REVIEW

Hormonal control of programmed cell death/apoptosis

Wieland Kiess and Brian Gallaher¹

Children's Hospital, University of Leipzig, Oststr. 21–25, D-04103 Leipzig, Germany and ¹Research Centre for Developmental Medicine and Biology, University of Auckland, School of Medicine, Auckland, Private Bag 92019, New Zealand

(Correspondence should be addressed to W Kiess)

(B G is now at the Children's Hospital, University of Leipzig, Oststr. 21–25, D-04103 Leipzig, Germany)

Abstract

Apoptosis or programmed cell death is a physiological form of cell death that occurs in embryonic development and during involution of organs. It is characterized by distinct biochemical and morphological changes such as DNA fragmentation, plasma membrane blebbing and cell volume shrinkage. Many hormones, cytokines and growth factors are known to act as general and/or tissue-specific survival factors preventing the onset of apoptosis. In addition, many hormones and growth factors are also capable of inducing or facilitating programmed cell death under physiological or pathological conditions, or both. Steroid hormones are potent regulators of apoptosis in steroid-dependent cell types and tissues such as the mammary gland, the prostate, the ovary and the testis. Growth factors such as epidermal growth factor, nerve growth factor, platelet-derived growth factor (PDGF) and insulin-like growth factor-I act as survival factors and inhibit apoptosis in a number of cell types such as haematopoietic cells, preovulatory follicles, the mammary gland, phaeochromocytoma cells and neurones. Conversely, apoptosis modulates the functioning and the functional integrity of many endocrine glands and of many cells that are capable of synthesizing and secreting hormones. In addition, exaggeration of the primarily natural process of apoptosis has a key role in the pathogenesis of diseases involving endocrine tissues. Most importantly, in autoimmune diseases such as autoimmune thyroid disease and type 1 diabetes mellitus, new data suggest that the immune system itself may not carry the final act of organ injury; rather, the target cells (i.e. thyrocytes and β cells of the islets) commit suicide through apoptosis. The understanding of how hormones influence programmed cell death and, conversely, of how apoptosis affects endocrine glands, is central to further design strategies to prevent and treat diseases that affect endocrine tissues. This short review summarizes the available evidence showing where and how hormones control apoptosis and where and how programmed cell death exerts modulating effects upon hormonally active tissues.

European Journal of Endocrinology 138 482–491

Defining apoptosis/programmed cell death

There are two major types of cell death, namely apoptosis and necrosis (1–9). Both apoptosis and necrosis involve a sequence of consecutive biochemical and morphological events ultimately leading to cell death (3, 4, 6). Necrosis is characterized by rupture of the cell membrane and leakage of cell plasma. It very often causes an acute inflammatory response and pathological tissue reaction involving groups of adjoining cells (1). In contrast, apoptosis or programmed cell death is a physiological form of cell death (8). Typically, cells from multicellular organisms self-destruct when they are no longer needed or have become damaged (1–9). To survive, all cells from multicellular animals depend upon the constant repression of this suicide programme by signals from other cells. Apoptosis

usually involves scattered individual cells in a tissue. It occurs in embryonic development (10–16) and also during involution of organs (17–24). It is characterized by distinct biochemical and morphological changes such as DNA fragmentation, plasma membrane blebbing and cell volume shrinkage. Many hormones, cytokines and growth factors are known to act as general or tissue-specific survival factors preventing the onset of apoptosis; conversely, many hormonal factors are known to induce or enhance apoptotic processes (Table 1).

Ultrastructural changes in apoptosis

Early apoptosis is characterized by compaction and segregation of chromatin in sharply circumscribed masses. There is convolution of the nuclear outline,

Table 1 Involvement of hormones, cytokines and growth factors in apoptosis/programmed cell death.

Known inhibitors of apoptosis
Testosterone
Oestradiol
Progesterone
Growth factors (EGF, IGF-I, NGF, PDGF)
Interleukins
Growth hormone
Prolactin
Gonadotrophins
Potential inducers of programmed cell death
Glucocorticoids
Progesterone
Thyroid hormones
Growth factor (IGF-I, EGF, PDGF, NGF) withdrawal
Transforming growth factor- β
Tumour necrosis factor
Fas ligand

condensation of the cytoplasm with preservation of the integrity of organelles, and convulsion of the cell surface (1, 3, 4, 6). In the next phase, the nucleus fragments and the cytoplasm condenses, with extensive protrusion of the cell surface. The separation of the surface protuberances produces membrane-bounded apoptotic bodies of various sizes and composition. These bodies are phagocytosed by nearby cells and degraded by lysosomal enzymes (3, 4, 6, 9). In summary, the consecutive and ordered appearance of condensed chromatin, budding of the nucleus and subsequent nuclear fragmentation is characteristic of apoptosis. In parallel, overall condensation also occurs in the cytoplasm. Extensive protrusion/blebbing of the cell surface follows, especially when thymocytes and lymphocytes become apoptotic (1, 3, 4, 6). In lymphocytes and other cell types, membrane-bounded apoptotic bodies appear, but cytoplasmic organelles remain intact. Whereas apoptotic bodies are often recognized by light microscopy, transmission electron microscopy or scanning electron microscopy have to be used to visualize the specific ultrastructural changes typical of programmed cell death (6, 9).

Incidence of apoptosis in health and disease

Apoptosis is a unique feature of multicellular organisms that enables continuous renewal of tissues by cell division while maintaining the steady-state level of the various histological compartments. Programmed cell death exists only rarely in prokaryotes or protozoa that proliferate and expand perpetually under optimal conditions; however, it has been described in a few unicellular organisms (1). This is consistent with the assumption that apoptosis has emerged during evolution as a series of steps providing a selective advantage to the best adapted offspring by preventing flawed

offspring from competing for resources (1). Programmed cell death is a constitutive feature during the normal development of mammals (14, 15). Cell deletion in regressing interdigital webs and during the development of the gut mucosa and of the retina are well studied examples of apoptotic cell death during the development of mammals (14). In amphibia, apoptosis is responsible for the involution of larval organs during metamorphosis. Steady-state kinetics are maintained in most healthy adult mammalian tissues by apoptosis occurring at a low rate that complements mitosis. In fact, the traditional emphasis placed on cell proliferation as the dominant parameter in cell population control is being tempered by the realization that cell deletion and cell death are also of cardinal importance (3). Importantly, normal involution of endocrine-dependent tissues induced by changes in blood concentrations of trophic hormones is mediated by apoptosis, as are other normal involutional processes such as ovarian follicular atresia (22–24).

