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IDENTIFICATION OF A BETA$_2$-ADRENERGIC RECEPTOR IN MAMMALIAN EPIDERMIS*

ELIZABETH A. DUELL

University of Michigan Medical School, Departments of Dermatology and Biological Chemistry, Ann Arbor, MI 48109, U.S.A.

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Abstract—The presence of a beta$_2$-adrenergic receptor in the epidermis has been demonstrated, based on the following pieces of information: (1) the addition of salbutamol, a beta$_2$-agonist, to slices of epidermal tissue increased the levels of cyclic AMP in the tissue above control levels in a dose-dependent manner with a maximum response after 5 min of incubation in 5×10^{-5} M salbutamol, (2) the addition of butoxamine, a beta$_2$-antagonist, in conjunction with isoproterenol or salbutamol reduced the epidermal cyclic AMP levels when compared to levels obtained with agonist alone, (3) practolol, a beta$_1$-antagonist, had little effect on the salbutamol-induced increases in the cyclic AMP levels and further elevated the levels of cyclic AMP obtained by the addition of isoproterenol, (4) the addition of propranolol to the tissue in conjunction with isoproterenol or salbutamol reduced the levels of cyclic AMP to near control values, and (5) Ro 20-1724, a cyclic nucleotide phosphodiesterase inhibitor, maintained the salbutamol-elevated cyclic AMP levels for a longer period of time.

Cyclic AMP has been implicated as an important modulator or control component in cellular proliferation [1, 2] and/or differentiation [3, 4] in a number of systems. The epidermis appears to be a suitable system for investigating the possible regulatory role for this compound since the epidermis is a tissue undergoing constant renewal wherein the cells normally progress through a differentiative process before sloughing into the environment. The presence of a beta-adrenergic responsive system in the epidermis has been established [2, 5, 6]. Since beta-adrenergic receptors have been subdivided further into β_1 and β_2, based on the response elicited from a series of sympathomimetic amines with respect to cardiac stimulation or bronchodilation and vasopressor activities [7], the further establishing of the β_1 or β_2 nature of the receptors in the epidermis was of interest from a scientific as well as a practical point of view. If cyclic AMP does have an important role in the control of proliferation or differentiation in the epidermis, then the type of receptor present in the epidermis would be important from the point of view of treating skin disease. It would seem to be advantageous to the patient to be able to alter the cyclic AMP levels in the epidermis with minimal involvement of cardiac function. Therefore, a β_1 and β_2-agonist, isoproterenol (IPR) [7], a β_2-agonist, salbutamol [8, 9], a β_1 and β_2-antagonist, propranolol [10], a β_1-antagonist, practolol [11], and a β_2-antagonist, butoxamine [10–12], were utilized in an attempt to delineate the nature of the receptor present in the epidermal tissue.

MATERIALS AND METHODS

Hairless mice (HRS/J) were obtained from Jackson Laboratories (Bar Harbor, ME), the Castroviejo keratome from the Storz Instrument Co. (St. Louis, MO), and isoproterenol, norepinephrine and propranolol from the Sigma Chemical Co. (St. Louis, MO). The following compounds were received as gifts: butoxamine from the Burroughs Wellcome Co. (Triangle Park, NC), papaverine hydrochloride from Eli Lilly & Co. (Indianapolis, IN), practolol from Ayerst Laboratories Inc. (New York, NY), QH-25B from Pharmacia As (Hillerød, Denmark), Ro 20-1724 from Hoffmann-LaRoche Inc. (Nutley, NJ), and salbutamol (albuterol) from the Schering Co. (Kenilworth, NJ).

Young adult male mice were killed by cervical dislocation, and epidermal strips were immediately removed with a Castroviejo keratome, adjusted to a depth of 0.1 mm. These epidermal strips were cut into small pieces and placed in beakers containing a Krebs–Ringer bicarbonate solution with added glucose. One mouse yields approximately 60–80 mg wet weight of epidermis, which is sufficient material for at least one time point.

