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Decreased Accumulation of Cyclic Adenosine 3′,5′-Monophosphate in "Ischemic" Skin after 12-0-Tetradecanoyl-phorbol-13-acetate Treatment

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The effect of 12-0-tetradecanoyl-phorbol-13-acetate (TPA) on cyclic adenosine 3′,5′-monophosphate (cyclic AMP) level in adult mouse skin in response to ischemia was examined. The incubation of skin pieces in a buffered salts medium at 37°C resulted in a rapid accumulation of cyclic AMP. In mouse skin pieces maximum accumulation (about 6 times the basal level) occurred after 2 min incubation and was followed by a rapid decline in the cyclic AMP level. This "ischemic" rise in epidermal cyclic AMP was greatly reduced if skin was used 16 hr after a single application of 17 nmoles of TPA. The effect of TPA on cyclic AMP accumulation in response to ischemia was first observed at 1 hr after TPA treatment and was maximal at 4 hr. The lack of "ischemic" response in TPA-treated skin was not related to an increase in the activity of cyclic AMP phosphodiesterase after TPA application. In addition, the accumulation of cyclic AMP in skin in response to both ischemia and exposure to isoproterenol, adenosine, histamine, or prostaglandin E_2 (PGE_2) was not observed in skin treated with the tumor promoter TPA.

The basal level of adenosine 3′,5′-monophosphate (cyclic AMP) increases markedly in several tissues [1–4] including pig [5] and mouse skin [6,7] after excision. This change in cyclic AMP level has been considered due to ischemia (cessation of blood flow). Despite several efforts made to understand the molecular mechanism of this accumulation of cyclic AMP, the reason for its occurrence remains unresolved. In a recent report [8] we have shown that the pulse in cyclic AMP levels is not due to the release of materials which activate adenylate cyclase after binding to cellular receptors. In addition, it was also shown that the functional interaction between the receptors for various stimulators and adenylate cyclase is maintained during the cyclic AMP accumulation in response to ischemia.

Phorbol ester tumor promoters such as 12-0-tetradecanoyl-phorbol-13-acetate (TPA) greatly depress the accumulation of cyclic AMP in mouse epidermis in response to β-stimulation [7,9,10]. It is believed that phorbol esters exert this effect by disrupting the coupling of adenylate cyclase molecules with the β-adrenergic receptors [11]. In the present study, we set out to understand the ischemic increase in cyclic AMP in mouse skin using TPA.

MATERIALS AND METHODS

Materials

[8-^{3}H]cyclic AMP (27.5 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, England. TPA was purchased from Cambrian Chemicals Ltd., Cryodon, England. For this study, female Swiss albino mice (75–80 days old) were housed and treated with TPA (17 nmole; 0.2 ml acetone) as described before [12].

Methods

The procedure for the preparation of the skin pieces used to study changes in cyclic AMP levels in response to ischemia has been described previously [6]. Cyclic AMP determinations were done after column purification of the acid extracts from the skin [12]. Cyclic AMP phosphodiesterase activity was determined in epidermal extracts according to the method of Marks and Raab [13].

RESULTS

Effect of TPA on Cyclic AMP Accumulation in Mouse Skin Pieces in Response to Ischemia

The cyclic AMP levels were determined in excised skin pieces that were incubated in Bullough's medium [14] for various times at 37°C. A maximum 6-fold increase in cyclic AMP levels in acetone-treated skin pieces was observed at 2 min. The cyclic AMP levels started declining after 2 min and reached near basal level by 7 min. It was also shown that the "ischemic" rise in cyclic AMP was markedly decreased if skin was used 16 hr after a single application of 17 nmol of TPA (see reference 6). The decline in cyclic AMP level in TPA-treated skin in response to ischemia started as early as 1 hr after TPA application and maximal effect on ischemic cyclic AMP accumulation was observed by 4 hr (see Fig 1). Acetone itself had-slight effect on "ischemic" cyclic AMP accumulation at early 1–2 hr time points. However skin treated with acetone recovered by 3 hr and showed a normal cyclic AMP response to ischemia. The zero time basal cyclic AMP levels in these experiments were obtained by collecting skin pieces into cold medium as described previously [6]. There was some indication of lower zero time basal cyclic AMP level in TPA-treated skin than acetone-treated animals at later time points. However, we feel that these apparent differences are due to artifactual accumulation of cyclic AMP due to onset of ischemia in acetone- but not in TPA-treated skin pieces in the cold medium [8].

Effect of TPA on Cyclic AMP Phosphodiesterase Activity in Adult Mouse Skin

A simple mechanism to explain the lack of the "ischemic" accumulation of cyclic AMP in TPA-treated skin would be an increase in the activity of cyclic AMP phosphodiesterase after TPA treatment. As shown in Table I, no increase in the activity of this enzyme was observed in skin up to 4 hr after treatment. The low affinity enzyme activity increased nearly 3-fold over control between 4–8 hr after TPA application confirming earlier finding of Verma, Froscio, and Murray [15].

