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REVIEW ARTICLE

Cell Life and Death in the Anterior Pituitary Gland: Role of Oestrogens

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Apoptotic processes play an important role in the maintenance of cell numbers in the anterior pituitary gland during physiological endocrine events. In this review, we summarise the regulation of apoptosis of anterior pituitary cells, particularly lactotrophs, somatotrophs and gonadotrophs, and analyse the possible mechanisms involved in oestrogen-induced apoptosis in anterior pituitary cells. Oestrogens exert apoptotic actions in several cell types and act as modulators of pituitary cell renewal, sensitising cells to both mitogenic and apoptotic signals. Local synthesis of growth factors and cytokines induced by oestradiol as well as changes in phenotypic features that enhance the responsiveness of anterior pituitary cells to pro-apoptotic factors may account for cyclical apoptotic activity in anterior pituitary cells during the oestrous cycle. Considering that tissue homeostasis results from a balance between cell proliferation and death and that mechanisms involved in apoptosis are tightly regulated, defects in cell death processes could have a considerable physiopathological impact.

Key words: apoptosis, pituitary, lactotroph, oestrogens, oestrous cycle.

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The maintenance of tissue homeostasis in the anterior pituitary gland is regulated mainly by hormones and neurotransmitters released from the hypothalamus and by systemic hormones secreted by target glands. Hormones, growth factors and cytokines locally synthesised in the pituitary gland also participate in this regulation, acting in an autocrine or paracrine manner (1, 2). It is well recognised that the anterior pituitary gland exhibits substantial plasticity during adult life. Transient and apparently unremarkable changes in the prevalence of mitotic and apoptotic events may have considerable impact on the dynamics of pituitary cell subpopulations (3, 4). The existence of several checkpoints in apoptosis indicates a finely regulated balance between cell life and death (5, 6). Cell death by apoptosis has been observed in lactotrophs, somatotrophs, gonadotrophs and corticotrophs (7). Apoptotic processes in the anterior pituitary play an important role in the maintenance of cell numbers during the oestrous cycle or during regression from a hyperplastic state (8, 9). It has been postulated that the proper functioning of the somatotroph subpopulation, and probably of other anterior pituitary cell subpopulations, depends on the fine tuning of the number, activity and positioning of these cells (10), suggesting that tissue remodelling may have an important role in the organisation of cellular networks to ensure a suitable response to physiological demands. Under basal conditions, daily cell turnover in the adult male pituitary gland is approximately 1.5% (4), whereas anterior pituitary mitotic activity in cycling females was reported to be two-fold greater than that in males (11).

Breaking with the current dogma that oestrogens are required for the growth and development of hormone-sensitive cancer, steroid hormones are now considered to mediate both cell proliferation and cell death (12–15). It is well established that oestrogens can induce apoptosis in certain cell types such as long-term oestrogen-deprived breast cancer cells, prostate cells, osteoclasts and macrophages, depending on the phenotype and genetic and epigenetic circumstances (12–18). The mitogenic effects of oestrogens on anterior pituitary cells as well as their role in pituitary tumorigenesis in rodents are well documented (19–21). However, only the Fischer 344 rat strain is especially sensitive to the induction of prolactinomas by oestrogen administration (9, 19). Recent evidence shows that mitotic response to oestrogen exposure is transient and does not impel persistent pituitary growth (22). Some studies have shown that 17β-oestradiol (E_2) can also exert antiproliferative and pro-apoptotic actions in rodent anterior pituitary cells (23–26). The apoptotic mechanisms of E_2 are considered to involve stimulation of both extrinsic and intrinsic pathways (9, 15, 27).