Slices were preincubated for 20 min at 37° in a shaking water bath. At zero time the test compounds were added to the experimental beakers and buffer was added to the control beakers. At the designated time points, tissue samples were removed and immediately placed in liquid nitrogen. The samples were weighed, pulverized under liquid nitrogen, and homogenized in 6% trichloroacetic acid containing tracer amounts of tritiated adenosine 3′,5′-cyclic monophosphate ([^{3}H]-cyclic AMP). The homogenates were centrifuged at 18,000 *g* for 20 min at 4°. The supernatant fractions containing cyclic AMP were extracted three times with 6 vol. of water-saturated ethyl ether. The pellets from the centrifugation step were saved for the DNA determination [13], using salmon sperm DNA as the standard, and for the protein determination [14], using crystalline bovine serum albumin as the standard.

The cyclic AMP present in the ether-extracted supernatant fractions was partially purified by col-

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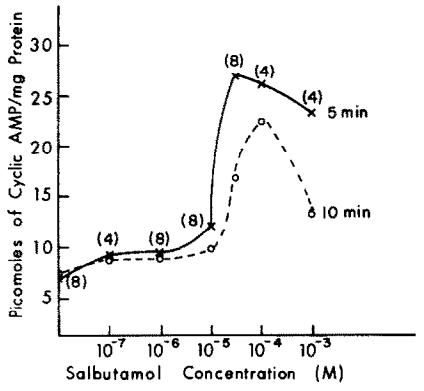

Fig. 1. Salbutamol-induced increases in cyclic AMP levels in incubated mouse epidermal slices. Keratomed mouse epidermal slices were preincubated for 20 min at 37° prior to drug addition. Tissue aliquots were removed at the time points indicated and immediately frozen in liquid nitrogen. Methods for cyclic AMP determination are given in Materials and Methods. The numbers in parentheses indicate the number of individual experiments represented at each data point.

umn chromatography of Bio-Rad AG 1-X2 resin in the chloride form. After application of the samples, the columns were washed with water, followed by elution of the cyclic AMP from the resin with a solution of 0.01 N HCl. The fractions containing the cyclic AMP were lyophilized, and subsequently the samples were resuspended in sodium acetate buffer, pH 4.4. An aliquot of the resuspended samples was counted to determine the percentage of the cyclic AMP recovered for assay. The amount of cyclic AMP in the samples was determined by the protein binding method [15] with each sample assayed in duplicate at three dilutions.

The human biopsy samples were obtained with a keratome setting of 0.1 mm from uninvolved areas and 0.25 to 0.45 mm for areas with lesions. The areas to be keratomed were infiltrated with 1% lidocaine without epinephrine just prior to the removal of the epidermis. All other procedures were identical to those listed for mouse tissue.

RESULTS

The curves shown in Fig. 1 indicate the levels of cyclic AMP obtained in the epidermal slices in response to increased concentrations of salbutamol in comparison to control samples after 5 and 10 min of incubation. The maximum accumulation of cyclic AMP (3.8-fold increase) occurred at a concentration of approximately 5×10^{-5} M salbutamol after 5 min of incubation, with a slightly lower accumulation (3-fold increase) after 10 min of incubation in 10^{-4} M salbutamol. The absence of a cyclic nucleotide phosphodiesterase (PDE) inhibitor in the experiment may account for the decrease in cyclic AMP levels after 10 min of incubation in comparison to the levels of cyclic AMP after 5 min of incubation.

In data not shown, the antagonists (practolol, propranolol or butoxamine) have no significant effect on the basal level of 6.9 ± 1.1 pmoles cyclic AMP/mg of protein present in the epidermal slices after 5 or 10 min of incubation. The effects of adding each of these antagonists to the epidermal slices in conjunction with salbutamol are demonstrated in Table 1. The addition of 5×10^{-5} M salbutamol to the slices resulted in a 3.8-fold increase in the cyclic AMP levels; this increase was reduced to control levels by the addition of 10^{-5} or 10^{-4} M propranolol to the tissue just prior to the addition of salbutamol.