Pathological atrophy of endocrine-dependent organs after pathological withdrawal of trophic hormonal stimulation is accompanied by a massive wave of apoptosis. This occurrence has been observed in the prostate after castration (25, 26) and in the adrenal cortex after suppression of secretion of adrenocorticotrophic hormone (ACTH) by administration of glucocorticoid (27). Various forms of cancer therapy, such as a variety of cancer-chemotherapeutic agents and moderate doses of radiation, induce or enhance extensive apoptosis in rapidly proliferating cell populations (5, 28). Apoptosis also occurs in many types of viral infection. T lymphocytes are known to induce apoptosis, as are natural killer and killer cells (29, 30). Apoptosis induced by immune cells may be involved in oncogenesis and autoimmune diseases such as autoimmune thyroid disease and diabetes mellitus (3, 4, 9).

Biochemical and molecular mechanisms

The process of programmed cell death involves an epigenetic reprogramming of the cell that results in an energy-dependent cascade of biochemical changes. These changes eventually lead to morphological changes within the cell, resulting in cell death and elimination. Changes in intracellular and, most importantly, intranuclear Ca^{2+} concentrations are likely to be involved in activating cleavage of DNA by nucleases during programmed cell death (31). In fact, the double-stranded linker DNA between nucleosomes is cleaved at regular inter-nucleosomal sites through the action of such Ca^{2+} – Mg^{2+} -sensitive neutral endonucleases (32). Zinc is a potent inhibitor of such enzymes (6).

Most apoptotic cells require synthesis of RNA and proteins (8, 9). Delay or abrogation of apoptosis by inhibition of macromolecular synthesis is well known. The dying cells express large amounts of mRNA for several enzymes. In addition, several degradative

Table 2 Classic signal transduction pathways of growth and proliferation that, among many others, are also known to be involved in the regulation of apoptosis.

Calcium channels
G-proteins
Tyrosine kinases
Tyrosine phosphatases
Protein kinase C
Heat-shock proteins
cAMP

enzymes become activated. Regulatory proteins maintain control over the apoptotic cascade (3).

Genes associated with programmed cell death

Genes responsible for the morphological and biochemical processes that fragment and phagocytose the dying cell without evoking an immune response have been identified: among these are classic components of signalling pathways (Table 2). Others, such as *fos*, *myc*, *hsp 70* and *p53*, seem to be of particular biological significance (Table 3) (11, 12, 33, 34). Often, such genes are recruited from other cellular functions, such as mitosis and differentiation. It is important to note that genes displaying a prolonged increase in the mRNA steady-state concentrations, such as *p53*, are most probably expressed in the surviving cells, whereas other genes might only be expressed by cells destined to die (9).

p53 mutations are frequent in some types of cancer, for example breast cancer. Whereas wild-type *p53* inhibits cell growth *in vitro*, *p53* is believed not to affect the induction of programmed cell death directly in many cells (35, 36). Rather, mutations in the *p53* gene can result in immortalization of cells and inhibition of apoptosis in transformed cells. A protein called *p21WAF1/CIP1* has been shown to be a critical downstream effector of *p53* and a potent inhibitor of cyclin-dependent kinases (37).

Table 3 Some of the genes and peptides of the apoptosis machinery.

Inhibitors
CED-9
Bcl-2 family
Bax and Bcl-2
(Bcl-x _L , Bcl-x _S)
Inducers
Interleukin-1-converting enzymes
CED-3
CPP32 cysteine proteases
Fas, fas ligand
c-rel
Transcription factors
c-Myc

Thyroid hormone is capable of initiating extensive programmed cell death at the onset of amphibian metamorphosis. Among the earliest genes that are induced by thyroxine and that are considered important for cell death are thyroxine receptors (16, 20, 38). Other cell-death-associated gene products, such as *TRPM-2/SGP-2* and *stromelysin-3*, have been identified. Thus genes that are known to be of key importance in regulating metabolism and diverse cellular functions also seem to be important for apoptosis. One gene/gene product that seems to be of key importance for apoptosis, *apo/fas*, has even been called 'death factor' (2, 28).

Among the genes known to prevent cell death, *Bcl-2* has been studied most vigorously (39–44). For example, *Bcl-2* protein is present at all stages of cerebellar granule neurone differentiation, and appears to be developmentally regulated. It is underexpressed in apoptotic granule neurones. In hypothyroid rats, the protein content of the cerebellum is drastically reduced. It is concluded that thyroid hormone, in conjunction with neurotrophins such as nerve growth factor (NGF) and neurotensin-3 (NT-3), has a permissive action on the developing central nervous system by reducing neuronal apoptosis and neuronal loss, and that *Bcl-2* has a crucial role in this process (41).

Signal transduction in programmed cell death

Apoptotic cell death can be triggered by calcium overload. Moreover, increases in cytoplasmic or nuclear ionized calcium (or both) have been implicated in all phases of apoptosis (Table 2). An increase in intracellular calcium pools very often precedes, and even can induce, internucleosomal DNA cleavage in the nucleus. Conversely, calcium chelators inhibit apoptosis. In general, Ca^{2+} signals are required for a number of cellular processes, including the activation of nuclear events such as gene transcription and cell cycle events. Intranuclear Ca^{2+} fluctuations are believed to affect chromatin organization, induce gene expression and activate cleavage of nuclear DNA by nucleases during apoptosis (31).

Increases in intracellular concentrations of cyclic AMP (cAMP) influence differentiation and luteinization of granulosa cells. Forskolin, a potent activator of adenylate cyclase, stimulated cell death in primary granulosa cells. Moreover, in such cells, blocking of the cellular phosphodiesterase activity in forskolin-stimulated cells by isobutylmethylxanthine, which maintains high concentrations of intracellular cAMP, led to further enhancement of cell death.

