Published in final edited form as:

Cancer Biomark. 2013 January 1; 13(3): 133–144. doi:10.3233/CBM-130323.

Effects of tobacco constituents and psychological stress on the beta-adrenergic regulation of non-small cell lung cancer and pancreatic cancer: implications for intervention

Hildegard M. Schuller

Experimental Oncology Laboratory, Department of Biomedical & Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN USA

Abstract

This review summarizes current preclinical and clinical evidence in support of the hypothesis that smoking and psychological stress have significant cancer promoting effects on non small cell lung cancer and pancreatic cancer via direct and indirect effects on nicotinic receptor-regulated beta-adrenergic signaling. Evidence is provided that targeted pharmacological interference with the resulting hyperactive cAMP-dependent signaling by beta-blockers or by γ -aminobutyric acid as well as positive psychological influences may be highly effective in preventing and improving clinical outcomes of these cancers, provided that appropriate diagnostic protocols are followed to monitor systemic levels of stress neurotransmitters and cAMP.

Introduction

Lung cancer is the leading cause of cancer deaths in developed countries. Due to differences in clinical behavior, lung cancer is commonly classified into non-small cell lung carcinoma (NSCLC), a family of several histological lung cancer types (adenocarcinoma, squamous cell carcinoma, large cell carcinoma) and small cell lung carcinoma (SCLC), with NSCLC being generally non-responsive to chemotherapy [1]. Smoking has been extensively documented as a leading risk factor for NSCLC and SCLC. Increased public awareness of the health risks associated with smoking has gradually decreased the number of smokers over the past three decades. However, contrary to expectations, this has not significantly reduced the number of overall lung cancer cases. Instead, a shift in the incidences of histological lung cancer types has been observed, with previously leading squamous cell carcinoma in the NSCLC family declining and adenocarcinoma rising [2, 3]. In fact, pulmonary adenocarcinoma (PAC) accounts for about 80% of NSCLC cases today. Interestingly, PAC is also the only type of lung cancer that develops in a significant number of never smokers and is particularly prevalent in women and African Americans [2–4].

Pancreatic ductal adenocarcinoma (PDAC), generally referred to as pancreatic cancer, is a relatively rare cancer with strong etiological association to smoking [5]. However, its unresponsiveness to existing cancer therapeutics renders this cancer the fourth leading cause of cancer deaths with a mortality rate near 100% within one year of diagnosis [6]. Despite of the significant decrease in smokers, neither the incidence nor mortality rate of pancreatic

Corresponding author: Hildegard M. Schuller, Experimental Oncology Laboratory, Department of Biomedical & Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37996, USA, Phone: 865-974-8217, Fax: 865-974-5616, hmsch@utk.edu.

Conflict of Interest: None to declare

cancer has significantly decreased. By contrast, there was even a significant increase in pancreatic cancer cases, particularly in women, over the past three decades [7, 8].

The disconnect between decreasing numbers of smokers and rise of PAC and PDAC as well as their unchanged mortality rates strongly suggests that factors other than smoking play significant roles in the development, progression and responsiveness to therapeutics of both cancers.

Chronic psychological stress has long been recognized as an important risk factor for cardiovascular disease [9]. In vitro investigations have shown two decades ago that classic agonists for beta-adrenergic receptors (β -ARs) stimulate the proliferation of PAC cells [10, 11] and it was shown in 2002 that these receptors also regulate the proliferation of PDAC cells [12]. The stress neurotransmitters norepinephrine and epinephrine are the physiological agonists for β -ARs and are released into the systemic circulation from the adrenal gland and nerve endings of the sympathetic in response to psychological stress. However, the potential stimulation of PAC and PDAC by psychological stress via interaction of stress neurotransmitters with the beta-adrenergic pathway has only very recently been investigated under controlled laboratory conditions [13, 14].

Smoking is not only a documented risk factor for most human cancers but also significantly increases the risk for the development of cardiovascular disease [9]. The adverse effects of smoking on the cardiovascular system are to a great extent caused by increased synthesis and release of norepinephrine and epinephrine in response to binding of nicotine to regulatory nicotinic acetylcholine receptors (nAChRs) in the adrenal gland and sympathetic nerves [9, 15]. While the resulting activation of beta-adrenergic signaling in the cardiovascular system and the associated increases in blood pressure and heart rate have been extensively studied, potential cancer stimulating effects of nicotine via the indirect activation of beta-adrenergic pathways expressed in PAC and PDAC have been given little attention. It has been recently reported that PAC and PDAC cells as well as the normal epithelia (small airway epithelium, pancreatic duct epithelium) in which these cancers arise produce their own stress neurotransmitters in response to nAChR stimulation by an agonist [16, 17]. These findings further underline the crucial importance of beta-adrenergic pathways for the regulation of both cancers.

The current review provides a critical analysis of the effects of tobacco constituents and psychological stress on the beta-adrenergic regulation of NSCLC and pancreatic cancer and their implications for cancer intervention.

Direct effects of the tobacco carcinogen NNK on beta-adrenergic signaling

The carcinogenic nitrosamine 4-methylnitrosamino)-1-(3-pyridyl)-1-butanone (N-nitroso-nicotine-ketone, NNK) is formed from nicotine by nitrosation in an acidic environment. While the amount of NNK per cigarette is much smaller than the amount of nicotine (pending on the type of cigarette on average by a factor of about 1,000), the additional conversion of nicotine to NNK in the mammalian organism can yield considerably higher concentrations of this potent carcinogen in certain organs. It has thus been reported that the pancreatic juice of smokers contains up to 3 μ mole/L of NNK [18] as opposed to theoretical picomolar to nanomolar systemic concentrations expected if the NNK contents per cigarette were evenly distributed within the human body.

Perceived structural similarities of NNK with stress neurotransmitters norepinephrine and epinephrine and with the site-selective beta-adrenergic agonist isoproterenol (Figure 1) prompted the hypothesis that NNK may be an agonist for β -ARs [19]. To test this hypothesis, receptor binding assays were conducted with cell membrane fractions from two

human PAC and four PDAC cell lines and from transfected Chinese hamster ovary cell lines selectively expressing either the human β -1 or the β -2-AR [12, 19]. Ascending concentrations of NNK, norepinephrine or epinephrine competed for beta-adrenergic binding sites with the radiolabelled β -AR-selective ligand [125 I]-cyanopindolol. These assays clearly identified NNK as an agonist for both types of β -ARs with an affinity 2,200 times greater than epinephrine to the β 2-AR and 600 times greater than norepinephrine to the β 1-AR [19, 20]. It is important to note that the observed ligand-binding of NNK was caused by binding of the unmetabolized parent NNK and not by one of its numerous metabolites, as the assay conditions included inhibitors of cytochrome P450, monoamine oxidase and cyclooxygenase. The prevention of metabolic breakdown of beta-adrenergic ligands is standard operating procedure for these assays as otherwise the ligands fail to reach steady state conditions.

