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Adaptation Physiology: the Functioning of Pituitary Melanotrope Cells during Background Adaptation of the Amphibian Xenopus laevis

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INTRODUCTION

The vertebrate hypothalamo-hypophyseal system is responsible for converting neuronal information into an endocrine output, the release of hormones into the blood. This process is known as neuroendocrine integration. A major source of neural input to the neuroendocrine system comes from sensory information concerning conditions in the environment (e.g. light, temperature, presence of stressors). Neuroendocrine transducer cells of the hypothalamo-hypophyseal system are responsible for integrating the diverse neuronal information to produce and release endocrine signals. These signals in turn coorrdinate the functioning of peripheral organs (e.g. reproductive organs, adrenal glands, thyroid gland) thus allowing the animal to adapt to changes in the environment.

An example of such a neuroendocrine transducer cell is the melanotrope cell of the pituitary pars intermedia. In amphibians this cell is responsible for converting neuronal information concerning color of background into an endocrine output. The cell produces the multifunctional precursor protein proopiomelanocortin (POMC), which is processed to give a number of peptides including melanophore-stimulating hormone (α -MSH). This peptide hormone stimulates the dispersion of the

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black pigment melanin in dermal melanophores thus causing skin darkening. The hormone is released in animals on a black background. Among amphibians, the South Afircan clawed toad Xenopus laevis shows a particularly strong adaptive response to background color. The skin pigment goes very rapidly from the fully aggregated to the fully dispersed state in animals transferred from a white to a black background, and rapidly aggregates upon transfer from black to white background (Fig. 1). The adaptation of Xenopus to a black background involves activation of biosynthetic and secretory processes in the melanotrope cells, whereas these processes inactivate during white-background adaptation [17, 22, 32, 65]. Therefore, by simply changing background color, it is possible to manipulate the activity of Xenopus melanotrope cells. This ease of manipulation is an attractive feature of these cells for studies aimed at elucidating cellular and molecular mechanisms of cell activation and inactivation. Indeed, Xenopus melanotrope cells have been the object of study in a number of laboratories over many years, starting with classic studies of Hogben and Slome (reviewed in [64]). This model system and related topics were extensively reviewed several years ago [23, 31, 39, 60]. The intention of this survey is to examine recent developments in the analysis of Xenopus intermediate lobe melanotrope cells, particularly in relation to the physiological functioning of these

cells.

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Dynamics of Background Adaptation: the Demonstration of Short-term and Long-term Adaptive Mechanisms

It has generally been thought that α -MSH is the only factor involved in stimulating pigment dispersion in dermal melanophores of *Xenopus* during black background adaptation. An analysis of events during white to black background transfer, however, has shown that there is a major discrepancy between the degree of pigment dispersion in dermal melanophores and plasma α -MSH levels [67]. The pigment becomes fully dispersed within a few hours of transfer to black background and yet it takes several days for plasma α -MSH to reach its maximum level (see Fig. 1). These results suggested that a factor other than α -MSH must be

responsible for the rapid pigment dispersion seen during the early stage of black background adaptation. The discovery that the rapid pigment dispersion can be blocked by the β -adrenergic receptor antagonist propranolol indicates that a β adrenergic receptor is involved [67]. In vitro experiments show that this receptor is working at the level of the dermal melanophore cells in causing pigment dispersion. Denervated skin explants lack the rapid short-term response, which indicates that the short-term mechanism depends on innervation of the skin. Probably, this innervation is a component of the autonomic nervous system releasing (nor)adrenalin in the vicinity of the melanophores [24]. In long-term-adapted animals (several days or longer on black background) the release of a-MSH from the pars intermedia is critical for maintaining pigment dispersion. This is shown by the fact that neurotransmitters such as



