Biochemical Pharmacology, Vol. 37, No. 15, pp. 3035–3037, 1988.
Printed in Great Britain.

0006–2952/88 $3.00 + 0.00
Pergamon Press plc

SHORT COMMUNICATIONS

Functional β-adrenergic receptors in a human mammary cell line (HBL-100)

(*Received* 10 *August* 1987; *accepted* 4 *January* 1988)

Cyclic AMP has been postulated to be an important "negative" regulator of mammary lactogenesis [1–4]. Adenylate cyclase activity and endogenous cyclic AMP levels increase in mammary tissue during pregnancy and then fall abruptly after parturition [2]. Phosphodiesterase levels change in the opposite manner [2]. The initiation of lactation is thought to be dependent upon the drop in cAMP levels, and studies in mammary tissue isolated from lactating rats indicate that, when endogenous cAMP levels are raised, a decrease in the formation of lactalbumin [3] and lactose [1, 3–5] results. The exact mechanism(s) by which cAMP exerts such changes is unknown. However, an inhibition of lactose formation may be due, at least in part, to an inhibition of glucose uptake by mammary cells [5].

Lactogenesis is apparently under adrenergic control since epinephrine-sensitive adenylate cyclase activity is present in mammary tissue [6]. Recently, two groups have demonstrated the presence of β-adrenergic receptors in preparations obtained from mammary glands of lactating rats [7, 8]. In this paper we describe the presence of a functional β-adrenergic receptor system in a human mammary cell line, HBL-100 [9, 10]. This is the first report of such a system in an immortal epithelial cell line which has been reported previously to possess lactogenic properties [11, 12]. The results presented here indicate that HBL-100 cells provide an *in vitro* source of β-adrenergic receptors which can be studied in terms of the regulation of lactogenesis, as well as other human mammary gland cell functions.

Materials and methods

Membrane preparation. HBL-100 cells (passage 28) were grown on 100-mm plates containing McCoy's 5A medium supplemented with 10% defined fetal bovine serum and 100 μg/ml each of penicillin G and streptomycin sulfate. Cell culture was carried out at 37° in a 5% CO_2 humidified atmosphere in air. Confluent monolayers (approximately 2×10^7 cells/100-mm dish) were used for membrane preparations. Following removal of the culture medium, monolayers were washed twice with phosphate-buffered saline (PBS), and then cells from two or three dishes were pooled by scraping them (10 ml of 50 mM Tris buffer, pH 7.4/100-mm plate) into a 50-ml plastic centrifuge tube. A 10-sec homogenization (at a setting of 5) was then carried out on ice using a Brinkmann Polytron homogenizer. The ice-cold homogenate was centrifuged at 50,000 *g* for 10 min, the supernatant fractions were removed, and the resulting pellets were then resuspended in the same volume of buffer and centrifuged again. Membrane pellets isolated from two dishes of cells were resuspended in about 5 ml of buffer. Aliquots of membrane suspension were stored at −80°, and thawed preparations were used only once for binding studies. It was necessary to resuspend the thawed membrane preparations with a 3-ml plastic syringe and an 18-gauge needle prior to use in binding studies.

Binding assay. Binding measurements were carried out at 37° in 12 × 75 mm borosilicate glass tubes. Typical incubation mixtures contained various concentrations of 1-[4,6-*propyl*-^{3}H]dihydroalprenolol ([^{3}H]DHA; 110 Ci/mmol, Amersham Corp., Arlington Heights, IL) and approximately 30 μg of membrane protein dissolved in 0.15 ml of Tris buffer. Unless otherwise indicated, when D,L-propranolol (DL-P; Sigma, St. Louis, MO) was included, the final concentration was 10 μM. Specific binding was considered to be the difference between the total amount of [^{3}H]DHA bound and the amount of [^{3}H]DHA bound in the presence of 10 μM DL-P. In some studies, various concentrations of atenolol (Stuart Pharmaceuticals, Wilmington, DE) or ICI 118, 551 (Imperial Chemical Industries, PLC, Macclesfield, England) were included in the incubation mixtures. Binding was initiated by the addition of membranes to the incubation medium. Incubations were terminated by the addition of 4 ml of ice-cold Tris buffer and immediate vacuum filtration of the samples on 24-mm GF/C filters (Whatman, Clifton, NJ). Incubation tubes received two additional rinses with ice-cold Tris buffer (4 ml/rinse) to ensure quantitative transfer of the samples onto the filters.