Survival and apoptosis

Apoptosis plays an important role in preservation of tissue homeostasis to ensure a balance between rates of cell proliferation and

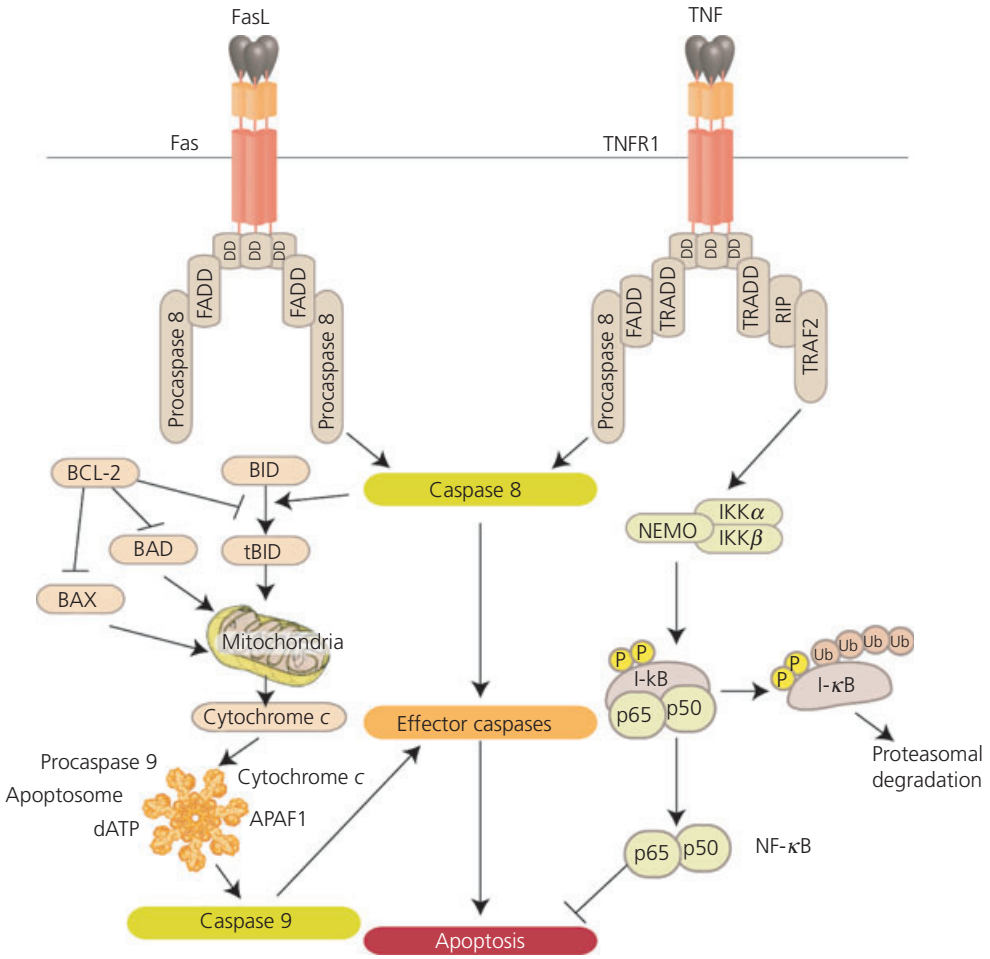

Fig. 1. Apoptosis signalling pathways. The extrinsic pathaway is triggered by binding of pro-apoptotic ligands to death receptors, members of the tumour necrosis factor receptor (TNFR) family with an intracellular death domain (DD), and requires TNFR-associated death domain (TRADD) and/or Fas associated death domain (FADD) mediated recruitment of procaspase 8. Processed caspase 8 can activate either effector caspases that cleave vital proteins within the cell or the BCL-2 family protein BID, resulting in translocation of truncated (t)BID to the mitochondria. The intrinsic (mitochondrial) pathway is arbitrated by interactions between pro- and anti-apoptotic proteins of the BCL-2 family (tBID, BAK, BAD) and involves mitochondrial membrane permeabilisation, resulting in the release of cytochrome *c* and formation of the apoptosome. In the presence of cytochrome *c* and dATP, apoptosis protease-activating factor-1 (APAF-1) binds and activates procaspase 9. Caspase 9 subsequently activates effector caspases. Through recruitment of TRADD, receptor-interacting protein (RIP)-1 and TNFR-associated factor-2 (TRAF-2), TNFR can activate the inhibitor of κB (IκB) kinase (IKK) complex. This complex phosphorylates IκB, leading to IκB degradation and allowing nuclear factor-kappa B (NF-κB) to move to the nucleus to regulate transcription.

