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Bromoacetylalprenololmenthane binding to β-receptors modulates the rate of hormone-induced internalization but not desensitization in S49 cells

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Bromoacetylalprenololmenthane was found to inhibit hormone-induced β-adrenergic receptor internalization in a dose-dependent fashion in S49 lymphoma cells, besides its known ability to bind to β-receptors irreversibly. This new found property of BAAM$^+$ was taken advantage of in studying whether receptor internalization is a necessary step in the desensitization of adenylate cyclase. BAAM-treated cells showed functional desensitization even when receptor internalization had been blocked substantially by 50–65%. This finding suggests that receptor internalization is not directly involved in desensitization.

β-Adrenergic receptor; Desensitization; Receptor internalization; Internalization blockade; (S49 wild type cell)

1. INTRODUCTION

β-Adrenergic agonists induce at least two distinguishable events at the cellular level: stimulation of intracellular cAMP accumulation and internalization of a proportion of the cell surface receptors. The rate of cAMP accumulation rapidly declines shortly after stimulation with hormone, the system becomes 'desensitized'. Receptor internalization has been proposed to play a significant part in desensitization [1,2]. However, recent evidence suggests that under certain conditions receptor internalization is not required for functional desensitization [3,4]. We show here that the β-antagonist BAAM which has been described to bind irreversibly to β-receptors [5], significantly modulates the rate of internalization of those receptors that remain accessible to hormone. In contrast, the observed agonist-induced decline in adenylate cyclase activity of intact cells was completely unaffected by the irreversible blockade of part of the receptors. We conclude therefore that receptor internalization is not directly involved in the desensitization of the enzyme activity.

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Abbreviations: BAAM, bromoacetylalprenololmenthane; CGP-12177, 4-(3-*tert*-butylamino-2-hydroxypropoxy)benzimidazole-2-one hydrochloride; DMEM, Dulbecco's modified Eagle's medium; IPR, (−)-isoproterenol

2. MATERIALS AND METHODS

S49 wild type cells were grown in DMEM containing 10% fetal calf serum in a growth cabinet (95:5 air/CO_2, v/v, 37°C) to a density of 1–2 × 10^6 cells per ml.

All reagents used were of standard purity as is commercially available. (−)-Isoproterenol bitartrate was obtained from Sigma, and BAAM was a gift from Dr J. Pitha, Bethesda, MD, USA.

To achieve an irreversible blockade of cell surface receptors, cells were treated with the antagonist BAAM in the following manner. Cells

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were incubated with BAAM added from an ethanolic stock solution at 4°C for 30 min, centrifuged (5 min, 1500 rpm), and washed in the cold six times with Hepes-buffered DMEM, pH 7.4 [6], containing 0.03% bovine serum albumin (Fluka) followed by one wash with Hepes-buffered DMEM. Cells were finally resuspended in a calculated volume of ice-cold Dulbecco medium and preequilibrated to the temperature at which the experiments were performed. Receptor numbers were determined by Scatchard analysis of saturation binding of [^{3}H]CGP-12177 (between 0.1 and 1.5 nM) at 30°C as described before [7]. Intracellular cAMP accumulation was measured at 37°C and was stopped by pipetting 0.5 ml of cell suspension into an equal volume of ice-cold trichloroacetic acid (10%). After centrifugation the supernatant was extracted 4 times with 1 ml of diethyl ether and the cAMP content in the aqueous phase was measured using the [^{3}H]cAMP assay kit from Amersham, England.

3. RESULTS AND DISCUSSION

Treatment of intact S49 cells with BAAM resulted in an irreversible blockade of β-receptors. This was established by Scatchard analysis of radioligand binding using ^{3}H-CGP-12177 which is specific for surface receptors [6] and by dose-response curves of IPR-dependent cAMP accumulation (not shown). The number of blocked receptors increased with increasing concentrations of BAAM applied. A concentration of 0.5–1.0 μM BAAM was required to block 50% of the receptors.

S49 cells carrying a reduced number of receptors accessible to hormone showed a decrease in the rate of hormone-induced receptor internalization