Intracellular cAMP determinations. Confluent monolayers of cells grown on 35-mm dishes were incubated under normal growth conditions for 15 min with various concentrations of isoproterenol and 0.1 mM 3-isobutyl-1-methylxanthine. After incubation, the medium was removed immediately and the monolayers were rapidly rinsed twice with 2 ml of ice-cold PBS. The cells were then dissolved in 2 ml of ice-cold acidic ethanol (1 ml of 1 N HCl dissolved in 100 ml of ethanol), and the extracts were allowed to remain at 0–4° for an additional 30 min. The extracts were then transferred (with two additional washings of 1 ml each of acidic ethanol) to glass tubes, and the precipitate was removed by centrifugation. Supernatant fractions were transferred to another set of glass tubes and dried at 40° under a stream of nitrogen. Dried samples were dissolved in 0.5 ml of Tris/EDTA buffer (0.05 M Tris–HCl, pH 7.5, containing 4 mM EDTA) and stored at −20°. cAMP determinations were carried out using a commercial cAMP assay kit (Amersham) as described by the manufacturer.

Other methods. Protein determinations were made according to Lowry *et al.* [13]. Analyses of binding data were made according to Scatchard [14] and Hill [15, 16].

Results and discussion

Previous studies indicate that β-adrenergic receptor systems play a significant role in mammary gland physiology, particularly with respect to lactogenesis. Since HBL-100 is an immortal human mammary cell line possessing lactogenic properties, we have looked for β-adrenergic receptors in these cells. As shown in Fig. 1, [^{3}H]DHA bound to HBL-100 membranes rapidly at 37°, with receptor saturation (specific binding) occurring within 2 min of incubation. Specific binding was also saturable with respect to [^{3}H]DHA concentration (Fig. 2, right panel), and at a [^{3}H]DHA concentration as high as 1 nM it accounted for approximately one-half of the total bound ligand. A Scatchard analysis (Fig. 2, left panel) revealed a single class of high affinity receptor sites (Hill coefficient = ~1.00) with a K_d of 0.68 nM and a B_{max} (maximum number of [^{3}H]DHA binding sites) of 78.4 fmol/mg membrane protein. A comparison of the data reveals that the β-adrenergic binding characteristics of HBL-100 cells differed from those reported for other mammary cell preparations [7, 8], i.e.

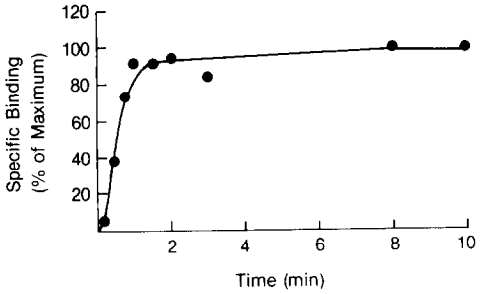

Fig. 1. Time course of specific binding of [^{3}H]DHA to HBL-100 membranes. Binding of [^{3}H]DHA (2.5 nM) was carried out at 37° as described in Materials and Methods. A representative experiment from three separate determinations is shown. Individual time points are an average in triplicate determinations. Maximum specific binding averaged 2.36 fmol (196 cpm) per sample.

HBL-100 cells contained (a) 3- to 5-fold fewer receptor sites per mg of protein, and (b) possessed receptors with an approximately 10-fold higher affinity for [^{3}H]DHA. These differences may be due to species variation, differences in *in vitro* versus *in vivo* growth conditions, and the fact that [^{3}H]DHA binding measurements on rat mammary acini preparations may reflect the binding characteristics of more than one cell type since these preparations are known to contain acinar secretory cells, myoepithelial cells, fibroblasts and other cellular elements [8].

A previous study reported that the β-adrenergic receptors of rat mammary acinar membranes are composed primarily of the β_2-subtype [8]. We have carried out a comparable study on HBL-100 cells and obtained similar results. β-Adrenergic subtype specificity was assessed by comparing the abilities of atenolol (a β_1-specific antagonist), ICI 118,551 (β_2-specific antagonist) and DL-P (not subtype specific) to inhibit the binding of [^{3}H]DHA to HBL-100 membranes. In this study, we compared the EC_{50} values (the concentration of competitor that effectively competes for 50% of the specific radioligand binding) of the various antagonists. As shown in Fig. 3, the EC_{50} of ICI 118,551 (3×10^{-9} M) was (a) substantially less than that of atenolol (7×10^{-7} M), and (b) similar to that obtained for DL-P (3×10^{-9} M). These results indicate that HBL-100 β-adrenergic receptors are primarily of the β_2-subtype, although the existence of β_1-receptors cannot be ruled out.