Similar to most other epithelial cancers, PDAC cell lines predominantly expressed β 2-ARs [12]. By contrast, the two investigated PAC cell lines and eight subsequently acquired PAC tissue samples prominently expressed β 1-ARs with either undetectable or weak expression levels of the β 2-AR [19, 21]. The observed predominance of β 1-ARs over β 2-ARs in PAC has been corroborated by immunostains of 48 PAC cases in a commercially available tissue microarray (Biomax Inc, Rockville, MD, USA), with every single one of the 48 PAC cases overexpressing the β 1-AR (Figure 2).

NNK as well as classic β -AR agonists significantly increased the proliferation of PAC and PDAC cells in vitro via cAMP-dependent intracellular signaling (Figure 3) that involved the activation of PKA and CREB as well as transactivation of the epidermal growth factor receptor (EGFR) pathway [22–24]. In analogy to the role of β -ARs as regulators of the synthesis and release of arachidonic acid (AA) in the cardiovascular system [25, 26], binding of NNK to β -ARs additionally caused the synthesis and release of AA from PAC [19] and PDAC [12] cells. Accordingly, NNK-induced cell proliferation was completely blocked by the broad-spectrum beta-blocker propranolol and partially inhibited by inhibitors of COX-2 and EGFR-specific tyrosine kinase [12, 19, 24]. In addition, the development of NNK-induced PAC or PDAC in hamsters was effectively prevented by the beta-blocker propranolol [27, 28]. These findings provide a mechanistic explanation for the frequent overexpression of the AA-metabolizing enzyme cyclooxygenase-2 (COX-2) and of the EGFR and its downstream effectors in PAC and PDAC tissues and identifies β -ARs as the upstream regulators of both signaling cascades. In addition, these studies suggested that the disappointing results of clinical trials that targeted the EGFR or AA pathway [6] may have been caused by the persistence of active signaling via cAMP/PKA/CREB under these protocols (Figure 3). In support of this interpretation, the development of NNK-induced PDAC [28] and PAC [27] in hamsters was completely blocked by treatment of the animals with propranolol whereas the cyclooxygenase inhibitor ibuprofen only partially prevented PDAC development in this animal model [29].

Indirect effects of nicotine and NNK on beta-adrenergic signaling

Receptor binding assays in SCLC cells were the first to identify NNK as an agonist for the α 7nAChR with a 1000 times higher affinity to this receptor than nicotine [30]. These findings were corroborated by another laboratory in oral keratinocytes and airway epithelial cells. The latter studies additionally showed that NNK also binds to nAChRs with subunits α 3 and α 5, albeit at lower affinities than to α 7 [31, 32]. As pointed out in the introduction, binding of an agonist to nAChRs in the adrenal gland and sympathetic nerves triggers the release of norepinephrine and epinephrine into the systemic circulation. Stimulating effects of nicotine observed on the growth of PDAC xenografts in mice [33] associated with increases in tumor cAMP, p-CREB and p-ERK were therefore initially interpreted as the beta-adrenergic responses to increased systemic levels in these neurotransmitters. However,

reports that cell lines from colon cancer [34] and gastric cancer [35] synthesize and release norepinephrine and epinephrine upon in vitro exposure to nicotine indicated that in addition to the nervous system and adrenal gland some epithelial cells have the machinery to produce their own stress neurotransmitters. In support of this hypothesis, in vitro studies showed that small airway epithelial cells [16] as well as pancreatic duct epithelia and PDAC cells [17] synthesized and released norepinephrine and epinephrine in response to nicotine or NNK (Figure 4), resulting in increased cell proliferation and migration that were reversed by the beta-blocker propranolol or by inhibition of adenylyl cyclase activation by γ -aminobutyric acid (GABA). Gene knockdown by siRNA transfections identified the $\alpha 7$ nAChR as an important regulator of this autocrine loop in all investigated cell lines [16, 17] while nAChRs $\alpha 3$ and $\alpha 5$ additionally participated in this activity in PDAC cells and pancreatic duct epithelia [30]. Chronic exposure of the cells to nicotine or NNK over a period of seven days increased the protein expression of all investigated nAChRs (Figure 5), a response accompanied by significantly increased production of norepinephrine and epinephrine in response to significantly lower nicotine or NNK concentrations [36]. These findings are consistent with sensitization of the involved nAChRs and lead to significantly increased proliferation and migration responses to nicotine or NNK [36]. In addition, these data suggest that the reactive desensitization of β -ARs typically observed after chronic exposure to agonist [37] was in these scenarios effectively counteracted by the increases in the production of their agonists, norepinephrine and epinephrine.

Contrary to the reported sensitization of nAChRs that regulate the synthesis and release of norepinephrine and epinephrine (above), nAChRs expressing the $\alpha 4$ subunit are desensitized by chronic nicotine [38]. Despite of reactive increase in receptor protein, this results in decreased synthesis and release of the inhibitory neurotransmitter γ -aminobutyric acid (GABA) regulated by this receptor. This phenomenon was initially described in the brain [38], but has recently also been described in PAC and PDAC cells [39]. The inhibitory actions of GABA are mediated by the $G\alpha_i$ -coupled GABA-B receptor [40, 41] that inhibits the activation of adenylyl cyclase. In addition, the synthesis of GABA from glutamate is suppressed by NNK-induced promoter hypermethylation of glutamate decarboxylase 2 [42]. Collectively, the resulting severe suppression of inhibitory GABA serves to significantly potentate the cancer stimulating effects of beta-adrenergic signaling in PAC and PDC of smokers (Figure 5). This interpretation is supported by findings that tissue microarrays from PACs and PDACs showed significant suppression of GAD and/or GABA in immunostains [40, 41] and is corroborated by experiments that have shown complete reversal of nicotine-induced PAC [43] and PDAC [33] progression in xenografts of mice treated with GABA.

The mutational potency of reactive NNK metabolites is well documented and includes activating point mutations in K-ras and inactivating mutations in the tumor suppressor gene p53 [44]. K-ras and p53 are also downstream effectors in multiple signaling cascades activated by β -ARs and thus indirectly enhance the cancer stimulating effects of the β -AR cascade. Both mutations are frequently found in PAC and PDAC and in a host of other human cancers. However, transfection of normal airway epithelial cells with either mutation alone or in combination in the presence and absence of additional mutations in EGFR-specific tyrosine kinases failed to transform these cells into cancer cells with the ability to grow as nude mouse xenografts [45]. Physiological feedback mechanisms typically balance the responses to beta-adrenergic receptors, with increases in downstream substrates triggering reactive desensitization of the upstream receptor [46]. Accordingly, in a normal cell artificially transfected with an activating mutation that is located downstream of nAChR-activated beta-adrenergic signaling cascade, the upstream β -ARs and nAChRs would be expected to adjust for this by reactive changes in expression and sensitivity, thus effectively counter-balancing the mutation-driven over stimulation. However, chronic exposure to nicotine or NNK disables this unique physiological feedback system by

sensitizing the regulatory $\alpha 7$ nAChR, causing excessive synthesis and release of the neurotransmitters (norepinephrine, epinephrine) that activate the beta-adrenergic signaling cascade. It would be intriguing to repeat the above-cited study to assess if chronic exposure to nicotine or NNK would facilitate the growth of mouse xenografts from normal airway epithelial cells transfected with mutations in K-ras or p53.

