Inflamm. res. 47, *Supplement 1* (1998) S42–S43
1023-3830/98/010S42-02 $ 1.50+0.20/0

Inflammation Research

Tiotidine, a classical H_2-antagonist, presents characteristics of an inverse agonist in U937 cell line

F. Monczor, B. L. Legnazzi, E. Rivera and C. Davio

Laboratorio de Radioisótopos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 PB, 1113 Buenos Aires, Argentina, Fax +54 1 962 5341, e-mail: Cardavio@huemul.ffyb.uba.ar

Introduction

It is generally accepted that the histamine H_2 receptor is coupled to the adenylyl cyclase system through direct interaction with a Gs protein family [1]. In the histiocytic lymphoma cell line U937, a histamine H_2 receptor was described that directly increases cyclic AMP production with a classical pharmacological profile [2, 3]. Recently it was found that drugs classically considered as antagonists, behave in some systems as inverse agonists [4]. The purpose of the present work was to study the tiotidine pharmacological characteristics in U937 cell line.

Materials and methods

Cell culture

U937 cells, obtained from the American Type Culture Collection (ATCC), were cultured at 37 °C in humidified atmosphere with 5% CO_2 in RPMI 1640 medium supplemented with 10% bovine fetal serum. Cells were routinely passed every 3 days and seeded at a density of 2.5×10^5 cells/ml.

Cyclic AMP assay

Cells were washed and resuspended in Hanks' balanced salt solution with 0.8 mM 3-isobutyl-1-methylxanthine (IBMX) at a density of 10^6 cells/ml, preincubated for 3 min at 37 °C and exposed to different drugs for 9 min at the indicated concentration. The reaction was stopped by centrifugation at 3000 g for 3 min. The pellet was resuspended in ethanol for cyclic AMP extraction and further centrifuged at 3000 g for 10 min. The ethanol phase was dried and resuspended in 50 mM Tris-HCl buffer. Cyclic AMP content was determined by means of competition with [^{3}H]cyclic AMP for protein kinase A, as previously described [5].

Results and discussion

Concentration-response curves with BU-E-75, a specific H_2 agonist, in the absence and presence of tiotidine were obtained (Fig. 1).The presence of tiotidine not only shifted the curve to the right increasing the EC_{50} (95 to 300 nM) but also diminished the maximal response and the basal values.

On the basis of these results, a concentration-response curve with tiotidine was performed and unexpectedly showed tiotidine to be a potent inverse agonist, capable of decreasing cAMP basal levels 3 to 4 fold (7.2 ± 1.2 to 2.0 ± 0.3 pmol/10^6cells) with an $EC_{50} = 95 \pm 10$ nM (Fig. 2). Taking into account that an inverse agonist could be mistaken for an antagonist due to the inadequate sensitivity of the screening system, the sensitivity was increased. A concentration-response curve in presence of forskolin (a direct adenylyl cyclase agonist) was obtained (Fig. 2), because when basal activity is higher, the negative effect of an inverse agonist becomes clearly evident [6]. With this experiment, tiotidine proved to be a powerful inverse agonist.

Drug and drug-receptor classifications are based on the identity of equilibrium dissociation constants for drug-receptor complexes (Kd). It is accepted that agonists may

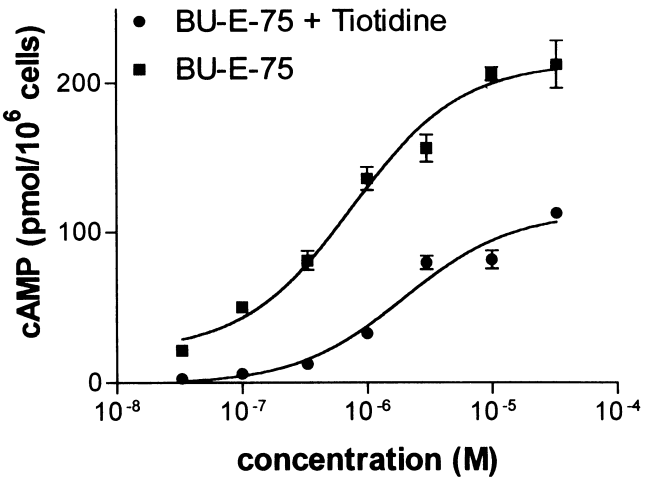

Fig. 1. Concentration-response curves of BU-E-75 on cAMP production in the presence (●) or absence (■) of 300 nM tiotidine. Each experiment represents four independent assays performed with different preparations of U937 cells. Each point is the mean ± SE of triplicate incubations.

