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Adenosine-Induced Alterations in the Adenosine 3′:5′-Monophosphate Levels in Mammalian Epidermis

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SUMMARY

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The addition of 0.5 mM adenosine to keratomed epidermal strips obtained from human volunteers or mice significantly increased the level of adenosine 3′:5′-monophosphate (cyclic AMP) in the tissue. The increase was dose dependent between 0.05 mM and 0.01 M adenosine. Theophylline decreased the adenosine-induced increase in cyclic AMP, while other cyclic nucleotide phosphodiesterase inhibitors such as papaverine or Ro 20-1724 augmented the increase. Adenosine 5′-monophosphate but not adenine increased the level of cyclic AMP in the epidermis. Epidermal basal cells in primary culture responded to the addition of adenosine and dipyridimole with a threefold increase in the cyclic AMP concentration in the cells. Adenosine increased the cyclic AMP levels in lesional and uninvolved areas of epidermis obtained from psoriatic patients.

INTRODUCTION

The initial investigation which associated increases in the cyclic AMP levels in brain with the addition of adenosine was carried out by Sattin and Rall (1). The observation was that adenosine and adenine nucleotides increased the cyclic AMP content of the tissue and that the adenosine effect could be blocked by the addition of methyl xanthines such as theophylline or caffeine. Since then a number of other tissues from various species have been shown to respond to the addition of adenosine with increased levels of cyclic AMP, probably due to the activation of adenylate cyclase (2–9). The methyl xanthines acted as competitive inhibitors of adenosine in several of these systems.

Adenosine is rapidly taken up by many tissues and converted into adenosine triphosphate. Initially there was a question as to whether the cell surface or an intracellular location was the site for the occurrence of the adenosine effect. The uptake of adenosine into cells can be greatly decreased or almost completely prevented by the addition of dipyridimole or hexobendine to the incubation solution (2, 3, 10). The data that provide evidence in support of the cell surface as the location for the initiation of the adenosine effect were provided by the augmentation of the increase in cyclic AMP intracellularly when dipyridimole or hexobendine was added to the incubation mixture with adenosine in comparison to the addition of adenosine alone (3, 6, 10).

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In this paper the results obtained by incubating the epidermis from psoriasis patients, adult mice, or neonatal mouse primary basal cell cultures with adenosine are presented.

MATERIALS AND METHODS

Materials. Adenine, adenosine, adenosine-5′-monophosphate (AMP), and L-isoproterenol-D-bitartrate (IPR) were obtained from Sigma Chemical Co. (St. Louis, Mo.), and theophylline was from Matheson, Coleman and Bell (Norwood, Ohio). Dipyridimole (Persantine) was a gift from Ciba-Geigy Pharmaceuticals (Ardsley, N. Y.), papaverine hydrochloride from Eli Lilly (Indianapolis, Ind.), and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) from Hoffmann LaRoche (Nutley, N. J.). Hairless mice (HRS/J) were obtained from Jackson Laboratories (Bar Harbor, Maine). The Castroviejo keratome was purchased from Storz Instrument Co. (St. Louis, Mo.).

Methods. Young adult male mice were sacrificed by cervical dislocation and the epidermis was removed immediately with a keratome set to a depth of 0.1 mm. The epidermal strips were placed on ice in Krebs–Ringer bicarbonate buffer with added glucose. Usually 60–80 mg of tissue was obtained from one mouse and this was sufficient material for one time point.

The tissue slices were incubated for 20 min in a 37°C shaking water bath in order to stabilize the cyclic AMP content. At zero time the compound(s) to be tested was added to some of the beakers containing the epidermal slices. However, if the required concentration of material

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was near the solubility limit of the test compound, the tissue slices were transferred to a beaker of Krebs–Ringer bicarbonate buffer containing the correct concentration of test drug. Approximately 60–100 mg of tissue was removed from the experimental or control beakers at the predesignated time point and immediately immersed in liquid nitrogen.

The frozen samples were weighed, were powdered with a mortar and pestle under liquid nitrogen, and were homogenized in 6% TCA containing tracer amounts of tritiated cyclic AMP in order to determine the recovery of the cyclic AMP to be assayed. The homogenates were centrifuged at 18,000*g* for 20 min. The pellets were set aside for protein and DNA determinations, the supernatant fractions were extracted with water-saturated ethyl ether, and the cyclic AMP in the supernatant fractions was subsequently partially purified by column chromatography on Bio-Rad AG 1-X2 resin. The fractions that contained cyclic AMP were pooled and lyophilized. The residues were resuspended in sodium acetate, pH 4.0, and were assayed for cyclic AMP content by the protein binding procedure (11). Each sample was assayed in duplicate at three dilutions. Protein content was determined by the method of Lowry *et al.* (12) using bovine serum albumin as standard. DNA content was determined by the method of Burton (13) with salmon sperm DNA as standard.

