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Advances in Brief

Norepinephrine-induced Migration of SW 480 Colon Carcinoma Cells Is Inhibited by β-Blockers[1]

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Abstract

β-Adrenoceptors are highly expressed on SW 480 colon carcinoma cells as was assessed by flow cytometry. We investigated the influence of norepinephrine on the migration of these cells using time-lapse videomicroscopy. Norepinephrine-treatment increased the locomotor activity within the population from 25% spontaneously locomoting cells to 65% locomoting cells. The β1/2-blocker propranolol but not the β1-blocker atenolol inhibited this increase. The intracellular signaling solely of norepinephrine-induced locomotion involved protein tyrosine kinase activity, whereas both spontaneous and norepinephrine-induced migration were reduced by inhibiting phospholipase Cγ and protein kinase Cα activity. In summary, norepinephrine-induced locomotion of SW 480 cells is β2-adrenoceptor mediated and distinct from spontaneous locomotion concerning the PTK involvement.

Introduction

Chemokines are well described substances that initiate the locomotion of leukocytes (1, 2). These peptides act on the cells via seven helices or serpentine receptors (3). Besides chemokines, other ligands of serpentine receptors have been described that regulate the migration of leukocytes, *e.g.*, formylated peptides (4). Recently, catecholamines have been described as being involved in the regulation of dendritic cell locomotion (5) and neutrophil granulocyte locomotion (6). Catecholamines act on the cells via adrenoceptors, which are members of the serpentine family. Serpentine receptors are on the intracellular side coupled to G protein-mediated signaling pathways (3) and to PTKs[3] via β-arrestin (7). Catecholamines have been reported to enhance the carcinogenic effect of tobacco-specific nitrosamine as was measured by the development of pulmonary adenocarcinoma in hamsters (8). Therefore, catecholamines have effects on both tumor cells and leukocytes. In our study we investigated whether catecholamines are involved in the regulation of tumor cell migration.

Materials and Methods

Cell Culture. The colon carcinoma cell line SW 480 (American Type Culture Collection, Rockville, MD) was maintained in antibiotic-free Leibovitz L-15 medium (PAA Laboratories GmbH, Linz, Austria), supplemented with 10% FCS (PAA Laboratories GmbH) in a humidified atmosphere without CO_2 addition.

Cell Migration Assay. Cultured cells were harvested using a trypsin/EDTA solution. Six $\times 10^4$ cells were mixed with 150 μl of buffered liquid collagen [1.63 mg/ml collagen type I (pH 7.4); Collagen Corporation, Fremont, CA) containing MEM (Sigma, Deisenhofen, Germany) as well as norepinephrine and the investigated pharmacological substances. Self-constructed glass chambers (9) were filled with this mixture. After polymerization of the collagen, the chambers were sealed, and cell locomotion within the three-dimensional collagen lattices was recorded by time-lapse videomicroscopy overnight at 37°C. For the analysis of migratory activity, 30 cells of each sample were randomly selected and two-dimensional projections of paths were calculated by computer-assisted cell tracking in 20-min step intervals.

Only the investigated substances were added to the collagen lattices; the cells were not incubated with any of the substances prior to the mixing of the cells with these substances and the buffered collagen. Norepinephrine was used at 1, 10, and 100 μM; either propranolol or atenolol was added in equimolar concentrations to norepinephrine. The src-specific PTK (3) inhibitor PP2 (10), the PLCγ-specific inhibitor U73122 (11), and the PKC inhibitor Go6976, specific for the α isotype (12), were added alone or in combination with 10 μM norepinephrine. All of these pharmacological substances were provided by Calbiochem-Novabiochem GmbH, Bad Soden, Germany. None of the substances was used in a concentration that was cytotoxic as was assessed by flow cytometry.

