accompanied by an increase in the biosynthesis of secretogranin II and by a decrease of chromogranin A. Previously we have already shown that large dense core vesicles of bovine sympathetic nerve when compared to adrenal chromaffin granules have a relatively higher content of secretogranin II versus the other chromogranins [23] and the same applies to brain tissue of the rat [32]. These data are consistent with a concept that the levels of secretogranin II are increased during differentiation of sympathetic cells to neurons.

The changes in the levels of two membrane components are more difficult to interpret. Their concomitant increase is smaller than that of the secretory protein secretogranin II. Does this indicate that more large dense core vesicles are formed and in addition, that each vesicle is filled with more soluble protein? On the other hand, the largest portion of these membrane components may be present in small translucent vesicles [26,27] and an increase might be due to more vesicles of this type. In any case we have established that the synthesis of these two membrane components is apparently not regulated separately during differentiation and compared with secretogranin II the increase in their synthesis is much smaller. This makes the TPA effect on secretogranin II expression even more specific and relevant.

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