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Short communication

Mitogenic effect of serotonin in human small cell lung carcinoma cells via both 5-HT_{1A} and 5-HT_{1D} receptors

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Abstract

We have recently shown that the mitogenic effect of serotonin (5-hydroxytryptamine, 5-HT) on human small cell lung carcinoma (SCLC) cells is at least partly due to stimulation of a 5-HT_{1D} receptor type We now report that the 5-HT_{1A} receptor agonist R(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) is also capable of stimulating [^{3}H]thymidine incorporation into SCLC GLC-8 cells, although with lower efficacy than 5-HT The simultaneous administration of maximal doses of 8-OH-DPAT and the 5-HT_{1D} receptor agonist sumatriptan reproduced the maximal [^{3}H]thymidine incorporation observed with 5-HT alone The 5-HT_{1A} receptor antagonists spiperone and SDZ 216-525 completely abolished the effect of 8-OH-DPAT (IC_{50} 30 nM for both drugs) behaving as pure antagonists Accordingly, the two drugs partially inhibited the mitogenic effect of 5-HT These data indicate that the mitogenic effect of 5-HT in SCLC cells involves both 5-HT_{1A} and 5-HT_{1D} receptor types

Keywords 5-HT_{1A} receptor, 5-HT_{1D} receptor, Cell proliferation , human, 5-HT receptor antagonist

1. Introduction

Small cell lung carcinoma (SCLC) is a very aggressive and metastatic tumor often associated with tobacco smoking SCLC cells express neuronal proteins like voltage-dependent Ca^{2+} channels (Codignola et al , 1993, Cattaneo et al , 1993a) and nicotinic receptors (Tarroni et al , 1992) They also produce and release peptides like bombesin and insulin-like growth factor 1 which can establish an autocrine mitogenic loop (Weynants et al , 1990) We have previously shown that 5-HT is contained in and released from the SCLC cell lines NCI-N-592 and GLC-8 (Cattaneo et al , 1993b) 5-HT is also mitogenic for SCLC cells and may therefore behave as an autocrine factor As this mitogenic effect of 5-HT might be important in controlling tumor growth, we began to characterize the 5-HT receptor subtype(s) present in SCLC cells We recently reported (Cattaneo et al , 1994) the involvement of 5-HT_{1D} receptors in 5-HT mitogenic effect We also showed that 5-HT and the 5-HT_{1D} receptor agonist sumatriptan inhibited adenylate cyclase activity in SCLC cell membranes In this study we further characterize the receptor subtypes involved in the action of 5-HT on SCLC cell cultures.

2. Materials and methods

2 1 Cell culture

GLC-8 cells were grown in Roswell Park Memorial Institute 1640 medium (RPMI-1640, Biochrom, Berlin, Germany) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA) The cells were kept at 37°C in a humidified atmosphere of 5% CO_2 in air

2 2 [^{3}H]Thymidine incorporation assay

GLC-8 cells were plated in RPMI-1640 medium without serum in 96-well microtiter plates at a density of $5–10 \times 10^3$ cells/well, and incubated with the various substances for 48 hrs Methyl[^{3}H]thymidine (1 μCi/well, specific activity 2 Ci/mmol, Amersham, UK) was added during the last 6 h of incubation The cells were then washed, lysed with distilled water and collected on filters

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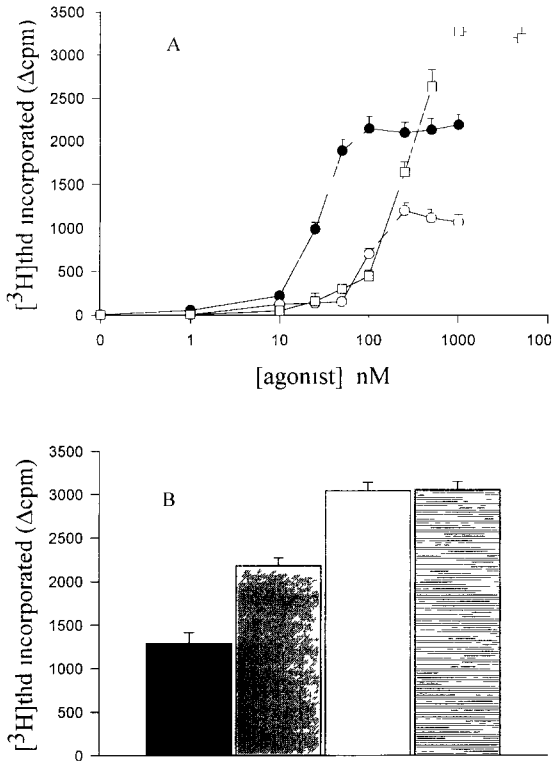