The primary epidermal cell cultures were prepared by the method of Marcelo *et al.* (14). Approximately 3 million cells were plated in T-25 flasks and were grown at 34°C for 72 h in Medium 199 with 13% fetal calf serum with streptomycin and penicillin in an atmosphere of 95% air, 5% CO_2.

At zero time the medium was removed from the T-flasks and 5 ml of fresh Medium 199 (without serum) containing the test compounds was added to each of the flasks. The flasks were then returned to the incubator. At the designated times, duplicate flasks were taken out of the incubator and the medium was removed. Three milliliters of 6% TCA containing tracer amounts of tritiated cyclic AMP was added to the flasks. The cells were scraped from the flasks and the flasks were rinsed with an additional 2 ml of TCA containing tracer cyclic AMP. The samples were then processed as indicated previously for the determination of the cyclic AMP, DNA, and protein contents of the samples.

RESULTS

The dose–response relationship between the concentration of adenosine in the incubation medium and the percentage increase in the cyclic AMP in the tissue after 10 min of incubation is shown in Fig. 1. Lower concentrations of adenosine (10^{-6} to 5×10^{-5} M) did not increase the levels of cyclic AMP in the epidermal slices. The basal level of cyclic AMP for the series of experiments was 7.1 ± 1.8 pmol of cyclic AMP/mg of protein. The highest increase in the cyclic AMP levels in this series of experiments occurred with the addition of 0.01 M adenosine after 10 min of incubation (327%), as shown in Fig. 1. Theophylline partially inhibited the increase in cyclic AMP levels up to the addition of 5×10^{-3} M adenosine.

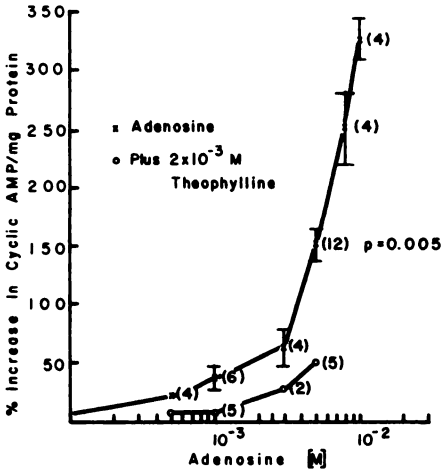

FIG. 1. *Cyclic AMP content of epidermal slices: Dose response to adenosine and blockade by theophylline*

Keratomed epidermal slices from hairless mice were incubated with various concentrations of adenosine in the presence or absence of theophylline. Aliquots of tissue were removed after 5, 10, and 15 min of incubation. The mean basal level of cyclic AMP was 7.1 pmol/mg of protein. The mean values and the SEM for the 10-min time points have been graphed. The numbers in parentheses indicate the number of different experiments for each drug concentration.

The lowest concentration of adenosine which gave a statistically significant increase of 80% ($P = 0.008$) in the cyclic AMP content after 5 min of incubation was 0.5 mM adenosine. This increase was blocked by the addition of 2 mM theophylline ($P = 0.001$).

Additional experiments were carried out to determine what effect, if any, would occur on the level of cyclic AMP in the treated tissue as a result of the simultaneous

TABLE 1

Effect of cyclic nucleotide phosphodiesterase inhibitors in combination with adenosine on the cyclic AMP levels in epidermal slices

All of the procedures are given under Methods. The incubations were carried out for 5 min. The results are expressed as the mean ± the standard error of the mean. *N* is the number of individual experiments for each data point.

Additions	pmol cyclic AMP/mg protein	*N*	*P* value
None[a]	6.5 ± 0.7	6	—
1 mM Adenosine[a,b]	11.1 ± 1.3	6	0.006
+ 2 mM Theophylline[b]	6.9 ± 0.7	6	0.001
+ 0.01 mM Papaverine[b]	17.7 ± 2.2	6	0.03
None[c]	7.2 ± 1.8	4	—
2 mM Theophylline[c]	8.4 ± 1.8	4	0.15
0.01 mM Papaverine[c]	8.1 ± 4.2	3	0.20
0.05 mM Ro 20-1724[c]	17.6 ± 2.8	3	$0.05 > P > 0.02$
5 mM Adenosine[c,d]	25.1 ± 1.3	3	$0.02 > P > 0.01$
+ 0.05 mM Ro 20-1724[d]	78.7 ± 19.0	3	0.02
+ 0.01 mM Papaverine[d]	41.1 ± 6.5	3	$0.01 > P > 0.001$

[a] Student's *t* test for paired data, control vs adenosine.