Flow Cytometry. The expression of α- and β-adrenoceptors of the SW 480 cells was determined using a FacsCalibur flow cytometer (Becton Dickinson, Heidelberg, Germany). Generally, 1×10^5 cells were incubated with 10 μg/ml primary antibody for 10 min at room temperature. The antibodies directed against the β1-, β2-, $\alpha2_B$-, and $\alpha2_C$-adrenoceptors were provided by Santa Cruz Biotechnology, Santa Cruz, CA; the antibodies directed against the α1- and $\alpha2_A$-adrenoceptors were provided by Dianova, Hamburg, Germany. After the incubation, the cells were washed and incubated with a FITC-conjugated secondary antibody (Fab fragment goat antimouse or antirabbit; Jackson ImmunoResearch Laboratories, West Grove, PA) under the same conditions. Nonspecific binding was determined by an unlabeled isotypic control antibody (Coulter-Immunotech, Hamburg, Germany). Additionally, flow cytometry was used to determine the cell viability subsequent to the migration experiments. The collagen matrices were digested using collagenase type I and IV (Worthington Biochemical Corp., Freehold, NJ), the cells were harvested and subjected to flow cytometry after propidium iodide staining. No changes of the cell viability attributable to treatment with the above mentioned inhibitors were observed throughout the experiments.

Results

Expression of Adrenoceptors. Flow cytometric analyses of the expression of α- and β-adrenoceptors subtypes revealed a faint expression of the α1- and $\alpha2_A$-adrenoceptors expression with a mean FITC-fluorescence intensity of 18.8 and 18.4, respectively, as compared with the isotypic control (14.8; Fig. 1). The $\alpha2_B$- and $\alpha2_C$-adrenoceptors mean FITC-fluorescence intensity was slightly higher (21.3 and 30.9, respectively), and the mean FITC-fluorescence intensity of the isotypic control was 5.8. In contrast to this low expression of α-adrenoceptors, a very high expression of β-adrenoceptors was observed. The mean FITC-fluorescence intensity was 315.7 for the β1-adrenoceptor and 325.7 for the β2-adrenoceptor, compared with 14.8 for the isotypic control.

Norepinephrine-induced Cell Migration. After incorporation within a three-dimensional collagen matrix, the SW 480 colon carcinoma cells developed a spontaneous locomotor activity of 25% locomoting cells (Fig. 2). This spontaneous locomotor activity of the SW 480 cells has been described previously by Kubens and Zanker (13).

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[3] The abbreviations used are: PTK, protein tyrosine kinase; PKC, protein kinase C; PLC, phospholipase C.

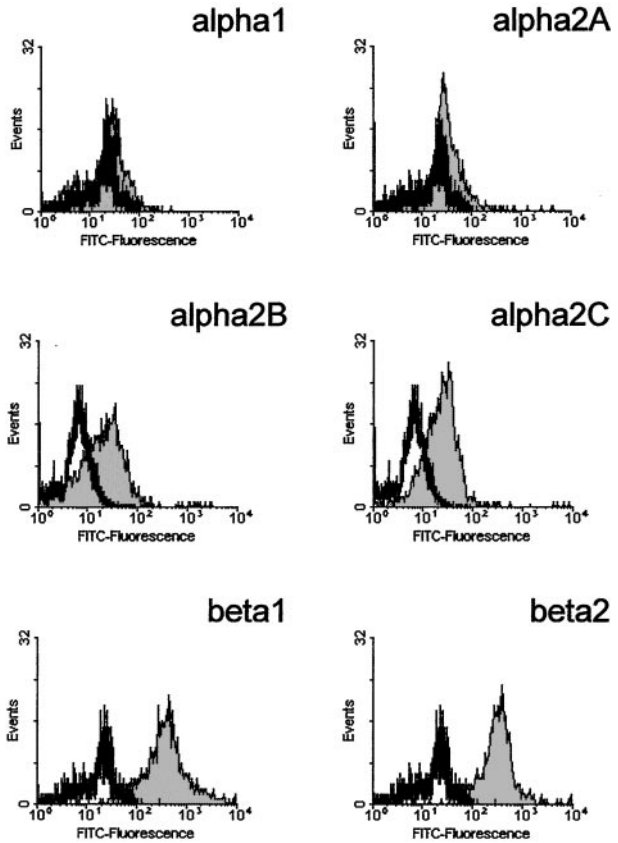

Fig. 1. Analyses of the α- and β-adrenoceptor expression of SW 480 colon carcinoma cells. The antibody bound FITC-fluorescence of specific antibodies (*filled gray*) was compared with an unspecific isotypic control (*black line*).

