16. ROLE OF ERGOT ALKALOIDS IN THE IMMUNE SYSTEM

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16.1. INTRODUCTION

The ergolines action on the immune system has been studied scarcely. Here, we will discuss the present state of knowledge on this field, and results concerning ergolines effect on certain subpopulations of lymphoid cells. The immunomodulatory effects of ergolines can be derived from the relationship between immunocytes and cells of the neuroendocrine compartment, the primary targets of ergoline compounds. Some of these data have been demonstrated in various *in vitro* and *in vivo* experimental systems. The recent research has been devoted predominantly to the immunosuppressive effect of dopaminergic and prolactin inhibitory ergopeptines. Studies from our department dealing with wide panel of ergolines (mostly prepared by Prof. Eich), and their direct action on lymphoid cells were provided by Šterzl *et al.* (1987)—modulation of B cells antibody production, and by Fišerová *et al.* (1991, 1995, 1996, 1997)—concerning modulation of cytotoxic (NK, T) cell effector functions. The ergolines cytostatic effect on tumour cells have been described in previous chapter by Eich and Pertz (Chapter 15). We will discuss here the effect of ergot alkaloids on tumour regression process from the immunological point of view, particularly the modulation of natural killer (NK) cell effector functions.

List of abbreviations: 5-HT—5-hydroxytryptamine (serotonin), CD—cluster of differentiation, CsA—cyclosporin A, CTL—cytotoxic T lymphocyte, cAMP—cyclic adenosin monophosphate, D_1R—dopamine D_1-type receptor, D_2R—dopamine D_2-type receptor, DTH—delayed type of hypersensitivity, GalNAc—N-acetyl-galactosamine, GlcNAc—N-acetyl-glucosamine, GvHR—graft versus host reaction, G-protein—guanine nucleotide binding protein, IFN—interferon, Ig—immunoglobulin, IL—interleukin, LAK—lymphokine activated killer, LPS—lipopolysaccharide, MAPK—mitogen-activated protein kinase, MHC—major histocompatibility complex, MLC—mixed lymphocyte culture, NK—natural killer, NKR-P1—natural killer rat protein 1, NO—nitric oxide, PBMC—peripheral blood mononuclear cells, PHA—phytohaemagglutinin, PKA—protein kinase A, PRL—prolactin, PRL-R—prolactin receptor, SRBC—sheep red blood cells, TCR—T cell receptor, TNF—tumour necrosis factor.

16.2. CONTEMPORARY KNOWLEDGE OF THE TOPIC—CLINICAL CORRELATES

Since the first observations regarding the central dopaminergic action of substituted ergolines, a number of compounds from this group have been tested using different psychopharmacological and neurochemical assays. Some of them have been introduced into the clinical therapies. Ergolines can serve as a physiologic agonists and/or antagonists at the α-adrenoceptors, dopamine and serotonin receptors (see Chapter 14 of Pertz and Eich).

Some of the clavine type of alkaloids e.g. elymoclavine, chanoclavine, and their analogues influence the monoamine turnover in the brain (Petkov and Konstantinova, 1986). Chanoclavine and its derivatives, as well as bromokryptine stimulating the dopamine D_2R expression (Watanabe *et al.,* 1987), are important for selective pharmacologic intervention in the treatment of various psychosis, hyperprolactinemia and PRL-producing tumours (Wu *et al.,* 1996). On the other hand, effects of ergot alkaloids on vasoconstriction, metabolic influences or smoth muscle cells contraction are mediated by α-adrenoceptors (Abrass *et al.,* 1985; Arnason *et al.,* 1988).