FIG. 1. A summary of the dynamics of various aspects of background adaptation by the amphibian *Xenopus laevis*. Changes in the degree of pigment dispersion in dermal melanophores, in plasma MSH levels and in the level of POMC biosynthesis in pars intermedia melanotrope cells are shown. The discrepancy between the rapid pigment dispersion and slow increase in plasma α -MSH (indicated by white arrows) led to studies that established the involvement of a β -adrenergic mechanism causing pigment dispersion during the early stages of black background adaptation [67]. The relatively slow decrease in POMC biosynthesis (black arrow) in black to white background adaptations is, in part, probably due to the fact that an enzymatic system for the degradation of RNA must first be synthesized [4]. See text for further discussion.

dopamine and GABA, which are known to inhibit in vitro release of α -MSH, cause skin whitening when injected into fully black-backgroundadapted animals [61, 63]. Treatment of long-term black-adapted animals with propranolol has no effect on pigment dispersion, which is in sharp contrast to the effect of this β -blocker in shortterm black-adapted animals [67]. The importance of a-MSH in long-term regulation of the melanophores is further illustrated by an analysis of plasma a-MSH levels in animals adapted for several weeks to white, light grey, dark grey and black backgrounds [52, 66]. Grey animals, which have only partially dispersed pigment in dermal melanophores, were found to have plasma a-MSH levels that are intermediate between the low levels found in white-adapted animals and the high levels of black-adapted animals.

Physiological Significance of Short-term and Longterm Mechanisms

It is generally accepted that in most amphibians pigment cells are regulated primarily by the endocrine system (α -MSH) while in fishes the nervous system is a more important regulatory system. This seems to fit the locomotory behavior of these animals as amphibians tend to be more stationary than fish and thus may not need rapid color adaptation. The presence of β -adrenergic receptors on amphibian melanophores, and thus the potential for nervous control of these cells, was established in early studies [14, 15, 30] but the physiological significance of these receptors remained obscure. The idea has been forwarded that the β -adrenergic receptor might be activated as a result of acute handling stress, thus causing transitory darkening, termed "excitement darkening" [6]. It is apparent that, in the species X. laevis at least, this receptor is also involved in the regulation of pigment migration during adaptation to a dark background.

In considering the significance of the β adrenergic regulatory mechanism in *Xenopus* it is important to realize that the capacity of melanotrope cells to synthesize α -MSH is acquired only very slowly, taking a number of days to reach maximum level during adaptation to black background [22, 32, 34, 44]. Hormone stores possessed by animals on white background are, however, depleted within 24 hr during adaptation to black background [22, 62]. For *Xenopus*, the regulation of dermal melanophores must be viewed as a cooperative effort; the β -adrenergic mechanism plays an important role in the short-term, during which time the melanotrope cells of the neuroendocrine system acquire the capacity to produce enough α -MSH to maintain pigment dispersion in the long-term. This cooperation ensures that *Xenopus* is capable of both rapid and sustained pigment dispersion.

Melanotrope Cell Morphology: the Demonstration of Cell Recruitment during Background Adaptations

There have been several studies on the morphology and ultrastructure of melanotrope cells of white- and black-background-adapted Xenopus [17, 21, 65]. These studies have shown that cells of white animals are small and inactive when compared to those of black animals. A question that remained was how melanotrope cells meet an intermediate (submaximal) demand for α -MSH, such as in animals on grey background. Two possible physiological mechanisms are: (1) all melanotropes are activated to an intermediate level of secretion to meet the submaximal demand for hormone, or (2) a subpopulation of melanotrope cells becomes fully acitvated to meet this demand, whereas the other cells remain relatively inactive. In the first case, the melanotrope cells act as a homogeneous population whereas in the latter situation they are heterogeneous. The results of an analysis at the light and electron microscopic level showed that the pars intermedia of grey-adapted animals is composed of a mixture of inactive and fully active melanotrope cells [52]. It was therefore concluded that melanotrope cells respond as a heterogeneous cell population to an increased demand for a-MSH, with progressively more cells being recruited to the active state as the physiological demand for α -MSH increases.