Effects of psychological stress on beta-adrenergic signaling

Psychological stress can be caused by a host of factors, including anxiety, type A personality, health problems, peer pressure, excessive exercise and socio-economic factors. Social stress caused by socio-economic factors is considered the most common form of psychological stress in today's society [47]. The classic paradigm for the regulation of responses to psychological stress (Figure 4) includes activation of the hypothalamo/pituitary/adrenal axis, resulting in acetylcholine binding to nAChRs that release stress neurotransmitters from nerves of the sympathetic and from the adrenal gland and hypothalamus-initiated release of cortisol from the adrenal glands [48]. The resulting elevated systemic levels of stress neurotransmitters would then provide a supportive environment for the development of cancers under beta-adrenergic regulation, including PAC and PDAC. In support of this hypothesis, recent experiments with mouse xenografts from PAC and PDAC have revealed significant increases in tumor progression associated with the activation of multiple signaling pathways downstream of β -ARs in mice exposed to social stress [13, 14]. In addition to the expected elevated systemic levels in stress neurotransmitters and cortisol, the stress-exposed mice also showed significantly decreased systemic GABA levels. In accord with these findings, xenograft tissues demonstrated upregulated protein levels of nAChRs $\alpha 3$, $\alpha 4$, $\alpha 5$, and $\alpha 7$ and increased norepinephrine and epinephrine while GABA and GAD were suppressed [13, 14]. These findings suggest that in addition to classic systemic stress responses the neurotransmitter production of the cancer cells was modulated by stress in a fashion similar to that observed in PAC and PDAC cells in vitro exposed to chronic nicotine or NNK [16, 36], (Fig. 4). The stress-induced changes in stimulatory and inhibitory neurotransmitters were accompanied by significant increases in tumor levels of downstream effectors of beta-adrenergic cAMP-dependent signaling, including phosphorylated CREB, ERK, Src and AKT as well as increases in vascular endothelial growth factor and prostaglandin E2 (PGE2). Treatment of the mice with GABA completely reversed all observed effects of social stress on PAC and PDAC xenografts, underlining the importance of cAMP signaling for the observed stress responses. In addition, the COX-2 inhibitor celecoxib showed strong reversal of all investigated social stress-induced PDAC promoting responses [49]. These findings indicate that the beta-adrenergic activation of the AA cascade is an important mediator of stress induced PDAC progression.

Additional evidence for the importance of beta-adrenergic signaling for the progression of PDAC comes from recent reports that norepinephrine stimulates the proliferation, migration and invasive potential of pancreatic cancer cells in vitro via activation of the P38/Map Protein Kinase pathway and that the broad-spectrum beta-blocker propranolol reversed these effects [50]. In accord with earlier findings that pancreatic cancer cell lines express predominantly the $\beta 2$ -AR [12], treatment of pancreatic cancer cell lines with the selective $\beta 2$ -AR antagonist ICI118,551 significantly inhibited the Ras/AKT/NFkB pathway, resulting in G1/S phase arrest and induction of apoptosis [50]. Selective pharmacological blockage of the $\beta 2$ -AR also significantly enhanced the anti-proliferative and pro-apoptotic actions of the cancer therapeutic gemcitabine in pancreatic cancer cells in vitro [51], suggesting that adjuvant therapy with a selective $\beta 2$ -blocker may increase clinical outcomes of gemcitabine therapy in PDAC patients.

Collectively, the experimental findings suggest strong cancer promoting effects of psychological stress via increased beta-adrenergic signaling in PAC and PDAC. This interpretation is supported by reports that PAC [52, 53] and PDAC [53] patients express particularly high levels of psychological stress among investigated cancer patients at the time of diagnosis and during years preceding diagnosis.

Implications for cancer intervention

Beta-blockers have been safely used for decades as long-term therapeutics for hypertension and heart disease. The idea to utilize this class of pharmaceuticals for the prevention and adjuvant to therapy of NSCLC and PDAC is therefore intriguing. However, it is important to remember, that the function of this class of drugs in cardiovascular patients is to return to physiological levels the excessive beta-adrenergic signaling that triggers this disease complex. Beta-blockers are only prescribed when high blood pressure and/or irregular heart function suggest beta-adrenergic hyper activity in the cardiovascular system and the proper adjustment of cardio-vascular function is regularly monitored during therapy. Clinical investigations that have reported beneficial effects of beta-blockers on breast cancer were derived from cancer patients treated with beta-blockers due to incidental cardiovascular disease. It would be ill advised to arbitrarily use beta-blockers for the prevention of cardiovascular disease in individuals without clinical symptoms of beta-adrenergic hyper function, as a drop in blood pressure and heart rate below physiological levels can be potentially life threatening. In analogy to this example, beta-blockers cannot realistically be proposed without appropriate diagnostic monitoring for the prevention of NSCLC or PDAC in individuals at risk for the development of these cancers or as adjuvant therapy to improve clinical outcomes.

As summarized in this review, currently available experimental evidence suggests a key role of cAMP-dependent signaling in the cancer promoting effects of beta-adrenergic signaling on NSCLC and PDAC. In addition, it has been shown that increases in tumor stress neurotransmitters and cAMP correlate with increases of these agents in serum and in the cellular fraction of blood in experimental animals [13, 14]. It therefore stands to reason that a diagnosis of above normal levels of stress neurotransmitters and cAMP in blood samples could be used to identify individuals that would benefit from cancer intervention with beta-blockers. Regular monitoring of these blood values would then need to ensure that levels do not drop below physiological levels. This is important not only because of potential undesirable drops in blood pressure and heart function but also because there do exist several cancers that are inhibited by beta-adrenergic signaling and cAMP [39]. It has thus been shown that the growth of small cell lung cancer cells in vitro is inhibited by the beta-adrenergic agonist isoproterenol [54] and by phosphodiesterase inhibitors [55] which block the enzymatic breakdown of cAMP. There is also evidence that Hodgkin's lymphoma may be inhibited by cAMP signaling [56]. In addition, the naturally occurring phosphodiesterase inhibitor theophylline as well as green tea that contained theophylline and caffeine prevented the development of SCLC in an animal model [57]. Similarly, beta-carotene and several retinoids inhibited the proliferation of cell lines from squamous cell carcinoma of the lung via the activation of non-genomic cAMP signaling [58], suggesting that increased cAMP downstream of β -ARs may have similar cancer inhibiting effects on this histological type of lung cancer. Moreover, a very recent study has reported significant cancer preventive effects of the phosphodiesterase inhibitor caffeine on basal cell carcinoma of the skin [59]. The goal of any attempts to prevent susceptible cancers by treatment with beta-blockers should therefore not be to block beta-adrenergic signaling but rather to return it to physiological levels, because otherwise the beta-blocker could potentially increase the risk for the development of cancers inhibited by cAMP and/or beta-adrenergic signaling.