The initial response to β-adrenergic stimulation is usually an increase in adenylate cyclase activity, resulting in an increased intracellular cAMP concentration [17]. cAMP then functions as a second messenger, eliciting a host of other responses. The data in Table 1 indicate that the β-adrenergic receptors of HBL-100 cells are functionally coupled to adenylate cyclase since stimulation by isoproterenol resulted in an increased intracellular cAMP content. This stimulation occurred in a dose-dependent manner, with the maximum increase in cAMP content (~20-fold) occurring at a concentration of approximately 10^{-7} M isoproterenol. In separate experiments (data not shown), we found that a smaller maximal isoproterenol response (approximately 1.5-fold) occurred if 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase inhibitor) was omitted during agonist stimulation. Similar results have been reported by others, i.e. stimulation of rat mammary β-receptors results in an approximately 20-fold increase in intracellular cAMP content when IBMX is included in the β-agonist incubation medium, and only about a 2-fold increase occurs when IBMX is omitted [8].

Studies in a variety of systems have shown that β-adrenergic receptors play important roles in various aspects of glandular functions. In salivary glands, for example, they regulate saliva formation by influencing protein and ion transport properties of acinar secretory endpieces [18] and ion transport properties of ductal epithelium [19]. In mammary glands their influence on milk formation is less well-studied, although β-adrenergic-related increases in cAMP formation undoubtedly influence lactose production by the

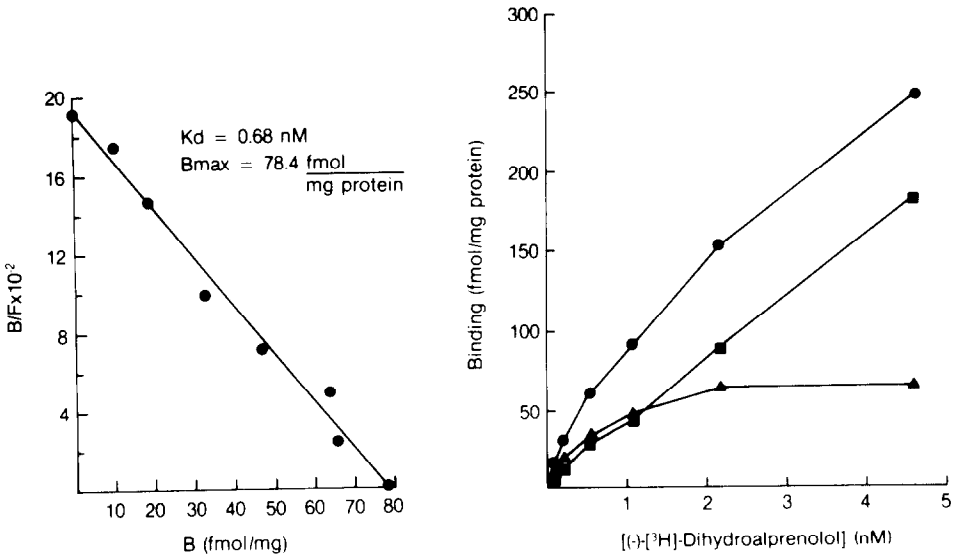

Fig. 2. [^{3}H]DHA concentration dependence on [^{3}H]DHA binding to HBL-100 membranes. The binding of [^{3}H]DHA to HBL-100 membranes was measured as described in Materials and Methods. Right panel: total binding (●—●), non-specific binding (■—■) and specific binding (▲—▲) of the radioligand to the membrane protein sample. A representative experiment from three separate determinations is shown. Individual concentration dependencies are the average of quadruplicate determinations. Left panel: Scatchard analysis of the specific binding curve.

Table 1. Effect of β-adrenergic stimulation on intracellular cAMP content in HBL-100 cells

Isoproterenol (M)	Experiment 1		Experiment 2	
	cAMP (pmol/dish)	relative cAMP concentration	cAMP (pmol/dish)	Relative cAMP concentration
0	14	1.00	9	1.00
10^{-9}	20	1.40	ND	ND
10^{-8}	98	7.00	46	51
10^{-7}	340	24.2	170	18.9
10^{-6}	290	20.7	170	18.9
10^{-5}	310	22.1	170	18.9
10^{-4}	220	15.7	ND	ND

Results of two separate experiments carried out in triplicate are shown. Isoproterenol treatment and cAMP determinations were carried out as described in Materials and Methods. Each 35-mm dish contained 2×10^6 cells. ND denotes results not determined. All cultures also received 0.1 mM 3-isobutyl-1-methylxanthine in the incubation medium during treatments with or without isoproterenol.