Bromokryptine was the first clinically used central dopaminergic prolactin inhibitor from ergoline-related compounds (Del Pozo *et al.,* 1972). Among the series of ergolines with amino group at the 8-α position the lisurid and tergurid showed considerable activity. The partial dopaminergic properties (D_2R agonist/antagonist) of tergurid, are effective in chronic treatment of Parkinson's disease (Mizokawa *et al.,* 1993; Schulzer *et al.,* 1992) and decreasing of extrapyramidal side effects of neuroleptic drugs (Filip *et al.,* 1992). Tergurid is efficient also in the treatment of stress or drug induced immunodeficiences (Rašková *et al.,* 1987). Tergurid's PRL inhibitory action (Valchar *et al.,* 1989), is utilized in the treatment of hyperprolactinemia (Mizokawa *et al.,* 1993), acromegaly (Dallabonzana *et al.,* 1986) and PRLproducing tumours like prolactinomas or breast cancer (Graf *et al.,* 1986; Anderson *et al.,* 1993). Dihydroergokryptine exhibit similar activity in treatment of microprolactinomas (Faglia *et al.,* 1987). The tumour growth is accompanied incidentally, by inefficient steroid negative feedback on PRL synthesis, downregulation of glucocorticoid receptors, and thus inhibition of the specific immune response. Bromokryptine treatment beside inhibition of PRL levels, induce also the restoration of glucocorticoid receptors (Piroli *et al.,* 1993). The dopamine agonists, except PRL producing tumours, may have an application in the treatment of B cell derived lymphoproliferative disorders arrested in an early differentiation stage (Braesch-Andersen *et al.,* 1992). Our preliminary experiments showed also the effectiveness of liposome-encapsulated agroclavine for *in vivo* treatment of syngeneic mouse mastocytoma. Agroclavine delay the tumour incidence, prolonged the survival time of tumour bearing animals (in 75%), and liposome encapsulation eliminate the extrapyramidal side effects (Fišerová *et al.,* 1996a).

16.3. THE IMMUNE AND NEUROENDOCRINE SYSTEMS COMMUNICATION

16.3.1. Basic Principles of the Immune Response

The cells of the immune system as a whole are responsible for two different, but interrelated forms of immunity. The nonadaptive, "innate" immunity, including monocytes, macrophages, granulocytes and NK cells, is fully functional before infectious agents or foreign antigens enter the body, and in some cases can be sufficient to destroy the pathogens by phagocytosis or soluble mediators (lymphokines) involved in inflammation. NK cells form a distinct lineage of lymphocytes, that kill a variety of targets including infected or malignantly transformed cells. The adaptive (aquired) immunity is responsible for the immunological memory, specificity and diversity of responses mediated by T and B lymphocytes.

16.3.2. The Regulatory Role of Biogenic Amines in Neuroendocrine Immune Axis

A variety of immune system products (cytokines, peptides) that function to coordinate the immune response may provide important signals for the neuroendocrine system. Those, currently known to have the most relevance for the neuroendocrine system are interleukins (IL) IL-1, IL-2, IL-6, tumour necrosis factors (TNF) and the interferons (IFN) their major role lies in the physiology of inflammation (infection, tumour growth). Lymphokines enhance the release of dopamine and norepinephrine from the adrenal medulla, the dopamine turnover in the brain and serve as endogeneous neurokines regulating striatal dopaminergic function (Zalcman *et al.,* 1994). Similarly, the dopamine is involved in the modulation of both TNFα and nitric oxide production by peritoneal macrophages, IL-6 release and inhibition of the TNFα synthesized by adrenal cells (Ritchie *et al.,* 1996).

On the other hand, mediators of the neuroendocrine system appear to facilitate or inhibit the functions of immune cells. It is recently well established that the balance between the pituitary prolactin and adrenal glucocorticoids regulate the immune response. The growth stimulatory PRL is a counterregulatory hormone to the anti-inflammatory glucocorticoids, which prevent the immune system overactivation. Their secretion is controled by the hypothalamic releasing or inhibitory hormones underlying the central dopaminergic control (Sinha, 1995). Thus, the nervous, endocrine and immune systems contribute to the maintenance of homeostasis. The dialog underlying this complex network of communication consist of signal molecules (neurotransmitters, neuropeptides, hormones, lymphokines) and related receptors. These are often shared by the immune and neuroedocrine cells and constitutes a biochemical information circuit between and within these systems. The catecholamine synthesis by non-neural cells support the hypothesis of autocrine and paracrine loops in the regulation of lymphocyte activity (Berquist

et al., 1994). An impaired hormonal or neurotransmitter function evoke a number of immunopathological situations (immunodeficiency, autoimmunity, malignant cell transformation).

