Adrenoceptors: Non Conventional Target for Breast Cancer?

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Abstract: Epinephrine and Norepinephrine, typically released during stress bind to nine different adrenoceptors (AR) which classically control the cardiovascular and respiratory systems. New targets were described for the many agonists and antagonists developed for these AR, as the central nervous system. During the last three decades, AR expression and action on the mammary gland/breast were extensively investigated. In the cow mammary gland, good milkability was associated with low density of β_2-AR and high density of α_2-AR. In the rat normal mammary gland, β-AR are expressed in the epithelial cells, alveoli, ducts, and adipocytes showing an exquisite regulation by steroid hormones and prolactin. In rat dimethylbenz(a)anthracene (DMBA) tumors, a close correlation was observed between tumor growth and β-AR concentration. β_2-AR were described in numerous human cell lines and breast tumors. The action of β-adrenergic compounds on cell proliferation is contradictory. While some authors found that β-agonists significantly inhibit cancer cell proliferation and tumor growth in mice, others described a significant reduction in DNA synthesis by β-blockers. Also, positive effects of β-AR on human carcinoma cell migration have been described.

α_2-AR are expressed in human breast cancer and non-cancer cell lines, their stimulation being associated with increased cell proliferation. *In vivo* clonidine increased tumor growth and α_2-adrenergic antagonists completely reversed this effect. When administered alone, rauwolscine inhibited tumor growth behaving as an inverse agonist. Therefore, the numerous adrenergic β- and α-AR agonists or antagonists could prove to be unexpected therapeutic options for mammary gland/ breast and mainly breast cancer.

Keywords: Beta-adrenoceptors, alpha-adrenoceptors, normal breast, breast cancer, mammary tumor.

INTRODUCTION

The endogenous stress catecholamines Epinephrine (E) and Norepinephrine (NE) are typical mediators of some of the physiological features associated with stress. As recently reviewed [1], environmental and psycho-social processes initiate a cascade of information-processing pathways, which trigger the fight-or-flight stress responses in the autonomic nervous system, or defeat/withdrawal responses produced by the hypothalamic-pituitary-adrenal axis. Cognitive and emotional feedback from cortical and limbic areas of the brain modulate the activity of hypothalamic and brain-stem structures that directly control the hypothalamic-pituitary-adrenal axis and the autonomic nervous system. The autonomic nervous system responses to stress are mediated primarily by activation of the sympathetic nervous system causing release of catecholamines (principally NE and E) from sympathetic neurons and the adrenal medulla respectively. Levels of catecholamines are increased in individuals who experience acute or chronic stress, and are responsible for the known stress effects on cardiac, respiratory, vascular and other system. The sympathetic nervous system originates from the brainstem, and the preganglionar neurons terminate in the ganglia near the spinal column. From these ganglia, postganglionar fibers, which release NE, run to the target organs. The adrenal medulla contains chromaffin cells, which release mainly E [1]. The endogenous E and NE transmit their biological signals *via* adrenoceptors (AR), G-protein-coupled receptors (GPCRs), which regulate a large number of physiologic and pathologic actions. GPCRs represent the single largest class of membrane proteins in the human genome, with over 800 GPCRs described [2]. GPCRs share a common structural signature of seven hydrophobic transmembrane segments, with an extracellular amino terminus and an intracellular carboxyl terminus, sharing the greatest homology within the transmembrane segments. The most variable structures among this receptor family are the carboxyl terminus, the intracellular loop spanning transmembrane domains TM5 and TM6, and the amino terminus. A very diverse sequence in the amino terminus is relatively short (10-50 amino acids) for monoamine and peptide receptors, and much larger (350-600 amino acids) for glycoprotein hormone receptors, and the glutamate family receptors, the largest being that of the adhesion family receptors [2]. For the vast majority of GPCRs, activation occurs when an agonist diffuses into an unliganded receptor. In many cases the unliganded receptor has some basal (constitutive) activity towards a G protein. Agonists are defined as ligands that fully activate the receptor. Partial agonists induce submaximal activation of the G protein even at saturating concentrations. Inverse agonists inhibit basal activity. Antagonists have no effect on basal activity, but competitively block access of other ligands [2].

ADRENOCEPTOR FUNCTIONS

The adrenergic system plays an important role in regulating body homeostasis in health and disease. A large number of drugs have been developed to modulate adrenergic signaling. Molecular cloning of AR has led to the identification of nine AR subtypes, which mediate the biological effects of E and NE. These AR may be divided into three groups, the α_1, α_2, and β-AR. Each of these types is composed of three receptor subtypes. The nine AR are α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3 [3]. Individual functions of the different receptor types and subtypes have been elucidated mainly by the generation of AR-deficient mice as reviewed in [4]. Mice lacking α_{1A}- or α_{1D}-AR were hypotensive under normal resting conditions, but deletion of the α_{1B} gene did not affect basal blood pressure. In the mouse, α_1-AR have been impli-

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cated in the development of hypertension and vascular hypertrophy. A critical role of α_{1B}-AR and noradrenergic transmission in the vulnerability to addiction has also been described. In contrast, transgenic overexpression of wild-type or constitutively active α_{1B}-AR under control of their own promoter resulted in an unexpected phenotype, which was characterized by severe neurological degeneration with Parkinson-like locomotor deficits and *grand mal* seizures [4]. The α_2-AR have also been analyzed. *In vivo*, α_{2A} and α_{2C} differentially control the release of NE from sympathetic nerves (α_{2A}) and E from the adrenal gland (α_{2C}). In mice lacking α_{2C}-AR, circulating and urine E levels were 2-fold higher than in wild-type mice. α_{2C}-AR deficient mice developed more pronounced cardiac hypertrophy and heart failure after cardiac pressure overload when compared to wild-type mice. The major function of the α_{2B}-AR subtype appears to be the control of vascular development in the mouse placenta [4]. The α_{2A}-AR subtype has been reported to be the predominant subtype involved in the antinociceptive, sedative, hypotensive, hypothermic, and behavioral actions of α_2-adrenergic agonists. Also, α_{2A}-AR may enhance cognitive functions of the prefrontal cortex. Stimulation of α_{2B}-AR in vascular smooth muscle leads to vasoconstriction, which causes an initial hypertension following rapid intravascular administration of α_2-adrenergic agonists. Although the α_{2A}-AR is the dominant subtype in most regions of the mouse central nervous system, the α_{2C} subtype is also widely distributed in the brain. Mice lacking all three α_2-AR subtypes did not survive beyond day 11.5 of embryonic development due to a defect in the formation of fetal blood vessels in yolk sac and placenta [4]. However, an independent colony of $\alpha_{2ABC}^{-/-}$ deficient mice could be established. By intercrossing of $\alpha_{2A}^{-/-}$, $\alpha_{2B}^{-/-}$ and $\alpha_{2C}^{-/-}$ mice, a small percentage survived a defect in placental development and was used to establish this colony [5]. With respect to β-AR, all three subtypes were functionally present in blood vessels and heart [4]. Cardiac β_1-AR mediate the chronotropic and inotropic effects of catecholamines, in contrast, deletion of the β_2-AR gene did not alter cardiac responsiveness to catecholamines. In addition to β_1 and β_2-AR, cardiac myocytes also contain the β_3-AR subtype. Transgenic mice with chronic activation of the β_1 subtype developed more severe cardiac hypertrophy, fibrosis, and heart failure than did the mice with β_2-AR overexpression. β_2-AR knockout mice have a lower body fat content and show a lower respiratory exchange ratio. Deletion of the β_3-AR resulted in a moderate increase in body fat content. β_{123} knockout mice were viable and fertile, had normal food intake, reduced metabolic rate, and increased leptin levels. When maintained on a high-fat diet, β_{123}-deficient mice developed massive obesity due to failure of diet-induced thermogenesis [4].