cell loss. Apoptosis is primarily executed by a family of cystein-proteases called caspases (28) and is achieved by two distinct but eventually converging signalling pathways: the extrinsic and the intrinsic pathways (29, 30). The extrinsic pathway is initiated by interaction of specific ligands to death receptors, members of the tumour necrosis factor receptor (TNFR) family, such as TNFR1, TRAIL and Fas (Fig. 1). Binding of death receptor ligands to their receptors rapidly causes the assembly of an intracellular death-inducing signalling complex (DISC) by recruitment of procaspase 8 and the adaptor protein Fas associated death domain (FADD). For TNFR1, binding of the protein TNFR-associated death domain (TRADD) is required for recruitment of FADD. Initiator caspase 8 can activate downstream effector caspases, such as caspases 3, 6 and 7. Fas activation also causes recruitment of caspase 10, which may contribute to the activation of effector caspases (30).

The intrinsic pathway relies on mitochondrial outer membrane permeabilisation, which allows the release of cytochrome *c* from the mitochondrial intermembrane space into the cytosol to form the apoptosome. Cytochrome *c*, together with dATP, facilitates a change in apoptosis protease-activating factor-1 (APAF-1) to enable procaspase 9 recruitment and processing (29). Active caspase 9 in turn activates effector caspases. The intrinsic pathway is controlled mainly by a balance between the antagonistic actions of pro-apoptotic and anti-apoptotic members of the Bcl-2 protein family (31, 32). The Bcl-2 family can register positive or negative signals and integrate them to decide cell fate. Because pro- and anti-apoptotic Bcl-2 family members can heterodimerise, their relative concentration functions as a rheostat for the apoptotic programme. Bcl-2 and Bcl-xL are anti-apoptotic proteins that protect cells from many cytotoxic insults. Other Bcl-2 relatives promote apoptosis and fall

into two distinct groups. Members of one pro-apoptotic group, such as Bax and Bak, are very similar to Bcl-2 in sequence and structure. The members of the other pro-apoptotic group, exemplified by Bik, Bad, Bim and PUMA, possess a Bcl-2 homology (BH) 3 domain, which is required for their function (31). Bid is a BH3-only protein that is both structurally and functionally related to multi-BH domain Bcl-2 family proteins (33). During apoptosis, Bid can be cleaved by caspase 8. Protease-cleaved Bid (tBid) migrates to the mitochondria, where it induces permeabilisation of the mitochondrial membrane, acting as a sentinel for death signals (33). The intrinsic pathway can also be regulated by certain stimuli such as reactive oxygen species and Ca^{2+}, which promote mitochondrial permeability transition (34).

Apoptosis is executed by effector caspases, which deactivate proteins that protect living cells from apoptosis, disassemble cell structures and cleave several proteins involved in cytoskeleton regulation, ultimately leading to cell death (35).