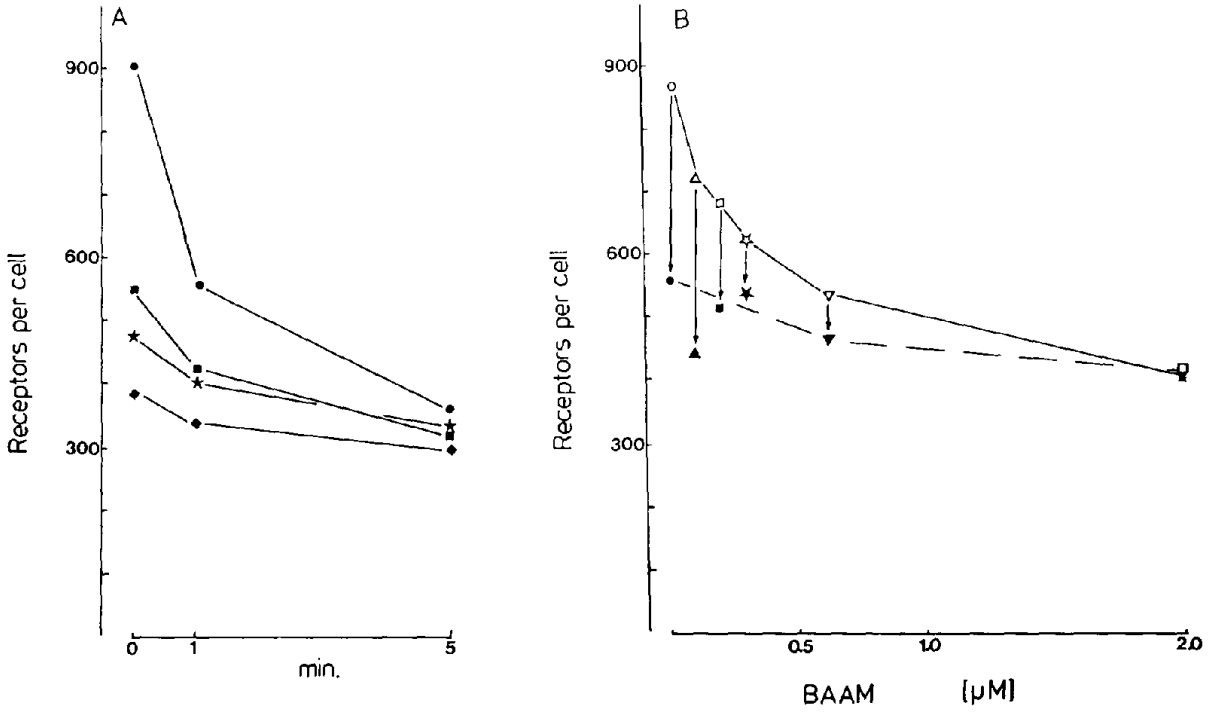

Fig.1. Comparison of the number of cell surface receptors before and after desensitization with 1×10^{-6} M IPR at 37°C in S49 cells carrying varied proportions of receptors blocked with BAAM. (A) Time course of internalization in cells pretreated with 0.25 μM (■), 0.63 μM (★), and 2.0 μM (◆) BAAM and non-treated control cells (●). Data points at time 0 denote receptor numbers before hormone addition. One out of two independent experiments shown. (B) Receptor number before (open symbols) and after (full symbols) 10 min desensitization in cells pretreated with five different concentrations of BAAM and non-treated control cells (○,●). Receptor numbers per cell were determined by Scatchard analysis of [^{3}H]CGP-12177 saturation binding curves.

(fig.1). In fig.1 the results of two types of experiments are shown. S49 cells were incubated with various amounts of BAAM as indicated and the numbers of unlabelled cell surface receptors were determined from Scatchard plots of saturation binding experiments with [^{3}H]CGP-12177. Fig.1A shows the time course of receptor internalization over 5 min at 1×10^{-6} M IPR (37°C) for 3 cell batches pretreated with different concentrations of BAAM and one untreated control. Fig.1B shows the degree of internalization as a function of BAAM concentration. The extent of receptor internalization achieved during a 10 min incubation with 1×10^{-6} M IPR at 37°C is plotted against the BAAM concentrations with which the cells had been pretreated beforehand. Both sets of experiments indicate that after BAAM treatment the rate of internalization slows down. The rate of internalization was reduced by 50–65% after application of 0.63 μM BAAM. Pretreatment with concentrations in excess of 2.0 μM BAAM completely prevented internalization, i.e. 1×10^{-6} M IPR produced no change in the number of receptors still accessible to CGP-12177 binding.

In contrast, treatment with BAAM did not seem to have any effect on desensitization (fig.2). Fig.2 shows the transient accumulation of intracellular cAMP after stimulation of BAAM (0.63 μM) pretreated and non-treated S49 cells with 1×10^{-6} M IPR at 37°C in the absence of phosphodiesterase inhibitor. BAAM-treated cells produced less cAMP than non-treated cells which is in accordance with the reduction in available receptor numbers [8]. The time courses of the rise and decline in cAMP content for control and pretreated cells run, however, in parallel. This is also the case after washing out the hormone and reincubation of an aliquot of cells with 1×10^{-6} M IPR (fig.2). In all cases, the maximal cAMP response is reached after about 2 min. The ratio between the maximal increases in cAMP before and after renewed hormone stimulation is also very similar for BAAM-treated cells and control cells.

Thus receptor blockade with BAAM alters the rate of internalization of non-blocked receptors but not the biological function of the transducing system, i.e. its desensitization. This indicates that internalization is not a sequential step in the desensitization process.