The surgical resection of cancer imposes considerable psychological stress on the patient, a fact that can potentially be exploited to reduce cancer relapse and metastasis by short-term peri-operative treatment with beta-blockers in PAC and PDAC patients undergoing these procedures. An experimental study in rats has thus shown that propranolol significantly reduced metastasis of intravenously injected mammary carcinoma cell lines that settled in the lungs and were then surgically resected [60]. A similar strategy could be employed to reduce the psychological stress associated with chemo- and radiation therapy of PAC and PDAC.

Chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema, are documented risk factors for lung cancer [61]. The prevailing therapeutics for this disease complex are epinephrine and structural analogues as well as phosphodiesterase inhibitors that increase cAMP by blocking its enzymatic breakdown. While the long-term use of each of these agents may potentially increase the risk for development of PAC and PDAC, broad-spectrum beta-blockers could not possibly be used in patients with COPD because of their broncho-constricting effects. Because broncho-constriction is regulated by β_2 -adrenergic receptors, cardiovascular patients with incidental COPD are usually treated with selective β_1 -AR antagonists. A similar strategy could potentially be followed to improve clinical outcomes in COPD patients with PAC because preclinical studies have identified β_1 -AR signaling as the major driving force in the development and progression of this cancer [19, 22, 23].

An inherent problem with the long-term use of beta-blockers is their ability to cause not only reactive sensitization to agonist of the very receptor that they block but also compensatory upregulation of unblocked β -ARs. Long-term treatment with a selective β_1 -blocker can thus cause the reactive sensitization of β_1 -ARs and compensatory upregulation of β_2 -ARs [62–64]. In light of the fact that cancer of the pancreas, colon, prostate and breast are under β_2 -AR control, this could potentially increase the risk for the development of cancer at these organ sites.

The preclinical data summarized in this review suggest that the increased systemic and tissue levels of norepinephrine and epinephrine caused by tobacco constituents and psychological stress via modulation in nAChR expression and function create an environment that selectively supports the development and progression of cancers regulated by beta-adrenergic signaling. Theoretically, nAChR antagonists could therefore also be used for the prevention and adjuvant therapy of these cancers. However, contrary to widely held belief that the α_7 nAChR is the cancer stimulating member of the nAChR family [65], recent in vitro data clearly show that this receptor can work in concert with nAChRs expressing the α_3 and α_5 subunits [17]. While the cooperation of these receptors has been shown in PDAC and pancreatic duct epithelial cells, polymorphisms in the genes encoding the α_3 and α_5 nAChRs have been associated with increased lung cancer risk, particularly PAC [66], suggesting important roles for these two nAChRs in addition to α_7 in this cancer as well. It therefore appears futile to pursue the development of α_7 nAChR antagonists for the prevention and treatment of NSCLC or PDAC.

A promising alternative to the blocking of beta-adrenergic or nicotinic receptors is offered by the strategy to raise the systemic levels of the amino acid neurotransmitter GABA instead [13, 14, 39–41]. GABA is the major inhibitory neurotransmitter in the nervous system where it counterbalances the G_{α_s} -mediated effects of excitatory neurotransmitters, including norepinephrine/epinephrine, by activating the $G_{\alpha i}$ -coupled GABA-B receptor [38]. In vitro studies with cell lines from cancer of the colon and mammary gland first reported reversal of beta-adrenergic norepinephrine-induced migration of cancer cells by GABA [67, 68]. More recent studies with PAC and PDAC cell lines in vitro and in mouse xenografts have

established that GABA treatment also reversed the cancer-stimulating effects of nicotine or psychological stress via $G\alpha_i$ -mediated inhibition of adenylyl cyclase activation [13, 14, 33, 40, 41, 43]. GABA has been safely used as a nutritional supplement for many years and could thus be easily used for cancer intervention. However, even when using this seemingly low risk approach, cAMP levels in blood samples would need to be carefully monitored before and during GABA treatment. It has been shown that GABA loses its inhibitory function and becomes an excitatory neurotransmitter in individuals who overexpress the π -subunit of the GABA-A receptor [69]. In individuals with this abnormality, GABA treatment would therefore fail to reduce the adenylyl cyclase-mediated formation of cAMP because it would preferentially bind to the upregulated stimulatory GABA-A receptor, an ion channel that could even potentially promote certain cancers. This interpretation is supported by findings that GABA stimulates the growth of PDAC cell lines that overexpress the GABA-A-R subunit π in vitro and in mouse xenografts [69].

Conclusions

Emerging preclinical and clinical evidence suggests a central role of beta-adrenergic signaling in the regulation of numerous cancers [70]. The regulatory role of nAChRs upstream of the beta-adrenergic signaling cascade highlighted in this review has been generally overlooked but is of key importance in both, smoking and stress-induced stimulation of beta-adrenergic signaling. In addition to NSCLC and PDAC addressed in this review, cancer stimulating effects of beta-adrenergic agonists and/or inhibitory actions of beta-blockers have been reported for cancer of the colon [34, 71], stomach [35], prostate [72, 73], breast [68, 72, 74, 75], and ovary [76] and inhibiting effects of GABA have been shown for colon cancer [67] and breast cancer [68]. A recent publication reported suppression of the tumor suppressor gene p53 in mice and several human cell lines in response to β_2 -AR activation by chronic treatment with epinephrine [77], thus further underlining the key role of beta-adrenergic signaling in human health and disease. The in vitro and in vivo experiments on PAC and PDAC stimulation by nicotine, NNK and psychological stress conducted in my laboratory and summarized in this review were conducted by my research team under identical conditions and with identical cell lines. Findings from these studies thus allow for a comparison of effects induced by these tobacco-specific agents and psychological stress. Based on these data, it cannot be overemphasized that the effects of chronic exposure to psychological stress on nAChR-regulated cancer-promoting beta-adrenergic signaling in vitro and in xenografts were virtually identical to those caused by chronic exposure to nicotine or NNK at concentrations within the range of their systemic levels in smokers. Accordingly, the world-wide economic problems and associated chronic socio-economic stress we have experienced particularly during the last 10 years may well account for the disturbing trends of rises in incidence of PAC and PDAC despite of significant decreases in smokers.