The glucocorticoid-induced apoptotic cell death in immature thymocytes is augmented by protein synthesis inhibitors, inhibitors of protein kinase C and heat-shock proteins (45). These data suggest that protein kinase C isoforms and heat-shock protein pathways

Figure 1 Serum factors induce apoptotic processes, as measured by DNA fragmentation in human SKNMC neuroblastoma cells. Cells were cultured in the presence of 10% fetal calf serum (FCS) for 48 h (t_0). SKNMC cells were then further cultured in the absence (–) or presence (+) of 10% FCS for up to 6 days. Fragmentation of cellular DNA was determined by nick labelling of DNA with phosphorus 32 and analysis of the DNA by gel electrophoresis.

have an important role in the signalling cascade of hormone-induced apoptotic cell death (Table 2).

The ligand-activated oestrogen receptor induces apoptosis in an erythroid cell line by binding to and inhibiting GATA-1, an erythroid transcription factor essential for survival and maturation of erythroid precursor cells. GATA-1 inhibition is reflected in the down-regulation of presumptive GATA-1 target genes (46). These data show that apoptosis can be regulated at the transcriptional level and directly involves the hormonal control of transcription factor expression.

Many enzymes that are involved in the regulation of growth and development, such as tyrosine kinases (47) and phosphatases, also modulate the progression towards cell death. In addition and most importantly, a family of proteases, also known as interleukin-1 converting enzymes, have a key role in apoptotic processes (1, 2, 6, 40, 48, 49).

Regulation of apoptosis

Regulation of apoptosis is mediated by many factors and on many levels of cell metabolism and cell biology. Viral infection, metabolic derangements such as sudden changes in glucose concentrations, heat, irradiation, toxins and drugs, all are capable of influencing the transition of a given cell to programmed cell death. In addition, the multiplicity of hormonal factors such as cytokines, steroids and peptide hormones or their withdrawal (as demonstrated in Fig. 1) are all involved in steering cells between death and proliferation (17, 50–55).

Role of growth factors/cytokines in apoptosis

Whereas it is well known that both epidermal growth factor (EGF) and the insulin-like growth factor (IGF)/insulin family stimulate the growth of many cell types by activating their respective tyrosine kinase receptors, it has also become apparent that both classes of growth factors and their receptors are capable of preventing apoptosis in many cells and cell types (Fig. 2) (47, 56–58). In human colorectal carcinoma cells, an anti-EGF receptor antibody induces apoptosis, whereas insulin delayed apoptotic DNA fragmentation. Signal transduction pathways shared by EGF and IGFs/insulin are thus believed to be involved in regulating apoptosis triggered

Figure 2 An antibody against the human IGF-I receptor increases the rate of apoptotic cell death of human SKNMC neuroblastoma cells. Cells were cultured until confluency, transferred to a serum-free medium and then further incubated in the presence or absence of recombinant human IGF-I (100 ng/ml) and monoclonal anti-IGF-I receptor antibody (αIR3) (1000 ng/ml) as indicated. Cell death was determined using a cell death detection kit (ELISA, Boehringer Mannheim, Mannheim, Germany).

by blockade of the EGF receptor. A number of genes, particularly c-Myc, are believed to have key roles in apoptosis triggered by growth factor withdrawal (35). C-Myc might have the potential to induce either cell death or proliferation; in the latter case, growth factors prevent the cell from proceeding on the death pathway, and will thus favour proliferation. Alternatively, cell cycle arrest, which normally occurs as a consequence of growth factor withdrawal, is overridden by c-Myc in such a way that the cell inappropriately continues to die by apoptosis (9, 36).

Some neurones are dependent on nerve- or target-derived neurotrophic factors. Absence of such factors or interruption of the continuous supply of neurotrophic factors such as NGF, eventually leads to the induction of apoptotic cell death and loss of neurones (59). Both axonal elongation and neuronal survival are maintained through the availability of growth factors and seem to be essential for peripheral nerve regeneration after injury (60).

In immature thymocytes and mature T cells, interleukins, such as IL-2 and IL-4, inhibit dexamethasone-induced apoptosis, whereas high doses of IL-2 are able to induce apoptosis both in thymocytes and in peripheral T cells (30, 45). A number of different growth factors and cytokines are involved in the regulation of apoptosis in the epidermis and in the regressing hair follicle (61).

The mechanisms by which growth factors modulate apoptosis might relate to their ability to influence the entry of a cell into the cell cycle: in fact, when tumour cells are forced out of the cell cycle into the quiescent state (G_0) they become insusceptible to programmed cell death. Growth factors prevent transit from G_1 to G_0 and thus increase the lytic susceptibility of a tumour cell. In summary, the susceptibility of a cell to the induction of cell death is believed to be a consequence of simultaneously activated growth stimulatory and growth inhibitory signalling pathways (55).

Role of steroid hormones in apoptosis

Steroid hormones have a major role in the regulation of growth, development, homeostasis and programmed cell death (17, 62). Together with other hormones and growth factors, steroids regulate both induction and inhibition of cell death, along with many other cellular functions, and the cellular composition of organs throughout the body (22, 63).

Glucocorticoid hormones induce apoptotic cell death in immature thymocytes and mature T cells through an active process, characterized by extensive DNA fragmentation (51). This process requires macromolecule synthesis and is inhibited by interleukins IL-2 and -4 (30, 45). Interestingly, it has become evident that adrenal steroids act on the brain: glucocorticoids are taken up and retained by the hippocampus. Glucocorticoid receptors have a role in containing programmed

cell death in the hippocampus and determine the rate of neurogenesis (60, 62). Low levels of adrenal steroids are also believed to enable development of cell death in the dentate gyrus. Thus glucocorticoids could also regulate apoptosis in the central nervous system (62, 64).