The results obtained by incubating the epidermal slices with a beta$_1$-antagonist practolol, in addition to the agonist salbutamol, are given in Table 1. The addition of 10^{-5} M practolol to the epidermal slices, in addition to salbutamol, resulted in a slight (12 per cent) decrease in the cyclic AMP levels in comparison to the values obtained in the presence of salbutamol alone. At a 10-fold higher concentration of practolol, a 49 per cent decrease in the amount of cyclic AMP accumulation occurred in comparison to salbutamol alone after 5 min of incubation. The increased accumulation of cyclic AMP in the epidermal slices after 5 min of incubation with salbutamol was decreased 60 per cent by the addition of 10^{-5} M butoxamine to the tissue. A 10-fold increase in the concentration of this antagonist resulted in a slight but significant

Table 1. Effects of propranolol, butoxamine and practolol on salbutanol-increased cyclic AMP levels in mouse epidermal tissue*

Additions	cAMP† (pmoles/mg protein)	p value‡	N
None	7.5 ± 0.4		8
5×10^{-5} M Salbutamol	28.1 ± 7.1	0.021	8
5×10^{-5} M Salbutamol + 1×10^{-5} M Propranolol	7.1 ± 0.4	0.010	7
5×10^{-5} M Salbutamol + 1×10^{-4} M Propranolol	9.3 ± 0.9	0.015	7
5×10^{-5} M Salbutamol + 1×10^{-5} M Butoxamine	12.9 ± 1.2	0.023	6
5×10^{-5} M Salbutamol + 1×10^{-4} M Butoxamine	11.6 ± 0.9	0.022	6
5×10^{-5} M Salbutamol + 1×10^{-5} M Practolol	24.8 ± 6.5	0.445	7
5×10^{-5} M Salbutamol + 1×10^{-4} M Practolol	14.4 ± 3.2	0.296	7

* Epidermal tissue slices were preincubated for 20 min at 37° in buffer. The antagonists and salbutamol were added simultaneously at zero time.

† Data are expressed as the mean ± standard error of the mean.

‡ The P value for salbutamol alone is in comparison to control. All other P values compare the salbutamol plus antagonist to salbutamol alone. Because of the interrelated nature of the three tests, a multiple comparison procedure should be employed. Using an overall α-level of 0.1, the three treatment comparisons are significant.

Table 2. Effects of a cyclic nucleotide phosphodiesterase inhibitor in the presence of salbutamol on cyclic AMP levels in mouse epidermal tissue*

Additions	cAMP† (pmoles/mg protein)
None	10.4 ± 2.5
5×10^{-4} Ro 20-1724	17.6 ± 2.8
5×10^{-5} M Salbutamol	24.1 ± 2.7
5×10^{-5} M Salbutamol + 5×10^{-4} M Ro 20-1724	74.8 ± 9.3

* Epidermal tissue slices were preincubated for 20 min in buffer at 37°. At zero time Ro 20-1724 and/or salbutamol were added to the various beakers containing tissue. After 5 min of incubation the tissue slices were removed and immediately frozen in liquid nitrogen.

† Data are expressed as the mean ± standard error of the mean; N = three experiments.

additional decrease in the cyclic AMP levels. Similar results were observed after 10 min of incubation.

In an attempt to obtain further information concerning the effectiveness of the beta$_2$-agonist salbutamol, the PDE inhibitor Ro 20-1724 was added to the incubation mixture in addition to the agonist. The addition of Ro 20-1724 (10^{-5} to 5×10^{-4} M) to the epidermal tissue slices resulted in a maximum increase of 84 per cent in the cyclic AMP levels above control values after 5 min of incubation (data not shown). As shown in Table 2, salbutamol increased the cyclic AMP levels 2.3-fold above control levels after 5 min of incubation. The addition of the PDE inhibitor and the agonist resulted in a synergistic 7.2-fold increase in the cyclic AMP levels.

The results obtained with the addition of the beta$_1$ and beta$_2$-agonist (IPR) to the epidermal tissue slices are given in Table 3. The samples were also treated

Table 3. Effects of butoxamine and practolol on cyclic AMP levels elevated by a β_1 and β_2-agonist*

Additions	cAMP† (pmoles/mg protein)
None	7.5 ± 0.9
10^{-5} M Isoproterenol	50.9 ± 7.1
10^{-5} M Isoproterenol + 10^{-5} M Practolol	82.4 ± 15.5
10^{-5} M Isoproterenol + 10^{-4} M Practolol	36.6 ± 7.0
10^{-5} M Isoproterenol + 10^{-5} M Butoxamine	40.0 ± 10.2
10^{-5} M Isoproterenol + 10^{-4} M Butoxamine	22.3 ± 4.1

* Keratomed epidermal slices were preincubated at 37° for 20 min in buffer solution. At zero time isoproterenol and/or antagonist were added to the tissue in the various beakers. After 5 min of incubation the tissue samples were removed and immediately frozen in liquid nitrogen.