Physiological Significance of Recruitment

Two general strategies may be used by endocrine cells to increase the rate of hormone release.

One is a simultaneous activation of the entire population of endocrine cells, the activation of oxytocin-producing neuroendocrine cells being a good example [49]. Here, the strength of stimulation (suckling) determines the degree of activation achieved in the homogeneous cell population. It appears that the heterogeneous response, exemplified by the intermediate lobe melanotrope cells of Xenopus, is the more general phenomenon among endocrine cells. Morphometric and biochemical evidence has been given that this mechanism holds for hormone secretion by gonadotropes [47, 48], lactotropes [35], pancreatic β cells [54] and follicular thyroid cells [11]. There may be an energetic advantage of this mechanism because an increased demand for hormone is met through activation of relatively few endocrine cells.

It appears from studies on mammalian lactotrope and somatotrope cells that the mechanism regulating cell recruitment involves differences in the sensitivity for regulatory factors among the individual endocrine cells. This has been discovered through an analysis of the effects of secretagogues on hormone release from individual cells, measured using the reverse-plaque assay [35, 47, 48] and, more recently, the sequential cell immunoblot assay [1, 26]. Using a modification of this latter assay it has been shown that *Xenopus* melanotropes display different sensitivities to dopamine, one of the α -MSH release-inhibiting factors [50].

Ultrastructure of Intermediate Lobe Nerve Terminals: Coexistence of Dopamine, GABA and Neuropeptide Y

The intermediate lobe melanotrope cells of X. laevis are regulated by multiple factors, both stimulatory and inhibitory (see Fig. 2). It had been assumed that each regulatory factor would be present in separate neuronal networks [23]. Analysis at the ultrastructural level, however, has revealed that GABA and NPY are present within



FIG. 2. A summary of the classical neurotransmitters and neuropeptides involved in the regulation of α-MSH secretion from the melanotrope cell of *Xenopus laevis*. Abbreviations: TRH, thyrotropin releasing hormone; CRH, corticotropin releasing hormone; NPY, neuropeptide Y; DA, dopamine; GABA, γ-aminobutyric acid; D₂, dopamine D₂ receptor; G_a, GABA_a receptor; G_b, GABA_b receptor. Stimulatory (+) or inhibitory (-) action on the secretory process is indicated. NPY, DA and GABA coexist in the same nerve terminals [51, 59] with NPY and DA being present within the same secretory vesicle [59].

the same nerve terminals within the intermediate lobe [51]; a recent study showed that these same varicosities also contain dopamine [59]. GABA is confined to small electron-lucent vesicles whereas dopamine and NPY are found in electron-dense vesicles. The varicosities have been shown to make close contact with both the melanotrope cells and the other major cell type of the pars intermedia, the so-called folliculo-stellate cells.

Physiological Significance of Coexistence

At present it is difficult to attribute a functional significance to the fact that the three α -MSH release inhibitory factors occur in the same nerve terminals. In another case of coexistence, that of acetylcholine and vasoactive intestinal peptide (VIP) in autonomic nerve terminals of the cat salivary gland, it has been shown that the transmitter substances involved can be differentially released [36]. Low frequency stimulation leads to release of acetylcholine alone while with high frequency stimulation both the neurotransmitter and the neuropeptide are released. Moreover, it has been shown that VIP potentiates the stimulatory action of acetylcholine on the salivary gland [36]. Concerning the Xenopus pars intermedia, the possibility of differential release of the coexisting inhibitory factors has yet to be examined. An analysis of the effect of combinations of these factors on secretion of a-MSH showed only additive effects [27], thus ruling out interactions leading to potentiation. Nonetheless, there is some evidence for differential action of these regulatory factors; inhibitions induced by GABA and dopamine are rapidly reversed when treatment with the factor is terminated whereas recovery from NPYinduced inhibition is achieved only very slowly [28]. Altogether these results suggest that dopamine and GABA are appropriate short-term inhibitors while NPY might be used for long-term inhibition of the melanotrope cell.

