Biochemical Pharmacology, Vol. 38, No. 19, pp. 3175–3178, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

IDENTIFICATION OF FUNCTIONAL BETA-ADRENERGIC RECEPTORS ON AC GLIOMA CELLS

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(*Received* 21 *October* 1988; *accepted* 8 *February* 1989)

Abstract—AC glioma cells, a clonal cell line derived from a rat glioma, responded to 1 mM dibutyryl-cyclic AMP and isobutylmethylxanthine with a change to stellate morphology. A concentration-related morphological change was induced by β_1- and β_2-adrenergic agonists with the order of potency being isoproterenol > soterenol > norepinephrine. Propranolol (nonselective β-antagonist), butoxamine (β_2-antagonist) and metoprolol (β_1-antagonist) significantly decreased the cell response to isoproterenol. Schild analysis of the response, using the competitive antagonist metoprolol, gave pA_2 values of 7.5 and 8.5 for the agonists norepinephrine and soterenol, respectively, with slopes of the curves being less than unity. These observations indicate that both β_1- and β_2-adrenergic receptors mediate the change in cellular morphology.

The role of glial cells in the central nervous system appears increasingly complex as numerous neurotransmitter receptors, ion channels, and metabolic processes are identified in glial cells. Neurotransmitter receptors on glial cells have been identified by analyzing the accumulation of intracellular second messengers and by radioligand binding techniques [1–4]. Several sources of glial cells have been used to study receptor function, including primary cultures, explants of fetal CNS, and clonal cell lines [5]. Cell lines derived from astroglial tumors have been demonstrated to be useful for the characterization of receptor and second messenger systems.

Igarashi and coworkers [6] described the rat AC glioma cell line which responded to dibutyryl-cyclic AMP and cholera toxin with a change to stellate morphology. The morphological change induced by dibutyryl-cyclic AMP is commonly observed in cultured astroglial cells [5]. Receptors for neurotransmitters have not been identified on the AC glioma cell line. The effects of dibutyryl-cyclic AMP and cholera toxin suggested that receptors which activate adenylate cyclase could be identified by monitoring the morphology of the cells upon exposure to an agonist. The objective of the present study was to characterize the receptors on AC glioma cells that are linked to adenylate cyclase by observing drug-induced morphological changes in the AC glioma cells.

MATERIALS AND METHODS

Cell culture. AC glioma cells were grown in Minimum Essential Medium (MEM) containing 10% fetal bovine serum. Cells were routinely cultured in 75 cm^2 flasks and passaged every 4 days. For morphological assays, cells were plated in 35 mm dishes at a density of 2×10^4 cells/dish and grown in 2 ml of medium for 24–48 hr at 37° in 5% CO_2.

Morphological analysis. Cells, plated in 35 mm dishes, were exposed to adrenergic agonists and antagonists by adding 50–100 μl of drug solution directly to the cultures containing 2 ml MEM. After incubating for 30 min, one randomly selected microscopic field was photographed per dish under 100× magnification. To determine the proportion of cells which displayed a stellate morphology, cells were counted and classified as uninduced if there was little or no cytoplasmic contraction, or induced if the cytoplasm was retracted and the cell bore multiple processes. Antagonists were added 10 min before an agonist. Control cells received the same volume of distilled water, which was used as the diluent for the drugs.

In a second series of studies, cells were pretreated with 50 μM phenoxybenzamine for 30 min at 37° to block α-adrenergic receptors and cellular uptake sites [7]. The treated cells were washed twice with 2 ml of medium to remove excess phenoxybenzamine before use in morphology studies. Schild plots and pA_2 values were determined according to the method of Arunlakshana and Schild [8] using concentration–response curves in the absence and presence of metoprolol.

Statistical analysis. Differences between mean values of treatment groups were determined by one-way ANOVA followed by a Newman–Keuls range test.