16.4. ERGOT ALKALOIDS IN EXPERIMENTAL IMMUNOLOGICAL STUDIES

The proliferation, cell-mediated cytotoxicity, and lymphokine production with respect to antitumour immune response represented our interest. The 30 natural or semisynthetic ergot alkaloids, and 30 glycosylated derivatives of ergolines have been screened. For the preparation of glycosylated derivatives, the dihydrolysergol and elymoclavine has been chosen (Křen *et al.,* 1996). These are the simplest structural representants of 9, 10-dihydroergolines and Δ-9, 10-ergolines used in the human therapy (e.g. nicergolin, pergolid, tergurid, dihydroergotamine, dihydroergotoxine).

The most important capability of properly functioning immune system is the clonal selection and subsequent proliferation of respective cells. Cell growth and activation in the immune system is regulated to a substantial degree by cytokine family of growth factors in correlation with neurophysiological, neurochemical, and neuroendocrine activities of the brain cells. Catecholamines produced by neuroendocrine or immune cells present in the inflammatory lesions, inhibit the lymphocyte proliferation and differentiation by triggering of apoptosis, and thus counteracting the chronicity of the disease (Josefson *et al.,* 1996). The effect of ergolines on cell proliferation is dependent on the activation (differentiation) state of individual cell subpopulations. In very low concentrations (10^{-7}M-10^{-10}M) ergot alkaloids increased the deprived cell mitotic frequency. In comparison to resting cells, previously activated lymphoblasts taken from IL-2, mitogens or allogeneic stimuli induced cultures, did not respond to the modulation by most of ergoline derivatives (Fišerová *et al.,* 1991). According to our results, synergistic effect was observed only on phytohaemagglutinin (PHA) induced proliferation of human peripheral blood mononuclear cells (PBMC) by dihydrolysergol and purified NK cells by agroclavine. This activation is due to induction of cytokines (IL-2, IFN-γ) release. In contrary, chanoclavine (D_2R agonist) exert a strong inhibitory effect on both IL-2 and PHA stimulated NK cells. The cell surface markers analysis showed that both clavines compete with IL-2 on IL-2 receptor, and thus block the proliferation of cells (Fišerová *et al.,* 1995a, 1997).

16.4.1. Modulation of Nonadaptive Immunity by Ergot Alkaloids

NK Cells as a Sensitive Targets for Ergot Alkaloids Action

NK cell-mediated cytotoxicity play an important role in the immunosurveillance against invanding pathogens control of tumour growth and regulation of the

hematopoiesis *in vivo* (Phillips *et al.,* 1992). High susceptibility to pituitary hormones and neurotransmitters, make them a suitable targets for ergolines immunomodulatory action. The ergot alkaloid interference with adrenergic, dopamine or serotonin receptors may influence also the immune responsiveness (Hellstrand and Hermodsson, 1987, 1989, 1990).

One of the major mechanisms by which NK activity is regulated *in vivo,* involves catecholamines released by the sympathetic nervous system, owing to the expression of both D_1 and D_2-type dopamine receptors on lymphocytes (Ovadia *et al.,* 1987; Ricci *et al.,* 1997). Dopaminergic regions in the brain is the effective site for modulation of immune parameters, partially the NK cell functions (Madden and Felten, 1995). Therefore lesions in the striatum or compression of the basal ganglia by brain tumours affect the proliferation of splenocytes and decrease NK cell activity (Imaya *et al.,* 1988; Deleplanque *et al.,* 1994). Central α-adrenergic involvement suppresses the NK cell activity, through the reduction of effector-target conjugates formation by cytolytic effector cells (Carr *et al.,* 1994). The serotoninergic regulation of cell-cell communications between NK cells and monocytes, as well as, the IFN-γ production is mediated through 5-HT_{1A}-type receptors. However, the MHC class I antigen expressiveness is regulated *via* 5-HT_2-type receptors (Hellstrand and Hermodsson, 1993).