A recent study has shown stunning decreases in systemic levels of stress neurotransmitters in a mouse model called "the calm mouse, an animal model of stress reduction" by the investigators, who provided the mice with larger cages, nesting materials and toys [78]. These animals which should be more appropriately called "happy mice" drive home an important message that has been too often overlooked in classic medicine: the mind is an extremely powerful force that can foster the development of numerous diseases, including cancer, and can potentially be utilized for cancer intervention. Whether or not cancer-stimulating cAMP signaling is reduced by beta-blockers that prevent the binding of stress neurotransmitters to β -ARs, GABA that inhibits activation of adenylyl cyclase, or by a significant decrease in systemic levels of stress neurotransmitters due to positive psychological influences such as "happiness" is basically inconsequential. Each of these methods effectively returns hyperactive cAMP signaling to physiological levels (Figure 5),

thereby effectively interrupting the vicious cycle of cancer-stimulating beta-adrenergic signaling.

Acknowledgments

Funding: Financially supported by PHS grants RO1CA088809, RO1CA096128, RO1CA042829, RO1CA130888, RC1CA144640 with the National Cancer Institute.

References

1. Bunn PA Jr, Thatcher N. Systemic treatment for advanced (stage IIIB/IV) non-small cell lung cancer: more treatment options; more things to consider Conclusion. *Oncologist*. 2008; (13 Suppl 1):37–46. [PubMed: 18263773]
2. Devesa SS, Bray F, Vizcaino AP, M D. Parkin, International lung cancer trends by histologic type: male:female differences diminishing and adenocarcinoma rates rising. *Int J Cancer*. 2005; 117:294–299. [PubMed: 15900604]
3. Toyoda Y, Nakayama T, Ioka A, Tsukuma H. Trends in lung cancer incidence by histological type in Osaka, Japan. *Jpn J Clin Oncol*. 2008; 38:534–539. [PubMed: 18689853]
4. Haiman CA, Stram DO, Wilkens LR, Pike MC, Kolonel LN, Henderson BE, Le Marchand L. Ethnic and racial differences in the smoking-related risk of lung cancer. *N Engl J Med*. 2006; 354:333–342. [PubMed: 16436765]
5. Lowenfels AB, Maisonneuve P. Risk factors for pancreatic cancer. *J Cell Biochem*. 2005; 95:649–656. [PubMed: 15849724]
6. Almhanna K, Philip PA. Defining new paradigms for the treatment of pancreatic cancer. *Curr Treat Options Oncol*. 2011; 12:111–125. [PubMed: 21461670]
7. Arfe A, Malvezzi M, Bertuccio P, Decarli A, La Vecchia C, Negri E. Cancer mortality trend analysis in Italy, 1970–2007. *Eur J Cancer Prev*. 2011; 20:364–374. [PubMed: 21499107]
8. Jooste V, Remontet L, Colonna M, Belot A, Launoy G, Binder F, Faivre J, Bouvier AM. Trends in the incidence of digestive cancers in France between 1980 and 2005 and projections for the year 2010. *Eur J Cancer Prev*. 2011; 20:375–380. [PubMed: 21478752]
9. Robinson JD, Cinciripini PM. The effects of stress and smoking on catecholaminergic and cardiovascular response. *Behav Med*. 2006; 32:13–18. [PubMed: 16637258]
10. Schuller HM, Cole B. Regulation of cell proliferation by beta-adrenergic receptors in a human lung adenocarcinoma cell line. *Carcinogenesis*. 1989; 10:1753–1755. [PubMed: 2569945]
11. Park PG, Merryman J, Orloff M, Schuller HM. Beta-adrenergic mitogenic signal transduction in peripheral lung adenocarcinoma: implications for individuals with preexisting chronic lung disease. *Cancer Res*. 1995; 55:3504–3508. [PubMed: 7627955]
12. Weddle DL, Tithoff P, Williams M, Schuller HM. Beta-adrenergic growth regulation of human cancer cell lines derived from pancreatic ductal carcinomas. *Carcinogenesis*. 2001; 22:473–479. [PubMed: 11238189]
13. Al-Wadei HAN, Plummer HK 3rd, Ullah MF, Unger B, Brody J, Schuller HM. Social stress promotes and γ -aminobutyric acid inhibits tumor growth in mouse models of non small cell lung cancer. *Cancer Prevent Res*. 2012; 5:189–196.
14. Schuller HM, Al-Wadei HA, Ullah MF, Plummer HK 3rd. Regulation of pancreatic cancer by neuropsychological stress responses: a novel target for intervention. *Carcinogenesis*. 2012; 33:191–196. [PubMed: 22072614]
15. Di Angelantonio S, Matteoni C, Fabbretti E, Nistri A. Molecular biology and electrophysiology of neuronal nicotinic receptors of rat chromaffin cells. *Eur J Neurosci*. 2003; 17:2313–2322. [PubMed: 12814364]
16. Al-Wadei HA, Al-Wadei MH, Masi T, Schuller HM. Chronic exposure to estrogen and the tobacco carcinogen NNK cooperatively modulates nicotinic receptors in small airway epithelial cells. *Lung Cancer*. 2010; 69:33–39. [PubMed: 19896235]
17. Al-Wadei MH, Al-Wadei HA, Schuller HM. Pancreatic Cancer Cells and Normal Pancreatic Duct Epithelial Cells Express an Autocrine Catecholamine Loop that Is Activated by Nicotinic