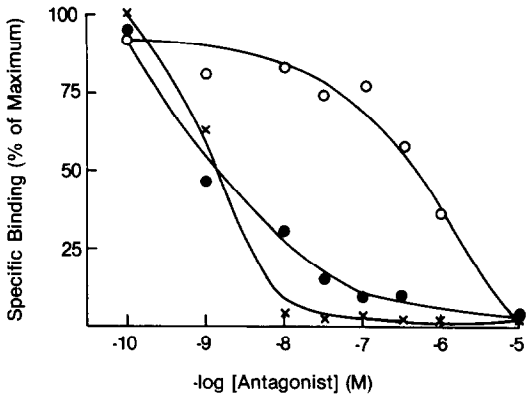

Fig. 3. Binding of [^{3}H]DHA to HBL-100 membranes in the presence of various concentrations of antagonist. Binding of [^{3}H]DHA (1.8 nM) to HBL-100 membranes was carried out in the presence of various concentrations of D,L-propranolol (●—●), ICI 118,551 (×—×) or atenolol (○—○). Specific binding determinations were made as described in Materials and Methods. A representative experiment of three separate determinations is shown. Individual concentration dependencies are an average of triplicate determinations.

acinar secretory endpieces. The results presented in this paper demonstrate that HBL-100 cells provide an *in vitro* system which can be used to investigate how lactose production, as well as other functions related to milk formation, may be regulated by a β-adrenergic receptor system in humans.

* Address correspondence to: Dr. Robert B. Wellner, National Institute of Dental Research, National Institutes of Health, Bldg. 10, Room 1A–01, Bethesda, MD 20892.

Clinical Investigations and Patient Care Branch
National Institute of Dental Research
National Institutes of Health
Bethesda, MD 20892, U.S.A.

ROBERT B. WELLNER*
XINJUN HE
YITZHAK MARMARY
BRUCE J. BAUM

REFERENCES

1. R. F. Loizzi, J. J. H. H. M. de Pont and S. L. Bonting, *Biochim. biophys. Acta* **392**, 20 (1975).
2. M. Sapag-Hagar and A. L. Greenbaum, *Eur. J. Biochem.* **47**, 303 (1974).
3. T. W. Perry and T. Oka, *Proc. natn. Acad. Sci. U.S.A.* **77**, 2093 (1974).
4. A. Munoz, S. Lavandero, E. Donoso, J. Puente and M. Sapag-Hagar, *Fedn Eur. Biochem. Soc. Lett.* **187**, 173 (1985).
5. C. J. Wilde and N. J. Kuhn, *Int. J. Biochem.* **13**, 311 (1981).
6. H. P. Bar, *Biochim. biophys. Acta* **321**, 397 (1973).
7. S. Lavandero, E. Donoso and M. Sapag-Hagar, *Biochem. Pharmac.* **34**, 2034 (1985).
8. R. A. Clegg and I. Mullaney *Biochem. J.* **230**, 239 (1985).
9. E. V. Gaffney, *Cell Tissue Res.* **227**, 563 (1982).
10. C. Caron de Fromental, P. C. Nardeux, T. Soussi, C. Lavialle, S. Estrade, G. Carloni, K. Chandrasekaran and R. Cassingena, *Expl cell Res.* **160**, 83 (1985).
11. F. P. Polanowski, E. V. Gaffney and R. E. Burke, *In Vitro* **12**, 328 (1976).
12. J. O'Connor, E. V. Chandrasekan and E. A. Davidson, *Fedn Proc.* **38**, 462 (1979).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. chem.* **193**, 265 (1951).
14. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
15. A. V. Hill, *Biochem. J.* **7**, 471 (1913).
16. A. Levitski, *Molecular Biology, Biochemistry,* and *Biophysics*, Vol. 28. Springer, New York (1978).
17. A. Levitski, *Physiol. Rev.* **66**, 819 (1986).
18. B. J. Baum, *J. dent. Res.* (Spec. Iss.) **66**, 628 (1987).
19. J. R. Martinez, *J. dent. Res.* (Spec. Iss.) **66**, 638 (1987).