[b] Student's *t* test for paired data, adenosine vs adenosine plus theophylline or papaverine.

[c] Student's *t* test for paired data, control sample vs addition of PDE inhibitors.

[d] Student's *t* test for paired data, adenosine vs adenosine plus PDE inhibitors.

addition of the cyclic nucleotide phosphodiesterase (PDE) inhibitors and adenosine. As shown in Table 1, the increase in cyclic AMP obtained by the addition of 1 mM adenosine was inhibited 50% by the addition of 2 mM theophylline ($P = 0.001$). In contrast, the other nonmethyl xanthine PDE inhibitors such as papaverine and Ro 20-1724 significantly augmented the adenosine-induced increases in the cyclic AMP content of the epidermis. The addition of either theophylline alone (2 mM) or papaverine alone (0.01 mM) had no effect on the endogenous levels of cyclic AMP in the epidermis after 5, 10, or 15 min of incubation. Ro 20-1724 alone (0.05 mM), however, was able to significantly increase the levels of cyclic AMP in the tissue at all three time points.

The data presented in Table 2 indicate that after 5 min of incubation, adenine did not significantly increase the levels of cyclic AMP, whereas adenosine 5'-monophosphate was approximately 64% (36.9 pmol cyclic AMP/mg protein) as effective as adenosine (57.9 pmol of cyclic AMP/mg protein) in increasing the levels of cyclic AMP in the epidermal slices. A synergistic increase (10-fold increase above control) in the levels of cyclic AMP resulted from the addition of 0.01 μM isoproterenol (IPR) in combination with 5 mM adenosine compared to the increases obtained with either adenosine (403%) or IPR (291%) alone. Similar results were obtained for the 10- and 15- min time points with all of the test compounds.

The data presented in Table 3 show that a primary epidermal cell culture obtained from neonatal mice responded to the addition of 5×10^{-3} M adenosine by exhibiting a marked increase in cyclic AMP (216%). The addition of dipyridimole alone to the cultures produced a slight increase (50%) in the cyclic AMP accumulation. The basal level was 6.8 picomol of cyclic AMP/mg of protein. The addition of both compounds to the primary cell cultures resulted in an elevation (263%) in the levels of cyclic AMP similar to that obtained with adenosine alone.

The effect of adenosine on the cyclic AMP levels in epidermal slices obtained from psoriasis patients is presented in Table 4. The 5 mM adenosine induced a statistically significant 133% increase in the uninvolved tissue and a statistically significant 150% increase in the involved tissue in comparison to control epidermis.

TABLE 2

Effect of adenine and adenosine containing compounds, alone, and combined with IPR on the cyclic AMP levels in epidermal slices

Procedures are the same as those given for Table 1.

Additions	pmol cyclic AMP/mg protein	N	P value
None[a]	11.5 ± 2.6	4	
5 mM Adenosine[a,b,c]	57.9 ± 7.1	4	<0.01
5 mM AMP[a,b]	36.9 ± 5.5	3	<0.001
5 mM Adenine[a]	12.1 ± 0.9	3	NS
0.1 μM IPR[a]	45.0 ± 4.5	3	$0.05 > P > 0.02$
5 mM Adenosine[c] + 0.1 μM IPR[c]	131.2 ± 66.97	3	0.05

[a] Student's *t* test for paired data, control vs added compounds.
[b] Student's *t* test for paired data, adenosine vs AMP, $P < 0.001$.
[c] Student's *t* test for paired data, adenosine vs adenosine plus IPR.

TABLE 3

Effects of adenosine and dipyridimole on the cyclic AMP levels in primary cultures of epidermal basal cells

Day 3 cultures were utilized in the experiments. Procedures are given under Methods. The incubations were carried out for 10 min. The data are expressed as the mean ± the standard error of the mean from two separate culture preparations.

Additions	pmol cyclic AMP/mg protein
None	6.8 ± 1.8
5 mM Adenosine	21.5 ± 3.7
0.01 mM Dipyridimole	10.3 ± 1.2
5 mM Adenosine + 0.01 mM dipyridimole	24.7 ± 5.8

DISCUSSION

The presence of an "adenosine receptor" in the epidermis in addition to a β-adrenergic receptor (15) may be important as a mechanism for altering the cyclic AMP levels in the epidermis. The exact function of the cyclic AMP in the epidermis is an area of active research at this time. There is some circumstantial evidence from disease tissue to indicate that cyclic AMP may play a role in the control of proliferation and differentiation.