The surveillance of apoptosis impacts on intracellular events that influence whether a cell will undergo apoptosis. One of the main signalling pathways involved in cell survival is nuclear factor-kappa B (NF-κB) (36). NF-κB is a family of transcription factors that exist as homo- or heterodimers of its members: c-Rel, RelA (p65), RelB, p105/p50 and p100/p52. When TNF-α binds to TFNR1, TRADD can recruit receptor-interacting protein 1 and TNFR-associated factor-2 to the DISC (Fig. 1). This complex can activate the NF-κB pathway by assembly of the inhibitor of κB (IκB) kinase (IKK) complex (30). The IKKβ catalytic subunit of the IKK complex phosphorylates the inhibitor of κB (IκB) family members bound to NFκB, leading to their ubiquitination and proteasomal degradation. This allows NF-κB to translocate to the nucleus, where it modulates the expression of target genes with a NF-κB consensus site in their promoter region. (36–38). NF-κB is involved in cell life and death decisions. Although NF-κB generally protects cells by inducing expression of prosurvival and pro-inflammatory proteins, it can also sensitise cells to pro-apoptotic factors, depending on the cell context (38). Activated NF-κB enters the nucleus resulting in transactivating death-antagonising genes but, when the NF-κB pathway is blocked, TNF-α induces apoptosis.

Regulation of apoptosis in anterior pituitary cells

Lactotrophs and somatotrophs

The anterior pituitary gland undergoes a process of remodelling under several physiological conditions. Mitosis and apoptosis occur in pituitary cells during pregnancy and lactation. (8, 9, 39). Lactotrophs undergo proliferation at the end of pregnancy and during lactation as a result of high oestrogen levels and suckling (39), whereas apoptotic processes reduce the size of the lactotroph subpopulation after lactation (40). The conversion of somatotrophs to lactotrophs was suggested to participate in the expansion of the lactotroph subpopulation during lactation in rats (8). However, in mice, only a small proportion of lactotrophs in the adult pituitary gland may develop from the somatotroph lineage (41). Also, an absence of lactotroph transdifferentiation was reported in the pituitary during pregnancy and lactation, suggesting that, under these physiological conditions in mice, changes in the lactotroph population may result from increased mitotic activity relative to cell death (42). During the oestrous cycle, cyclic changes in proliferation and cell death support lactotroph, somatotroph and gonadotroph functions (8, 9).

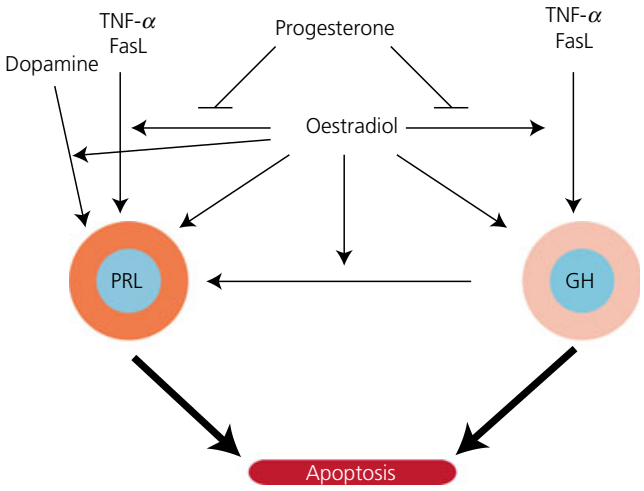

Fig. 2. Proapoptotic actions of oestrogens in lactotrophs and somatotrophs. GH, Somatotroph; PRL, Lactotroph; TNF, tumour necrosis factor.

Lactotrophs are the anterior pituitary cell subpopulation with the highest rate of proliferation followed by somatotrophs, corticotrophs and gonadotrophs (11). During the oestrous cycle, proliferative activity in anterior pituitary cells occurs only during oestrus and requires stimulation by hypothalamic factors and/or the sensitising action of E_2 (2, 8, 39). Approximately 2.5% of lactotrophs proliferate at this stage of the oestrous cycle (43) and therefore a similar number of these cells might die to maintain the size of the lactotroph subpopulation. The highest rate of apoptosis of anterior pituitary cells is observed at pro-oestrus (23, 44). Lactotroph proliferation at oestrus may prepare this pituitary subpopulation for the requirements of prolactin pro-oestrous surge. Apoptosis may reduce the increased number of lactotrophs at the following pro-oestrus when pregnancy does not occur, thereby allowing pituitary remodelling in each oestrous cycle. Evidence indicates that the increase in apoptosis at pro-oestrus depends on high levels of circulating oestrogen at this stage of the oestrous cycle because E_2 promotes apoptosis of anterior pituitary cells in primary culture (23). Also, we demonstrated that E_2 exerts a rapid pro-apoptotic action on lactotrophs and somatotrophs, probably through oestrogen receptors associated with the cell membrane (24) (Fig. 2). Chronic oestrogen treatment also induces apoptosis in the anterior pituitary gland (23, 45).