Natural killer cell activity is suppressed by several dopamine mixed D_1/D_2R or D_2R antagonists (thiothixine, fluphenazine, pimozide, haloperidol) in PRLindependent manner. In comparison, the bromokryptine decreases it *via* PRL reduction (Won *et al.,* 1995; Nozaki *et al.,* 1996). On the other hand, activation of dopamine (D_1R) neurotransmission has an immunoenhancing effect. The DiR specificity of ergoline compounds is very rare, however, agroclavine exhibit D_1R agonistic/antagonistic activity, as proved by inhibition of specific D_1R antagonist [^{3}H] SCH 23.390 binding (pIC_{50}–6.02) on rat striatal neurones. The detection of D_1R specifity of agroclavine on lymphoid cells in our experimental procedure was unsuccessful, even if the specific binding of [^{3}H]-agroclavine to lymphocytes was detected (Fišerová *et al.,* 1996b).

The essential role of PRL in NK cell proliferation, lymphokine activated killer (LAK) cell maturation, IL-2R expression, induction of genes for growth factors, perforins and membrane associated recognition molecules have been described (Matera *et al.,* 1996; Gilmour and Reich, 1994). Concurrently, reduction of NK cell-mediated cytotoxity during pathological hyperprolactinemia, is restored by bromocryptine therapy (Gerli *et al.,* 1986).

The knowledge of the NK cell receptors having affinity to carbohydrates (e.g. NKR-P1 lectin), enable us the search for its ligands involved in cytotoxic effector function with help of glycosylated ergot alkaloids. The carbohydrate part of the ergoline glycosides corresponds to the most common ligands of NKR-P1 receptor expressed on rat NK cytotoxic effectors, represented by either N-acetyl-galactosamine (GalNAc) and N-acetyl-glucosamine (GlcNAc) monosacharides or naturally occurring oligosaccharides containing such terminal moieties (Bezouška *et al.,* 1994a, b).

Using different concentrations of natural and semisynthetic ergolines in 4 hours ^{51}Cr-release human NK cell cytotoxicity assay, a dose-dependent effect was noted. When ergolines applied at high doses (10^{-6}M), inhibition of NK cell activity against sensitive target cells (K562) was observed, whereas at the low doses stimulatory ones. In contrary, when NK-resistant (RAJI) target cells were used, the most potent killing effect of PBMC was detected only in the presence of ergolines concentration from 10-^{6}M. Interestingly, the human PBMC maintained in 5-day cultures in the presence of either agroclavine, 10-α-methoxy-dihydro lysergol or nicergoline enhanced the cytotoxic potency of NK cells against K562 from 4 to 8-fold. However, when IL-2 activated lymphocytes were used, the stimulating effect of ergoline compounds have been found limited (Fišerová *et al.* in preparation).

Elymoclavine and dihydrolysergol profoundly changed the level of NK cell activity during *in vitro* assay. The cytotoxicity of human PBMC against NK-resistant (RAJI) target cells was stimulated predominantly in the presence of elymoclavine glycoconjugates, whereas the cytotoxicity against NK sensitive (K562) cells was stimulated in the presence of dihydro-lysergol glycoconjugates. On the contrary, the cytotoxic effector function of mouse splenocytes (Balb/c and Balb/c-nu/nu), is inhibited by glycoconjugates of elymoclavine and particularly by dihydrolysergol, especially in nude mice. It is noteworthy, that strong stimulatory effect of *ß*-glucopyranosid and sialylated N-acetyl-lactosamine derivatives of elymoclavine in human PBMC is completely contradictory to their effects in mouse cell system (Fišerová *et al.,* 1995b; Křen *et al.,* 1996).

Our understanding, if the sensitivity to the immunomodulation by glycosylated ergolines is due to effector or target cells, another experimental system represented by rat NK cell line (RNK16) was used. The rat target cells of astrocyte origin (C-6 glioma), mouse T lymphoma (YAC1) and mouse mastocytoma (P815) were chosen. The highest NK activity in short term (0–4 hours) assay was shown against YAC1 target cells in the presence of GalNAc and GlcNAc derivatives of elymoclavine. The P815 and C-6 target cells were sensitive to killing capability of NK cells after prolonged incubation period (20 hours). This effect was elicited especially by GalNAc derivative of elymoclavine, that was in correlation with its binding specificity to NKR-P1 protein at 10^{-8}M concentration. Generally, we found a weak stimulatory effect of glycosylated ergolines on effector cells, but substantially promoted killing effect with preincubated YAC1 target cells was demonstrated. (Fišerová *et al.,* 1997).