- Acetylcholine Receptors alpha3, alpha5, and alpha7. *Mol Cancer Res.* 2012; 10:239–249. [PubMed: 22188668]
18. Prokopczyk B, Hoffmann D, Bologna M, Cunningham AJ, Trushin N, Akerkar S, Boyiri T, Amin S, Desai D, Colosimo S, Pittman B, Leder G, Ramadani M, Henne-Bruns D, Beger HG, El-Bayoumy K. Identification of tobacco-derived compounds in human pancreatic juice. *Chem Res Toxicol.* 2002; 15:677–685. [PubMed: 12018989]
 19. Schuller HM, Tithof PK, Williams M, Plummer H 3rd. The tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is a beta-adrenergic agonist and stimulates DNA synthesis in lung adenocarcinoma via beta-adrenergic receptor-mediated release of arachidonic acid. *Cancer Res.* 1999; 59:4510–4515. [PubMed: 10493497]
 20. Schuller HM. Mechanisms of smoking-related lung and pancreatic adenocarcinoma development. *Nat Rev Cancer.* 2002; 2:455–463. [PubMed: 12189387]
 21. Schuller HM, Plummer HK 3rd, Bochsler PN, Dudric P, Bell JL, Harris RE. Co-expression of beta-adrenergic receptors and cyclooxygenase-2 in pulmonary adenocarcinoma. *Int J Oncol.* 2001; 19:445–449. [PubMed: 11494020]
 22. Laag E, Majidi M, Cekanova M, Masi T, Takahashi T, Schuller HM. NNK activates ERK1/2 and CREB/ATF-1 via beta-1-AR and EGFR signaling in human lung adenocarcinoma and small airway epithelial cells. *Int J Cancer.* 2006; 119:1547–1552. [PubMed: 16671086]
 23. Majidi M, Al-Wadei HA, Takahashi T, Schuller HM. Nongenomic beta estrogen receptors enhance beta1 adrenergic signaling induced by the nicotine-derived carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in human small airway epithelial cells. *Cancer Res.* 2007; 67:6863–6871. [PubMed: 17638897]
 24. Askari MD, Tsao MS, Schuller HM. The tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone stimulates proliferation of immortalized human pancreatic duct epithelia through beta-adrenergic transactivation of EGF receptors. *J Cancer Res Clin Oncol.* 2005; 131:639–648. [PubMed: 16091975]
 25. Pavoine C, Magne S, Sauvadet A, Pecker F. Evidence for a beta2-adrenergic/arachidonic acid pathway in ventricular cardiomyocytes Regulation by the beta1-adrenergic/camp pathway. *J Biol Chem.* 1999; 274:628–637. [PubMed: 9872996]
 26. LaBelle EF, Polyak E. Norepinephrine stimulates arachidonic acid release from vascular smooth muscle via activation of cPLA2. *Am J Physiol.* 1998; 274:C1129–C1137. [PubMed: 9575810]
 27. Schuller HM, Porter B, Riechert A. Beta-adrenergic modulation of NNK-induced lung carcinogenesis in hamsters. *J Cancer Res Clin Oncol.* 2000; 126:624–630. [PubMed: 11079726]
 28. Al-Wadei HA, Al-Wadei MH, Schuller HM. Prevention of pancreatic cancer by the beta-blocker propranolol. *Anticancer Drugs.* 2009; 20:477–482. [PubMed: 19387337]
 29. Schuller HM, Zhang L, Weddle DL, Castonguay A, Walker K, Miller MS. The cyclooxygenase inhibitor ibuprofen and the FLAP inhibitor MK886 inhibit pancreatic carcinogenesis induced in hamsters by transplacental exposure to ethanol and the tobacco carcinogen NNK. *J Cancer Res Clin Oncol.* 2002; 128:525–532. [PubMed: 12384795]
 30. Schuller HM, Orloff M. Tobacco-specific carcinogenic nitrosamines Ligands for nicotinic acetylcholine receptors in human lung cancer cells. *Biochem Pharmacol.* 1998; 55:1377–1384. [PubMed: 10076528]
 31. Arredondo J, Chernyavsky AI, Grando SA. The nicotinic receptor antagonists abolish pathobiologic effects of tobacco-derived nitrosamines on BEP2D cells. *J Cancer Res Clin Oncol.* 2006; 132:653–663. [PubMed: 16835749]
 32. Arredondo J, Chernyavsky AI, Grando SA. Nicotinic receptors mediate tumorigenic action of tobacco-derived nitrosamines on immortalized oral epithelial cells. *Cancer Biol Ther.* 2006; 5:511–517. [PubMed: 16582591]
 33. Al-Wadei HA, Plummer HK 3rd, Schuller HM. Nicotine stimulates pancreatic cancer xenografts by systemic increase in stress neurotransmitters and suppression of the inhibitory neurotransmitter gamma-aminobutyric acid. *Carcinogenesis.* 2009; 30:506–511. [PubMed: 19131543]
 34. Wong HP, Yu L, Lam EK, Tai EK, Wu WK, Cho CH. Nicotine promotes cell proliferation via alpha7-nicotinic acetylcholine receptor and catecholamine-synthesizing enzymes-mediated

- pathway in human colon adenocarcinoma HT-29 cells. *Toxicol Appl Pharmacol.* 2007; 221:261–267. [PubMed: 17498763]
35. Shin VY, Wu WK, Chu KM, Koo MW, Wong HP, Lam EK, Tai EK, Cho CH. Functional role of beta-adrenergic receptors in the mitogenic action of nicotine on gastric cancer cells. *Toxicol Sci.* 2007; 96:21–29. [PubMed: 17003101]
 36. Al-Wadei MH, Al-Wadei HA, Schuller HM. Effects of chronic nicotine on the autocrine regulation of pancreatic cancer cells and pancreatic duct epithelial cells by stimulatory and inhibitory neurotransmitters. *Carcinogenesis.*
 37. Lefkowitz RJ. The superfamily of heptahelical receptors. *Nat Cell Biol.* 2000; 2:E133–E136. [PubMed: 10878827]
 38. Markou A. Review. Neurobiology of nicotine dependence. *Philos Trans R Soc Lond B Biol Sci.* 2008; 363:3159–3168. [PubMed: 18640919]
 39. Schuller HM. Is cancer triggered by altered signalling of nicotinic acetylcholine receptors? *Nat Rev Cancer.* 2009; 9:195–205. [PubMed: 19194381]
 40. Schuller HM, Al-Wadei HA, Majidi M. Gamma-aminobutyric acid, a potential tumor suppressor for small airway-derived lung adenocarcinoma. *Carcinogenesis.* 2008; 29:1979–1985. [PubMed: 18310090]
 41. Schuller HM, Al-Wadei HA, Majidi M. GABA B receptor is a novel drug target for pancreatic cancer. *Cancer.* 2008; 112:767–778. [PubMed: 18098271]
 42. Bernert, H.; Plummer, HK., III; Schuller, HM. Chronic nicotine and NNK exposure causes promoter hypermethylation of GAD2 in human pancreatic duct epithelial cells; Orlando, FL, USA. AACR 102nd Annual Meeting; 2011. p. 3816
 43. Al-Wadei HA, Al-Wadei MH, Ullah MF, Schuller HM. Gamma-amino butyric acid inhibits the nicotine-imposed stimulatory challenge in xenograft models of non-small cell lung carcinoma. *Curr Cancer Drug Targets.* 2012; 12:97–106. [PubMed: 22165966]
 44. Hecht SS. DNA adduct formation from tobacco-specific N-nitrosamines. *Mutat Res.* 1999; 424:127–142. [PubMed: 10064856]
 45. Sato M, Vaughan MB, Girard L, Peyton M, Lee W, Shames DS, Ramirez RD, Sunaga N, Gazdar AF, Shay JW, Minna JD. Multiple oncogenic changes (K-RAS(V12), p53 knockdown, mutant EGFRs, p16 bypass, telomerase) are not sufficient to confer a full malignant phenotype on human bronchial epithelial cells. *Cancer Res.* 2006; 66:2116–2128. [PubMed: 16489012]
 46. Liu R, Ramani B, Soto D, De Arcangelis V, Xiang Y. Agonist dose-dependent phosphorylation by protein kinase A and G protein-coupled receptor kinase regulates beta2 adrenoceptor coupling to G(i) proteins in cardiomyocytes. *J Biol Chem.* 2009; 284:32279–32287. [PubMed: 19706594]
 47. Sterlemann V, Ganea K, Liebl C, Harbich D, Alam S, Holsboer F, Muller MB, Schmidt MV. Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: implications for stress-related disorders. *Horm Behav.* 2008; 53:386–394. [PubMed: 18096163]
 48. McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.* 2000; 886:172–189. [PubMed: 11119695]
 49. Al-Wadei HA, Al-wadei MH, Ullah MF, Schuller HM. Celecoxib and GABA cooperatively prevent the progression of pancreatic cancer om vitro and in xenograft models of stress-free and stress-exposed mice. *Plos One.* 2012 in press.
 50. Huang XY, Wang HC, Yuan Z, Huang J, Zheng Q. Norepinephrine stimulates pancreatic cancer cell proliferation, migration and invasion via beta-adrenergic receptor-dependent activation of P38/MAPK pathway. *Hepatogastroenterology.* 2012; 59:889–893. [PubMed: 22020907]
 51. Shan T, Ma Q, Zhang D, Guo K, Liu H, Wang F, Wu E. beta2-adrenoceptor blocker synergizes with gemcitabine to inhibit the proliferation of pancreatic cancer cells via apoptosis induction. *Eur J Pharmacol.* 2011; 665:1–7. [PubMed: 21570961]
 52. Hamer M, Chida Y, Molloy GJ. Psychological distress and cancer mortality. *J Psychosom Res.* 2009; 66:255–258. [PubMed: 19232239]
 53. Zabora J, BrintzenhofeSzoc K, Curbow B, Hooker C, Piantadosi S. The prevalence of psychological distress by cancer site. *Psychooncology.* 2001; 10:19–28. [PubMed: 11180574]