ADRENOCEPTOR STRUCTURE

The β_2-AR is one of the most extensively characterized members of the large GPCR family of membrane proteins. The sites of interactions between agonists and the receptor have been characterized by mutagenesis studies, and biophysical methods have been used to study the conformational changes associated with agonist binding and activation as recently reviewed [6]. Kobilka´s group has extensively studied the relations between structure and function for this receptor. By fluorescence lifetime analysis of a reporter fluorophore covalently attached to the G protein-coupling domain of the β_2-AR [7] they found that, in the absence of ligands, this domain oscillated around a single detectable conformation. Binding to an antagonist did not change this conformation but reduced the flexibility of the domain. However, when the β_2-AR was bound to a full agonist, the G-protein coupling domain existed in two distinct conformations and the conformations induced by a full agonist could be distinguished from those induced by partial agonists. They also reported [8] based on FRET (fluorescence resonance energy transfer) studies that the C-terminus of the β_2-AR is an extended, relatively flexible, and possibly disordered structure. Nevertheless, ligand-specific changes can be detected in the distance between the C-terminus and the cytoplasmic end of TM6 transmembrane domain. Catecholamine agonists induced changes in FRET similar to those induced by an inverse agonist. They suggested that this may reflect a conformational change necessary for arrestin-dependent activation of the mitogen-activated protein kinase (MAPK) pathway. The β_2-AR have been recently crystallized by two groups (although several authors are coincident), and its structure was determined bound to the inverse agonist carazolol, as commented in [9]. In one of the reports [6], β_2-AR were embedded in a lipid environment (bilayers of fatty molecules similar to those that constitute cell membranes). To provide a large, polar surface suitable for crystal formation, the β_2-AR molecules were obtained as complexes with an antibody fragment that specifically binds to the intracellular third loop. The antibody fragment did not interfere with agonist binding or with the conformational changes that accompanied receptor activation. The diffraction data were obtained by high-brilliance microcrystallography and the structure determined at 3.4 Å / 3.7 Å resolution. The ligand-binding site could be identified by an extended flat feature close to the extracellular side of the transmembrane helices. This region corresponds to the retinal-binding site of rhodopsin. As compared to rhodopsin receptor, the β_2-AR has a more open structure. Rhodopsin has no detectable basal activity, a feature essential for vision. In contrast, even when bound to the inverse agonist carazolol, the comparatively high basal activity of the β_2-AR was suppressed by only 50%. The β_2-AR structure differs from rhodopsin in having weaker interactions between the cytoplasmic ends of transmembrane domains TM3 and TM6 [6]. The other group [10] replaced the intracellular third loop of β_2-AR with the small T4 phage lysozyme stable protein. This protein promoted crystal formation. The modified receptor, also bound to carazolol, was crystallized in a cholesterol-doped lipidic cubic phase that stabilized the receptor in a natural membrane-like environment and the micrometer-size transparent crystals then were evaluated with a 10-μm x-ray beam. They concluded that the ligand-binding site of the β_2-AR was located in a position similar to that of the covalently bound ligand of rhodopsin. However, key differences from rhodopsin were also observed. Particularly in several of the kinked transmembrane helices and in the second extracellular loop, which in the β_2-AR contains an unusual pair of disulfide bonds and an extra helix. They suggested that this loop and the absence of structure in the N-terminal region of the receptor may be important for ligand binding [10]. The structure of the β_2-AR provide new insights into the process of

activation [11]. One example is the presence of several amino acids where mutations lead to elevated basal activity (constitutive activity mutants). In the crystal structure, these amino acids form packing interactions with side-chains of one or more adjacent transmembrane segments, thereby stabilizing an inactive state. Several of these constitutive activity mutants are linked through packing interactions with amino acids that are essential for receptor activation. These observations suggest that the process of activation involves side-chain rearrangement of many amino acids on adjacent transmembrane domains extending from the ligand binding site to cytoplasmic domains [11].

The α_2-AR structure have also been studied in detail [12]. When testing binding models for several natural and synthetic drugs, the two-site models were superior to the one-site models for several compounds. This group suggested several interactions for α_{2A}-AR with agonists. The charged amine group would be optimally coordinated to one side chain oxygen of Asp113 in TM3. Additionally, α_{2A}-AR binds (*R*)-E with a higher binding affinity in comparison to (*R*)-NE, and they attribute this to the additional contacts formed between the *N*-methyl group of E and the hydrophobic residues Phe411 and Phe412 in TM7. Also, as the *R*-isomers are better for binding than the S-isomers or Dopamine, they suggested that the β-OH group would form an hydrogen bond with an oxygen of Asp113. The α_{2A}-AR binding site is rich in aromatic residues. In their model, a network of aromatic interactions could form if small adjustments took place in the orientations of the side chains of these residues. They also suggested that the catechol OH groups have a more important role in receptor activation than in ligand binding, being able to form intimate contacts with Ser200 and Ser204 in TM5, if TM5 rotates clockwise [12].

ADRENOCEPTOR SIGNALING

An interesting feature is the coupling of the AR to different G proteins. The α_1-AR, after binding to the agonist, activates Gq that leads to the activation of phospholipase C (PLC). PLC hydrolyses phosphatidylinositol-4,5-bisphosphate to produce inositol-1,4,5-trisphosphate (IP3) and diacylglycerol. IP3 binds to its receptor and mobilizes intracellular calcium, and diacylglycerol activates protein kinase C (PKC). Both calcium and activated PKC regulate related gene programs and respective physiological functions. In addition to the well-known PLC signaling pathways, α_1-AR have also been shown to couple to MAPK, signal transducer and activator of transcription (STATS), and small GTPases [13].

With respect to α_2-AR, extensive work has been performed. In addition to the classical activation of the inhibitory Gi protein by α_2-AR which leads to the inhibition of adenylyl cyclase, thus resulting in decreased cellular cyclic AMP (cAMP) levels, many other signaling pathways have been described [14]. PC12 neuronal cells do not express endogenously AR. However, using clones of these cells stably transfected with the human α_2-AR genes, it has been shown [15] that the stimulation of all three subtypes also caused activation of PI3K and phosphorylation of extracellular signal-regulated kinase (Erk 1/2). Further investigation of the signaling pathways in these cells provided evidence for a putative pathway by which α_2-AR activate Erk 1/2 and Akt. This pathway involves the stimulation of PLC, arachidonic acid release, arachidonic acid metabolism by cytochrome P450-dependent epoxygenase, stimulation of matrix metalloproteinases and subtype-specific transactivation of epidermal growth factor receptor (EGFR) through Src activation and heparin-binding epidermal growth factor (EGF)-like growth factor release [16]. Also, the treatment of the colon cancer cells expressing the human α_{2A}-AR CaCo2-3B with the α_2-AR agonist UK 14304 induced not only Erk, but also Akt phosphorylation. Both effects were strongly attenuated by inhibition or desensitization of EGF receptor, matrix metalloproteinase blockade, heparin-binding-EGF neutralization or phosphatidylinositol 3-kinase inhibitors. Increased cell spreading and/or migration was suggested [17].

β-AR signaling is also complex. Several reports as reviewed [4] demonstrated that β_1, β_2, and β_3-AR differ in their intracellular signaling pathways and subcellular localization. While the positive chronotropic effects of β_1-AR activation were clearly mediated *via* the stimulatory G protein (Gs) in myocytes, dual coupling of β_2-AR to Gs and inhibitory Gi proteins was evident in cardiac myocytes from newborn mice. β_2-AR were normally confined to caveolae in cardiac myocyte membranes and this localization was essential for physiological signaling of this receptor subtype. Dual coupling of the β_3 subtype to both Gs and Gi, with the Gi component dominating, was described as reviewed [4].