Ergolines Action on Macrophages

Dopamine receptors, present on peritoneal macrophages play an important role in lipopolysaccharide (LPS)-induced TNF-α and nitric oxide (NO) production. Pretreatment with D_2R agonists, bromocryptine or quinpirole, caused the blunting of both the TNF-α and NO responses to LPS injected intraperitoneally. Sulpiride, the D_2R antagonist, has an inhibitory action on

both TNF-α and NO production by peritoneal macrophages. However, antagonists of D_1R inhibited only the LPS-induced NO release and did not alter the TNF-α levels (Hasko *et al.,* 1996). These findings may be of clinical relevance, since most of the therapeutically used ergoline compounds posses effect on D_2R. The oxidative burst of macrophages was significantly stimulated also by agroclavine, elymoclavine and nicergoline (Šůla *et al.,* 1991).

16.4.2. Modulation of Adaptive Immunity by Ergot Alkaloids

Ergolines Action on T Lymphocyte Effector Functions

The neuroendocrine control of the thymus (as a source of T lymphocytes) appears to be extremly complex, with the apparent existence of intrathymic biological circuitry involving the *in situ* production of pituitary hormones and neuropeptides, as well as, the expression of their respective receptors by thymic cells. Thus, in addition to the classical endocrine pathway, paracrine and autocrine mechanisms are probably implicated in the generation of T cell repertoire. The germline rearrangements of the TCR (T cell receptor) are not completly random, and can be modulated by certain hormones (prolactin, estradiol), cytokines (IL-4) and also drugs like cyclosporine A or bromocryptine (Dardenne and Savino, 1994). Bromocryptine changes the developmental pattern of thymocytes by enhancement of differentiation (Thyl.2, CD4 antigens expression), and inhibition of proliferative rate (Russel *et al.,* 1988; Aboussaouira *et al.,* 1989).

The suppression of pituitary PRL release diminishes a variety of immune responses, including activation of resting T cells by mitogens, IL-2 or alloantigens, T cell-dependent primary IgM response and T cell-dependent macrophage activation. Bromocryptine suppresses all of the mentioned T lymphocyte functions through inhibition of PRL secretion, followed by blocking of IL-2, IFN-γ production and IL-2 receptor (CD25) expression (Bernton *et al.,* 1988, 1992). Moreover, bromocryptine potenciates the immunosuppressive effect of cyclosporin A (CsA) on T cell proliferation and CD25 antigen expression (Bernton *et al.,* 1992). The additive effect is done by two factors: (i) CsA compete with PRL for common binding site on the cell surface, and (ii) bromocryptine decrease the number of circulating PRL molecules (Hiestand *et al.,* 1986; Vidaller *et al.,* 1986; Morikawa *et al.,* 1994). On the other hand, the percentage of $PRL\text{-}R^+$ lymphocytes is not influenced by bromokryptine, since the PRL-R expression is regulated by other factors, than pituitary PRL level (Leite de Moraes *et al.,* 1995). According to Blank *et al.* (1995), the immunosuppressive effect of bromocryptine may be added to induction of nonspecific suppressoric factor produced by $CD8^+$ T cells.

The ergolines with various carbohydrate moities strongly inhibit (at 10^{-11} M) the mitotic activity of resting and PHA-activated lymphocytes. Among them the β-glucopyranosides of all tested ergolines elicited the most pronounced inhibition. These findings are in correlation with carbohydrate binding specificity

of lectins important in regulation of cell growth and differentiation. The target population influenced by ergoline ß-glucopyranosides are T cells as proved in comparative experiments with athymic nude mice, and their euthymic littermates (Fišerová, unpublished results).