TNF-α and FasL, which are cytokines belonging to the TNF superfamily, induce apoptosis of lactotrophs and somatotrophs but not gonadotrophs or corticotrophs. The promotion of apoptosis of these cell subpopulations by death receptor activation is triggered in cells from rats at pro-oestrus, when circulating E_2 levels are highest, but not at dioestrus (7, 46, 47, Jaita G et al, unpublished data). This process appears to be oestrogen-dependent because TNFR and Fas

activation requires the presence of E_2 to induce apoptosis of lactotrophs and somatotrophs from ovariectomised rats (7, 46, 47, Jaita G et al, unpublished data). The lack of apoptotic response to death receptor activation in these cell types at dioestrus could result from the action of progesterone, whose circulating levels peaks at this stage of the oestrous cycle. Indeed, the permissive action of oestrogens to TNF-α- and FasL-induced apoptosis is abolished by progesterone (46, Jaita G et al, unpublished data), probably through a paracrine factor because the expression of progesterone receptors is confined to gonadotrophs (8) (Fig. 2).

Apoptosis was reported to be induced by D_2 dopamine receptor agonists in anterior pituitary glands from female rats exposed to oestrogens and in prolactinomas (48, 49). However, dopamine was demonstrated to trigger apoptosis in GH3 cells, comprising a somatolactotroph cell line lacking D_2 receptors, by a mechanism involving dopamine transporter and oxidative stress. This apoptotic process was suggested to play a role in lactotroph regression in post-lactating rats but not in virgin or lactating rats (50, 51). D_2 activation was also shown to mediate apoptotic action of dopamine in lactotrophs. The presence of E_2 was required to bring about dopamine-induced apoptosis, suggesting that dopamine may be one of the signals contributing to apoptosis of lactotrophs previously sensitised by high levels of oestrogens at pro-oestrus (52).

Oestrogens not only induce apoptosis of anterior pituitary cells and sensitise them to pro-apoptotic stimuli, but also exert opposing actions on proliferation of lactotrophs depending upon the mitogen context. E_2 alone or in combination with forskolin stimulates proliferation of lactotrophs, whereas it inhibits proliferation in combination with insulin or insulin-like growth factor (IGF)-I (25). The mitogenic and antimitogenic actions of oestrogens may be exerted directly on lactotrophs because they do not require paracrine signals from other pituitary cell types for these actions (53). Also, E_2 was reported to inhibit early mitogenic activity promoted by insulin and IGF-I in lactotrophs by a mechanism mediated by membrane-associated oestrogen receptors (26). IGF-1 prevents apoptosis in anterior pituitary cells, lactotrophs in particular, through activation of the phosphatidylinositol 3-kinase/Akt pathway (54). Although oestrogen-responsive pituitary cell lines may not behave exactly like normal anterior pituitary cells, EGF cross-talks with E_2 to positively stimulate cell proliferation in GH3 lactotrophs cells (55) but induces cell death in GH4C1 cells (56). The oestrogen dependency of both induction of apoptosis and inhibition of proliferation may result in a reduction of the lactotroph population at a specific stage of the oestrous cycle, providing one means for terminating the pro-oestrous prolactin surge.