RESULTS

AC glioma cells exhibit a flat fibroepithelioid morphology under normal conditions, but when exposed to dibutyryl-cyclic AMP the cells become spherical with an abundance of cytoplasmic processes (stellate morphology); this response is maximal at a dibutyryl-cyclic AMP concentration of 1 mM [6]. This change was also induced by the adrenergic receptor agonists isoproterenol, soterenol, norepinephrine and terbutaline. The phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) at 10^{-4} M produced a

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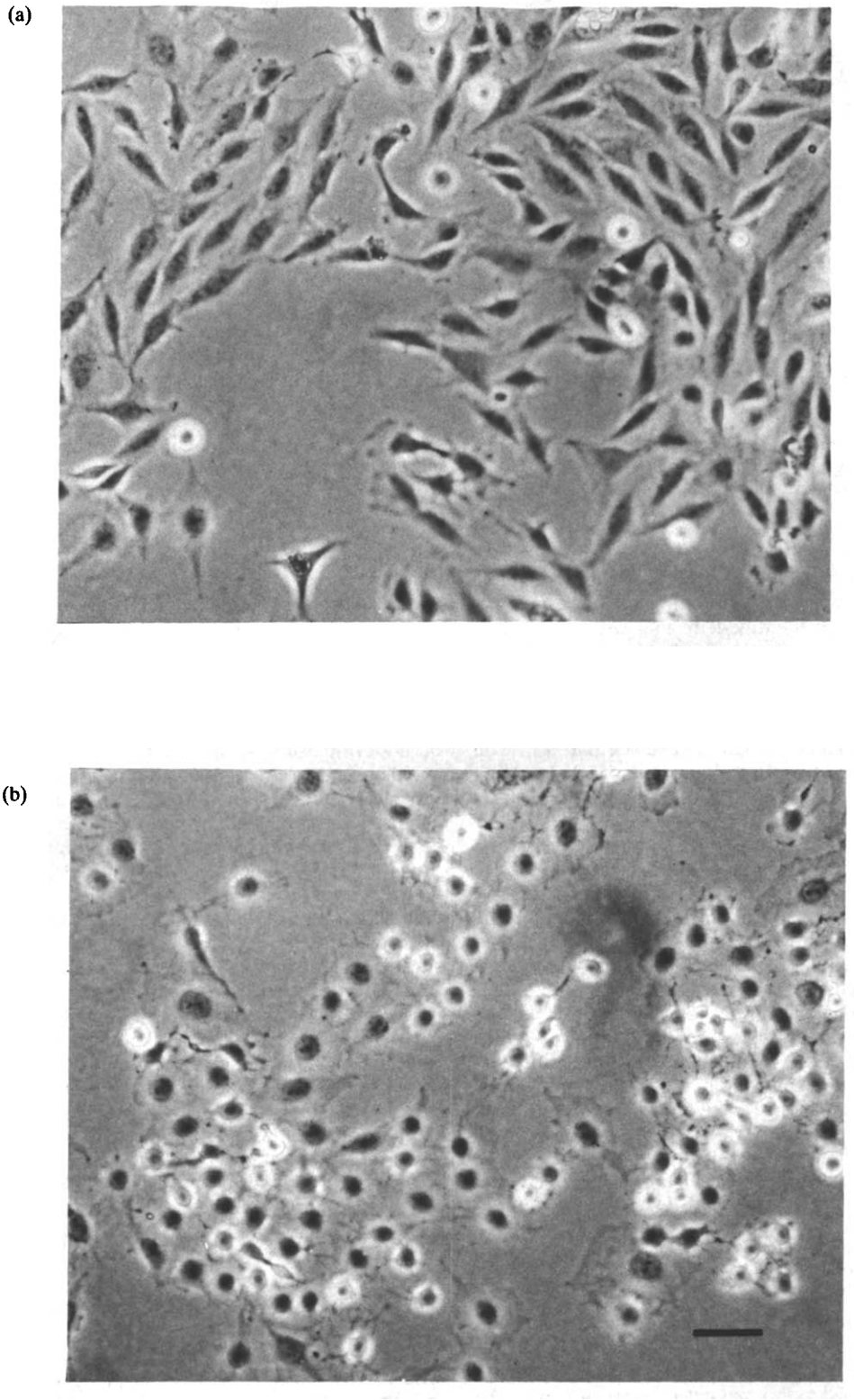