The group of 30 natural and semisynthetic ergot alkaloids were assayed on T cell-dependent immune responses either *in vitro* (MLC, specific cytotoxic T cell activity—CTL) or *in vivo* (graft versus host reaction—GvHR). T lymphocyte response to alloantigens in MLC was inhibited only in the presence of ergopeptine alkaloids, α- and ß-ergokryptine, nicergoline and lisuride (at 10^{-6}M). The natural and semisynthetic ergolines, or ergopeptines like ergometrine, ergotamine and terguride do not influence the MLC reactivity. The CTL lytic activity against stimulator (allogeneic) cells induced in the presence of ergoline compounds is enhanced by lysergol, dihydrolysergol and nicergoline, and inhibited by ß-ergocryptine, ergometrine, ergocristine and nicergoline. GvHR do not show any significant changes, even if high doses (5–20 mg/kg) of ergolines were applied. More sensitive to immunomodulation by ergolines was the delayed type of hypersensitivity (DTH) reaction. The ergopeptine alkaloids—ergometrine, tergurid, lisurid, nicergoline and some of the natural and semisynthetic ergolines (lysergol, chanoclavine, 10α-methoxy-dihydrolysergol) showed significant immunosuppressive effect. Agroclavine, elymoclavine, and dihydrolysergol do not influence the DTH reaction (Šůla *et al.,* 1991). There are contradictory results from the work of Urbanová *et al.* (1990), who found tergurid as an inducer of the DTH reaction in stressed calves, caused by changes in catecholamine receptor expression and release of neuroendocrine mediators during stress.

Ergolines Action on B Lymphocytes

The presence of D_2-like dopamine receptors on B lymphocytes (Santambrogio *et al.,* 1993), suggesting a functional role of dopamine in mitogen-induced B-cell activation and immunoglobulin synthesis. Experiments provided by Kaňková-Vančurová *et al.* (1989) have shown that polyclonal activation of splenocytes in the presence of ergot alkaloids (dihydroergocornine, dihydroergocristine, dihydro-α and ß-ergocryptine), increased number of antibody producing cells.

Various neurotransmitters and related compounds (e.g. ergot alkaloids) acting in the central nervous system, can affect the B lymphocyte responsiveness and alter the antibody production against T cell-dependent antigens (SRBC—sheep red blood cells and other alloantigens). This is due to the expression of adrenergic (predominantly of ß-type), dopamine (D_2R) and sertonin receptors by B cells (Santambrogio *et al.,* 1993; Smejkal-Jagar and Boranic, 1994; Genaro *et al.,* 1986). The conversion of L-DOPA (L-dihydroxyphenylalanine) to dopamine in the organism could selectively affect B lymphocyte effector functions to T-dependent antigens, whereas the antibody production induced by bacterial antigens remains unchanged (Boukhris *et al.,* 1987). Similarly, bromocryptine suppresses through dopamine

agonistic, and dihydroergosine *via* serotonin antagonistic properties the immunoglobulin generation by activated B cells to SRBC (Morikawa *et al.,* 1993; Smejkal-Jagar *et al.,* 1993).

The screening of 33 natural and semisynthetic ergot alkaloid provided in Malbrook system for *in vitro* antibody production (to SRBC) was shown by Šterzl *et al.* (1987). Ergopeptines and alkaloids interacting with α-adrenoceptors on lymphocytes negatively affected the number of antibody producing cells. Compounds derived from lysergic acid were completely inactive in this respect. In contrary, the application of natural and semisynthetic ergolines *in vivo* to Balb/c mice showed only slight decrease of B lymphocyte antibody production in most of the tested ergolines. Marked inhibition were seen only in the IgG production by derivatives of lysergic acid—dihydrolysergol, 10-α-methoxy-dihydrolysergol, and lisurid (Šůla *et al.,* 1991). These findings demonstrate that ergolines can influence the B lymphocytes directly (*in vitro* experiments) or by means of neuroendocrine system (*in vivo* experiments) and both of these activities are inhibitory.

The recent investigations fulfil the major regulatory role of PRL also in autoimmune diseases. PRL serves as a co-mitogen of lymphocytes in pathogenesis of some rheumatic diseases e.g. systemic lupus erythematosus, rheumatoid arthritis (collagen type II arthritis), Sjögren's syndrome, Reiter's syndrome, and psoriatic arthritis. High PRL levels in these patients are effectively treated by bromokryptine, that is accompanied also by restoration of immune disturbancies (Gutierez *et al.,* 1994). Another autoimmunities like antiphospholipid syndrome and experimental allergic encephalomyelitis (adequate to multiple sclerosis in man), under PRL control, are also effectively improved by bromocryptine therapy (Shoenfeld, 1994).