Augmentation of Ischemic Cyclic AMP Accumulation in Skin Pieces by Isoproterenol, Adenosine, Histamine or PGE_2

Several known stimulators of adenylate cyclase were tested for their effects on "ischemic" cyclic AMP levels. Fig 2 demonstrates the augmentation of "ischemic" cyclic AMP levels in skin pieces incubated for 2 min with various concentrations of isoproterenol. Maximal enhancement of "ischemic" cyclic AMP was obtained at 10 μM isoproterenol concentration. When this maximally active concentration of isoproterenol was added to the flask containing skin pieces, "ischemic" cyclic AMP accu-

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Abbreviations:
cyclic AMP: cyclic adenosine 3′,5′-monophosphate
PGE_2: prostaglandin E_2
TPA: 12-0-tetradecanoyl-phorbol-13-acetate

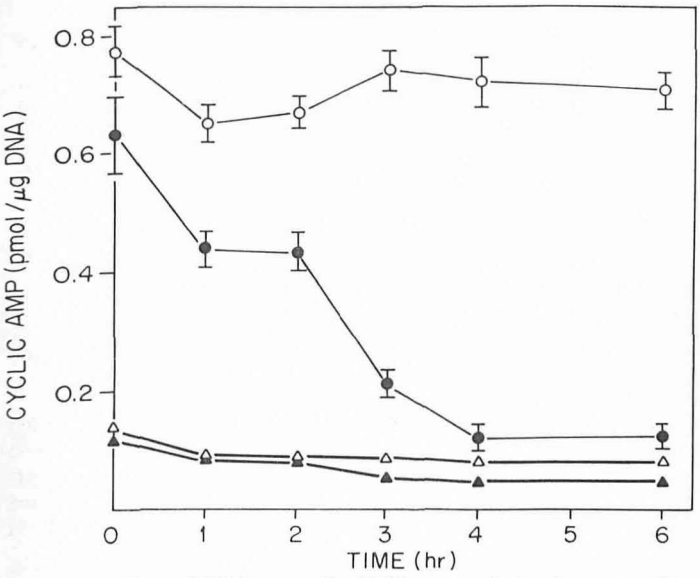

FIG 1. Effect of TPA on cyclic AMP accumulation in mouse skin pieces in response to ischemia. Mice were treated with acetone or 17 nmol of TPA as described in Methods. At various times after treatment, animals were killed and the cyclic AMP determinations in acetone (○) or TPA-treated (●) skin pieces were done after a 2 min incubation at 37°C. The basal levels of cyclic AMP in acetone (△) and TPA-treated (▲) skin were determined as described previously (6). Each *point* is the mean ± SE of determinations carried out on 6 skin pieces from 3 animals.

TABLE I. *Effect of TPA on cyclic AMP phosphodiesterase activity in adult mouse epidermis*[a]

Time after application (hr)	Cyclic AMP phosphodiesterase activity (p moles cyclic AMP/min/mg protein)			
	2 μM		400 μM	
	Acetone	TPA	Acetone	TPA
2	19.91 ± 2.10	23.64 ± 2.01	380 ± 25	417 ± 26
4	25.00 ± 1.80	24.36 ± 2.13	513 ± 40	586 ± 35
8	44.90 ± 6.78	41.05 ± 4.20	523 ± 42	1500 ± 72

[a] At various times after acetone (0.2 ml) or TPA (17 nmol/mouse in 0.2 ml acetone) treatment epidermal extracts were prepared and phosphodiesterase activity was measured at 2 μM and 400 μM cyclic AMP concentrations as described in Methods. Each value is the mean ± SE of determination done on 6 samples from 3 animals.

mulation in acetone-treated skin but not in TPA-treated skin was augmented by about 2.5 times. The enhanced cyclic AMP levels by incubation of adult mouse skin pieces for 2 min at 37°C, were further elevated by the addition of optimally active concentrations of either adenosine, histamine or PGE_2 [4,8]. All these compounds mentioned above did not induce 'ischemic' cyclic AMP levels in TPA-treated skin pieces (Table II).