Lefkowitz´s group has extensively studied β-AR desensitization for the last decades. The β_2-AR stimulation with an agonist results in a transient pulse of cAMP, persisting for less than 5 minutes. This group analyzed the contributions of cAMP-dependent kinase, GPCR kinases, and β-arrestin to the regulation of β_2-AR signal kinetics by using small molecule inhibitors, small interfering RNAs, and mouse embryonic fibroblasts. They found that the cAMP response is restricted in duration by two distinct mechanisms in HEK-293 cells: GPCR kinase (GRK6)-mediated receptor phosphorylation leading to β-arrestin mediated receptor inactivation and cAMP-dependent kinase-mediated induction of cAMP metabolism by phosphodiesterases. By the means of a mathematical model of signal kinetics, they concluded that direct receptor inactivation by cAMP-dependent kinase is insignificant but that GRK6/β-arrestin-mediated inactivation is rapid and profound, occurring with a half-time of 70 seconds [18].

ADRENOCEPTORS AND CANCER

Adrenoceptors have been described in several cancer cells. Some examples will be shown in this review. Both β_1- and β_2-AR have been described in HKESC-1 esophageal cancer cells [19]. Stimulation of these receptors by E significantly increased both cell proliferation and cAMP levels, and this effect was abolished by the simultaneous incubation with β-adrenergic antagonists. The incubation with E also stimulated Erk 1/2 phosphorylation, cyclooxigenase-2 (COX-2) and cytosolic phospholipase A2 expression. The stimulation of cyclin D1, cyclin E2, cyclin-dependent kinase (CDK)-4, CDK-6, and retinoblastoma protein phosphorylation at Ser807/811 were abrogated by β_1-AR antagonists. However, E increased the expression of vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR)-1 and -2

by stimulation of β_2-AR, mitogen-activated protein kinase/ Erk kinase (MEK)-, and COX-2 [19]. Moreover, it has been demonstrated that β-AR signaling could be up-regulated at multiple levels by EGFR activation in esophageal cancer cells and that EGF-induced enhanced cell proliferation required transactivation of β-AR in these cells [20].

A very interesting investigation in the cultured colon cancer cell line HT-29 [21] showed that the proliferative response of these cells to a tobacco-specific nitrosamine was abolished by a β_1- and β_2-selective antagonists. Cell growth was also stimulated by the nonselective adrenergic agonist NE and more effectively by the β-selective agonist isoproterenol (ISO). A concomitant increase in cAMP and COX-2 expression, cytosolic phospholipase A2 expression, and prostaglandin E2 release were found [21]. The influence of NE on the migration of SW 480 colon carcinoma cells has been investigated using time-lapse videomicroscopy [22]. NE-treatment increased the locomotor activity within the population from 25% spontaneously locomoting cells to 65% locomoting cells, while the non subtype specific β-blocker propranolol inhibited this increase [22].

In has been described [23] that exogenous β_2-AR agonists may improve the antitumor effect of chemotherapy by cisplatin in a human non-small cell lung cancer cell line [23].

Functional α_{2C}-AR have been described in human neuroblastoma SH-SY5Y cells [24].

The α_2-AR antagonist, yohimbine was shown to inhibit the proliferation of human pancreatic PC-2 and PC-3 cell lines and induce their apoptosis [25].

In prostate cancer the quinazoline-based α_1-AR antagonists, as doxazosin and terazosin can induce apoptosis, inhibit invasion and migration of prostate cancer and endothelial cells and reduce their adhesion potential to extracellular matrix components, thus enhancing their susceptibility to anoikis as reviewed in [26]. Immortalized benign prostate epithelial RWPE cells have been transfected with short hairpin RNA constructs targeting β_2-AR [27]. In this model, the inhibition of β_2-AR confered increased invasion, without affecting cell proliferation. When the effect of β-AR agonists was analyzed *in vivo* by subcutaneously injecting native DU145 prostate cancer cells into nude mice, the ISO treated group developed significantly smaller tumors [27]. Furthermore, to assess β_2-AR expression during human prostate cancer progression, six benign prostate tissue samples, seven clinically localized prostate cancers and six metastatic prostate cancers from a previous prostate cancer microarray study from the group [28] were compared. The β_2-AR transcript was strongly repressed in the metastatic samples [27].

In an elegant work on ovarian cancer [29], chronic behavioral stress resulted in higher levels of tissue catecholamines, greater tumor burden and more invasive growth of ovarian carcinoma cells. This work used the *in vivo* orthotopic mouse model in which human ovarian carcinoma cells are inoculated into the peritoneal cavity of nude mice. Tumors in stressed animals showed markedly increased vascularization and enhanced expression of VEGF, MMP2 and MMP9 through activation of the tumor cell cAMP–PKA signaling pathway by the β_2-AR [29].

NORMAL BREAST AND BREAST CANCER

The development of the breast has been extensively studied by Russo´s group [30]. The breast progressively develops from infancy to puberty under the main stimuli of pituitary and ovarian hormones. During pregnancy, under the stimulus of new endocrine organs, the placenta and the developing fetus, the breast parenchyma branches profusely, leading to the formation of secretory lobular structures. During the first and second trimesters of pregnancy, the structures rapidly progress to lobules which are composed of more numerous and smaller alveoli per lobule. During the last trimester of pregnancy, active milk secretion supervenes, the alveoli become distended and the breast acquires a fully differentiated condition. After weaning, all the secretory units of the breast regress. Also, after menopause, all remaining differentiated lobular structures regress, acquiring the appearance of the lobules of nulliparous women, from which they seem morphologically indistinguishable, although differences in cell proliferation are evident. Breast cancer originates in the undifferentiated terminal ducts (which contain stem cells), the site of origin of ductal carcinomas. The susceptibility of the undifferentiated lobules to undergo neoplastic transformation has been attributed to their high rate of cell proliferation and carcinogen binding to the DNA and low reparative activity [30].

Breast cancer is by far the most frequent cancer among women (23% of all cancers), with an estimated 1.15 million new cases in 2002, ranking second overall when both sexes are considered together. The prognosis (the ratio between prevalence and incidence) from breast cancer is generally rather good, the average in developed countries is 73% and in developing countries 57%. As a result, breast cancer ranks as the fifth cause of death from cancer overall, although still the leading cause of cancer mortality in women in most countries (the 411,000 annual deaths represent 14% of female cancer deaths). Because of its high incidence and relatively good prognosis, breast cancer is the most prevalent cancer in the world today; there are an estimated 4.4 million women alive who have had breast cancer diagnosed within the last 5 years [31].

β-ADRENOCEPTORS IN BREAST/MAMMARY STRUCTURES

The first descriptions concerning adrenergic stimulation of the mammary gland and AR implied the normal bovine mammary gland because of their importance in milk production. Bovine teats contain longitudinal smooth muscles in the wall of the teat cistern and circular muscles around the teat canal. β-AR present in smooth muscles of the cistern wall of teats of lactating cows were identified and characterized by [^{3}H]-dihydroalprenolol (DHA) binding [32]. Activation of β-AR resulted in their relaxation [33]. More details are given when α-AR are analyzed in the following section. Afterwards, Bruckmaier's group extensively studied AR expression in the bovine mammary gland first by binding assays [34] and then by quantitative real-time reverse transcription polymerase chain reaction (qPCR) [35]. The density of β-AR, as tested by binding of the non subtype selective β-adrenergic antagonist [^{3}H]-DHA, was similar in the teat wall

(123.3 ± 21.2 fmol/mg protein) and the large mammary ducts (119.1 ± 21.8 fmol/mg protein), but much lower in the parenchyma (23.7 ± 5.3 fmol/mg protein) [34]. When analyzing AR expression by qPCR, they found that the β_2-AR subtype was most highly expressed, followed by β_1 and β_3, which showed the lowest expression of all the AR in this assay [35].