16.4.3. Lymphokine Production by T and NK Cells

The important effect of ergolines on humoral immune mechanisms concerns the induction of lymphokine production mediated by both T lymphocytes and NK cells. The liberation of IFN-γ release following mitogenic stimulation is significantly elevated after treatment of patients by lisurid (Baier and Poehlau, 1994; Poehlau *et al,* 1994).

Our initial experiments indicated, that ergoline compounds modulate in cooperation with other stimulators, as a second signal messengers (IL-2, lectins, alloantigens), the lymphokine production (IFN-γ, TNF-*ß,* IL-2) of human PBMC, as well as NK cells. Elevated production of IFN-γ and TNF-*ß* has been found in ergoline stimulated cultures in the presence of PHA. Significant enhancement of IFN-γ release was induced preferentialy by agroclavine, and TNF-*ß* release by chanoclavine and ergometrine. Agroclavine, elymoclavine and ergometrine, markedly increased the IL-2 production in alloantigen (MLC) stimulated cultures (Fišerová *et al.,* 1995a).

16.5. INTRACELLULAR EVENTS ASSOCIATED WITH ERGOLINES ACTION

16.5.1. Signal Transduction

The lymphoid cells possess membrane receptors for a variety of hormones and neurotransmitters that affect the DNA synthesis and proliferation through adenylate cyclase system (Remaury *et al.,* 1993). Neurotransmitter and peptide hormone receptors, the target structures for ergolines interaction with cell membrane, are coupled to intracellular effector enzymes by GTP-binding proteins (G-proteins). Intracellular induction of adenylate cyclase system by ergolines evoke the transduction of signal to the nucleus with changes in appropriate genes is accompanied by cell membrane receptors expression.

Involvement of PRL and dopamine in the signaling events of lymphoid, as well as, tumour cells is important, in relation to the dopaminergic and PRL inhibitory action of engaged ergolines. PRL can induce a variety of signaling pathways for transmission of the growth signal to cell nucleus, followed by changes of the cell shape and mitotic activity. The common feature, on the top of this cascade, is the activation of the *ras* oncogene. The p21ras-GTP activation by PRL coupled to mitogen-activated protein kinase (MAPK) (Das and Vonderhaar, 1996), accompany the growth and progression of tumour cells. The mechanisms involved in dopamine-mediated inhibition of PRL release and subsequent inhibition of tumour growth, are still not fully understood.

Dopamine receptors are coupled to G-protein α subunits associated with cAMP inhibition (D_2R) or activation (D_1R), and phospholipase C activity (D_1R). Stimulation of mitotic activity is associated with decrease in cAMP content, and the inhibition with cAMP increase. In addition, adenylate cyclase system is involved in protein kinase A (PKA) activation and subsequent PKA phosphorylation of Raf-1, that causes blockage in the Ras pathway (Marx, 1993). It means, that substances elevating cAMP levels in cells (forskolin, D_1R agonists e.g. agroclavine), suppresse *ras* oncogene, therefore can modulate also the carcinogenesis (Suh *et al.,* 1992). Moreover, D_1R is linked to cellular intermediate early gene systems *(c-fos)* producing a phosphoprotein (Fos), involved in the control of class I. MHC antigen expression during the early stages of differentiation. This fact is of great importance in relation to their role in the process of tumour recognition and lysis by cytotoxic (T, NK) cells, and slow the tumour promotion and metastatic spread (Kushtai *et al.,* 1990).

Involvement of Ergolines in Signaling Pathways of NK, T Cells

Biological support for the notion that ergolines participate in the signal transduction of NK cells, was provided by agroclavine. The engagement of appropriate NK cell membrane receptors by agroclavine cause an indirect enhancement of adenylate cyclase through inhibition of G-protein αi/1,2-subunit (Fišerová *et al.,* 1997). Agroclavine induced in purified population of NK cells the intracellular accumulation of Ca^{2+} and inositolphosphates production,

suggesting the phospholipase C-Ca^{2+} pathway activation. Similar activities were not inducible in T cell population (Fišerová *et al.*, 1996b). The involvement of the adenylate cyclase system in the ergolines action was demonstrated by the reduction of both prolactin release and cAMP accumulation produced by dihydroergokryptine and dihydroergocristine (Fiore *et al.*, 1987).