Treatment of the SW 480 cells with 10 μM norepinephrine augmented the locomotor activity to 65%. Treatment of the cells with 1 μM norepinephrine increased the locomotor activity to a lesser extend, whereas 100 μM norepinephrine led to results similar to those with 10 μM norepinephrine (not shown). The addition of the β-adrenoceptor blocker propranolol had no effect on the spontaneous locomotor activity (Fig. 2*A*), but reduced the norepinephrine-induced locomotion to the activity of the untreated control. In contrast, the β1-specific adrenoceptor blocker atenolol only marginally influenced the norepinephrine-induced locomotion (Fig. 2*B*).

Signaling Cascade of Norepinephrine-induced Migration. In search of the signal transduction underlying the spontaneous and norepinephrine-induced locomotion of the SW 480 cells, we inhibited key regulatory molecules using specific inhibitors. The activity of PTKs of the src-family was inhibited using PP2 (Fig. 3*A*). This inhibitor led to a reduction of the norepinephrine-induced locomotion from 70% locomoting cells to the level of spontaneous locomotor activity (20% locomoting cells) within the course of the experiment. The spontaneous locomotion was not reduced by this inhibitor. In contrast, inhibition of the PLCγ using the specific inhibitor U73122 (Fig. 3*B*) and inhibition of the PKCα using the specific inhibitor Go6976 (Fig. 3*C*) led to a reduction of both spontaneous and norepinephrine-induced locomotion as well.

Discussion

We have reported previously that the intracellular signaling that regulates the migration of neutrophil granulocytes and T lymphocytes differs between spontaneous locomotion and the migration induced by chemokines (14). Herein we used norepinephrine to investigate whether the engagement of serpentine receptors might initiate a type of tumor cell migration that differs from the spontaneous migration. Spontaneous locomotor activity is supposed to be initiated by integrin binding to the extracellular matrix (2). Using MV3 melanoma cells, we showed that the blocking of the collagen-binding $\alpha_2\beta_1$ integrin inhibited spontaneous locomotor activity (15). Our investigation proved that norepinephrine induces migration in addition to the spontaneous migration of the SW 480 colon carcinoma cells. This migratory type is different from the spontaneous locomotion with regard to the involvement of src-PTKs (Fig. 4). The functional link of the adrenoceptors to a PTK signaling is given by β-arrestin (7). Downstream from the signal transduction, the PLCγ is activated by tyrosine phosphorylation caused by PTKs (16). The activity of this phospholipase is an integrating signal for the regulatory signal transduction of both spontaneous (*i.e.,* matrix-induced) and norepinephrine-induced locomotion, because both types of migration are reduced by an inhibitor specific for this enzyme (Fig. 4). The enzymatic activity of the PLCγ generates the second messenger diacylglycerol, which is an activator for the PKCα. We have shown previously that the PKCα is the isozyme needed for colon carcinoma cells migration (17). The PKCα plays an important role even in untransformed, normal colon epithelium. Frey *et al.* (18) have shown, that the PKCα in nontransformed intestinal epithelial cells regulates the growth via modulation of Cip/Kip family cyclin-dependent kinase inhibitors and the retinoblastoma suppressor protein. Furthermore, the PKCα is located in focal adhesions of rat embryo fibroblasts (19). Focal adhesions are