β-AR were also characterized in the normal mammary gland of experimental animals. In the rat, the radioautographic localization of [^{125}I] cyanopindolol (CYP), a potent β-AR antagonist, revealed the presence of specific β-AR in the epithelial cells, alveoli, ducts, as well as adipocytes. [^{125}I] CYP bound specifically to membranes prepared from lactating mammary glands. Scatchard analysis of the binding data showed the presence of a single class of high affinity sites, with an apparent Kd value of 25.0 ± 0.4 pM and a binding capacity of 32.5 ± 1.2 fmol/mg protein in lactating mammary glands at random stages of lactation. The order of potency of a series of agonists to compete for [^{125}I]-CYP binding was consistent with the interactions with a β_2-AR. β-AR binding showed a 2- to 3-fold increase during pregnancy. Such a result correlated with parallel increases in stimulation of adenylate cyclase activity, the cytosolic progesterone receptor (PR) concentration, as well as plasma 17 β-estradiol (E_2) and progesterone levels. At parturition, a sharp decline in β-AR concentration was observed, a finding concomitant with a drop in PR levels as well as plasma E_2 and progesterone concentrations. However, during midlactation, β-AR reached their maximal levels [36]. β-AR levels fluctuated in the rat mammary gland during the estrous cycle, with higher receptor numbers during the proestrous and estrous phases of the cycle. Ovariectomy caused an almost 50% loss of β-AR concentration in the mammary gland of virgin rats. Treatment of ovariectomized animals with E_2 or progesterone alone or in combination for 3 weeks induced a marked increase in β-AR concentration. Ovariectomy of lactating animals decreased β-AR number to approximately 30% of the value in intact controls, while combined withdrawal of circulating ovarian hormones and inhibition of plasma prolactin levels caused an almost complete inhibition of β-AR concentration. Scatchard analysis of the binding data revealed that the observed alterations in β-AR resulted from changes in the number of binding sites, with no change in binding affinities [37].

The same group analyzed in detail the β-AR in mammary tumors induced by dimethylbenz(a)anthracene (DMBA) administration in the rat [38]. The binding of [^{125}I]-CYP to membrane preparations of DMBA-induced rat mammary tumors was rapid at room temperature, reaching half-maximal specific binding at 30 minutes of incubation. Scatchard analysis of the data indicated that [^{125}I]-CYP bound to a single class of high affinity sites (114 ± 2.1 fmoles/mg protein) at an apparent Kd value of 38.0 ± 0.3 pM. The order of potency of a series of agonists to compete for [^{125}I]-CYP binding was consistent with interaction with a β_2-AR: zinterol > (-) ISO > (-) E >> (-) NE. The presence of high levels of β-AR in carcinogen-induced mammary tumors, the marked decrease in their number following ovariectomy accompanied by parallel changes in PR concentration, suggest a high hormonal sensitivity of tumor β_2-AR. Moreover, a close correlation was observed between progressing, static, and regressing tumors after ovariectomy and β-AR concentration in membranes prepared from DMBA tumors. Moreover, spontaneous mammary tumors of aging rats also expressed high levels of β-AR [38]. This group also reported that when the β-AR binding assays were performed with membrane preparations from these tumors, β-AR concentration was sharply reduced 28 days following ovariectomy (20.5 ± 0.5 fmol/mg protein vs. 178 ± 3.4 fmol/mg protein in the control) or treatment with LHRH (17.2 ± 2.2 fmol/ mg protein). This effect on the β-AR population in the tumor was accompanied by the well known regression of tumor growth (0.87 ± 0.41 cm^2 in ovariectomized rats and 0.56 ± 0.24 cm^2 in LHRH-treated vs. 9.69 ± 2.29 cm^2 in the control ones) and a significant decrease in PR concentration. Treatment of ovariectomized rats with E_2 alone or in combination with progesterone caused a highly significant increase in β-AR and PR levels, as well as in tumor growth. Increase in endogenous prolactin levels by pituitary implant under the kidney capsule resulted in a parallel increase of these three parameters [39].

In human breast cancer cell lines, β-AR have also been identified. Using [^{3}H]-DHA, a β-adrenergic antagonist, β-AR were described in human breast cancer MDA-MB-231, MCF-7, VHB-1, T47-D and BT-20 cell lines, comparing them with the HBL-100 cells. Every cell line tested showed high affinity (Kd 1-9 nM). However, while MDA-MB-231 exhibited high β-AR binding (300 ± 42 fmol/mg protein), and HBL-100 a rather high binding too (126 ± 50 fmol/mg protein), all the other cell lines ranged from 1 to 10 fmol/mg protein. Binding was rapid at 37°C and showed a single binding site and the incubation with agonists induced cAMP production [40]. The same group has reported in human breast tumors the presence of β-AR with high affinity (Kd 1-3 nM) by Scatchard analysis. They were mainly of the β_2-subtype by competition studies with several agonists and antagonists. Moreover, they normally coupled with G-protein. A slight correlation ($0.05<p<0.1$) was observed between AR and PR, and no correlation between AR and estrogen receptor (ER) [41]. Also, β-AR were described in CG-5 breast cancer cells using a radiometric assay [42]. CG-5 cells were derived from a casual contamination by MCF-7 cells of a primary culture from a pleural effusion of a patient with postmenopausal advanced breast cancer. β_2-AR concentrations (107 ± 5 fmol/mg membrane protein) were significantly higher than β_1-AR concentrations (38 ± 3 fmol/ mg membrane protein). These β-AR were functional as demonstrated by the significant increase in cAMP production induced by different concentrations of ISO.

With respect to β-adrenergic action, contradictory results have been presented. On the one hand, cell proliferation inhibitory effects of agonists have been described. MDA-MB-231 human breast cancer cells expressed high β-AR levels, predominantly of the β_2 subtype [43]. Receptor stimulation by ISO, beginning with concentrations as low as 1 nM evoked immediate and robust reductions in DNA synthesis which were completely blocked by propranolol and were of the same magnitude as the effects elicited by high concentrations of 8-Br-cAMP. ISO-induced inhibition of DNA synthesis was maximal at 100 nM and maintained throughout several days of exposure, resulting in a decrement in total cell number and an even greater effect was seen when cAMP breakdown was inhibited by theophylline. A concentration-

dependent reduction in receptor binding was evident within 1 h, with nearly complete down-regulation by 24 h. Receptor binding then remained at 5-10% of control values throughout 72 h of exposure. Treatment of cells with 1mM ISO for 2 h, which caused approximately a 25% reduction in β-AR binding, also elicited a comparable loss of the membrane response of adenylyl cyclase to ISO. However, changes at the level of G-protein function were evident [43]. Moreover, it has been demonstrated that stimulation of the β_2-AR induced significant tumor growth suppression and tumor regression in mice bearing established MDA-MB-231 human breast tumors under the mammary fat pads [44]. This group used a chemical biology approach by screening a 2000 small molecule compound library from the National Cancer Institute for pharmacological agents capable of suppressing the high levels of phosphorylated Erk 1/2 (p-Erk 1/2) in the human breast cancer cell line MDA-MB-231. One of these compounds, called ARA-211 suppressed potently the levels of p-Erk 1/2 without affecting the levels of total Erk 1/2. ARA-211 inhibited Raf-1 and Mek-1 kinase activities when intact cells were treated. ISO, a specific β-AR agonist, was also able to stimulate the formation of cAMP and to decrease the levels of p-Erk 1/2. This stimulation resulted in the inactivation of the Raf-1/Mek-1/Erk 1/2 pathway by a cAMP-dependent activation of protein kinase A, but not by the Rap1 guanine exchange factor exchange protein activated by cAMP (EPAC). ARA-211 potently inhibited anchorage-dependent and -independent growth, and induced apoptosis in cell lines where it stimulated the β_2-AR and inhibited p-Erk 1/2. The treatment with intraperitoneal ARA-211 of mice bearing established MDA-MB-231 breast tumors under the mammary fat pads, suppressed growth at lower doses and caused tumor regression at higher ones. In the same MDA-MB-231 mammary fat pad model, ISO at the same high dose did not cause tumor regression and inhibited tumor growth by 89% [44].