The Folliculo-Stellate Cell has an Inhibitory Action on the Release of α-MSH from the Melanotrope Cell

Folliculo-stellate cells are glial-like cells structurally related to the astrocytes of the central nervous system. They are evenly distributed

throughout the pars intermedia and constitute about 5% of the cell content of the lobe. These cells possess long extensions that make intimate contacts with virtually every melanotrope cell [53, 56]. The inhibitory action of folliculo-stellate cells on the secretory process of melanotropes was demonstrated in studies with 7-day cultured neurointermediate lobes of Xenopus [28]. The cultured tissue is devoid of neuronal influences due to the degradation of nerve terminals, as demonstrated ultrastructurally and confirmed by immunocytochemical analysis showing a complete lack of dopamine, GABA and NPY. Giving such tissue a depolarizing pulse of K⁺ in superfusion gives rise to a biphasic response with respect to the secretion of a-MSH. The short duration stimulatory phase could be attributed to a direct K⁺evoked depolarization of the melanotrope cell. The subsequent inhibitory phase, however, can only be attributed to K⁺-induced activation of an inhibitory mechanism emanating from another cell type within the cultured lobes. Ultrastructural analyses show that in such tissue the folliculostellate cell remains viable and it was therefore concluded that this cell type must be the source of the K⁺-induced inhibition. The mechanism of action of the folliculo-stellate cell on the melanotrope cells has still to be established. The stellate cell could release an α -MSH release inhibiting factor. Alternatively, it could inhibit the secretory activity of the melanotrope cells by inducing changes in the extracellular ionic environment of the melanotropes. Precedent for the first option can be found in the literature. Folliculo-stellate cells of the mammalian pars distalis have been reported to produce and secrete paracrine factors that regulate secretory activity of endocrine cells in this organ [5, 12, 13]. In support of the second option is the observation that bovine intermediate lobe folliculo-stellate cells are involved in ion transport [10]. Further, a high level of staining indicative of Na⁺/K⁺-ATPase activity has been observed between melanotropes and folliculostellate cells of the frog pars intermedia [56]. It was suggested that this activity might reflect stellate cell regulation of the extracellular ionic environment of the melanotropes.

Physiological Significance of the Inhibition by Folliculo-Stellate Cells

In view of the structural relationship between folliculo-stellate cells and the melanotropes, activation of an inhibitory mechanism via the folliculo-stellate cell would be expected to affect a number of melanotrope cells simultaneously. Possibly, this indirect mechanism of inhibition is used when a general inhibition of the pars intermedia is required, as opposed to the recruitment mode of regulation where cells are individually activated or inactivated. The question arises as to how the inhibitory mechanism emanating from the folliculo-stellate cell is itself activated and inactivated. While we initially thought that NPY might be of importance in this respect [28], subsequent analyses have shown that NPY can act directly on the melanotrope cell to inhibit a-MSH release. Further research will be required to fully understand the role of the folliculo-stellate cell in the regulation of the secretory process.

Analysis of Secretion: the Demonstration of Fast and Slow Secretory Pathways

The biosynthetic and secretory activity of melanotrope cells of Xenopus can be set to a very high level simply by placing the animal for several days on black background. This fact allows the use of in vitro pulse-chase labelling methods to follow the biosynthetic and secretory process of Xenopus melanotropes. A number of studies have been devoted to an analysis of the release of radiolabelled peptides [27, 33, 43, 57]. In a pulse-chase labelling experiment with Xenopus neurointermediate lobes, conducted in combination with tissue superfusion methods to analyze the dynamics of the secretory process, it was shown that there are two phases in the release of radiolabelled products [43]. The first phase comprises a rapid increase (reaching a maximum 3 hr after the pulse) followed by a rapid decrease; the second phase is a low basal secretion that persists for many hours of superfusion. In a subsequent analysis, using dual-labelling protocols to follow simultaneously the release associated with both phases, it was concluded that each phase reflects the functioning of a distinct pathway of secretion [68]. The first, termed the *fast pathway*, concerns peptides that are released within 6 hr of their biosynthesis. The second pathway, designated the *slow pathway*, pertains to peptides remaining in the melanotrope cells for up to 2 days before being released. It was shown that in the presence of dopamine the secretory peptides of the *fast pathway* are shunted to the *slow pathway* and are subsequently released from this latter pathway when the dopamine treatment is terminated.