In cultured MCF-7 human breast cancer cells, tumour necrosis factor (TNF)- α exerted a dose-dependent inhibition of cell growth. Although an earlier cytostatic effect was apparent, nuclear shrinkage and cytolysis of the cells suggest that growth inhibition is mediated via an increase in programmed cell death *in vitro*. 17β -Oestradiol sensitized the cells to both the cytostatic and the cytolytic effects of TNF- α . Chun *et al.* (21) have suggested that sex steroids have an important role in the regulation of apoptotic cell death in the ovary: whereas oestrogens inhibit ovarian granulosa cell apoptosis, androgens seem to enhance ovarian DNA fragmentation. These conclusions were drawn from experiments in hypophysectomized rats treated with diethylstilboestrol, oestradiol, oestradiol benzoate or testosterone. *In vitro*, 17β -oestradiol protected neurones from oxidative-stress-induced cell death. This effect was oestrogen receptor dependent (19).

Quantitative light and electron microscopic data indicate that antiprogestins initiate terminal differentiation, which eventually leads to apoptotic cell death in breast cancer cells. In contrast, progesterone is a potent inhibitor of programmed cell death in such cells (65).

Extensive programmed cell death is initiated at the onset of amphibian metamorphosis, resulting in 100% of cells dying in some larval tissues. All cell death during metamorphosis is under hormonal control: thyroid hormone is able to initiate apoptosis and to lead to tail regression in amphibian development (16). Bernal & Nunez (20) have suggested that thyroid hormone also has a role in oligodendroglial and neuronal differentiation and cell death.

Role of peptide hormones in programmed cell death

Whereas, as noted above, thyroid hormone is able to initiate the extensive programmed cell death that occurs at the onset of amphibian metamorphosis, prolactin (PRL), when given exogenously, prevents both natural and thyroxine-induced metamorphosis (16). In addition, lactogenic hormones such as PRL, human growth hormone and human placental lactogen inhibit dexamethasone-induced cytolysis of Nb2 lymphoma cells. The effect of PRL has been shown to be a direct inhibition of dexamethasone-induced DNA fragmentation, indicative of programmed cell death. Thus PRL has the capacity to protect the cell against glucocorticoid-receptor-mediated induction of apoptosis (52). A similar anti-apoptotic effect of PRL has been shown in Burkitt lymphoma cells.

Mullerian inhibiting substance (MIS) is a differentiating and antiproliferative peptide. It is tightly regulated

by transacting factors such as SRY at the transcriptional level, and by testosterone post-translationally. MIS acts at specific sites through activating a localized programme of cell death that is initiated via receptor-mediated events. MIS-induced apoptosis is a crucial component of the machinery involved in sexual differentiation (13).

In a study designed to elucidate the role of gonadotrophins in regulating steroid metabolism in human epithelial ovarian carcinoma cells, human chorionic gonadotrophin (hCG), but not follicle-stimulating hormone (FSH), produced apoptotic cell death when added to cell cultures for 72 h in the presence of cAMP (63). However, the effect of the peptide hormone seems to be indirect and most probably was due to its ability to increase steroid hormone secretion in the cells studied.

Using *in situ* end-labelling of DNA breaks (TUNEL, *in situ* transferase mediated dUTP nick end labelling) Ceccatelli *et al.* (27) investigated the occurrence of apoptosis in the adrenal cortex of hypophysectomized rats. Apoptosis was detected in the zonae fasciculata and reticularis 3 days after hypophysectomy. With the exception of the zona reticularis, apoptotic cell death was largely prevented by ACTH replacement in 3-day hypophysectomized animals. Their results demonstrate that ACTH, and probably other pituitary factors, may be involved in preventing apoptosis in the adrenal cortex (27).

Parathyroid-hormone-related peptide mediates cellular growth and differentiation. Recently, nucleolar localization of the peptide has been demonstrated in chondrocytic cells. Expression of parathyroid-hormone-related peptide by these cells delayed apoptosis induced by serum deprivation; this effect depended on the presence of the intact nucleolar targeting signal (66). These findings not only demonstrate that parathyroid-hormone-related peptide serves as a regulator of apoptosis in chondrocytes, but also demonstrate a novel mechanism by which this peptide may modulate programmed cell death.

Hormonal control of apoptosis in the testis

Dimethane sulphonate is a Leydig cell toxin and greatly facilitates apoptosis in adult rat seminiferous epithelium. Testosterone was shown both to inhibit and to induce apoptosis in rat seminiferous tubules in a stage-specific manner after administration of ethane dimethane sulphonate. In particular, testosterone withdrawal induced cell death in most stages of the cell cycle. However, at later stages of the cell cycle, testosterone promoted apoptosis in premeiotic cells. These data point to an intricate timing of hormonal effects on programmed cell death in the testis (53). The role of the basement membrane for Sertoli cell survival is known. Dirami *et al.* (67) have shown recently that a combination of peptide and steroid hormones and growth factors

was able significantly to reduce apoptosis and increase cell survival of Sertoli cells in culture. However, in the absence of basement membrane, FSH and other regulators of Sertoli cell function cannot prevent Sertoli cell apoptosis (67).

Hormonal control of apoptotic cell death in the prostate

In hormone target tissues, such as the prostate, apoptosis can be induced by ablation of the appropriate hormone. In fact, the epithelial cells of the prostate undergo apoptosis after castration (9). Both in normal and in neoplastic prostatic cells, androgens serve as cell-type-specific endogenous regulators of programmed cell death. Androgens simultaneously influence DNA synthesis and induce the synthesis of substances with mitogenic effects in prostate cells. They counterbalance these agonistic effects on proliferation with an antagonistic effect on programmed cell death. Conversely, oestrogens exert direct and indirect opposing effects on cell death in the prostate (26).

Gonadectomy leads to apoptotic cell death in the mouse reproductive tract (44, 68). After the apoptotic events, down-regulation of Bcl-2 and new expression of Fas were observed. Using Fas-lacking mice, it was demonstrated that the regression of the male reproductive tract is triggered by Fas-mediated apoptosis. Suzuki *et al.* (44) concluded from their experiments that, in the mouse prostate, the apoptotic death signal involves a number of steps, the first and most important of which was down-regulation of Bcl-2.

