interesting in relation to its hyperglycaemic action that the increased supply of glutamine from muscle is complemented by a stimulation of glutamine uptake and conversion to glucose by the kidney *in vitro* by 3-aminopicolinate [18, 19]. In contrast, Chen and Lardy [3] apparently discount any contribution of renal gluconeogenesis to the hyperglycaemic action of 3-aminopicolinate, since functional renalectomy did not impair the hyperglycaemic response to the agent. In addition to the kidney, the small intestine is a major site of glutamine utilization and the product of this metabolism is alanine [20], which may then serve as a gluconeogenic precursor in the liver. Thus, the increased muscle glutamine production induced by 3-aminopicolinate in the present study could stimulate gluconeogenesis directly in the kidney, or the liver, or indirectly via conversion to alanine by the small intestine.

In summary, the present work has shown that the hyperglycaemic agent, 3-aminopicolinate, has no effect on phosphoenolpyruvate carboxykinase in crude extracts of soleus and EDL muscles. Muscle lactate dehydrogenase was inhibited by the agent and this leads to interference with assay procedures dependent on this enzyme. In muscle incubations with valine, 3-aminopicolinate had no effect on alanine production or on net protein breakdown (tyrosine production), but markedly stimulated glutamine release. The increased glutamine release may serve directly, or indirectly via intestinal conversion to alanine, as a gluconeogenic precursor. Its increased peripheral supply by muscle may contribute to the hyperglycaemic action of the agent *in vivo*.

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Biochemical Pharmacology, Vol. 34, No. 11, pp. 2034–2036, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

β-Adrenergic receptors in rat mammary gland

(*Received* 10 *April* 1984; *accepted* 18 *October* 1984)

It has been shown by several authors that mammary gland responds to catecholamine hormones, assessed by the inhibition of lactose production in guinea pig and mouse mammary slices [1, 2], and that mammary tissue has an adrenaline-responsive adenylate cyclase [3]. It is also known from our studies and those from other laboratories [4–6] that the second messenger cyclic AMP is an important negative regulator of lactogenesis.

Since there are no studies on β-adrenergic receptors in mammary gland, we have attempted to identify and characterize such receptors in the rat mammary secretory tissue with the object of establishing their physiological significance for lactogenesis through regulation of cyclic AMP levels.

In the present work, we reported the results obtained by the use of (−)-[^{3}H]dihydroalprenolol [(−)-[^{3}H]DHA], a potent β-adrenergic antagonist radioligand, to identify, characterize and count β-adrenergic receptors in epithelial cell membranes from lactating rat mammary gland tissue and mammary acini.

Materials and methods

Materials were obtained from the following sources: (−)-[^{3}H]dihydroalprenolol (35.6 Ci/mmole) and [2,8-^{3}H]cyclic AMP (31.5 Ci/mmole) from New England Nuclear, Boston, MA, U.S.A.

All other reagents were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A.

Mammary gland membrane preparation. Membranes were prepared from abdominal mammary glands from lactating Sprague–Dawley rats (9–15 days) by using the method described for heart and other tissues [7]. Protein concentration was determined by the method of Lowry *et al.* [8].

Radioligand binding assay. Binding of (−)-[^{3}H]DHA was determined according to Williams *et al.* [7]. In brief, (−)-[^{3}H]DHA and membrane suspension (0.5 mg protein) were incubated for 15 min with shaking at 37° in 50 mM Tris–HCl buffer (pH 7.5) containing 10 mM $MgCl_2$ to a final volume of 150 μl. The reaction was terminated by dilution to 2 ml with ice-cold incubation buffer. Total binding for

(−)-[^{3}H]DHA was determined by measuring the amount of radioactivity retained on the Whatman GF/C filters. For non-specific binding assay, the incubations were performed in the presence of 10 μM (±)-propanolol. Substraction of non-specific binding from total binding yielded specific binding.

Isoproterenol-stimulated adenylate cyclase activity was determined measuring cyclic AMP production with the radioisotope dilution assay described by Ayad and Foster [9].

Data analyses were performed according to Williams *et al.* [7, 10], Scatchard [11] and Cheng and Prusoff [12].

