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ACCUMULATION OF CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE IN ADULT AND NEWBORN MOUSE SKIN: RESPONSES TO ISCHEMIA AND ISOPROTERENOL

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Levels of cyclic adenosine 3′,5′-monophosphate in adult mouse skin pieces were rapidly increased on incubation at 37°C, or on exposure to isoproterenol. Accumulation of the cyclic nucleotide under both conditions was greatly decreased in newborn mouse skin, or in adult skin treated with the tumor promotor 12-0-tetradecanoyl-phorbol-13-acetate.

The basal level of cyclic adenosine 3′,5′-monophosphate (cAMP) in pig skin has been shown to increase rapidly following its removal from the body [1]. This effect has been attributed to ischemia and has also been reported for heart and cerebellum (see [1] for references). The physiologic basis for the rise in cAMP levels is unknown, but its occurrence has obvious implications for studies on cyclic nucleotide metabolism in skin pieces. In the present paper, this phenomenon has been studied in skin from newborn mice and in adult mouse skin treated with the tumor promoter 12-0-tetradecanoyl-phorbol-13-acetate (TPA). This latter treatment has previously been shown to lead to a decreased response to the β-adrenergic stimulator isoproterenol [2,3] and to both elevated and decreased levels of epidermal cAMP [3,4].

MATERIALS AND METHODS

Female Swiss albino mice were housed and treated with TPA (20 nmole; 0.2 ml in acetone) as described before [5]. [8-^{3}H]cAMP (sp act 27.5 Ci/mmole) was obtained from the Radiochemical Centre, Amersham, England. TPA was obtained from Cambrian Chemicals Ltd., Croydon, England.

All operations for the preparation of skin pieces for the study of cAMP changes during ischemia were carried out in a 4°C room. Mice were killed by cervical dislocation; the skin was excised and wetted thoroughly with cold Bullough's [6] medium. The dermis was rapidly scraped off with a scalpel blade, the skin cut into two pieces (approximately 1 cm × 1.5 cm each) and placed into cold Bullough's buffer. Skin pieces were collected into cold buffer from 3 animals for each time point, transferred to the laboratory, and placed in a flask containing 20 ml of buffer prewarmed to 37°C. The total time taken to collect 6 skin pieces at 4°C and transfer them to buffer at 37°C varied between 2 and 2.5 min. After an appropriate incubation time, skin pieces were removed to liquid nitrogen, individually ground in a prechilled mortar, and extracted with cold 5% trichloroacetic acid, as described previously [5]. cAMP determinations were carried out after column purification of the acid extracts [5].

It is recognized that the procedure of scraping used was somewhat imprecise and that the skin pieces will have had a variable amount of dermal tissue associated with them. Although histologic examination of scraped skin pieces indicated only a relatively small contamination with dermal material, its contribution to the results presented is not known. In our hands dermal contamination was more variable when sampling was attempted using a keratotome. In preliminary experiments it was demonstrated that ischemic rises in cAMP (see below) also occurred in epidermal scrapings of skin pieces [5] made after incubation at 37°C.

The procedure for the incubation of skin pieces in experiments with isoproterenol has been described [5]. The pieces were incubated at 37°C for 10 min before the addition of isoproterenol to the flasks. Samples were prepared for cAMP determination as described above.

RESULTS

As shown in the Table, the incubation of skin pieces from control (acetone-treated) adult mice in Bullough's medium at 37°C resulted in a rapid accumulation of cAMP. The zero time points in these experiments were obtained by collecting skin pieces into cold medium as described in *Materials and Methods*. A markedly decreased accumulation of cAMP was observed 16 hr after the application of the tumor promoter TPA. As shown in Figure 1, skin from 3- and 7-day-old mice also accumulated less cAMP during ischemia when compared with skin from 21- or 75-day-old animals. In these experiments there was some indication of higher zero time basal levels of cAMP in the skin of older animals. However, we feel that there is a strong possibility that these apparent differences are due to artifactual accumulation of cAMP following the death of adult animals and the collection of skin pieces into cold buffer. Thus, in a separate experiment, zero time cAMP levels were

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Abbreviations:
cAMP: cyclic adenosine 3′,5′-monophosphate
TPA: 12-0-tetradecanoyl-phorbol-13-acetate

TABLE. *Effect of TPA treatment on the accumulation of cyclic AMP in incubated skin pieces from adult mice*

Skin pieces were incubated at 37°C as described in *Materials and Methods*. Each point represents the mean ± SE of determinations carried out on 6 skin pieces from 3 animals.