Ro 20-1724 and papaverine are inhibitors of the cyclic nucleotide phosphodiesterase(s) present in the epidermis (16). *In vitro* these compounds increase the levels of cyclic AMP in involved and uninvolved epidermal tissue obtained from psoriasis patients (16). The topical application of a cream containing either of these cyclic nucleotide phosphodiesterase inhibitors but not the cream alone improved the lesional areas in psoriasis (17, 18). Since psoriasis is a proliferative skin disease and since the topical application of compounds that can elevate cyclic AMP is efficacious in the treatment of psoriasis, then the levels of cyclic AMP may be important in the control of proliferation in epidermal basal cells.

Additional circumstantial evidence that implicates cyclic AMP in the control of proliferation comes from studies with β-adrenergic antagonists. Experimentally, increased proliferation occurred, as judged by the labeling index, when propranolol was injected intradermally into uninvolved epidermal areas of psoriasis patients, but saline injections did not produce this effect (19). Clinically, psoriasiform eruptions occurred after the treatment of patients with the β_1-antagonist practolol (20, 21). Thus the presence of antagonists capable of blocking the β-

TABLE 4

Elevation in cyclic AMP levels in uninvolved and involved epidermis from psoriasis patients after 10 min of incubation with adenosine

The procedures and incubations were carried out as indicated in Methods. The results are expressed as the mean ± the standard error of the mean. *N* was six patients.

Additions	pmol cyclic AMP/μg DNA	
	Uninvolved	Involved
None	0.3 ± 0.1	0.4 ± 0.1
5 mM Adenosine	0.7 ± 0.2	1.0 ± 0.3
P value[a]	<0.01	<0.01

[a] Student's *t* test for paired data, control vs drug addition.

adrenergic receptors, resulting in a possible decrease in cyclic AMP levels, increased the rate of proliferation.

The presence of an adenosine receptor in the epidermis permits an alternative means of increasing the cyclic AMP levels in the the tissue. The response of the epidermis to the addition of adenosine or 5′-AMP *in vitro* but not adenine resulted in an increase in the levels of cyclic AMP, and this response is similar to that observed in other tissues such as brain (1, 4), cell cultures of transformed cells derived from brain (5, 6, 9, 10), and heart (2, 3).

The adenosine-induced increase in the cyclic AMP content of the epidermis was blocked or decreased by the addition of theophylline. A competitive type of inhibition with methyl xanthines such as theophylline of this increase in the cyclic AMP level is characteristic of a P_1 receptor of the purinergic system as described by Burnstock (22). The P_2 purinergic receptor has a reverse order of potency of the agonists, namely, adenosine triphosphate > adenosine diphosphate > 5′-AMP > adenosine, and no increase in the cyclic AMP level is observed. Methyl xanthines have no effect on the P_2 receptor response. In the purinergic system adenosine triphosphate is released from the nerve terminals and serves as the transmitter or messenger of the nerve impulse. If the entire purinergic system exists in the epidermis, the release of adenosine triphosphate from the nerve terminal and the subsequent nucleotidase activity present in the epidermis could release sufficient adenosine or 5′-AMP to activate the P_1 type (adenosine) receptor.

Other cyclic phosphodiesterase inhibitors such as Ro 20-1724 or papaverine augmented the increase in cyclic AMP in epidermal tissue. This result is consistent with the known action of these compounds, namely, the prevention of the hydrolysis of cyclic AMP without interfering with the binding of adenosine to its receptor.

Both the uninvolved and the lesional epidermal tissues obtained from psoriasis patients respond to the *in vitro* addition of adenosine with an increase in the cyclic AMP content in the tissue. The tissue from the lesional area did not show a diminution in its capacity to respond to the addition of adenosine with an increase in the cyclic AMP in the tissue. These data may be useful in the future in the treatment of such a disease.

The primary cultures of epidermal basal cells obtained from neonatal mice respond to the addition of adenosine to the culture medium with an increase in the cyclic AMP level in the cells. The cell surface nature of the adenosine response is indicated by the dipyridimole effect in the cell culture system. The simultaneous addition of dipyridimole, which blocks the uptake of adenosine into the cells, with adenosine produced a slightly higher elevation in the cyclic AMP levels in the cell culture system in comparison to the addition of adenosine by itself. For the period of time required in these experiments, dipyridimole had no effect on the cyclic AMP levels.

The results presented in this paper indicate that the epidermal tissues from both normal and abnormal sources respond to the addition of adenosine in a manner indicative of the presence of an adenosine type of receptor on the tissue. The receptor may be similar to, if not identical to, the P_1 receptor of the purinergic system. Further investigation of the epidermal tissue will be necessary in order to determine how effective the adenosine receptor or purinergic system may be in alleviating the hyperproliferative state of the epidermis that occurs in various diseases.

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