DISCUSSION

The incubation of mouse skin pieces in Bullough's medium at 37°C causes a rapid accumulation of cyclic AMP in this tissue and maximal rise in cyclic AMP level was seen at 2 min after incubation. Mufson, Simsiman, and Boutwell [7] also reported similar increase in cyclic AMP levels in mouse skin at 1 min after excision by simply maintaining the tissue at room temperature. An identical phenomenon of cyclic AMP increase has also been observed in brain and heart [4]. Such a rapid and transient increase in cyclic AMP level has been considered due to ischemic response. The "ischemic" rise is not observed if skin is used 4–16 hr after a single application of the tumor promoter TPA (see Fig 1 and reference 6). It should also be noted that TPA does not cause any shift in the accumulation of cyclic AMP in response to ischemia [6]. An increase in the activity of epidermal cyclic AMP phosphodiesterase is not likely to be a primary reason for the TPA effect. Thus, although TPA induces an increase in the activity of low affinity cyclic AMP phosphodiesterase after 4–8 hr, the effect of TPA on the "ischemic" accumulation of cyclic AMP starts within an hour and fully develops by 4 hr.

Addition of either adenosine, histamine, isoproterenol, or PGE_2 resulted in an additional cyclic AMP accumulation in acetone-treated skin pieces in response to ischemia suggesting that the functional relationship between the receptors for several activators is maintained well during ischemic response. All these stimulators of cyclase were totally ineffective in enhancing cyclic AMP accumulation in TPA-treated skin. This observation indicates that both the ischemic and normal receptor-cyclase mechanism were blocked in skin following TPA application. If "ischemic" cyclic AMP accumulation is dissociated from the normal receptor-cyclase activation [8], the mechanism by which TPA exerts its effect on "ischemic" cyclic AMP response may not be related to the property of TPA to induce defective coupling between cellular receptors and cyclase molecules [11], which results in a negative response of several

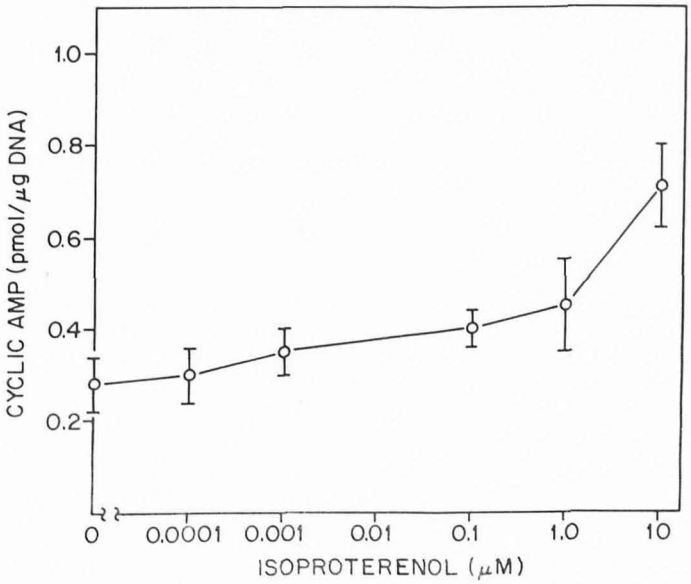

FIG 2. Agumentation of "ischemic" cyclic AMP acculation by various concentrations of isoproterenol. Cyclic AMP determinations were done after a 2 min incubation at 37°C with various concentrations of isoproterenol. Basal level of cyclic AMP (prior to 2 min incubation) was 0.09 ± 0.01 pmol/μg DNA. Each *point* is the mean ± SE of determinations carried out on 6 skin pieces from 3 animals.

TABLE II. *Stimulation of cyclic AMP accumulation by either adenosine, histamine, PGE_2 or isoproterenol in incubated skin pieces from TPA-treated adult mice*[a]

Treatment	Cyclic AMP (p moles/μg DNA)	
	Acetone	TPA
None	0.66 ± 0.11	0.11 ± 0.01
Adenosine (0.5 mM)	1.16 ± 0.17	0.13 ± 0.02
Histamine (0.2 mM)	1.00 ± 0.09	0.08 ± 0.01
PGE2 (3 μM)	1.14 ± 0.21	0.07 ± 0.01
Isoproterenol (10 μM)	1.70 ± 0.21	0.11 ± 0.02

[a] Mice were treated with 0.2 ml acetone or TPA (17 nmol in 0.2 ml acetone/mouse). After 17 hr, skin pieces from these mice were incubated at 37°C for 2 min in the presence of various stimulators of adenylate cyclase. Each value is the mean ± SE of determinations carried out on 6 skin pieces from 3 animals.

Basal levels of cyclic AMP in acetone and TPA treated skin pieces were 0.16 ± 0.01 and 0.10 ± 0.01 p mol/μg DNA, respectively.

stimulators of adenylate cyclase. We feel that TPA can also trigger some mechanism which affects the total cyclase activity under either "ischemic," receptor-cyclase, or both controls.

Finally, we propose that the "ischemic" cyclic AMP pulse is probably sensitive to the redox state changes of the cells. Such redox changes can be induced by TPA through the generation of free radicals [16–19] such as superoxide anion and hydroxyl radicals. A similar mechanism involving the redox state in the "ischemic" cyclic AMP accumulation in mouse skin has been proposed earlier [8].

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