Fig. 1. AC glioma cells (a) as they appear under normal conditions and (b) 30 min after exposure to 10^{-9} M isoproterenol. Calibration bar, 50 μm.

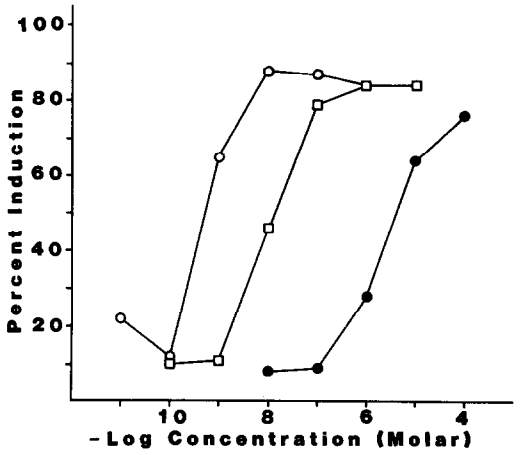

Fig. 2. Concentration–response curves for the morphological change (induction) in AC glioma cells induced by isoproterenol (○), norepinephrine (●), and soterenol (□). Data are expressed as percent of cells with stellate morphology. Each point is the mean of five observations.

maximal morphological response that was qualitatively indistinguishable from that produced by adrenergic agonists (results not shown). Responses to adrenergic agonists occurred within 15 min of adding the drug, and the maximal effect was seen at 30 min and persisted for at least 60 min. Figure 1 shows the morphological response of the AC glioma cells induced by isoproterenol at 10^{-9} M after a 30-min incubation.

The response of the AC glioma cells to isoproterenol, soterenol, and norepinephrine was quantitated using drug concentrations ranging from 10^{-11} to 10^{-4} M (Fig. 2). The concentration of agonist which produced 50% of maximal induction of the cells (EC_{50}) was calculated from these data. The nonselective β-adrenergic agonist isoproterenol was the most potent with an EC_{50} of 7.7×10^{-10} M, the β_2-selective agonist soterenol had an EC_{50} of 1.5×10^{-8} M, and the β_1-selective agonist norepinephrine was the least potent with a value of 1.8×10^{-6} M. Isoproterenol and soterenol produced equivalent maximal responses, whereas norepinephrine did not produce a maximal response even at 10^{-4} M. The lower potency of norepinephrine was not attributable to α-adrenergic stimulation or cellular uptake, since these effects would have been blocked by the phenoxybenzamine pretreatment. Removal of phenoxybenzamine from the assay did not change the EC_{50} for norepinephrine.

Adrenergic antagonists relatively selective for β_1- or β_2-receptors significantly ($p < 0.05$) inhibited the responsiveness of the AC glioma cells to 10^{-9} M isoproterenol (Table 1). Propranolol, a nonselective β-antagonist, also reduced the response of the cells to isoproterenol.

pA_2 values for the β_1-selective antagonist metoprolol were estimated by using agonists with different β_1- or β_2-receptor selectivity. Concentration–response relationships were determined for soterenol (β_2-selective) and norepinephrine (β_1-selective) in the absence and presence of three concentrations

Table 1. Inhibition of the morphological response of AC glioma cells to isoproterenol by β-adrenergic antagonists

Treatment*	Percentage of cells with stellate morphology†
ISO	65 ± 14
ISO + propranolol, 10^{-9} M	20 ± 6‡
ISO + butoxamine, 10^{-7} M	36 ± 10‡
ISO + metoprolol, 10^{-8} M	33 ± 3‡

* Isoproterenol (ISO, 10^{-9} M) was added to the cells, and 30 min later the cells were photographed. The percentage of cells which exhibited stellate morphology was determined from the photographs. Antagonists were added 10 min before isoproterenol.