On the other hand, inhibitory cell proliferation effects have been described with β-blockers instead of agonists. A significant reduction in DNA synthesis has been described by [^{3}H]-thymidine incorporation assays in three estrogen-responsive (MCF-7, ZR-75, MDA-MB-361) and three estrogen non-responsive (MDA-MB-453, MDA-MB-435, MDA-MB-468) cell lines derived from human breast cancers by β-blockers. Responsiveness to propranolol varied among the cell lines with no apparent association with the presence or absence of estrogen-responsiveness. The selective β_1-adrenergic antagonist atenolol or the β_2-adrenergic antagonist ICI 118,551 each caused significant inhibition of DNA synthesis in all cell lines tested, with ICI 118,551 generally having the greater effect. However, the β-adrenergic agonist ISO caused significant stimulation of DNA synthesis only in two of the estrogen non-responsive cell lines (MDA-MB-435 and MDA-MB-453). None of the estrogen-responsive cell lines responded to ISO with an increase in DNA synthesis [45]. Although it has been recently described that all of the currently available stocks of MDA-MB-435 cells are derived from the M14 melanoma cell line [46], all the breast cancer cell lines studied showed a similar effect of β-blockers. Also the same group described that the exposure of MDA-MB-453 cells for 6 days to 1 μM of the β-blocker propranolol decreased β_2-AR mRNA levels, while treatment for 30 minutes daily for 7 days had no effect. A high affinity agonist for β-AR, called formoterol, stimulated activation of Erk 1/2 in MDA-MB-453 cells, but only at 150 minutes [47].

With respect to the effect of β-AR on cell migration, using a time-lapse videomicroscopy and computer-assisted cell tracking, Entschladen´s group found that MDA-MB-468 human breast carcinoma cells exhibited positive chemotaxis towards NE [48]. They have also described [49] that this migration can be inhibited by using specific β_2-AR blocker ICI 118,771. NE significantly activated the cAMP response element binding protein (CREB). Microarray analysis revealed changes of gene expression toward a highly motile tumor cell type, among them, the collagen receptor α2-integrin. Concomitantly, two actin-binding proteins (gelsolin and the myristoylated alanine-rich C kinase substrate (MARCKS)) were significantly downregulated in MDA-MB-468 cells [49].

α_1- AND α_2-ADRENOCEPTORS BREAST/MAMMARY STRUCTURES

α_1- and α_2-AR have also been described in the bovine mammary gland. Activation of α-AR in teat muscle preparations induced their contraction [33]. Roets and coworkers stated that the smooth muscles in the teats of cows are innervated by efferent, motor, sympathetic nerves. NE released at the synapse acts upon α_1-AR in the cell membrane of the smooth muscles. In between milking periods, tone of sympathetic nerves is high causing both longitudinal and sphincter muscles of the teat to contract to prevent loss of milk. During teat manipulation and milking, the sympathetic tone decreases by reflex, causing the teat to lengthen and the sphincter to relax. Presynaptic α_2- and β_2-AR present in the outer surface of adrenergic nerve endings are stimulated by NE released in the synaptic cleft. Activation of these α_2-AR induces an inhibition of transmitter release, whereas activation of the β_2-AR causes an increase of release. They suggested that good milkability is associated with a low density of β_2-AR and a high density of α_2-AR, the latter being the major regulatory mechanism [33]. Bruckmaier's group extensively studied AR expression in the cow mammary gland first by binding assays [34] and then by qPCR [35]. The number of α_1- and α_2-AR, as measured by binding to the selective antagonists [^{3}H]-prazosin and [^{3}H]-rauwolscine respectively, decreased from the teat to the mammary ducts to the parenchyma. Most of the α_1- and α_2-AR were found in the teat wall (126.4 ± 25.4 fmol/mg protein for prazosin binding and 130.1 ± 21.6 fmol/mg protein for rauwolscine). The binding to the mammary ducts was less important (38.8 ± 6.3 fmol/mg protein for prazosin binding and 23.4 ± 9.4 fmol/mg protein for rauwolscine). In the parenchyma binding was barely detectable for prazosin binding (7.8 ± 3.4 fmol/mg protein) and non detectable for rauwolscine [34]. Based on these results, the same group [50] suggested the hypothesis that the effects of α- and β-AR stimulation in the bovine mammary gland are mainly mediated by reactions of the large mammary ducts. Contraction and relaxation of smooth muscles in the teat wall and sphincter after administration of α- and β-agonists seem to have less influence on milkability and milk flow than reactions of smooth muscles around the large mammary ducts [50]. When analyzing the mRNA ex-

pression by qPCR for α_2-AR, the α_{2A}-subtype was the highest, followed by α_{2B} and α_{2C}. Within the α_1-AR type, mRNA of the α_{1A}-subtype was most frequent, followed by α_{1B}-. However, the expression of the latter subtypes were higher than the α_2-AR [35].

NE contractile action on the human internal mammary artery was mediated (to a major part) by α_{1B}- and (to a minor part) by α_{1A}-AR [51]. Similarly, the bovine mammary artery has been tested for the response to adrenergic stimulation. NE and the α_1-agonist phenylephrine gave sigmoidal dose-response curves with pEC_{50} ($-logEC_{50}$) values of 5.97 ± 0.07 and 6.21 ± 0.32 respectively. Several agonists and antagonists were tested and the results suggested major involvement of the α_{1B}-AR subtype in contraction of this artery [52].

It has also been reported [53] that E upregulated mdr1 gene expression in MCF-7 breast cancer cells *via* α_2-AR in a dose-dependent manner. This Mdr1 upregulation could be specifically inhibited by pretreatment with small interfering siRNA. Consequently, adrenergic stimulation enhanced the pump function of P-glycoprotein and confered resistance of MCF-7 cells to paclitaxel. *In vivo*, restraint stress increased mdr1 gene expression in the MCF-7 cancer cells that were inoculated subcutaneously into the SCID mice and provoked resistance to doxorubicin in the implanted tumors. This effect could be blocked by yohimbine injection but not by corticoid blockers [53].

The α_2-AR action in human breast cancer cells was described by our group [54]. (-) E, (-) NE and the specific α_2-adrenergic agonist clonidine (Clo) significantly stimulated tritiated thymidine incorporation to MCF-7 cells at very low concentrations. The α_2-adrenergic antagonist yohimbine reverted the stimulation of all agonists. Clo caused a clear and significant inhibition of stimulated cAMP levels both in the intracellular and the extracellular fractions. This inhibition on cAMP levels caused by Clo was completely reversed by the α_2-antagonist yohimbine [54]. We have then described and characterized α_2-AR in several breast tumor and non tumor cell lines [55] by RT-PCR, immunocytochemistry and binding studies. The cancer IBH-6, IBH-7, developed in our laboratory [56], the MCF-7 and the non-tumor at low passages HBL-100 cell lines, expressed both α_{2B}- and α_{2C}-AR-subtypes. A single subtype was expressed in malignant HS-578T (α_{2A}), MDA-MB-231 and non-tumor MCF-10A cells (α_{2B} for the latter two cell lines). All cell lines exhibited significant binding to the specific antagonist [^{3}H]-rauwolscine. The α-, α_2-, and the α_1-compounds with known affinity for α_2-AR, including E, NE, yohimbine, Clo, rauwolscine and prazosin, competed significantly with binding in MCF-7 cells. In addition, IBH-6, IBH-7 and MCF-7 cells showed significant staining with specific antibodies against α_{2B}- and α_{2C}-AR subtypes, when tested by immunocytochemistry. In all cell lines, the specific agonists Clo or oxymetazoline stimulated [^{3}H]-thymidine incorporation. EC_{50} values were in the range of 20-50 fM for IBH-6, IBH-7, and HS-578T; 0.14 pM for MCF-7; 2-82 pM for HBL-100 and MCF-10A cells, and a biphasic behavior with a maximum value at 38.0 pM, was observed for MDA-MB-231 cells. The specific α_2-adrenergic antagonist rauwolscine always reversed this stimulation at 0.1 nM. This study described for the first time the expression of α_2-AR in human epithelial breast cell lines. Moreover, the activation of these receptors was associated with an enhancement of cell proliferation [55].