At present, it is not possible to say whether the two secretory pathways of Xenopus melanotrope cells occur in one cell type or, alternatively, reflect the functioning of two different cell types within the tissue. There is morphological evidence for both possibilities. In support of the one cell-type explanation is the observation that there are two types of secretory granules in Xenopus melanotropes, electron-dense granules and somewhat larger, fibrous, electron-lucent granules [17]. In the biosynthetically active melanotrope cells of blackadapted animals the dense granules dominate but there also are, within these cells, lucent granules; in the inactive cells of white-adapted animals the granules are almost exclusively of the lucent type. Morphological indication that the pathways might reflect the activity of two cell types is provided by the observation that the melanotrope cell population of the pars intermedia of black-adapted animals is heterogeneous; the tissue possesses both active and inactive malonatrope cells [52].

Physiological Significance of Two Secretory Pathways

The existence of multiple secretory pathways in endocrine cells seems to be a general phenomenon, although the physiological significance of these pathways is unknown. Multiple pathways have been reported for cells producing thyrotropin [25], prolactin [7, 45], somatotropin [7, 8, 58] and parathyroid hormone [16]. An important criterion for attributing physiological significance to such pathways is to establish that they are independently regulated. This would appear to be the case for the pathways in prolactin cells [9] and parathyroid hormone producing cells [16, 46]. In both cases the pathways concern newly synthesized hormone *versus* hormone sequestered in mature secretory compartments. For the prolactin cell the mature pathway is cyclic-AMP-dependent while release of newly synthesized hormone is independent of the cyclic nucleotide. A similar situation might exist for *Xenopus* melanotrope cells; the *slow pathway* appears to be more sensitive to stimulation by 8-bromo-cyclic-AMP than the *fast pathway* [68]. Interestingly, in mouse melanotrope cells 8bromo-cyclic-AMP gives clear differential effects, the cyclic nucleotide analogue being more effective in stimulating mature peptides than newly synthesized peptides [29].

In considering the physiological significance of independently regulated secretory pathways it is worthwhile to focus on cells that produce multifunctional precurgor proteins such as POMC. The existence of independently regulated pathways in POMC-producing cells might endow the cells with the ability to manipulate the peptide composition, and thus the physiological effect(s) of their secretory signal. In this way the cell itself could be multifunctional, responding with different sets of peptides to different physiological demands. Studies with the melanotrope cell of *X. laevis* may help to establish the full potential of POMC-producing cells to participate in multiple regulatory processes.

Analysis of POMC Gene Expression: Regulation is at the Level of both Gene Transcription and mRNA Stability

POMC gene expression in the pars intermedia is 20 to 30 times higher in animals adapted to a black background than in animals adapted to a white background. Using a steady-state kinetic model for mRNA degradation, the half-life of POMC mRNA in *Xenopus* melanotrope cells was determined during the process of background adaptation [4]. During induction of the POMC gene (i.e. following white to black background transfer) the half-life of POMC mRNA was found to be 3- to 4-fold longer than during de-induction of the gene (transfer from a black to a white background). This difference in the stability of POMC mRNA is not sufficient to account for the 20- to 30-fold differences in the steady-state levels of POMC

mRNA in fully black- *versus* fully white-adapated animals. Therefore, it seems that background adaptation involves not only changes in the stability of POMC mRNA but also changes in the transcriptional activity of the POMC gene.