The accepted model of development of bihormonal somatomamotrophs as obligatory precursors for lactotrophs and somatotrophs was questioned by recent studies in transgenic mice suggesting that the majority monohormonal lactotrophs and somatotrophs arise independently (41, 42). The somatomammotrophs express oestrogen receptors and a subpopulation of somatotrophs also express luteinising hormome and follicle-stimulating hormone mRNAs (8). Oestrogens *per se* induce apoptosis of somatotrophs and sensitise them to TNF-α- and FasL-induced apoptosis (24, 46, Jaita G et al, unpublished data). It is possible that part of the somatotroph subpopulation that dies by apoptosis may be bihormonal or multihormonal cells and that this process could help to restrain somatotroph expansion.

Gonadotrophs and other anterior pituitary cell types

Recruitment and maturation of gonadotrophs together with an increase in the proportion of lactogonadotrophs and somatogonadotrophs at pro-oestrus can contribute to rapid expansion of cells bearing gonadotrophin transcripts during the oestrous cycle (8). Several factors such as gonadotrophin-releasing hormone (GnRH), oestradiol and growth factors are involved in the increase in the proportion of gonadotrophin-bearing cells, especially after gonadectomy (8). GnRH enhances IGF-1-induced, mitogen-activated protein kinase (MAPK)-extracelleular-regulated kinase 1/2 (ERK 1/2) and protein kinase C (PKC) signalling pathway-mediated cell proliferation in a gonadotroph cell line, whereas it strongly diminishes the anti-apoptotic effect of IGF-1 by inhibiting PKCα-mediated Akt phosphorylation (57). Although GnRH promotes proliferation of pituitary cells, *in vitro* studies have shown that GnRH induces cell cycle arrest, stimulates apoptosis and limits the mitogenic and anti-apoptotic effects of IGF-1 (54, 57). Yin and Arita (44) reported that gonadotrophs undergo a cyclic change in apoptotic cell death during the oestrous cycle and suggested that inhibition of apoptosis at oestrus could be a result, at least in part, to the pro-oestrous surge of GnRH, helping to maintain the population of gonadotrophs needed for the next cycle.

Concerning other anterior pituitary cell subpopulations, Nolan *et al.* (4) reported that dexamethasone treatment of adrenalectomised rats reduces mitosis and increases apoptosis of anterior pituitary cells. These authors reported that glucocorticoid withdrawal exerts direct pro-apoptotic action in the pituitary, largely confined to cells that have recently entered the cell cycle (58). Considering that adrenalectomy and gonadectomy induce non-additive pituitary mitotic and apoptotic responses, it was suggested that the same progenitor cell responds mitotically to both conditions (59). It was also reported that oestrogens, besides stimulating prolactin-bearing cells, provide mitotic stimulus to several other prolactin-negative pituitary subpopulations (60).

Signalling pathways involved in oestrogen-induced apoptosis

E_2 plays a role in cell turnover in both normal and transformed cells by modifying the expression of oestrogen-responsive genes involved in the cell cycle and/or apoptosis (12, 15). In the classical pathway of oestrogen action, oestrogens bind to nuclear oestrogen receptors ERα and ERβ, which are ligand-dependent transcription factors. The levels of specific ER isoform variants, along with receptor coactivator, corepressor and integrator proteins, modulate ER activity directly (61). It is now recognised that the actions of E_2 are also exerted through at least three extranuclear steroid pathways: (i) interaction of ER with other transcription factors, such as Sp-1, AP-1 and NF-κB, that bind to their response elements; (ii) ligand-independent ER signalling mediated by second-messenger pathways that phosphorylate ER and (iii) nongenomic pathways initiated by

membrane-associated ER (62). The anterior pituitary of adult female rats expresses ERα and ERβ. The expression of ERα is the highest at pro-oestrus and localised mainly in prolactin-, growth hormone- and gonadotrophin-bearing cells (63). Several variant isoforms of ER mRNA are also synthesised in the adult rat pituitary. A unique truncated form of ERα, TERP-1 whose expression peaks at pro-oestrus cannot bind DNA but modulates effects of full-length ERα and ERβ through protein–protein interactions (61). In addition, a membrane-associated ERα has been localised in lactotrophs and somatotrophs (24, 26, 64, 65).