Hormonal control of apoptosis in the ovary

It is now established that atresia of ovarian follicles in mammals is initiated by apoptotic cell death of the granulosa cells in response to hormonal deprivation (9, 14, 17, 21, 69). Only a small proportion of follicles escape programmed cell death. Growth factors and oestrogens have been identified as follicle survival factors; androgens and gonadotrophin releasing hormone potentiate apoptosis of the follicle (14).

Granulosa cells are the main producers of the female sex steroid hormones that are responsible for the cyclicity in ovarian function. Programmed cell death in the ovary has a crucial role in limiting the number of follicles that can ovulate and thus prevents the development of more embryos than can successfully complete pregnancy (17). *In vivo* studies on the hormonal regulation of follicle atresia have been difficult because of the presence of heterogeneous population of follicles in the ovary. However, using serum-free cultures of preovulatory follicles, Chun *et al.* (21) were able to study the regulation of apoptosis by gonadotrophins, IGF-I and IGF-binding protein (IGFBP)-3. A spontaneous increase in apoptotic DNA

fragmentation occurred after 24 h of culture in the absence of hormones, whereas hCG or FSH suppressed follicular apoptosis in a dose-dependent manner by as much as 60–62%. Treatment of follicles with IGF-I or insulin also inhibited follicular atresia, whereas IGFBP-3 reversed the inhibitory effect of both hCG and IGF-I on apoptosis. It was concluded that endogenously produced IGF-I in part mediates the effect of gonadotrophins to inhibit follicular apoptosis (21). In addition, these studies by Chun *et al.* have suggested that sex steroids have an important role in the regulation of apoptotic cell death in the ovary. It is believed that oestrogens inhibit and androgens enhance endonuclease activity in ovarian granulosa cells.

Hormonal control of apoptotic cell death in the mammary gland

Interaction of the retinoic acid receptor type of receptors and an array of lactogenic and mammogenic hormones such as EGF and hydrocortisone seems to be of utmost importance for mammary gland development (38).

Growth of the normal mammary gland involves proliferation, differentiation, programmed cell death and remodelling of the basement membrane throughout the cyclic ovarian stimulation of the menstrual cycle and during the pregnancy and lactation cycle. The regulation of these processes involves a concerted action of both oestrogen and progesterone.

Regression of the lactating mammary gland is induced by the decreasing prolactin and glucocorticoid hormone concentrations associated with weaning (9). The hormone dependency of breast cancer has been recognized for nearly a century. Cyclical patterns of cell division and cell death are observed also in normal breast tissue; the influence on disease progression of cyclical hormonal concentrations among premenopausal women is now being elucidated. Withdrawal of lactogenic hormones triggers a programme of epithelial cell death that is characterized by decreased gene expression for the milk protein casein, and increased expression of apoptosis-associated genes such as transforming growth factor- β (18).

Hormonal control of apoptotic cell death in the uterus

Discontinuation of oestrogen stimulation results in apoptotic cell death in the uterine epithelium of neonatal mice, but not in the stroma (54). In addition, oestrogen, progesterone, dihydrotestosterone and dexamethasone all inhibit programmed cell death of uterine epithelium when administered to female newborn mice for 4 days from the day of birth (54). Endometria of rhesus monkeys treated with RU486, an antiprogesterin, had significantly more epithelial cell death by apoptosis, increased stromal compaction and less proliferating stromal cells than had endometria from animals treated

with oestradiol (68). It was concluded that, in the primate endometrium, progesterone and oestradiol have prolifeferative or anti-apoptotic effects, or both (68). However, after prolonged exposure of the endometrial stroma to progesterone, apoptosis occurs in this tissue, and may contribute to breakthrough bleeding.

Molecular mechanisms controlling cell death during blastocyst implantation and decidualization have been studied recently. In fact, progesterone and oestrogen control endometrial differentiation and apoptosis by controlling Bcl-2 gene family expression. Expression of Bcl-2 decreased after hormone treatment and decidualization, whereas Bax protein increased during decidualization. In summary, the balance between Bax and Bcl-2 expression is altered during stromal cell differentiation. Increased expression of Bax precedes nucleosomal DNA fragmentation and apoptosis. These processes are believed to play a significant part in placental development (39).

Hormones and apoptosis in disease states

As has been briefly outlined above, the 'decision' of a cell to undergo programmed cell death can be influenced by a wide variety of regulatory stimuli, including many hormones. Recent evidence suggests that alterations in cell survival contribute to the pathogenesis of a number of human diseases, including cancer, viral infections, autoimmune diseases, neurodegenerative disorders, and acquired immunodeficiency syndrome (AIDS). Treatments designed to regulate the time frame during or extent to which a cell undergoes apoptosis may have the potential to change the natural progression of some of these diseases (3, 8, 28, 70).

Recent experimental evidence suggests that apoptosis has an important role in regulation of tumour growth and tumour response to cancer therapy. Apoptosis develops rapidly after cytotoxic treatments and is dose dependent. The apoptotic response correlated well with the antitumour efficacy of radiation or chemotherapy, or a combination thereof, which makes it a candidate predictor of tumour treatment response (5). Tumours vary in their apoptotic response to cytotoxic drugs and irradiation. In addition to this intertumour heterogeneity, there is also a significant intratumour heterogeneity in induction of apoptosis, consistent with the idea that the propensity for apoptosis is genetically and hormonally regulated. Within the prostate, for example, androgens are capable of both stimulating proliferation and inhibiting the rate of the glandular epithelial cell death (25). An imbalance in programmed cell death is believed to occur during prostatic cancer progression (25). Regulating apoptosis might therefore be an effective and natural way to improve antitumour therapy: therapeutic gain would be achieved by increasing the apoptotic response of cancerous tissues, or by inhibiting the apoptotic response of normal tissues (5).

In polycystic renal disease, normal renal tissue is lost in association with progressive deterioration of renal function. Apoptotic DNA fragmentation was detected in polycystic kidneys, and even more often in polycystic kidneys from patients with renal failure, whereas no apoptotic DNA fragmentation was present in kidneys from patients without renal disease.