† Each value is the mean ± SE of three plates of cells.

‡ Significantly different ($P < 0.05$) from the experimental group exposed to isoproterenol alone.

Table 2. pA_2 values for metoprolol using norepinephrine and soterenol as agonists

Agonist	pA_2	Slope of Schild plot
Norepinephrine	7.5 ± 0.3	0.44
Soterenol	8.5 ± 0.3	0.53

Metoprolol (10^{-8}, 10^{-7} and 10^{-6} M) was incubated with norepinephrine (10^{-8} to 10^{-4} M) or soterenol (10^{-9} to 10^{-5} M), in three plates of cells per treatment, and the morphological response of AC glioma cells was quantitated 30 min later.

* Values are means ± SE (N = 3).

of metoprolol (10^{-8}, 10^{-7} and 10^{-6} M). Metoprolol shifted the concentration–response curves to the right, suggesting competitive antagonism. The concentration–response curves were then subjected to Schild analysis (Table 2). The pA_2 values for norepinephrine and soterenol were different, and the slopes of the Schild plots were significantly less than one. A slope of less than one is expected if the tissue expresses multiple functional receptor subtypes (β_1 and β_2).

DISCUSSION

AC glioma cells, a rat clonal cell line first described by Igarashi *et al.* [6], respond to β-adrenergic agonists with a reversible change in cellular morphology. This morphological change, initially reported to be induced by dibutyryl-cyclic AMP and cholera toxin, can be stimulated by either β_1- or β_2-adrenergic agonists and is apparently mediated by an elevation in intracellular cyclic AMP.

The cyclic AMP-induced change in morphology, as observed in AC glioma cells, has been reported for a variety of glial cell lines and primary cultures [5]. The intracellular mediator of the response is generally held to be cyclic AMP [9], although this view has been questioned recently [10]. In the present study, the role of cyclic AMP in mediating

the response of the AC glioma cells to β-adrenergic agonists was demonstrated indirectly. Since the stellate morphology is induced by dibutyryl-cyclic AMP and by agents known to elevate intracellular cyclic AMP, cyclic AMP is a likely intracellular mediator of the response.

In the present study, characterization of β-adrenergic agonist and antagonist responses indicates that both β_1 and β_2 subtypes were present in AC glioma cells. This is in contrast to results obtained with purified astroglia from rat cerebral cortex, which possess β_1-adrenergic receptors [3]. The human astrocytoma cell line 1321N1 possesses primarily β_2-adrenergic receptors, whereas rat C6 glioma cells possess both β_1- and β_2-adrenergic receptor subtypes [3]. The conclusion that both receptor subtypes are present in AC glioma cells is supported by the observation that the morphological response to isoproterenol can be partly inhibited by either the relatively β_1-selective antagonist metoprolol or the β_2-selective antagonist butoxamine. Also, the Schild plots had slopes of less than unity, suggesting the presence of multiple receptor subtypes. However, a Schild plot with a slope of less than one can be accounted for by cellular uptake of the drug [11]. This was discounted by the inability of phenoxybenzamine, an uptake inhibitor, to alter the EC_{50} of norepinephrine.

In summary, the AC glioma cell line expressed functional β_1- and β_2-adrenergic receptors that mediated a cellular morphological change, and this appeared to be related to intracellular cyclic AMP. The AC glioma cell line gave a predictable, concentration-related response to beta-agonists and is a useful model for the study of glial β-adrenergic receptors.

Acknowledgement—This research was supported in part by PHS Grants NS 19584, 5T32ES07039 and S07RR05586.

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