54. Plummer HK 3rd, Dhar MS, Cekanova M, Schuller HM. Expression of G-protein inwardly rectifying potassium channels (GIRKs) in lung cancer cell lines. *BMC Cancer*. 2005; 5:104. [PubMed: 16109170]
55. Shafer SH, Phelps SH, Williams CL. Reduced DNA synthesis and cell viability in small cell lung carcinoma by treatment with cyclic AMP phosphodiesterase inhibitors. *Biochem Pharmacol*. 1998; 56:1229–1236. [PubMed: 9802335]
56. Aleksijevic A, Lugnier C, Giron C, Mayer S, Stoclet JC, Lang JM. Cyclic AMP and cyclic GMP phosphodiesterase activities in Hodgkin's disease lymphocytes. *Int J Immunopharmacol*. 1987; 9:525–531. [PubMed: 3040609]
57. Schuller HM, Porter B, Riechert A, Walker K, Schmoyer R. Neuroendocrine lung carcinogenesis in hamsters is inhibited by green tea or theophylline while the development of adenocarcinomas is promoted: implications for chemoprevention in smokers. *Lung Cancer*. 2004; 45:11–18. [PubMed: 15196729]
58. Al-Wadei HA, Schuller HM. Non-genomic inhibitory signaling of beta-carotene in squamous cell carcinoma of the lungs. *Int J Oncol*. 2009; 34:1093–1098. [PubMed: 19287967]
59. Song F, Qureshi AA, Han J. Increased caffeine intake is associated with reduced risk of Basal cell carcinoma of the skin. *Cancer Res*. 2012; 72:3282–3289. [PubMed: 22752299]
60. Goldfarb Y, Sorski L, Benish M, Levi B, Melamed R, Ben-Eliyahu S. Improving postoperative immune status and resistance to cancer metastasis: a combined perioperative approach of immunostimulation and prevention of excessive surgical stress responses. *Ann Surg*. 2011; 253:798–810. [PubMed: 21475023]
61. Celli BR. Chronic obstructive pulmonary disease and lung cancer: common pathogenesis, shared clinical challenges. *Proc Am Thorac Soc*. 2012; 9:74–79. [PubMed: 22550249]
62. Maki T, Kontula K, Harkonen M. The beta-adrenergic system in man: physiological pathophysiological response Regulation of receptor density and functioning. *Scand J Clin Lab Invest Suppl*. 1990; 201:25–43. [PubMed: 1978755]
63. Rosendorff C. Beta-blocking agents with vasodilator activity. *J Hypertens Suppl*. 1993; 11:S37–S40. [PubMed: 8104240]
64. Lund-Johansen P, Omvik P. Acute and chronic hemodynamic effects of drugs with different actions on adrenergic receptors: a comparison between alpha blockers and different types of beta blockers with and without vasodilating effect. *Cardiovasc Drugs Ther*. 1991; 5:605–615. [PubMed: 1678963]
65. Cesario A, Russo P, Nastrucci C, Granone P. Is alpha7-nAChR a possible target for lung cancer and malignant pleural mesothelioma treatment? *Curr Drug Targets*. 2012; 13:688–694. [PubMed: 22300036]
66. Amos CI, Gorlov IP, Dong Q, Wu X, Zhang H, Lu EY, Scheet P, Greisinger AJ, Mills GB, Spitz MR. Nicotinic acetylcholine receptor region on chromosome 15q25 and lung cancer risk among African Americans: a case-control study. *J Natl Cancer Inst*. 2010; 102:1199–1205. [PubMed: 20554942]
67. Joseph J, Niggemann B, Zaenker KS, Entschladen F. The neurotransmitter gamma-aminobutyric acid is an inhibitory regulator for the migration of SW 480 colon carcinoma cells. *Cancer Res*. 2002; 62:6467–6469. [PubMed: 12438237]
68. Drell, TLt; Joseph, J.; Lang, K.; Niggemann, B.; Zaenker, KS.; Entschladen, F. Effects of neurotransmitters on the chemokinesis and chemotaxis of MDA-MB-468 human breast carcinoma cells. *Breast Cancer Res Treat*. 2003; 80:63–70. [PubMed: 12889599]
69. Takehara A, Hosokawa M, Eguchi H, Ohigashi H, Ishikawa O, Nakamura Y, Nakagawa H. Gamma-aminobutyric acid (GABA) stimulates pancreatic cancer growth through overexpressing GABAA receptor pi subunit. *Cancer Res*. 2007; 67:9704–9712. [PubMed: 17942900]
70. Antoni MH, Lutgendorf SK, Cole SW, Dhabhar FS, Sephton SE, McDonald PG, Stefanek M, Sood AK. The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. *Nat Rev Cancer*. 2006; 6:240–248. [PubMed: 16498446]
71. Masur K, Niggemann B, Zanker KS, Entschladen F. Norepinephrine-induced migration of SW 480 colon carcinoma cells is inhibited by beta-blockers. *Cancer Res*. 2001; 61:2866–2869. [PubMed: 11306460]