Physiological Significance of Dual Control Sites in the Regulation of POMC Gene Expression

The stabilization of POMC mRNA in animals on a black background would seem physiologically relevant. This mechanism will help maintain a high level of POMC mRNA under this environmental condition, and thus would add to the efficiency of the POMC expression system. Likewise, the decreased stability of POMC mRNA in animals transferred from a black to a white background seems a physiologically appropriate response to the new environmental situation. The shutting down of the POMC expression system, viewed at the level of POMC biosynthesis, is a slow process (see Fig. 1). Part of the reason for this may be that in the animals adapting to a white background RNA degrading enzymes must first be synthesized. This is indicated by the fact that treatment of animals with mRNA synthesis inhibitors block the degradation of POMC mRNA observed after black to white background transfers [4].

Analysis using Differential Hybridization: a Battery of Genes is Coexpressed with the POMC Gene

Proper functioning of a peptide-secreting cell requires not only production of precursor proteins for secretory peptides but also expression of genes associated with the secretory function. Such genes include those encoding for precursor-processing enzymes and for structural and regulatory proteins involved in the translocation, sorting, packaging and release of secretory products. To identify genes coexpressed with the POMC gene in Xenopus melanotrope cells advantage has been taken of the fact that the transcriptional process is very active in cells of black-adapted animals but inactive in white-adapted animals. The method used, termed differential hybridization, involved screening of a Xenopus pituitary cDNA library using single-stranded cDNA probes synthesized from



FIG. 3. An overview of the differential screening approach being used to find genes important to the transducer function of the melanotrope cell of *Xenopus laevis*. The approach is based on the premiss that non-POMC genes that are more highly expressed in black- than in white-adapted animals would be important in supporting the regulated secretory pathway leading to the release of POMC-derived peptides.

RNA of biosynthetically active and inactive melanotropes, i.e. from black and white animals, respectively (Fig. 3). Screening approximately 20,000 colonies of the library resulted in the identification of 130 differentially hybridized clones [41]. Of these, 92% were found to be related to POMC mRNA and discarded from further analysis. The 10 remaining clones presumably code for proteins essential for the secretory activity of the Xenopus melanotrope cell. Thus far only one of these clones has been fully characterized. It has proved to be highly similar (97% identical) to a partially sequenced porcine and human pituitary protein named 7B2 [18, 55]. The Xenopus 7B2 cDNA clone was used to isolated a human pituitary cDNA clone encoding the entire structure of human 7B2 [40]. A comparison of the amino acid sequences of Xenopus and human 7B2 shows that the 7B2 protein, and in particular the N-terminal region, is remarkably well conserved during 350 million years of vertebrate evolution. The overall degree of amino acid sequence identity between *Xenopus* and human 7B2 is 83%, with most differences being conservative amino acid substitutions. This degree of conservation is much higher than that between the POMC proteins of these two species (55%) [42].

While POMC and 7B2 are clearly coexpressed, there are some differences in the nature of the expression of these proteins in *Xenopus* melanotrope cells. First, there are large differences in the steady-state levels of mRNA for the two proteins; in black-adapted animals the amount of mRNA coding for POMC in the pars intermedia is about 25-fold higher than that coding for 7B2 [41]. Moreover, the differences in steady-state levels of 7B2 and POMC mRNA between black- and whiteadapted animals are about 8-fold and 25-fold, respectively [2]. From an analysis of the time course in the expression of the genes in animals transferred from a white to a black background it is evident that there are also important differences in the dynamics of the expression of the two genes. The level of POMC mRNA was significantly increased within 16 hours of transfer whereas it took over 40 hours for significant increases in the level of 7B2 mRNA to be detected [2]. It will be interesting to investigate the molecular mechanism(s) underlying the differences in activity between the 7B2 and POMC genes. It could be that the POMC promotor contains elements that are responsible for the very high level of POMC gene expression in the intermediate lobe. Alternatively, it is possible that POMC mRNA contains sequences responsible for a long half-life in this tissue while 7B2 mRNA is less stable because it lacks such sequences.