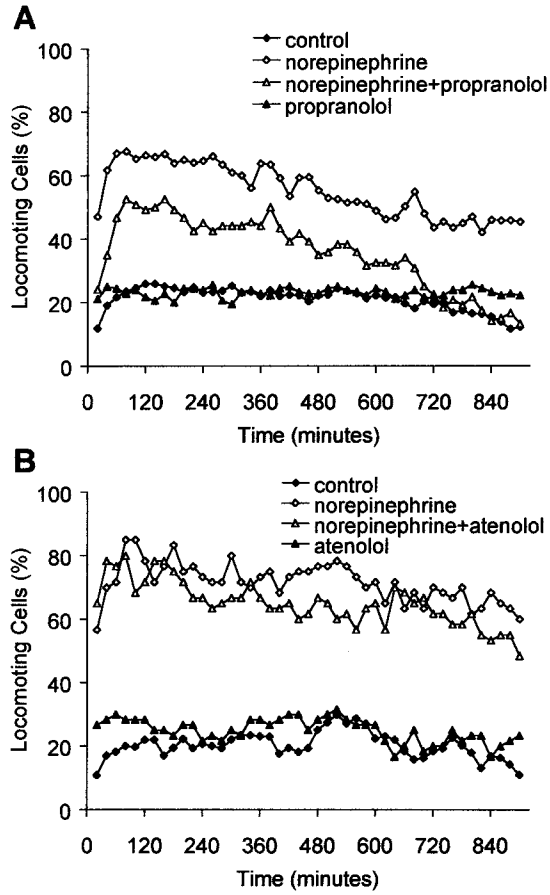

Fig. 2. Inhibition of the promigratory catecholamine effect in SW 480 cells by propranolol but not by atenolol. Migration of the cells was induced by the addition of 10 μM norepinephrine. β-adrenoceptors were blocked by the addition of either 10 μM propranolol (*A*) or 10 μM atenolol (*B*). The graphs show mean values of three independent experiments (90 cells were analyzed per sample).

multiprotein complexes that are essential for the migration of tumor cells (2).

Norepinephrine is a neurotransmitter that is also released in stress reactions. The long-lasting elevation of catecholamines attributable to chronic stress is known to be a risk factor for heart as well as for cancer diseases (20). Thus, we provide molecular evidence for a functional link between psychoneurological events namely the increasing release of the neurotransmitter norepinephrine *in vivo* and its promigratory influence on tumor cells *in vitro*. This finding gets even more relevant when taking the immune system into consideration: we have shown that two important effector cells of the immune system, the T lymphocytes and the neutrophil granulocytes, are inhibited in their migratory activity by the presence of epinephrine or norepinephrine.[4] The inhibiting effect of catecholamines on the formyl-methionyl-leucyl-phenylalanine-induced neutrophil granulocyte migration is caused by an increase of cellular cAMP as was reviewed by Elferink and VanUffelen (6).