Correspondence to: C. Davio

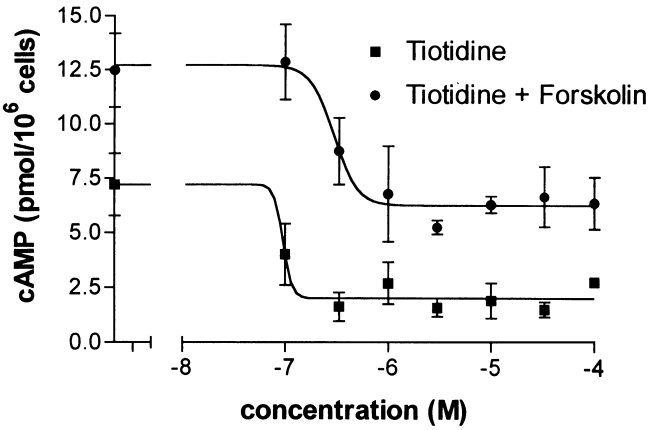

Fig. 2. Concentration-response curves of tiotidine on cAMP production in the presence (■) or absence (●) of 20 μM forskolin. Each experiment represents four independent assays performed with different preparations of U937 cells. Each point is the mean of triplicate incubations. The EC_{50} value is 95 ± 10 nM (mean ± SEM).

display Kd values that are not dependent solely upon the nature of the agonist and receptor but also upon the process of stimulus transduction [7]. For this reason, pharmacologists base receptor classifications primarily on antagonist Kd values, and use these to compare those present in natural physiological systems.

The key upon which all of these classifications rest is the assumption that the reference antagonists bind only to the receptor and do not, in any way, alter it. Traditionally, tiotidine is the reference antagonist for the H_2 receptor characterization [8]. However, at least in this system, tiotidine generates a pharmacological response corresponding to an inverse agonist. The concept of inverse agonism is currently a subject of discussion. If a ligand decreases the probability of receptor-transducer association or destabilizes the pre-existing association of these entities, then the ligand can be thought of as an inverse agonist. This is the case for tiotidine in this system. Therefore the implication of these results is that tiotidine should not be considered the reference antagonist for H_2 receptor classification.

Acknowledgments. This work was supported by grants from the Antorchas Foundation (A-13218/1-000026) and the Buenos Aires University (FA 005).

References

[1] Kuhn B, Schmid A, Harteneck C, Gudermann T, Schultz G. G proteins of the G(q) family couple the H_2 histamine receptor to phospholipase C. Mol Endocrinol 1996;10:1697–707.
[2] Smit M, Leurs R, Shukrula S, Bast A, Timmerman H. Rapid desensitization of the histamine H_2 receptor on the human monocytic cell line U937. Eur J Pharmacol 1994;288:17–25.
[3] Shayo C, Davio C, Brodsky A, Mladovan A, Lemos B, Rivera E, et al. Histamine modulates the expression of c-fos through cyclic AMP production via the H_2 receptor in the human promonocytic cell line U937. Mol Pharmacol 1997;51:983–90.
[4] Smit MJ, Leurs R, Alewijnse AE, Blauw J, Amerongen GPV, Vandevrede Y, et al. Inverse agonism of histamine H_2 antagonists accounts for upregulation of spontaneously active histamine H_2 receptors. Proc Natl Acad Sci USA 1996;93:6802–7.
[5] Davio C, Cricco G, Bergoc R, Rivera E. H_1 and H_2 histamine receptors in N-nitroso-N-methyluera (NMU)-induced carcinomas with atypical coupling to signal transducers. Biochem Pharmacol 1995;50:91–6.
[6] Kenakin T, Morgan P, Lutz M. On the importance of the 'antagonist assumption' to how receptors express themselves. Biochem Pharmacol 1995;50:17–26.
[7] Weiss J, Morgan P, Lutz M, Kenakin T. The cubic ternary complex III. Resurrecting efficacy. J Theor Biol 1996;181:381–97.
[8] Gajtkowski GA, Norris DB, Rising TJ, Wood TP. Specific binding of 3H-tiotidine to histamine H_2 receptors in guinea pig cerebral cortex. Nature 1983;304:65–7.