Most importantly, in autoimmune diseases such as autoimmune thyroid disease and type 1 diabetes mellitus, new data suggest that the immune system itself may not carry out the final act of organ injury; rather, the target cells (i.e. thyrocytes and β cells of the islets) commit suicide through apoptosis (71). To date, no autoimmune diseases in humans have been directly linked to genes involved in the control of apoptosis; however, investigations into the role of apoptosis in the development of autoimmune diseases such as type 1 diabetes are now beginning to be undertaken. Alterations in the susceptibility of lymphocytes to death by apoptosis *in vitro* have been reported in autoimmune disease. In addition, increased apoptotic rates in islets of Langerhans have been demonstrated in the pancreas of diabetic subjects.

Autoimmune diseases are characterized by the proliferative expansion of lymphocytes reactive to self antigens. It has been shown that repetitive treatment with antigen can result in the selective death of antigen-reactive lymphocytes *in vivo*. Specific deletion of lymphocytes by repetitive treatment with a disease-associated autoantigen has been shown to be effective in the treatment of experimental autoimmune disease. Such treatments may prove effective in human autoimmune disease such as type 1 diabetes and Grave's disease if the specific antigens involved in the autoimmune reaction are to be identified. The primary location of apoptotic cells in rheumatoid arthritis synovial tissue is at the level of the synovial lining. Local invasiveness and tissue destruction in rheumatoid arthritis seem to be controlled by proto-oncogenes, c-jun, c-fos and c-myc. From the findings of ovarian studies, it is apparent that oestrogens generally prevent and androgens induce apoptosis. In fact, the expression of c-myc in primary cultures of synovial macrophages treated with oestrogens is increased (51). Further studies of the influence of gonadal steroids on synoviocyte apoptosis and proto-oncogene expression could offer new perspectives on the pathogenesis and therapy of synovitis in rheumatoid arthritis and other rheumatic diseases (51).

As in amphibian morphogenesis, apoptosis is a key component of developmental processes in mammalian tissues. Failure to induce cell death might hence lead to failure of organogenesis and to organ malformations. MIS, for example, is important for sexual differentiation. Failure of MIS to induce localized programmed cell death may be associated with tumourigenesis, infertility and, most importantly, with sexual ambiguity (13). An exaggerated programmed cell death during embryonal development may cause developmental abnormalities.

Certain viruses can inhibit apoptosis, and metabolic stress or damage of cell structures can induce apoptosis. Therefore, not only viral infections, but also drugs and chemical and physical injuries during embryogenesis may interfere with balanced programmed cell death and thus induce malformations. Drugs and therapy designs directed to modulate the apoptotic process will offer new approaches to the prevention of congenital malformations (14).

Conclusions

This review has aimed to summarize some of the available evidence showing where and how hormones control apoptosis and where and how programmed cell death exerts modulating effects upon hormonally active tissues. The understanding of how hormones influence programmed cell death and, conversely, of how apoptosis affects endocrine glands, is central to further design strategies to prevent and treat diseases that affect endocrine tissues.

Acknowledgements

This work was partly supported by grants from the Alexander von Humboldt Foundation, Bonn, Germany (to B G), the Deutsche Krebshilfe, Bonn, Germany (to W K) and BIOMED 2 program, Brussels (W K).

References

- 1 Ameisen JC. The origin of programmed cell death. *Science* 1996 272 1278–1279.
- 2 Barinaga M. Forging a path to cell death. *Science* 1996 273 735–737.
- 3 Lavin M & Watters D (Eds). *Programmed cell death – the cellular and molecular biology of apoptosis*. Chur, Switzerland: Harwood Academic Publ., 1993.
- 4 Mihich E & Schimke RT (Eds). *Apoptosis. Pezcoller Foundation Symposia 5*. New York: Plenum Press, 1994.
- 5 Milas L, Stephens LC & Meyn RE. Relation of apoptosis to cancer therapy. *In Vivo* 1994 8 665–673.
- 6 Sen S. Programmed cell death: concept, mechanism and control. *Biological Review of the Cambridge Philosophical Society* 1992 67 287–319.
- 7 Steller H. Mechanisms and genes of cellular suicide. *Science* 1995 267 1445–1449.
- 8 Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995 267 1456–1462.
- 9 Tomei LD & Cope FO (Eds). *Apoptosis II: the molecular basis of apoptosis in disease. Current Communications in Cell and Molecular Biology 8*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1994.
- 10 Roodman GD. Advances in bone biology: the osteoclast. *Endocrine Reviews* 1996 17 308–332.
- 11 Abbadié C, Kabrun N, Bouali F, Smardova J, Stehelin D, Vandenbunder B *et al.* High levels of c-rel expression are associated with programmed cell death in the developing avian embryo and in bone marrow cells *in vitro*. *Cell* 1993 75 899–912.
- 12 Coucouvanis EC, Martin GR & Nadeau JH. Genetic approaches for studying programmed cell death during development of the laboratory mouse. *Methods in Cell Biology* 1995 46 387–440.
- 13 Catlin EA, MacLaughlin DT & Donahoe PK. Mullerian inhibiting