Results and discussion

(−)-[^{3}H]DHA, a potent β-adrenergic antagonist, has been used to identify β-adrenergic receptors in membrane preparations from several tissues and also for verifying their affinity and specificity as true β-adrenergic receptors [7, 13–15]. Using direct binding methods, the number and affinity of (−)-[^{3}H]DHA binding sites in mammary gland membranes of lactating rats were assessed from binding isotherms and Scatchard analysis. (−)-[^{3}H]DHA binding to lactating mammary gland membranes was saturable with an apparent saturation at 220 fmoles/mg protein. Half-maximal saturation occurred at approximately 8 nM (−)-[^{3}H]DHA, which corresponds to an estimate of the equilibrium dissociation constant (K_d) (Fig. 1). Scatchard analysis of the binding isotherm revealed only one class of binding sites (Fig. 1, inset). In three independent experiments, each in duplicate, the mean K_d for binding of (−)-[^{3}H]DHA to membranes was 9.0 ± 1.2 nM (±S.E.M.) and n, the maximum number of (−)-[^{3}H]DHA binding sites, was 240.3 ± 23.9 fmoles/mg protein (±S.E.M.). Similar results were obtained utilizing membrane from purified acini. These acini were prepared according to Flint [16], by a controlled collagenase-hyaluronidase digestion of mammary gland lactating animals (results not shown).

Competition studies for (−)-[^{3}H]DHA binding sites by stereoisomers of isoproterenol demonstrate the appropriate stereoselectivity for β-adrenergic receptors. (−)-Isoproterenol was 10-fold more potent than the racemic stereoisomer and 100-fold than the corresponding dextro form (Fig. 2).

From these specificity binding studies it is possible to conclude that the adrenergic agonists competed for the (−)-[^{3}H]DHA binding sites in the following order of potency: (−)-isoproterenol > (−)-epinephrine ≫ (−)-norepinephrine (Fig. 2). This order is identical with the well-established order of potency for these agents in producing physiological β-adrenergic responses [17]. The adrenergic agonists had high affinity for the mammary gland binding site, with the following K_d values: 1.3 μM for (−)-isoproterenol, 4.1 μM for (−)-epinephrine and 41.2 μM for (−)-norepinephrine. These K_d values from the radioligand binding assay are in agreement with the (−)-epinephrine concentration for half-maximal stimulation of adenylate cyclase (K_a) from lactating rabbit mammary gland [3].

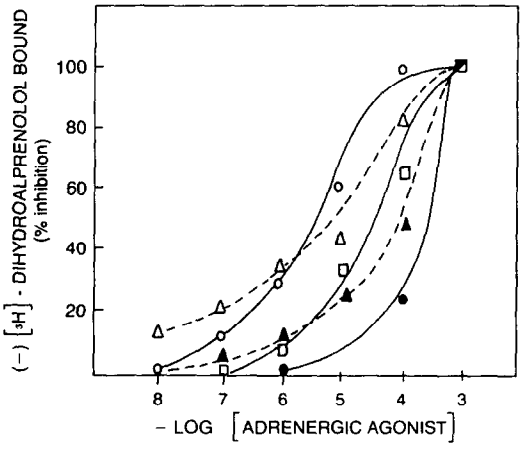

Fig. 2. Inhibition of specific (−)-[^{3}H]DHA binding to mammary gland membranes by adrenergic agonists. Mammary gland membranes (13 days of lactation) were incubated with (−)-[^{3}H]DHA (20 nM) as described under Materials and Methods, in the absence and presence of the following agonists: (−)-isoproterenol (○); (+)-isoproterenol (●); (±)-isoproterenol (□); (−)-epinephrine (△); and (−)-norepinephrine (▲). Each value represents the mean of duplicate assays.