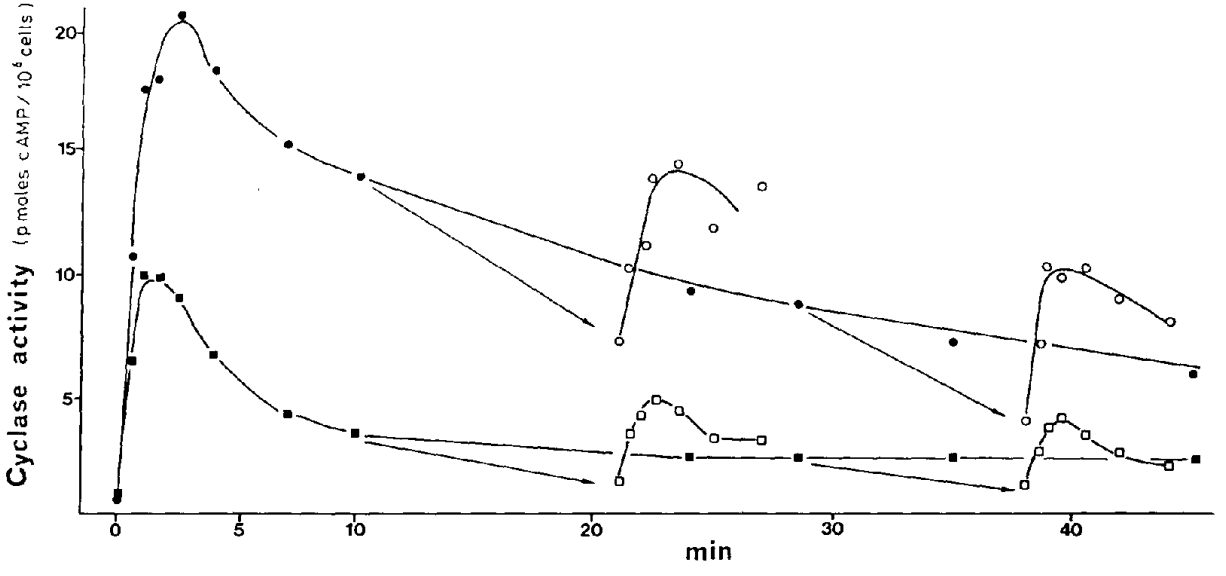

Fig.2. Time course of desensitization of adenylate cyclase activity in S49 cells pretreated with 0.63 μM BAAM (■, □) and in non-treated S49 cells (●,○). Cells were incubated with 1×10^{-6} M IPR at 37°C in DMEM containing no phosphodiesterase inhibitor. Aliquots were taken and assayed for cyclase activity (full symbols). At the times indicated further aliquots were washed with hormone-free medium and were resuspended in an identical volume of DMEM. The cells were then again incubated with 1×10^{-6} M IPR at the times denoted by the arrow tips and cAMP accumulation measured as before (open symbols). One typical experiment out of three performed is shown.

Although BAAM has not previously been reported to block internalization, it is not unique in doing so. Phenylarsine oxide [9], concanavalin A [10] or the reduction of the cellular ATP content [11] also prevent internalization. So far, only one study (with phenylarsine oxide) also shows a functional aspect of desensitization, i.e. the agonist-induced reduction in adenylate cyclase activation, to be unrelated to receptor internalization [4]. But none of the cited workers reports a dose-related modulation of receptor internalization as is seen with BAAM. BAAM might therefore prove useful in future studies of the mechanism of receptor internalization.

We investigated further whether the BAAM-mediated reduction in hormone-accessible receptors was responsible for the observed inhibition of internalization. We chose to approach this question by reducing the number of surface receptors in a different manner. We pretreated S49 cells in growth conditions (see section 2) with the agonist terbutaline (3×10^{-6} M) for 16 h. After this incubation, cells had down-regulated the number of surface receptors per cell (CGP-12177 binding) to 243 compared to an untreated control with 716 receptors per cell. The pretreated cells were washed free of terbutaline and then incubated with 1×10^{-6} M IPR for 10 min at 37°C. Interestingly, the extent of internalization was found to be 58% with 101 remaining surface receptors in comparison to 63% of control cells with 265 receptors remaining on the surface. Two other experiments using pretreatment with 1×10^{-7} M and 5×10^{-7} M terbutaline over 16 h yielded similar results, i.e. 57 and 61% internalized receptors, respectively.

Receptor density per surface area does therefore not seem to be of importance for receptor internalization in the absence of prior BAAM treatment. Alternative explanations for the observed effect of BAAM could be that either the chemical modification of receptors prevents the internalization of non-modified receptors or that the labelling of other sites, e.g. lipids, leads to the obstruction of internalization.

Whatever the molecular basis for the reduction in the rate of internalization may be, we have shown that desensitization proceeds normally despite alterations in the rate of receptor internalization.

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