- substance: new perspectives and future directions. *Microscopy Research and Technique* 1993 **25** 121–133.
- 14 Haanen C & Vermes I. Apoptosis: programmed cell death in fetal development. *European Journal of Obstetrics, Gynecology and Reproductive Biology* 1996 **64** 129–133.
 - 15 Lang RA. Apoptosis in mammalian eye development: lens morphogenesis, vascular regression and immune privilege. *Cell Death and Differentiation* 1997 **4** 12–20.
 - 16 Tata JR. Hormonal regulation of programmed cell death during amphibian metamorphosis. *Biochemistry and Cell Biology* 1994 **72** 581–588.
 - 17 Amsterdam A, Dantes A, Selvaraj N & Aharoni D. Apoptosis in steroidogenic cells: structure–function analysis. *Steroids* 1997 **62** 207–211.
 - 18 Atwood CS, Ikeda M & Vonderhaar BK. Involution of mouse mammary glands in whole organ culture: a model for studying programmed cell death. *Biochemical and Biophysical Research Communications* 1995 **207** 860–867.
 - 19 Behl C, Widmann M, Trapp T & Holsboer E. 17-Beta estradiol protects neurons from oxidative stress-induced cell death *in vitro*. *Biochemical and Biophysical Research Communications* 1995 **216** 473–482.
 - 20 Bernal J & Nunez J. Thyroid hormones and brain development. *European Journal of Endocrinology* 1995 **133** 390–398.
 - 21 Chun S-Y, Eisenhauer KM, Minami S, Billig H, Perlas E & Hsueh AJW. Hormonal regulation of apoptosis in early antral follicles. FSH as a major survival factor. *Endocrinology* 1996 **137** 1447–1456.
 - 22 Evans-Storms RB & Cidlowski JA. Regulation of apoptosis by steroid hormones. *Journal of Steroid Biochemistry and Molecular Biology* 1995 **53** 1–8.
 - 23 Hsueh AJW, Billig H & Tsafiri A. Ovarian follicle atresia. A hormonally controlled apoptotic process. *Endocrine Reviews* 1994 **15** 707–724.
 - 24 Moulton BC, Motz J, Serdoncillo C, Akcali KC & Khan SA. Progesterone withdrawal and RU-486 treatment stimulate apoptosis in specific uterine decidual cells. *Cell Death and Differentiation* 1997 **4** 76–81.
 - 25 Denmeade SR, Lin XS & Isaacs JT. Role of programmed apoptotic cell death during the progression and therapy for prostate cancer. *Prostate* 1996 **28** 251–265.
 - 26 Sinowatz F, Amselgruber W, Plendl J, Kolle S, Neumuller C & Boos G. Effects of hormones on the prostate in adult and aging men and animals. *Microscopy Research Technique* 1995 **30** 282–292.
 - 27 Ceccatelli S, Diana A, Villar MJ & Nicotera P. Adrenocortical apoptosis in hypophysectomized rats is selectively reduced by ACTH. *Neuroreport* 1995 **6** 342–344.
 - 28 Nagata S. Fas ligand and immune evasion. *Nature Medicine* 1996 **2** 1306.
 - 29 Le Deist F, Emile JF, Rieux-Laucat F, Benkerrou M, Roberts I, Brousse N & Fischer A. Clinical, immunological, and pathological consequences of Fas-deficient conditions. *Lancet* 1996 **348** 719–723.
 - 30 Migliorati G, Nicoletti I, D'Adamo F, Spreca A, Pagliacci MC & Riccardi C. Dexamethasone induces apoptosis in mouse natural killer cells and cytotoxic T lymphocytes. *Immunology* 1994 **81** 21–26.
 - 31 Nicotera P & Rossi AD. Nuclear Ca^{2+} : physiological regulation and role in apoptosis. *Molecular and Cellular Biochemistry* 1994 **135** 89–98.
 - 32 Galli C, Meucci O, Scorziello A, Werge TM, Calissano P & Schettini G. Apoptosis in cerebellar granule cells is blocked by high KCl, forskolin, and IGF-I through distinct mechanisms of action: the involvement of intracellular calcium and RNA synthesis. *Journal of Neuroscience* 1995 **15** 1172–1179.
 - 33 Gimmi CD, Morrison BW, Mainprice BA, Gribben JG, Boussiotis VA, Freeman GJ *et al*. Breast-cancer-associated antigen, DF3/MUC1, induces apoptosis of activated human T cells. *Nature Medicine* 1996 **2** 1367–1370.
 - 34 Halevy O. Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD. *Science* 1995 **267** 1018–1021.
 - 35 Harrington EA, Bennett MR, Fanidi A & Evan GE. c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO Journal* 1994 **13** 3286–3295.
 - 36 Hermeking H & Eick D. Mediation of c-Myc-induced apoptosis by p53. *Science* 1994 **265** 2091–2093.
 - 37 Sheikh MS, Rochefort H & Garcia M. Overexpression of p21WAF1/CIP1 induces growth arrest, giant cell formation and apoptosis in human breast carcinoma cell lines. *Oncogene* 1995 **11** 1899–1905.
 - 38 Lee PP, Darcy KM, Shudo K & Ip MM. Interaction of retinoids with steroid and peptide hormones in modulating morphological and functional differentiation of normal rat mammary epithelial cells. *Endocrinology* 1995 **136** 1718–1730.
 - 39 Akcali KC, Khan SA & Moulton BC. Effect of decidualization on the expression of bax and bcl-2 in the rat uterine endometrium. *Endocrinology* 1996 **137** 3123–3131.
 - 40 Kumar S. The Bcl-2 family of proteins and activation of the ICE-CED-3 family of proteases: a balancing act in apoptosis? *Cell Death and Differentiation* 1997 **4** 2–3.
 - 41 Muller Y, Rocchi E, Lazaro JB & Clos J. Thyroid hormone promotes BCL-2 expression and prevents apoptosis of early differentiating cerebellar granule neurons. *International Journal of Developmental Neuroscience* 1995 **13** 871–885.
 - 42 Parrizas M & LeRoith D. IGF-I inhibition of apoptosis is associated with increased expression of the bcl-xL gene product. *Endocrinology* 1997 **138** 1355–1358.
 - 43 Strobel T, Swanson L, Korsmeyer S & Cannistra SA. BAX enhances paclitaxel-induced apoptosis through a p53-independent pathway. *Proceedings of the National Academy of Sciences of the USA* 1996 **93** 14094–14099.
 - 44 Suzuki A, Matsuzawa A & Iguchi T. Down regulation of Bcl-2 is the first step in Fas-mediated apoptosis of male reproductive tract. *Oncogene* 1996 **13** 31–37.
 - 45 Migliorati G, Nicoletti I, Nocentini G, Pagliacci MC & Riccardi C. Dexamethasone and interleukins modulate apoptosis of murine thymocytes and peripheral T-lymphocytes. *Pharmacological Research* 1994 **30** 43–52.
 - 46 Blobel GA & Orkin SH. Estrogen-induced apoptosis by inhibition of the erythroid transcription factor GATA-1. *Molecular and Cellular Biology* 1996 **16** 1687–1694.
 - 47 Xia Z, Dickens M, Raingeaud J, Davis RJ & Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 1995 **270** 1326–1331.
 - 48 Pronk GJ, Ramer K, Amiri P & Williams LT. Requirement of an ICE-like protease for induction of apoptosis and ceramide generation by REAPER. *Science* 1996 **271** 808–810.
 - 49 Boudreau N, Symptom CJ, Werb Z, Bissell MJ *et al*. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. *Science* 1995 **267** 891–893.
 - 50 Cutolo M, Sulli A, Barone A, Serio B & Accardo S. Sex hormones, proto-oncogene expression and apoptosis: their effects on rheumatoid synovial tissue. *Clinical and Experimental Rheumatology* 1996 **14** 87–94.
 - 51 Fletcher-Chiappini SE, Compton MM, La Voie HA, Day EB, Witorsch RJ & Compton MM. Glucocorticoid–prolactin interactions in Nb2 lymphoma cells: antiproliferative versus anticytolytic effects. *Proceedings of the Society for Experimental Biology and Medicine* 1993 **202** 345–352.
 - 52 Henriksen K, Hakovirta H & Parvinen M. Testosterone inhibits and induces apoptosis in rat seminiferous tubules in a stage-specific manner: *in situ* quantification in squash preparations after administration of ethane dimethane sulfonate. *Endocrinology* 1995 **136** 3285–3291.
 - 53 Iwahashi H, Hanafusa T, Eguchi Y, Nakajima H, Miyagawa J, Itoh N, Tomita K, Namba M, Kuwajima, Noguchi T, Tsujimoto Y & Matsuzawa Y. Cytokine-induced apoptotic cell death in a mouse pancreatic beta/cell line: inhibition by Bcl/2. *Diabetologia* 1996 **39** 530–536.
 - 54 Jo T, Terada N, Saji F & Tanizawa O. Inhibitory effects of estrogen, progesterone, androgen and glucocorticoid on death of neonatal