† Data are expressed as the mean ± standard error of the mean; N = eight experiments.

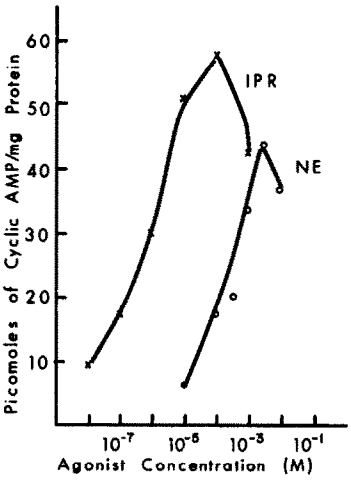

Fig. 2. Dose–response curves obtained by incubating mouse epidermal tissue slices with various concentrations of isoproterenol or norepinephrine. Keratomed mouse epidermal slices were preincubated for 20 min at 37° prior to the addition of isoproterenol (IPR) or norepinephrine (NE). Tissue samples were removed after 5 min of incubation and immediately frozen in liquid nitrogen. The procedures for cyclic AMP determination are given in Materials and Methods. Each data point represents the mean of two separate experiments.

with the specific antagonists to determine the effects that these compounds have on the stimulation of cyclic AMP accumulation by IPR. IPR increased the levels of cyclic AMP in the tissue 6.8-fold after 5 min of incubation. After 10 min of incubation in the absence of a PDE inhibitor, this decreased to 2.9-fold. If the beta$_1$-antagonist was added to the tissue just prior to the addition of the IPR, there was an increase in cyclic AMP accumulation to almost 10-fold by the addition of 10^{-5} M practolol. A 10-fold higher concentration in the practolol levels decreased the initial elevation to 4.9-fold. In contrast, the addition of 10^{-5} M butoxamine to the tissue samples just prior to the addition of IPR resulted in a decrease in the levels of cyclic AMP accumulation to 5.3-fold. A 10-fold increase in the beta$_2$-antagonist levels reduced cyclic AMP accumulation to 2.2-fold.

The dose–response curves obtained by the addition of a series of concentrations of IPR or norepinephrine (NE) to epidermal slices are shown in Fig. 2. IPR is more potent in that 10^{-4} M IPR produced an 8.3-fold increase in the cyclic AMP levels in the epidermal slices, while 5×10^{-3} M NE is required to produce a 6.5-fold increase in the cyclic AMP levels.

The results obtained with human biopsy samples are shown in Table 4. The addition of QH-25B (10^{-5} M), a putative beta$_2$-agonist, to involved psoriasis tissues or normal-appearing uninvolved tissues resulted in a 2-fold increase in the amount of cyclic AMP accumulated in the tissue samples compared to control samples. The same results were obtained whether DNA or protein content was used as the data base for expressing the cyclic AMP concentrations.

Table 4. Effect of lipophilic β_2-agonist on cyclic AMP levels in human psoriatic and uninvolved epidermis*

	cAMP† (pmoles/μg DNA)	cAMP† (pmoles/mg protein)
Uninvolved epidermis	0.7 ± 0.04	15.2 ± 0.4
+ 10^{-5} M QH-25B	1.5 ± 0.04	30.0 ± 1.4
Psoriatic epidermis	0.7 ± 0.12	10.6 ± 2.9
+ 10^{-5} M QH-25B	1.4 ± 0.07	22.9 ± 3.3

* Both involved (lesional) and uninvolved epidermal areas were removed with a keratome from each patient. The epidermal strips were cut into 3 mm squares and preincubated for 20 min in Krebs–Ringer bicarbonate buffer. At zero time QH-25B was added to the experimental beakers containing tissue. After 5 min of incubation the tissue samples were removed and immediately frozen in liquid nitrogen.