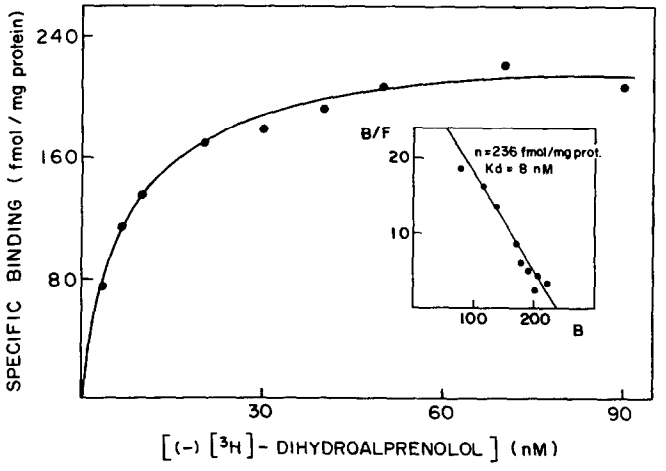

Fig. 1. Saturation curve and corresponding Scatchard analysis of (−)-[^{3}H]-dihydroalprenolol binding to mammary gland plasma membranes (9 days lactation). Mammary gland membranes were incubated with increasing concentrations of (−)-[^{3}H]DHA in the absence (total binding) or presence (non-specific binding) of 10 μM (±)-propanolol. Each value is the mean of duplicate determinations. This is a representative experiment which was independently performed three times. Inset: Scatchard plot of (−)-[^{3}H]DHA binding to mammary gland membranes.

Also, it could be demonstrated that compounds which are structurally related to catecholamines, such pyrocatechol and 3,4-dihydroxymandelic acid, but devoid of β-adrenergic activity, did not compete for the binding sites. At 1 mM concentration, these compounds inhibited the binding of (−)-[^{3}H]DHA to mammary membranes by less than 6.6 and 17.2% respectively.

Finally, a functional response to mammary β-adrenergic receptor triggering was verified by measuring the increase of cyclic AMP production after isoproterenol stimulation of adenylate cyclase in lactating mammary gland membranes (Fig. 3). These experiments confirmed the inhibition of lactose production by epinephrine, isoproterenol and dibutyryl cyclic AMP, reported by Loizzi *et al.* [1].

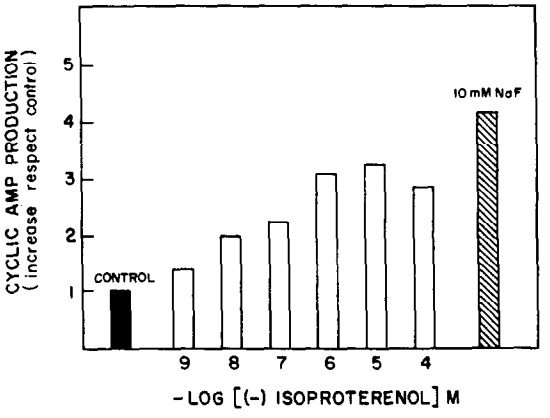

Fig. 3. Increase in cyclic AMP production after isoproterenol stimulation of adenylate cyclase in lactating mammary gland membranes (17 days). Mammary gland membranes were incubated in the presence of an ATP regenerator system and increasing concentrations of (−)-isoproterenol (ranging from 10^{-4} to 10^{-9} M) for 15 min at 37°. The data show the mean from two experiments with triplicate incubations and duplicate determinations of cAMP.

It may be concluded that the rat mammary gland has β-adrenergic receptors which are functionally operative and which have specificity and affinity characteristics similar to those described for other cells and tissues. Studies on the changes in the characteristics of the β-adrenergic receptors and of the number and distribution of the α-adrenergic receptors in relation to the lactation cycle are now in progress in order to establish their role in the process of lactogenesis.

Acknowledgements—This work was supported by Grant B 1138–8333 from the Departmento de Desarrollo de la Investigación, Universidad de Chile. We are indebted to Prof. A. L. Greenbaum, University College London, for his help in the preparation of this manuscript.

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Biochemical Pharmacology, Vol. 34, No. 11, pp. 2036–2040, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Microsomal interactions and inhibition of lipid peroxidation by etoposide (VP-16, 213): Implications for mode of action*

(*Received* 18 *July* 1984; *accepted* 15 *October* 1984)

The etoposide VP-16, 213 (NSC 141540, Fig. 1), a semi-synthetic podophyllotoxin derivative, is a promising anti-tumour agent for the treatment of small cell lung carcinoma [1, 2] and currently is undergoing extensive clinical evaluation. While the parent podophyllotoxin (PDP, Fig. 1) binds to microtubulin protein and arrests cells in metaphase [3], VP-16 has been shown to induce single-stranded breaks in DNA of HeLa cells [4] and to inhibit incorporation of nucleic acid precursors [5]. Recent studies by Wozniak and

* Presented, in part, at the American Association for Cancer Research, Toronto, Canada, May 9–12, 1984.