Fig 1 (A) Effect of 5-HT (□), sumatriptan (●) and 8-OH-DPAT (○) on [^{3}H]thymidine incorporation in GLC-8 cells Results are given as differences (Δcpm) over basal values (1143 ± 118 cpm) (B) GLC-8 cells were stimulated with 500 nM 8-OH-DPAT (solid black column), sumatriptan 100 nM (solid gray column), 500 nM 8-OH-DPAT + 100 nM sumatriptan (open column) or 1 μM 5-HT (horizontal lines) Results are expressed as in panel A Each point/column is the mean ± S E of 3 independent experiments performed in quadruplicate

with an automatic cell harvester (Titerteck, Flow laboratories, Rockville, MD, USA) The filters were placed in filter counter scintillation fluid (Packard, Downers Grove, IL, USA) and counted using standard procedures

2 3 Drugs

The drugs used were the following 5-hydroxytryptamine creatine-sulphate (Sigma Chemicals, St Louis, MO, USA), R(+)-8-hydroxy-dipropylaminotetralin (8-OH-DPAT) and spiperone (RBI, Natick, MA, USA), methyl 4-{4-[4-(1,1,3-trioxo-2H-1,2-benzoisothiazol-2-yl)butyl]-1-piperazinyl} 1H-indole-2-carboxilate (SDZ 216-525, Sandoz Italia, Milan, Italy) Sumatriptan for medical use was employed (Permicran, Ellem, Milan, Italy)

3. Results

3 1 8-OH-DPAT effect on DNA synthesis in GLC-8 cells

Fig 1A compares the effect of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on [^{3}H]thymidine incorporation by GLC-8 cells, with the effects of 5-HT and of the 5-HT$_{1D}$ receptor agonist sumatriptan 8-OH-DPAT is less efficacious but more potent than 5-HT (EC_{50} 90 nM and 250 nM, respectively) in stimulating DNA synthesis, whereas it is less efficacious and less potent than sumatriptan (EC_{50} 25 nM) The maximum effect of 8-OH-DPAT and sumatriptan appears to account for approximately 35% and 65% of the 5-HT maximum effect, respectively

Maximal doses of 8-OH-DPAT and sumatriptan, when applied together to GLC-8 cells, produce the same extent of [^{3}H]thymidine incorporation as maximal doses of 5-HT alone (Fig 1B) confirming the additivity of the effects produced by the activation of the two receptor subtypes

3 2 Effect of antagonists on DNA synthesis induced by the various stimuli

Experiments with selective antagonists were performed using putative 5-HT$_{1A}$ (spiperone and SDZ 216-525, Lum and Pierce, 1988, Hoyer et al , 1992) and 5-HT$_7$ (mianserine and chlorpromazine) receptor antagonists 5-HT$_7$ is a recently cloned 5-HT receptor which displays quite a high affinity for 8-OH-DPAT (Ruat et al , 1993) Fig 2 shows that spiperone completely inhibited the incorporation of [3H]thymidine produced by 8-OH-DPAT (IC_{50} 30 nM) whereas it did not influence the effect of sumatriptan Spiperone was devoid of intrinsic activity (up to 5 μM 1009 ± 100 cpm, control cells 885 ± 183 cpm) and can therefore be considered as pure antagonist in this system Spiperone produced a 45% inhibition (IC_{50} 400 nM) of [^{3}H]thymidine incorporation stimulated by 5-HT (1 μM) The same kind of experiments were carried out using the 5-HT$_{1A}$ receptor antagonist SDZ 216-525 and almost identical results were obtained (not shown) Neither chlorpromazine nor mianserine displayed any activity on