We have then studied if the proliferative action of catecholestrogens in breast cancer cells could be mediated by the α_2-AR [57]. In mammalian species, catecholestrogen formation from E_2 is quantitatively the most important metabolic pathway of this endogenous sex hormone, rivaling the parent estrogens in concentration [58]. The formation of the catecholestrogens is catalyzed by specific cytochrome P450 isoforms, including CYP1A1/1A2, CYP1B1, and CYP3A4 [59]. The hydroxylation of 17β-E_2 and estrone occurs at either the C-2 or C-4 position, rendering the catecholestrogen 2-hydroxy-estradiol (2-OH-E_2), 4-hydroxy-estradiol (4-OH-E_2), 2-hydroxy-estrone (2-OH-E_1) and 4-hydroxy-estrone (4-OH-E_1). We assessed if the catechol group of these estrogens could make them suitable ligands for AR. In competition studies in human breast cancer MCF-7 cells, high concentrations of 2-OH-E_2, 2-OH-E_1 and 4-OH-E_1 competed for [^{3}H]-rauwolscine binding, whereas 4-OH-E_2 did not. The contribution of α_2-AR and ER in proliferation enhancement was analyzed with specific antagonists. The specific α_2-adrenergic antagonist yohimbine partially reversed the effect of catecholestrogens except 4-OH-E_2. The selective ER downregulator ICI-182780 or fulvestrant partially or totally reversed the effect of all hydroxylated catecholestrogens. As can be seen in Fig. (**1**), panel a, when analyzing the effect of the combination of both antagonists in MCF-7, the contribution of the α_2-AR and ER for 2-OH-E_2, 2-OH-E_1 and 4-OH-E_1 was mixed, whereas for 4-OH-E_2, the only receptor implied was an ER. In MDA-MB-231 cells (ERα-negative) the proliferation was stimulated by these three catecholestrogens and reversed by the adrenergic antagonist (Fig. **1**, panel b). It can be concluded from this work that α_2-AR contribute at least in part to the mitogenic effect of 2-OH-E_2, 2-OH-E_1 and 4-OH-E_1 [57]. When comparing the molecular structures of the natural catecholamines (Fig. **2**) with the catecholestrogen (Fig. **3**), the similarity seems more pronounced with the 2-OH-catecholestrogen than with the 4-OH-catecholestrogen. And in fact, 2-OH-E_1 was the most efficient competitor for α_2-AR. Although with lesser affinity, 2-OH-E_2 bound to the α_2-AR while 4-OH-E_2 did not. On the other hand, the ketone function in 17 seemed to provoke a better configuration of the molecules for α_2-AR binding than the 17β-OH because the estrones are much more efficient than the estradiols in competition studies. Curiously, this 17-keto function reduces the binding to the ER [60]. As the catecholestrogen are larger molecules than the catecholamines, a direct comparison cannot be performed, but probably the catecholestrones have a spatial conformation more related to catecholamines than the catecholestradiols.

Finally, we have tested [61] the *in vivo* influence of α_2-adrenergic agonists and antagonists on mouse mammary transplantable tumors originally induced by medroxyprogesterone (MPA) [62]. This was the first stage in order to consider the α-AR as potential targets for therapy for breast cancer. The incubation for two days with the α_2-AR agonists Clo and dexmedetomidine significantly enhanced mouse mammary tumor cell line MC4-L5 [63] proliferation with an exquisite sensitivity. These agonists also significantly stimulated tumor growth *in vivo*. As shown in Fig. (**4**), panels a and b, Clo significantly stimulated tumor growth in the

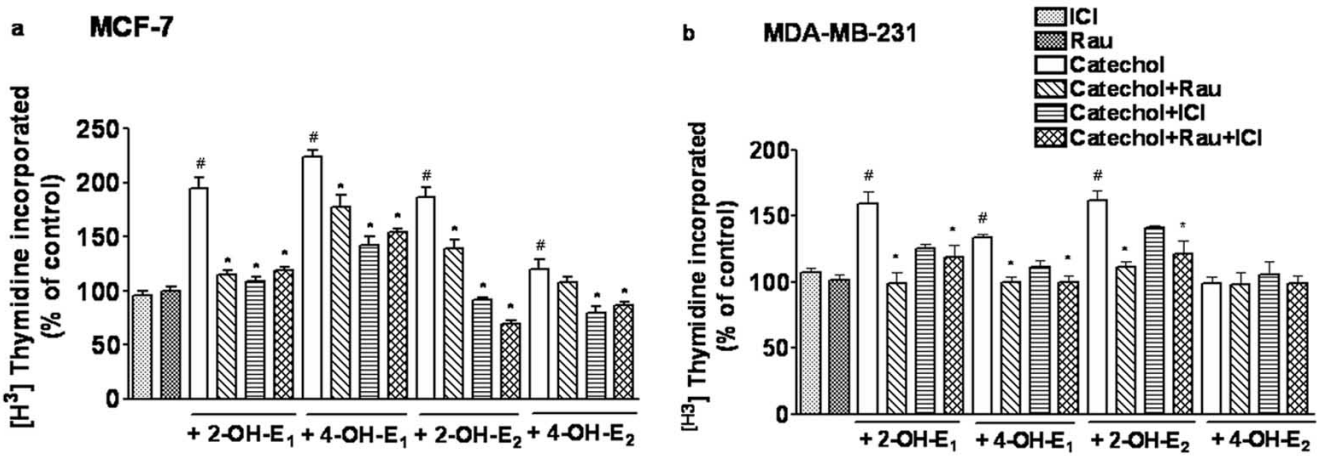

Fig. (1). Effect of the specific α_2-adrenergic antagonist rauwolscine and/or the selective estrogen receptor downregulator ICI 182780 on cell proliferation induced by the catecholestrogens 2-hydroxy-estrone (2-OH-E_1), 2-hydroxy-estradiol (2-OH-E_2), 4-hydroxy-estrone (4-OH-E_1) and 4-hydroxy-estradiol (4-OH-E_2) in MCF-7 (**a**) and MDA-MB-231 cells (**b**).

This Figure was modified from [57]. The experiments were performed with a constant concentration of the corresponding catecholestrogen (30 nM) and a constant concentration of the antagonist (1μM). # $p < 0.001$ with respect to the control in the absence of any compound (except in the case of 4-OH-E_2, in which p value was <0.05 in (a)) *$p < 0.001$ with respect to the incubation in the presence of the corresponding catecholestrogen in (**a**); $p < 0.05$ in (**b**), as analyzed by ANOVA followed by Tukey-Kramer comparison test. The experiment was repeated with similar results. Mean ± S.E.M. is shown.

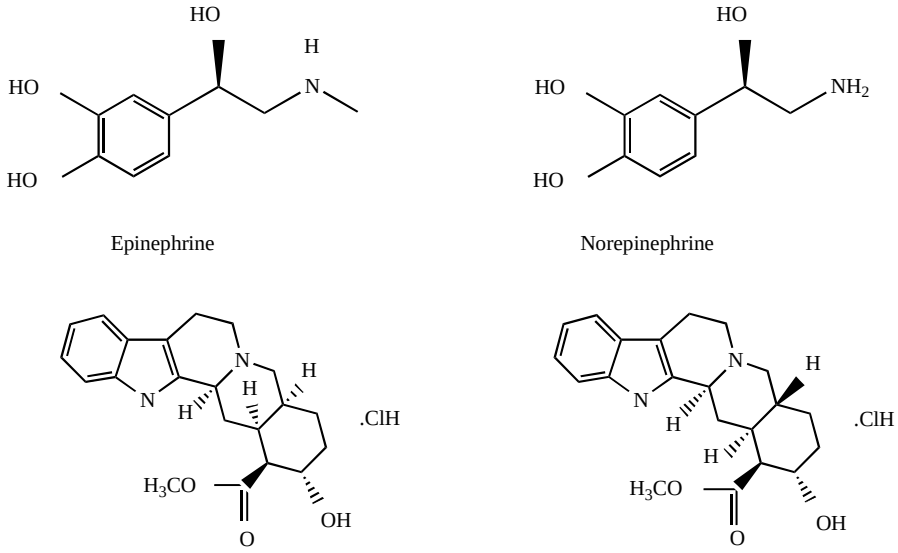

Fig. (2). Structures of the catecholamines norepinephrine and epinephrine and the α_2-adrenergic antagonists rauwolscine and yohimbine.