- mouse uterine epithelial cells induced to proliferate by estrogen. *Journal of Steroid Biochemistry and Molecular Biology* 1993 **46** 25–32.
- 55 van der Bosch J, Horn D, Ruller S & Schlaak M. Modulation of tumor cell susceptibility to cytokine-induced cell death by hormones, growth factors, and cell density. *Journal of Cellular Physiology* 1992 **151** 395–404.
 - 56 Resnicoff M, D Abraham, Yutanawiboonchai W, Rotman HL, Kajstura J, Rubin R, Zoltick P & Baserga R. The IGF-I receptor protects tumor cells from apoptosis *in vivo*. *Cancer Research* 1995 **55** 2463–2469.
 - 57 Singleton JR, Dixit VM & Feldman EL. Type I IGF receptor activation regulates apoptotic proteins. *Journal of Biological Chemistry* 1996 **271** 31791–31794.
 - 58 Rubin R & Baserga R. Biology of disease: IGF-I receptor. Its role in cell proliferation, apoptosis, and tumorigenicity. *Laboratory Investigation* 1995 **73** 311–331.
 - 59 Liuzzi FJ & Tedeschi B. Peripheral nerve regeneration. *Neurosurgery Clinics of North America* 1991 **2** 31–42.
 - 60 McEwen BS Corticosteroids and hippocampal plasticity. *Annals of the New York Academy of Science* 1994 **746** 134–142.
 - 61 Paus R, Rosenbach T, Hass N & Czarnetzki BM. Patterns of cell death: the significance of apoptosis for dermatology. *Experimental Dermatology* 1993 **2** 3–11.
 - 62 Gould E, Wooley CS & McEwen BS. Adrenal steroids regulate postnatal development of the rat dentate gyrus: I. Effects of glucocorticoids on cell death. *Journal of Comparative Neurology* 1991 **313** 479–485.
 - 63 Wimalasena J, Meehan D & Cavallo C. Human epithelial ovarian cancer cell steroid secretion and its control by gonadotropins. *Gynecologic Oncology* 1991 **41** 56–63.
 - 64 Wolozin B, Iwasaki K, Vito P, Ganjei JK, Lacana E, Sunderland T, Zhao B, Kusiak JW, Wasco W & D'Adamio L. Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation. *Science* 1996 **274** 1710–1713.
 - 65 Michna H, Nishino Y, Neef G, McGuire WL & Schneider MR. Progesterone antagonists tumor-inhibiting potential and mechanism of action. *Journal of Steroid Biochemistry and Molecular Biology* 1992 **41** 339–348.
 - 66 Henderson JE, Amizuka N, Warshawsky H, Biasotto D, Lanske BM, Goltzman D & Karaplis AC. Nucleolar localization of parathyroid hormone-related peptide enhances survival of chondrocytes under conditions that promote apoptotic cell death. *Molecular and Cellular Biology* 1995 **15** 4064–4075.
 - 67 Dirami G, Ravindranath N, Kleinman HK & Dym M. Evidence that basement membrane prevents apoptosis of Sertoli cells *in vitro* in the absence of known regulators of Sertoli cell function. *Endocrinology* 1995 **136** 4439–4447.
 - 68 Slayden OD, Hirst JJ & Brenner RM. Estrogen action in the reproductive tract of rhesus monkeys during antiprogesterin treatment. *Endocrinology* 1993 **132** 1845–1856.
 - 69 Eisenhauer KM, Chun S-Y, Billig H & Hsueh AJW. GH suppression of ovarian follicle apoptosis and partial neutralization by IGFBP. *Biology of Reproduction* 1995 **53** 13–20.
 - 70 Gibbons GH & Dzau VJ. Molecular therapies for vascular diseases. *Science* 1996 **272** 689–693.
 - 71 Li W, Liu X, Yanoff M, Cohen S & Ye X. Cultured retinal capillary pericytes die by apoptosis after an abrupt fluctuation from high to low glucose levels. A comparative study with retinal capillary endothelial cells. *Diabetologia* 1996 **39** 537–547.

Received 23 December 1997

Accepted 5 February 1998