Time of incubation (*min*)	Cyclic AMP (pmole/μg DNA)	
	Acetone	TPA
Zero time	0.162 ± 0.044	0.076 ± 0.010
2	0.970 ± 0.154	0.110 ± 0.015
5	0.424 ± 0.130	0.036 ± 0.004
7	0.210 ± 0.028	0.043 ± 0.010

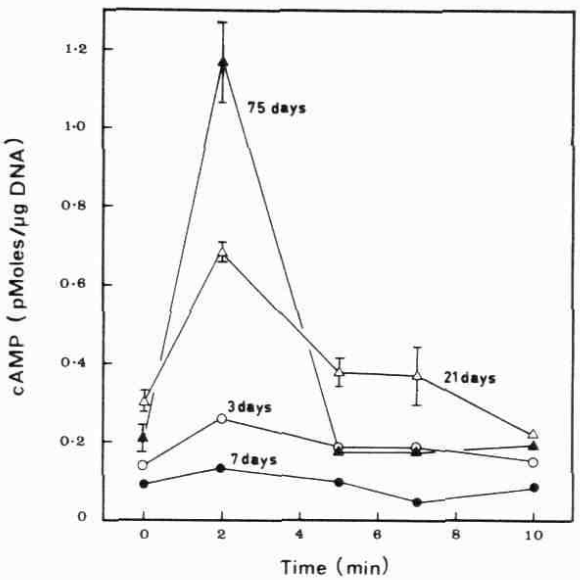

FIG. 1. Accumulation of cAMP in mouse skin pieces during ischemia. Skin pieces were collected from mice at 3 (○), 7 (●), 21 (△), and 75 (▲) days of age and incubated at 37°C as described in *Materials and Methods*. Each point represents the mean ± SE of determinations carried out on 6 skin pieces collected from 3 animals.

determined on skin pieces immediately transferred to liquid nitrogen following removal from the animal. The levels obtained with 3-day and 75- to 80-day-old mice were 0.165 ± 0.005 and 0.169 ± 0.006 pmole/μg DNA, respectively.

The ability of croton oil or TPA to greatly depress the accumulation of cAMP in mouse epidermis in response to isoproterenol both in vivo and in vitro has been well documented [2,3,5]. Similarly, no accumulation of cAMP in epidermis was observed after the intraperitoneal injection of isoproterenol into 3-day-old mice. The injection of isoproterenol into adult mice (0.01 μmole/gm body weight) increased the basal cAMP levels from 0.29 ± 0.05 pmole/μg DNA (saline-injected controls) to 2.9 ± 0.05 pmole/μg DNA over a 10-min period. Values in saline- and isoproterenol (0.04 μmole/gm body weight)-injected 3-day-old mice were 0.16 ± 0.009 and 0.18 ± 0.005 pmole/μg DNA, respectively. Similar results were obtained when skin pieces from 3-day-old and adult mice were incubated in the presence of 10^{-5} M isoproterenol (Fig. 2). In this case some limited accumulation of cAMP was observed in skin from 3-day-old mice in the presence of isoproterenol. The basal initial