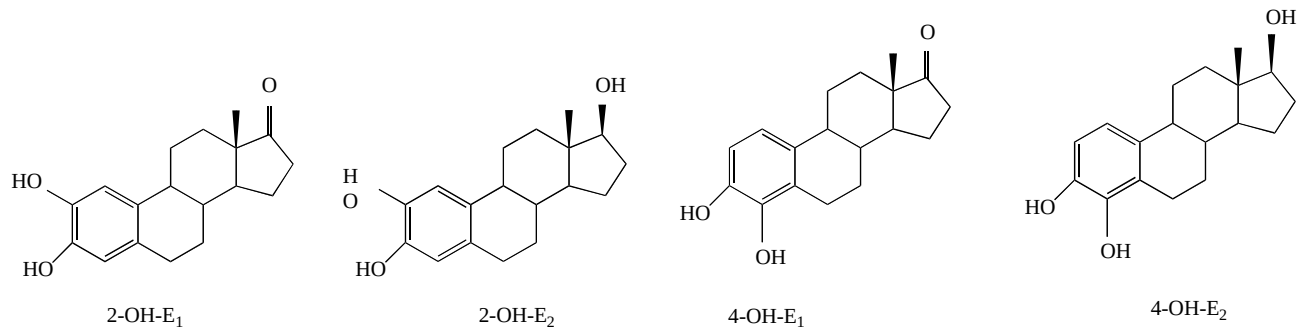

Fig. (3). Structures of the catecholestrogens 2-hydroxy-estrone (2-OH-E_1), 2-hydroxy-estradiol (2-OH-E_2), 4-hydroxy-estrone (4-OH-E_1) and 4-hydroxy-estradiol (4-OH-E_2).

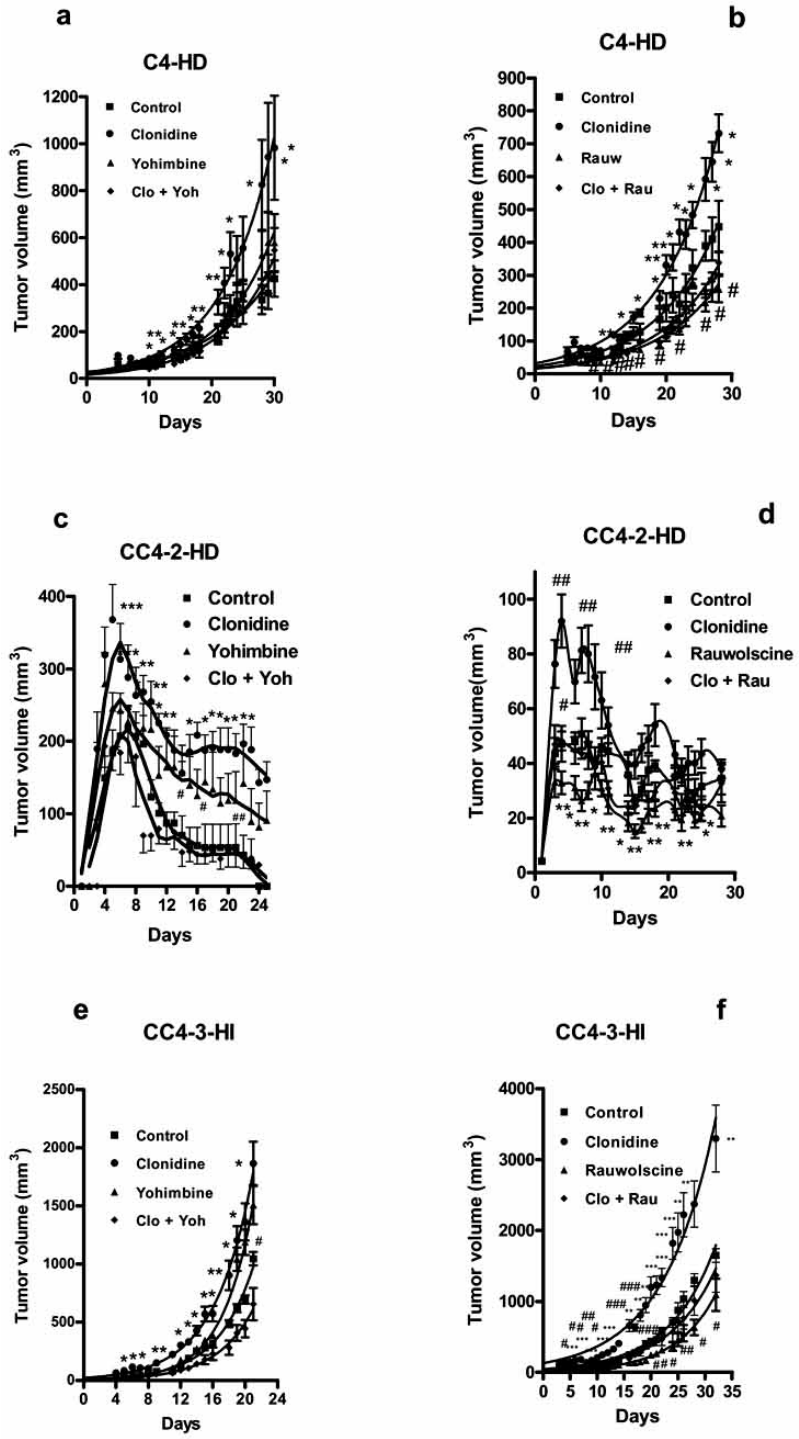

Fig. (4). Effect of the α_2-adrenergic antagonists yohimbine and rauwolscine in different mouse mammary tumors.

This Figure was modified from [61]. Tumors were subcutaneously inoculated and measured daily. Animals were injected daily with clonidine (0.1mg/kg), yohimbine (0.5mg/kg), both of them simultaneously or vehicle (saline solution) in (**a**, **c** and **e**) or clonidine (0.1 mg/kg), rauwolscine (0.5mg/kg), both of them simultaneously or vehicle (saline solution) in (**b**, **d** and **f**). A pellet of MPA (depot preparation) was given to groups in (**a** and **b**). Points represent the mean ± S.E.M. The significantly different points (as analyzed by ANOVA followed by Tukey-Kramer multiple test) are marked in the graph (* or #, $p<0.05$, **, ##, $p< 0.01$ and *** and ###, $p<0.001$ with respect to control). The experiment was performed twice with similar results. In **a** and **b** the progestin-dependent C4-HD mouse mammary tumor was used, in **c** and **d**, the less progestin- dependent CC4-2-HD and in **e** and **f**, the progestin-independent CC4-3-HI was analyzed.

progestin-dependent C4-HD tumor (even in the presence of MPA), in the less progestin-dependent CC4-2-HD (Fig. **4**, panels c and d) and in the progestin-independent CC4-3-HI (Fig. **4**, panels e and f) (the latter two in the absence of MPA). The α_2-AR antagonists yohimbine and rauwolscine completely reversed the agonist´s effect. The most interesting feature in this investigation was that the mice receiving yohimbine (chemical structure in Fig. **2**) alone showed a non-significant but constant increase of tumor growth (Fig. **4**, panels a, c and e). On the other hand, rauwolscine, a stereo-isomer of yohimbine also called α-yohimbine [64] (chemical structure in Fig. **2**) alone diminished significantly tumor growth, behaving as an inverse agonist (Fig. **4**, panels b, d and f). Inverse agonists are thought to reduce the functional activity of the receptors below the baseline activity observed in the absence of any ligand [65]. In different *in vitro* models, rauwolscine was [66-68] or was not [65] found to exert inverse agonist actions. To our knowledge, this was the first report of rauwolscine acting as an *in vivo* inverse agonist [61]. In CC4-3HI tumors, rauwolscine treatment enhanced apoptosis and diminished the mitotic index while clonidine had the inverse effect [61].