The involvement of the Fas/FasL pathway in the apoptotic effects of E_2 was demonstrated in cell lines derived from breast cancer with long-term oestrogen deprivation (12), neurones (66), endothelial cells (67) and other cell types (15). An oestrogen-responsive element has been identified in the promoter region of Fas and FasL genes. Also, transactivating factors Sp-1 and AP-1, which are crucial effectors of E_2 signals, regulate Fas and FasL promoters (15, 68, 69). Oestrogens upregulate Fas expression in lactotrophs and somatotrophs and also increase FasL expression in lactotrophs (47). Oestrogens also stimulate TNF-α expression, an effect possibly involved in TNF-α release from pituitary cells at pro-oestrus (70). Therefore, increased expression of death ligands and death receptors in the morning of pro-oestrus would lead to apoptosis of anterior pituitary cells at this stage of the oestrous cycle.

It was suggested that the apparently paradoxical effect of oestrogens on apoptosis in different cell types may likely involve the differential modulation of expression of Bcl-2 family proteins (12, 32). Both a dominant negative form of ERα and over-expression of wild-type ERα induce apoptosis in GH4 lactotroph cells by decreasing Bcl-2 expression and increasing phosphorylation of p38 MAPK (71). *In vivo* and *in vitro* studies demonstrated that oestrogens increase the Bax/Bcl-2 ratio, a crucial determinant of cell susceptibility to apoptosis, suggesting that E_2 may be directly involved in the increased Bax/Bcl-2 ratio in the pituitary gland at pro-oestrus (23). Lactotroph apoptosis at the termination of lactation is also related to increased expression of Bcl-2 (40).

E_2 could also exert antiproliferative and pro-apoptotic actions by inhibiting the anti-apoptotic/prosurvival factor NF-κB (15). Strong evidence indicates that E_2 down-regulates NF-κB activity in breast cancer cells, suggesting that the inhibition of NF-κB is implicated in the pro-apoptotic effects of oestrogens (37, 72). In GH3 pituitary cells, the NF-κB pathway inhibitors reduce cell proliferation and viability, suggesting that NF-κB may promote cell growth and survival in pituitary tumours (73). We observed that inhibition of the NF-κB pathway induces apoptosis of anterior pituitary cells and sensitises them to the pro-apoptotic action of TNF-α, suggesting that oestrogens would likely modify pituitary responsiveness to pro-apoptotic stimuli by inhibiting NF-κB activity (Eijo G, unpublished data).

Although many cues for oestrogenic regulation of cell proliferation and apoptosis in the pituitary are still unknown, the nature of the effects of oestrogen is likely to involve both classical and non-classical oestrogen mechanisms of action. The activation of membrane-associated ERα in anterior pituitary cells could be a mechanism for rapid adaptation to variations in circulating levels of gonadal steroids (27).

Conclusions

In summary, oestrogens modulate apoptosis in the anterior pituitary gland by modifying its responsiveness to pro-apoptotic signals (Fig. 2). The apoptotic mechanisms of oestradiol in anterior pituitary cells may involve both death receptor as well as mitochondrial pathways. Specific molecular events include the activation of the TNF-α/TNFR1 and Fas/FasL systems, changes in the balance of pro- and anti-apoptotic Bcl-2 proteins, and inhibition of the NF-κB pathway. The E_2–oestrogen receptor complex may interpret the cell context to determine cell life or death. Fluctuations in circulating levels of gonadal steroids during the oestrous cycle could turn several cell pathways on or off to allow tissue remodelling. Even though the percentage of anterior pituitary cells renewed in each oestrous cycle is low, small changes in the number of mitotic and apoptotic cells may have remarkable consequences for tissue homeostasis.

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