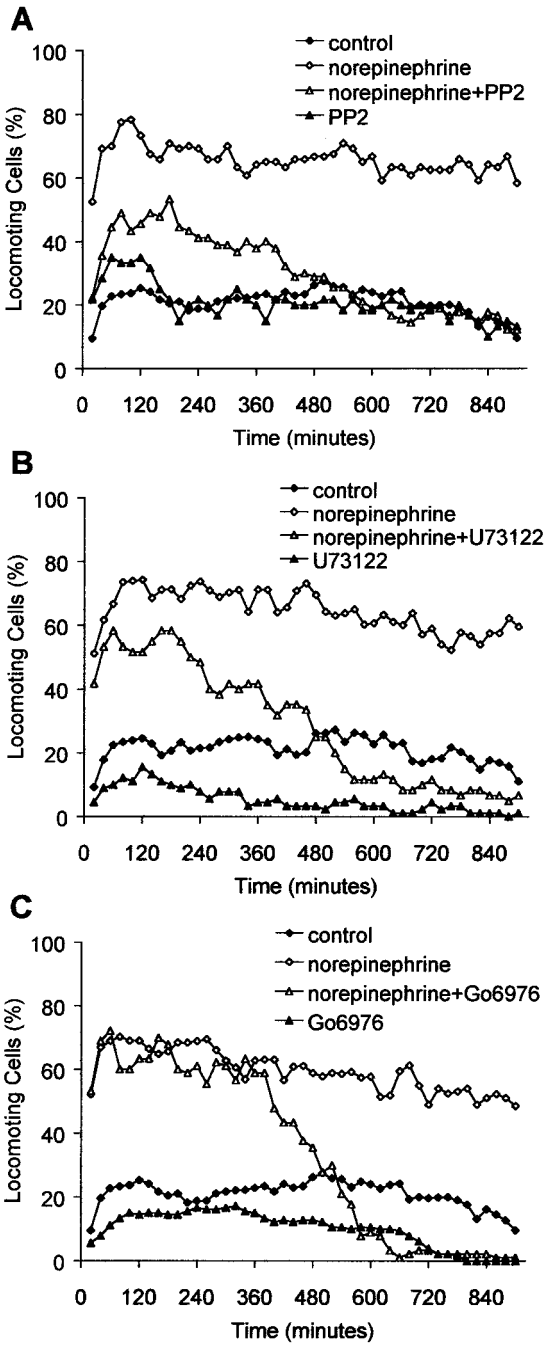

Fig. 3. Signal transduction of the catecholamine-induced SW 480 cell migration. Migration of the cells was induced by addition of 10 μM norepinephrine. The activity of src-PTKs was inhibited by 10 nM PP2 (*A*), the PLCγ was inhibited by 4 μM U73122 (*B*), and the PKCα was inhibited by 6 nM Go6976 (*C*). The graphs show mean values of three independent experiments (90 cells were analyzed per sample).

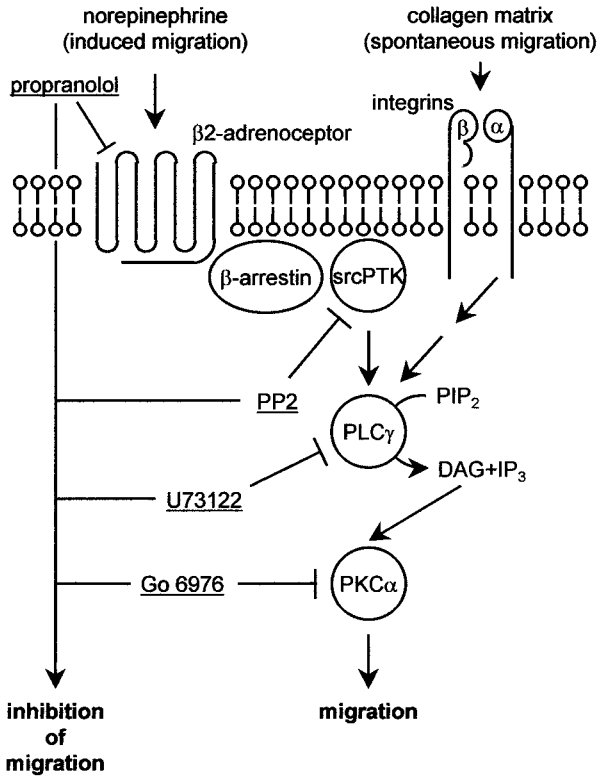

Fig. 4. Model of the signal transduction pathway regulating spontaneous (matrix-induced) and norepinephrine-induced locomotion of SW 480 colon carcinoma cells.

The promigratory effect of norepinephrine on the migration of SW480 colon carcinoma cells was inhibited by the β-adrenoceptor-blocking agent propranolol at pharmacological dosages relevant for human beings. More interestingly, atenolol, a specific β1-adrenoceptor-blocking agent, did only marginally influence migration. This might suggest the use of β2-blocking, non-heart active pharmaceuticals for the preventive treatment in a diagnosed colon carcinoma to inhibit metastatogenesis in the progress of the cancer disease by following preventive clinical trials. Furthermore, ligands of serpentines in the immune system (*i.e.*, chemokines) not only initiate migration but also cause directed migration within a gradient (14). If a gradient of catecholamines could similarly cause a directed migration of colon carcinoma cells, this would have considerable consequences for the view on the pattern of metastases occurring in this special type of cancer. Therefore, our findings might open new pharmacological possibilities for the preventive treatment of a colon carcinoma, to delay or to inhibit the progression of the disease with regard to invasion and the development of metastases.

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[4] Unpublished observations.

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