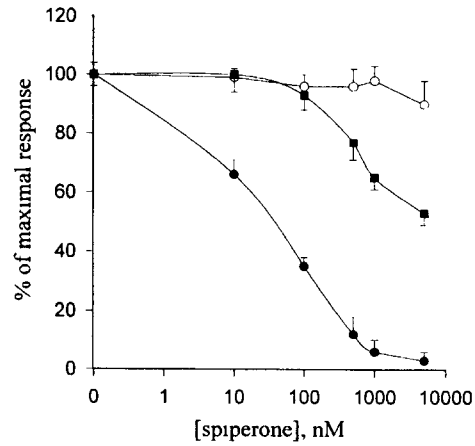

Fig 2 Effect of spiperone (5 min preincubation) on [^{3}H]thymidine incorporation induced by 500 nM 8-OH-DPAT (●), 100 nM sumatriptan (○) and 1 μM 5-HT (■) Results are given as percent of the maximal responses observed with the agonists in the absence of spiperone Each point is the mean ± S E of 3 independent experiments performed in quadruplicate

8-OH-DPAT-stimulated DNA synthesis up to a concentration of 2 μM (not shown)

4. Discussion

We have previously shown that 5-HT_{1D} receptor subtype is at least partly responsible for the mitogenic effect of 5-HT in the human SCLC cell line GLC-8 (Cattaneo et al., 1993b, Cattaneo et al., 1994) Here we report that the 5-HT_{1A} receptor agonist 8-OH-DPAT also induces increased [^{3}H]thymidine incorporation in the same cell line at concentrations in reasonable agreement with affinity values estimated by radioligand binding studies in vitro, especially if one considers the complexity of the phenomenon studied here The experiments with the 5-HT_{1A} and 5-HT_7 receptor antagonists strongly indicate that 8-OH-DPAT exerts its mitogenic effect by stimulating the 5-HT_{1A} receptor subtype The maximal effect of 5-HT_{1A} receptor activation on [^{3}H]thymidine incorporation appears to account for about 35% of 5-HT maximal effect, whereas activation of the 5-HT_{1D} receptor subtype (by the selective agonist sumatriptan) accounts for the remaining 65% Furthermore, the effect of the combined application of the two drugs accounts for 100% of 5-HT effect, thus, the activation of the two receptor subtypes by selective agonists appears to be additive

The involvement of 5-HT_{1A} receptor subtype is confirmed by the experiments with 5-HT receptor antagonists reported here 5-HT_{1A} receptor antagonists like spiperone and SDZ 216-525 (Lum and Pierce, 1988, Hoyer et al., 1992) completely and selectively inhibit the effect of 8-OH-DPAT, without affecting sumatriptan's action and only partially interfering with 5-HT effect on [^{3}]thymidine incorporation These findings indicate that in our cell system the two drugs act as selective 5-HT_{1A} antagonists and do not interfere with 5-HT_{1D} receptor function

It is known that several 5-HT receptor ligands behave as either antagonists or partial agonists, depending on the extent of the receptor reserve, on the efficiency of the receptor-effector coupling and on the cellular location of the receptor they act upon (pre vs post synaptic receptors) (Hoyer and Boddeke, 1993) In the cell system investigated here, spiperone and SDZ 216-525 display no agonistic activity We therefore suggest that in SCLC cells there is no relevant 5-HT receptor reserve and the receptors might be considered to be of the post-synaptic type

In conclusion, based on the data presented, we suggest that the mitogenic actions of 5-HT in this cell line are mediated by the activation of both 5-HT_{1A} and 5-HT_{1D} receptors Although the activation of the two receptor subtypes appears to be additive, it is not clear whether the two pathways are completely independent Indeed, Fig 2 shows that, in order to affect the 5-HT_{1A} component of serotonin action, 5-HT_{1A} receptor antagonists must be employed at concentrations higher than those effective on 8-OH-DPAT We are now investigating whether this phenomenon arises from interactions between the two receptor subtypes or from other causes

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