Biosynthetic studies have revealed that 7B2 is a precursor protein in Xenopus [3]. During pulse incubations there was synthesis of a 25 kDa form of 7B2 which was converted, during subsequent chase incubations, to an 18 kDa protein. Chemical cleavage and peptide mapping have shown that processing takes place in the C-terminal region [3], a region that possesses three pairs of basic amino acid residues [40]. Such pairs often function as cleavage sites in the enzymatic processing of precursor proteins. Xenopus 7B2 is apparently sequestered in the regulated secretory pathway of the melanotrope cell and is possibly stored within the same granules as the POMC-derived peptides. This is shown by the observation that the 18 kDa form of the protein is released [3] and that this release can be inhibited or stimulated by factors established to inhibit or stimulate, respectively, the secretion of POMC-derived peptides from Xenopus melanotrope cells [2]. Therefore, 7B2 is not only coexpressed with POMC but it is also, at the level of secretion, coordinately regulated with POMC-derived peptides. The concept of coordinate regulation can be extended to the level of gene expression. Long-term treatment (3 days) of neurointermediate lobes with apomorphine (D₂ dopamine receptor agonist), GABA or NPY leads to decreases not only in the level of POMC mRNA but also to concomitant decreases in the level of mRNA coding for the 7B2 protein [2].

Physiological Significance of the Coexpressed 7B2 Protein

The function of the 7B2 protein is unknown. In mammalian tissues it has been shown to be present in cells containing secretory granules, such as neurons and endocrine cells [20, 38]. Ultrastructural studies show this protein to be stored within secretory granules [37, 38]. Therefore, it is conceivable that 7B2 has an important function in the secretory pathway of these cells, such as being a component of the exocytotic machinery or a sortase directing secretory proteins to the regulated pathway of secretion. The suggestion has been made that the 7B2 protein is a member of the granin (chromogranin-secretogranin) family [19]. On the other hand, the fact that 7B2-derived products are released makes an extracellular function, i.e. as an endocrine or paracrine factor, also a distinct possibility.

Concluding Remarks

The analysis of the pituitary melanotrope of Xenopus shows that this cell, in fulfilling its neuroendocrine integrative function, is extremely complex. The cell acts as a neuroendocrine interface under the control of multiple factors, some of which coexist within the same nerve terminals. The regulatory factors clearly have differential effects on the melanotrope cells, some of these factors stimulating the melanotrope cell secretory process (viz. the neuropeptides CRH and TRH) while others (dopamine, GABA, NPY) inhibit secretion (see Fig. 2). Even among the inhibitory factors there appears to be differential action. Dopamine and GABA give rise to rapid and reversible inhibition of secretion, and might thus be appropriate inhibitors for short-term adaptations. Physiological background adaptations have been shown to involve the activation or inactivation of individual melanotrope cells, depending on the demand for α -MSH. Because both dopamine and GABA are delivered to the melanotrope cells via synaptic communication, they could very well be involved in regulating the (de)recruitment of individual cells. NPY, in contrast, may have a more general effect on the pars intermedia. This

neuropeptide acts more slowly than dopamine and GABA in inducing inhibition and, once established, this inhibition is more sustained than that induced by the two classical neurotransmitters.

There are several challenging areas for future research. One area of interest concerns the physiological significance of the multiple secretory pathways of the amphibian melanotrope cell. A first step in examining this will be to determine the peptide content of the pathways. This will give insight into the potential biological activities associated with these secretory signals. Also of immediate interest will be studies on the effect of the various regulatory factors on the pathways, to determine if they are independently regulated. On the molecular front, further characterization of genes co-expressed with the POMC gene in the Xenopus melanotrope should ultimately reveal a host of gene products that are essential to the proper physiological functioning of this neuroendocrine transducer cell.

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