CONCLUSION AND FUTURE DIRECTIONS

The main conclusions of this review are shown in Table **1**.

In the cow mammary gland, it has been suggested that good milkability is associated with a low density of β_2-AR and a high density of α_2-AR, the latter being the major regulatory mechanism. The AR subtypes are distributed at the mRNA level as follows: $\alpha_{1A} > \alpha_{1B}$, $\alpha_{2A} > \alpha_{2B} > \alpha_{2C}$, and $\beta_2 > \beta_1 > \beta_3$.

In the rat normal mammary gland, β-AR were expressed in the epithelial cells, alveoli, ducts, as well as adipocytes. β_2-AR binding showed a 2- to 3-fold increase during pregnancy, declining sharply at parturition. However, during midlactation, β-AR reached their maximal levels. In tumor mammary tissue, a close correlation was observed between progressing, static, and regressing tumors after ovariectomy and β-AR concentration in membranes prepared from DMBA tumors.

In human breast cancer cell lines, β_2-AR were described in human cancer MDA-MB-231, MCF-7, VHB-1, T47-D and BT-20 cell lines, comparing them with the non-tumor HBL-100 cells. Every cell line tested showed high affinity. β_2-AR have also been found in human breast tumors.

The action of β-adrenergic compounds on cell proliferation is contradictory. It has been found that the β-agonists significantly inhibited cell proliferation in MDA-MB-231 human breast cancer cells, which expressed high β-AR levels. Moreover, in mice bearing the same cells, significant tumor growth suppression and tumor regression were described with a compound called ARA-211, which stimulated β_2-AR. However a significant reduction in DNA synthesis has been described in three estrogen-responsive (MCF-7, ZR-75, MDA-MB-361) and three estrogen non-responsive (MDA-MB-453, MDA-MB-435 and MDA-MB-468) cell lines derived from human breast cancers by β-blockers instead of antagonists.

Also, significant positive effects of β-AR on MDA-MB-468 human breast carcinoma cell migration have been described using a time-lapse videomicroscopy and computer-assisted cell tracking.

The α_2-AR are expressed in human breast cancer and non-cancer cell lines. Their stimulation was always associated with increased cell proliferation. When analyzing the effect of these compounds *in vivo* in a model of mouse mammary tumors originally induced by MPA, Clo increased tumor growth in every tumor independently of their responsiveness to this progestin. On the other hand the α_2-adrenergic antagonists yohimbine and rauwolscine were able to completely reverse this effect. However, when administered alone, yohimbine slightly increased tumor growth while its stereoisomer rauwolscine alone inhibited it, behaving as an inverse agonist.

From all these work here reviewed it can be concluded that E and NE released during both acute and chronic stress can affect cell proliferation in the normal breast/mammary gland and even tumor growth in breast cancer. The interaction of these natural catecholamines with their receptors is complex, binding to nine different AR subtypes with diverse and even opposite actions. However, the possibility of pharmacological intervention is very tempting. It is nevertheless

Table 1. Summary

Model	Results
Bovine normal mammary gland	Milkability associated with low concentrations of β_2-adrenoceptors. Milkability associated with high concentrations of α_2-adrenoceptors.
Rodent normal and tumor mammary gland	β_2-adrenoceptors are expressed in epithelial cells, alveoli, ducts as well as adipocytes. Positive correlation between β_2-adrenoceptors and DMBA tumor growth. α_2- inverse agonist rauwolscine inhibits cell proliferation and tumor growth.
Human tumor cells *in vitro*	α_2- and β_2-adrenoceptors are expressed in human breast cancer cell lines. Inhibition of cell proliferation by β_2-agonists or antagonists. Increased cell motility and chemotaxis towards epinephrine by β_2-adrenoceptors. α_2-adrenoceptor activation is associated with increased cell proliferation.
Human breast cancer cells *in vivo*	β_2-adrenoceptos are expressed in human tumors. Inhibition of tumor growth (in nude mice) by the β_2- agonist ARA-211.

too early to begin clinical tests. As it has been described that some breast cancer cells exhibit increased cell migration through β-AR, the progression of tumors in the long run must be analyzed. Although much work must be performed yet, two drugs appear as eventually possible therapeutic for breast cancer, the β-adrenergic agonist ARA-211 and the α_2-inverse agonist rauwolscine.

The research on adrenergic effect on breast cancer has focused mainly on the epithelial cancer cells. However, another aspect of great importance is the contribution of stroma cells in tumors *in vivo.* In invasive breast cancer, the basal membrane is normally lost, and tumor cells are in direct contact with an activated interstitial stroma composed mainly by fibroblasts and myofibroblasts, tumor vasculature and a considerable number of infiltrating immune cells, such as lymphocytes, macrophages and mast cells [69]. An adrenergic effect on dermal fibroblast migration has been described [70]. In this model, the activation of two different β_2-AR-mediated signaling pathways has been described. One of them is a Src-dependent pro-migratory pathway, transduced through the EGFR and Erk, and a PKA-dependent pro-proliferative pathway. β_2-AR activation attenuates collagen gel contraction and alters the actin cytoskeleton and focal adhesion distribution signaling through PKA [70]. In human benign prostatic hyperplasia, retrospective analysis of the effect of α_1-antagonists [26] has implicated the apoptotic effect of these compounds on the stroma. In fact, stomal components, mainly smooth muscle cells, correlated with improved lower urinary tract symptoms of this benign disease [26]. The study of the implication of stromal fibroblast on α_2-effect on cell proliferation and tumor growth is advanced in our laboratory. The stromal compartment also includes mast cells, macrophages and T-cell subtypes [69]. Recent studies showed that T cells, mast cells and the normal macrophages are able to synthesize catecholamines, as well as expressing several AR [71]. However, catecholamine synthesis, expression of AR and even more important, catecholamine biological effect on tumor-associated macrophages, a major component of tumor stroma [69], is still pending. Another possible mechanism of action for adrenergic compounds is their effect on angiogenesis. Both α_1-AR [72] and α_{2B}-AR [73] have been described to be important regulators of normal developmental angiogenesis. β_2-AR mediated enhanced tumor angiogenesis in an orthotopic model of ovary cancer [29]. However, more studies must be performed in order to analyze the effect of all adrenergic compounds on cancer angiogenesis.

In order to analyze the possibility of a therapeutic action based on targeting the adrenoceptors, either by agonists or by antagonists, the effect of these adrenergic compounds on the immune system should also be considered. In a series of studies in rats, Ben-Eliyahu and Page have shown (reviewed on [74]) that endogenous systemic release of catecholamines, after stress or surgery, suppressed circulating and marginating-pulmonary natural killer cells (NK). This suppression was also shown *in vivo* and was related to increased susceptibility to metastasis of the MADB106 mammary adenocarcinoma in the F344 rat [74]. Moreover [75], when these rats underwent laparotomy and where intravenously inoculated with the same cancer cells for assessing lung tumor retention, surgery showed a deleterious impact on this parameter. The administration of a single pre-operative dose of the β-blocker propanolol attenuated this effect, as well as partially, the decrease of NK cytotoxicity [75].

A wide range of possible therapeutic resources that have been developed and already accepted for treatment on other pathologies, mainly cardiovascular and air ways diseases, open new avenues in the research for breast cancer treatment. On the other way, the chronic use of adrenergic compounds on these pathologies should be analyzed in the light of their effect on several cancers.

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