† Data are expressed as the mean ± standard error of the mean; N = three separate patient biopsies from involved and uninvolved areas.

DISCUSSION

The presence of a functional beta-adrenergic receptor in the epidermis has been demonstrated, as determined by an increase in the levels of cyclic AMP in the epidermis in response to the addition of IPR or epinephrine to the tissue [2, 5, 6]. What effect such an increase in the levels of cyclic AMP may have with respect to the control of proliferation or differentiation in this tissue remains an area of active research.

There are clinical data that suggest that beta antagonists can alter epidermal proliferation and differentiation [16–18]. Experimentally, the intradermal injections of propranolol can induce increased rates of proliferation in uninvolved skin of psoriatic patients in comparison to control population [16]. The data can be interpreted to indicate that a blockade of the beta receptor and a possible decrease in cyclic AMP levels may lead to increased proliferation. Practolol (a beta$_1$-antagonist), when administered clinically, can induce psoriasiform lesions [17, 18] in individuals. These data are more difficult to interpret in light of the data presented in this paper which indicate that the receptor in the epidermis is beta$_2$ in nature. The practolol effects may be non-specific or an indirect effect via a dermal component that alters the epidermal cyclic AMP system.

In the isolated epidermal system employed in this study, the keratomed epidermal strips contained less than 10 per cent dermal contamination. The epidermal strips responded to the beta$_2$-antagonist salbutamol with an increase in the levels of cyclic AMP in the tissue. The increase in cyclic AMP could be diminished by the addition of the beta$_2$-agonist butoxamine but not by the beta$_1$-antagonist practolol. Similar results were obtained with IPR, in that the increased levels of cyclic AMP were lessened with butoxamine addition, but the control levels were not achieved. Practolol did not decrease the IPR-induced increase in cyclic AMP levels; in fact, the cyclic AMP levels were increased. Also isoproterenol is a better agonist than norepinephrine in increasing the levels of cyclic AMP in the epidermal slices. From previous experiments, epinephrine is known to be a weak agonist. These data would also suggest that a beta$_2$ receptor occurs in the epidermis. Thus, in the isolated system relatively free of dermis, the receptor appears to correspond to the beta$_2$-adrenergic receptor. The beta receptor may have a significant input into the regulation of epidermal proliferation and differentiation by regulating the levels of cyclic AMP in the tissue.

The incubation of the patient material with the lipophilic beta-agonist QH-25B indicates that the compound is capable of increasing the levels of cyclic AMP in human tissue and may be efficacious clinically, due to its lipophilic nature. Ro 20-1724 and papaverine are both inhibitors of the cyclic AMP PDE activity that is present in the epidermis [19]. *In vitro*, these compounds increase the levels of cyclic AMP in involved and uninvolved epidermal tissue obtained from psoriasis patients. The topical application of a cream containing either of these compounds, but not cream alone, improves lesional areas of psoriasis [20, 21]. These data support the contention that cyclic AMP may be a critical component in the control of proliferation and differentiation in epidermal basal cells.

Additional information in the literature which relates to the role of cyclic AMP in the epidermis is obtained from tissue culture systems. In primary epidermal basal cell cultures obtained from neonatal mice, the addition of 8-bromo-cyclic AMP or cholera toxin to the cultures results in an intracellular increase in the cyclic AMP levels and an increase in the rate of proliferation without apparent loss of differentiative function [22]. In the adult guinea pig epidermal cell cultures, the addition of dibutyryl cyclic AMP or isoproterenol to the cultures inhibited cell proliferation, as determined by DNA synthesis [23]. In a G_2 assay system, the addition of dibutyryl cyclic AMP, isoproterenol, or other compounds capable of increasing cyclic AMP levels in human epidermal cultures inhibited epidermal mitosis [24]. Recent studies utilizing adult human primary epidermal cell cultures indicated that cyclic AMP at very low concentrations stimulated epidermal cell proliferation as measured by tritiated thymidine incorporation into DNA, but at moderate or high levels cyclic AMP inhibited cell proliferation [25].

Consequently, the beta-adrenergic system in the

epidermis and skin from various age groups and species needs to be defined more closely to determine the effects that this system may have on the maintenance of normal rates of proliferation and differentiation.

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