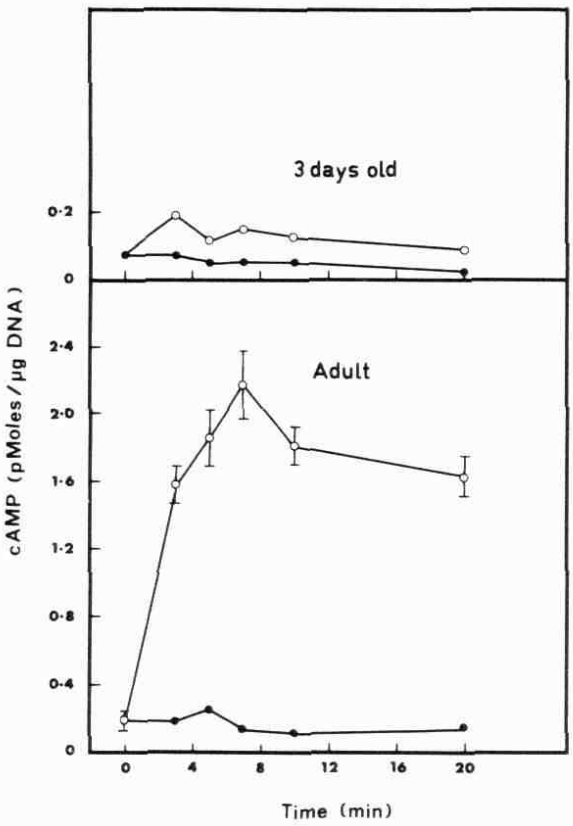

FIG. 2. Accumulation of cAMP in mouse skin pieces induced by isoproterenol. Skin pieces from adult (75 days) and 3-day-old mice were incubated as described in *Materials and Methods* in the absence (●) and presence (○) of 10^{-5} M isoproterenol. Each point represents the mean ± SE of determinations carried out on 6 skin pieces from 3 animals.

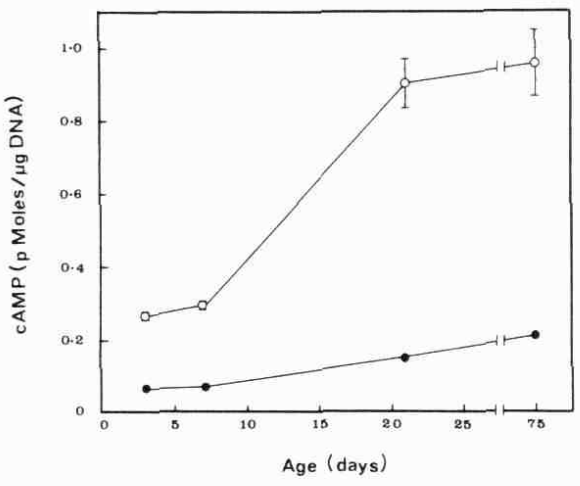

FIG. 3. Accumulation of cAMP induced by isoproterenol in skin pieces from mice of varying ages. Skin pieces were incubated for 7 min in the absence (●) or presence (○) of 10^{-5} M isoproterenol as described in *Materials and Methods*. Each point represents the mean ± SE of determinations carried out on 6 skin pieces from 3 animals.

levels of cAMP were also higher in adult mouse skin, but this may, at least partially, have been due to differences in accumulation during ischemia (Tab.).

The ability to accumulate cAMP in response to isoproterenol developed between 7 and 21 days of age (Fig. 3). Again, there was an increase in the basal levels of cAMP in skin pieces from older mice, which probably reflected the development of an ischemic response as discussed above.

DISCUSSION

The present results have confirmed that incubation of adult mouse skin at 37°C leads to a rapid but transient increase in cAMP levels. The mechanism for this increase is totally unexplained. Previous experiments with pig skin have indicated that β-adrenergic stimulation is not involved [1]. In preliminary experiments (unpublished) we have shown that accumulation of cAMP in mouse skin is not inhibited by either α- or β-adrenergic antagonists. Skin from adult mice which is incubated at 37°C for 10 min, and in which cAMP levels had reverted to near-control values, accumulated cAMP rapidly in the presence of isoproterenol. This accumulation is mediated via β-adrenergic receptors and has been reported in a number of laboratories [3,5,9,12]. Other possible stimulators which are known to elevate cAMP levels in skin are PGE_1 and PGE_2 [7–9], histamine [10], and adenosine [11]. It is, of course, quite possible that cAMP accumulation is not mediated through a receptor-adenylate cyclase interaction, but by some change in the internal milieu of the cell.

The molecular basis for the lack of cAMP accumulation during ischemia or in response to isoproterenol following TPA treatment or in newborn animals is unknown. An increase in the activity of epidermal cyclic nucleotide phosphodiesterases is not likely to be a primary cause for the TPA effects. Thus, although TPA induces an increase in the activity of low-affinity phosphodiesterases after 6 hr [4,9], the lack of response to isoproterenol develops within 2 hr [3]. Similarly, the effect of TPA on the ischemic accumulation of cAMP develops within 2 to 3 hr (unpublished data). Other possibilities include alterations in the number and/or properties of cellular receptors or in the interaction between receptors and adenylate cyclase.

The observations with skin from 3-day-old mice are of particular interest as they add to the list of apparent similarities between newborn mouse skin and adult skin that has been treated with tumor promoters [13]. The data are therefore consistent with models proposing that tumor promoters induce a dedifferentiation of epidermal cells [13,14].

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