72. Lang K, Drell TLt, Lindecke A, Niggemann B, Kaltschmidt C, Zaenker KS, Entschladen F. Induction of a metastatogenic tumor cell type by neurotransmitters and its pharmacological inhibition by established drugs. *Int J Cancer*. 2004; 112:231–238. [PubMed: 15352035]
73. Palm D, Lang K, Niggemann B, Drell TLt, Masur K, Zaenker KS, Entschladen F. The norepinephrine-driven metastasis development of PC-3 human prostate cancer cells in BALB/c nude mice is inhibited by beta-blockers. *Int J Cancer*. 2006; 118:2744–2749. [PubMed: 16381019]
74. Powe DG, Voss MJ, Zanker KS, Habashy HO, Green AR, Ellis IO, Entschladen F. Beta-blocker drug therapy reduces secondary cancer formation in breast cancer and improves cancer specific survival. *Oncotarget*. 2010; 1:628–638. [PubMed: 21317458]
75. Melhem-Bertrandt A, Chavez-Macgregor M, Lei X, Brown EN, Lee RT, Meric-Bernstam F, Sood AK, Conzen SD, Hortobagyi GN, Gonzalez-Angulo AM. Beta-blocker use is associated with improved relapse-free survival in patients with triple-negative breast cancer. *J Clin Oncol*. 2011; 29:2645–2652. [PubMed: 21632501]
76. Sood AK, Bhatti R, Kamat AA, Landen CN, Han L, Thaker PH, Li Y, Gershenson DM, Lutgendorf S, Cole SW. Stress hormone-mediated invasion of ovarian cancer cells. *Clin Cancer Res*. 2006; 12:369–375. [PubMed: 16428474]
77. Hara MR, Kovacs JJ, Whalen EJ, Rajagopal S, Strachan RT, Grant W, Towers AJ, Williams B, Lam CM, Xiao K, Shenoy SK, Gregory SG, Ahn S, Duckett DR, Lefkowitz RJ. A stress response pathway regulates DNA damage through beta2-adrenoreceptors and beta-arrestin-1. *Nature*. 477:349–353. [PubMed: 21857681]
78. Gurfein BT, Stamm AW, Bacchetti P, Dallman MF, Nadkarni NA, Milush JM, Touma C, Palme R, Di Borgo CP, Fromentin G, Lown-Hecht R, Konsman JP, Acree M, Premenko-Lanier M, Darcel N, Hecht FM, Nixon DF. The calm mouse: an animal model of stress reduction. *Mol Med*. 2012; 18:606–617. [PubMed: 22398685]

Norepinephrine **Epinephrine** **Isoproterenol** **NNK**

Figure 1.

The stress neurotransmitters norepinephrine and epinephrine are the physiological agonists for β -ARs. Both agents bind to α and β -ARs, with norepinephrine having higher affinity for the β_1 -AR and epinephrine for the β_2 -AR. They each contain a catechol ring and an aliphatic side chain with a nitrogen atom. When the steric bulk of the side chain is increased (as in isoproterenol), the ligand binds selectively to β -ARs. The pyridine ring of the nicotine-derived carcinogenic nitrosamine NNK with its aliphatic side chain containing the N-Nitroso group (N-N=O) resembles isoproterenol, rendering the affinity of NNK 2,200 times greater than epinephrine to the β_2 -AR and 600 times greater than norepinephrine to the β_1 -AR [19, 20].

Figure 2. Photomicrographs exemplifying immunostains for the expression of the $\beta 1$ - and $\beta 2$ -AR in lung adenocarcinoma tissues on tissue microarray LC1002 (Biomax Inc., Rockville, MD, USA), containing 48 cases of lung adenocarcinoma. Each of these cases showed prominent expression of the $\beta 1$ -AR whereas only faint positive immunoreactivity was detected for the $\beta 2$ -AR. Immunostains were conducted using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) with exposure to primary antibodies (Santa Cruz Biotechnologies, Santa Cruz, CA, USA) at a dilution of 1: 25 overnight in a moist chamber at 4°C.

Figure 3.

Working model of the direct effects of NNK on cancer promoting beta-adrenergic signaling in pulmonary adenocarcinoma and pancreatic ductal adenocarcinoma. Binding of NNK to the β_1 - and β_2 -ARs activates the stimulatory G-protein $G\alpha_s$, leading to the activation of adenylyl cyclase, the single rate-limiting step for the formation of cAMP from ATP. In turn, cAMP and its effector activated PKA activate multiple intracellular signaling cascades that have been shown to increase cell proliferation, migration and angiogenesis while inhibiting apoptosis in PAC and PDAC. This entire cancer-promoting cascade is successfully interrupted by beta-blockers that prevent binding of NNK to β -ARs or by GABA that inhibits the activation of adenylyl cyclase.

Figure 4.

The tobacco constituents nicotine and NNK as well as psychological stress cause the release of the stress neurotransmitters norepinephrine and epinephrine from sympathetic nerves and from the adrenal medulla into the systemic circulation [9, 48]. In addition, they stimulate the synthesis and release of both stress neurotransmitters from small airway epithelial cells and from pancreatic duct epithelium, in which these agents act as autocrine growth factors [16, 17].

Figure 5.

Working model of the indirect effects of chronic psychological stress, chronic NNK and nicotine on cancer promoting beta-adrenergic signaling via nicotinic receptor-mediated synthesis and release of stress neurotransmitters and suppression of GABA. Responses to psychological stress are initiated by binding of the neurotransmitter acetylcholine to nAChRs whereas nicotine and NNK in smokers replace acetylcholine as nAChR ligands due to their higher affinity to these receptors. Upon chronic exposure to either of these agonists, protein expression of nAChRs $\alpha 7$, $\alpha 5$, and $\alpha 3$ is increased without concomitant desensitization, causing increased synthesis and release of norepinephrine and epinephrine that drive cancer promoting beta-adrenergic signaling. By contrast, the same nAChR

agonists desensitize the $\alpha_4\beta_2$ nAChR and despite of its reactive increase in receptor protein synthesis and release of its effector GABA is suppressed. Promoter hypermethylation of the enzyme glutamate decarboxylase (GAD), which catalyzes the formation of GABA from glutamate, additionally suppresses GABA synthesis. The resulting hyperactive cAMP-dependent signaling downstream of β -ARs can be reduced to physiological levels by beta-blockers, GABA supplementation of positive psychological influences, such as happiness.