16.5.2. Nuclear Affinity of Clavines

Many informations concerning the affinity of some ergolines to the nuclear structures, have been obtained from the studies on molecular level. The interaction of lysergic acid diethylamide (LSD) with DNA, followed by chromosomal breaks, is well documented. Juranic *et al.* (1987) described the interaction of other lysergic acid derivatives—ergosine, ergosinine and dihydroergosine with calf thymus DNA. The intrinsic binding constants were higher for the interaction of these alkaloids with single stranded DNA, and is dependent on D-ring steric conformation of the ergoline molecule. Deoxyribose or deoxyribonucleotides are involved in this interaction. The preferential binding to the nuclear structures have been detected also in the case of agroclavine on the series of tumour cell lines and lymphocytes. However, the direct interaction with salmon sperm or calf thymus DNA was not detected (Fišerová *et al.*, 1996b). Festuclavine derivatives, particularly the 13-bromo-1-cyclopropyl-methyl-festuclavine, inhibiting the DNA and RNA syntheses, are due to depressed transport of nucleosides into the human lymphoid leukemia Molt 4B cells (Hibasami *et al.*, 1990).

16.6. CONCLUSIONS

It is well known that ergot alkaloids and their derivatives exert diverse pharmacological effects in relation to small changes in their chemical structure. Ergoline compounds affect the neuroendocrine system by interaction with membrane receptors for number of biogenic amines. Such a direct interaction of ergolines with neurotransmitter receptors on lymphoid cells till has not been proved. However, we can hypothesise distinct mechanisms of ergot alkaloid interaction with lymphoid and tumour cells, based on indirect experimental findings: (i) expression of dopamine, serotonin or α-adrenoceptors on lymphocytes, that can be involved in action of ergopeptines; (ii) inhibition of signaling pathways through intracellular enzymes, (serin/threonin kinases-Ras-MAPK) leading to mitogenesis by activation of adenylate cyclase system (D_2R agonistic, PRL inhibitory ergolines); (iii) interaction with the nuclear structures or direct binding with DNA, include derivatives of agroclavine, festuclavine, and several ergopeptines—LSD, ergosine, ergosinine, dihydroergosine.

The presented findings showed different effect of ergolines on proliferation of resting and activated lymphoid cells which may be added to their prolactin

release regulatory action. Contradictory effects of ergolines on cell-mediated (mostly stimulatory) and humoral (inhibitory) immune response probably involve different type of neuroendocrine regulation in these cell populations.

Generally, we can deduce that the functional activity of glycosylated derivatives of elymoclavine and dihydrolysergol is dependent on both the alkaloid and terminal saccharide part of the molecule. Nevertheless, the status of effector cells (resting activated or stressed), as well as, the sensitivity and origin of the target cells are involved. It could be supposed that ergot alkaloids elymoclavine and dihydrolysergol, play a quantitatively different role in the lytic processes. Increased killing capability of effector cells pretreated by elymoclavine prevails to its protective effect on targets, and glycosylation pronouced these effects. In contrary, dihydrolysergol enhance the susceptibility of pretreated target cells, and cut down the lytic capability of effectors. Again, glycosylation emphasize immunomodulative properties of ergoline molecule.

Thus, the crucial question arising from all these experiments, concerns the nature of receptors, signal transduction and mechanism(s) involved in effector-target interaction. Considering the cytotoxicity and related experiments (binding studies—not shown), appears that ergot alkaloids may influence not only effectors but also tumour targets. Under such a conditions, the mechanism is other, than that involved in direct triggering of effector cells. This is in agreement with the fact, that cytotoxic effect on C-6 targets occurs after 20 hours, i.e. later than on the lymphoid targets.

Using different assays, our studies have confirmed that as many drugs, involving ergot alkaloids in common clinical use, could have potential immunomodulatory properties due to central or peripheral dopaminergic action. Principal evidence leads to the regulation of prolactin release and modification of glucocorticosteroid effects on immune and other target tissues. Apparent involvement of ergot alkaloids in recognition and signaling events of lymphocytes is more complex than appreciated, likely due to the till limited informations.

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