POSSIBLE INVOLVEMENT OF CYCLIC AMP IN INFLAMMATION INDUCED BY A SURFACTANT

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Abstract—Alkyl dimethylbenzylammonium chloride (alkyl-DBAC), a cationic surfactant, produces acute exudative inflammation accompanied by an enhancement of energy metabolism. The mechanism of metabolic changes, consequently the cyclic AMP level and the effects of certain drugs were determined in the gastrocnemius muscles of rats in which acute exudative inflammation had been induced by intramuscular injections of alkyl-DBAC. A transient increase in the cyclic AMP level was noted at 15 to 30 minutes after the injection of alkyl-DBAC, and this elevation was antagonized by chlorpromazine, diphenhydramine, promethazine, aspirin and indomethacin. The time course of increasing tendency in the cyclic AMP level after the injection of histamine closely paralleled that of alkyl-DBAC. These results suggest that cyclic AMP may be involved in the metabolic changes with inflammation induced by alkyl-DBAC.

Enhancement of energy metabolism in acute exudative inflammation is reportedly induced by alkyl dimethylbenzylammonium chloride (alkyl-DBAC), a cationic surfactant, the principal effects of which were an increase in vascular permeability, edema and leukocyte migration (1).

Cyclic AMP is considered to be involved in the metabolism of ATP (2, 3), glycogen and lipids, but unrelated to glycogenolysis arising from muscle contraction (4). Other workers have reported data indicating anti-inflammatory action of cyclic AMP and suggested the inhibitory effects on degranulation of mast cells (5-7), platelets (8-10) and neutrophiles (11, 12), vascular permeability increase (7), edema (13, 14) and granuloma formation (13).

If cyclic AMP does indeed play certain roles in inflammatory responses, then there should be evidence that the level is being influenced in the inflamed tissues, and assuming that changes in the cyclic AMP level are antagonized by anti-inflammatory agents, it is plausible that this cyclic nucleotide perhaps plays certain roles in inflammation processes.

The cyclic AMP level and effects of some anti-histaminics and anti-inflammatory agents, all of which were determined in the gastrocnemius muscles of rats in which acute exudative inflammation had been induced by alkyl-DBAC were investigated and the findings are reported herein.

MATERIALS AND METHODS

Materials

Dodecyl-DBAC (Kao Soap Co., Ltd., Tokyo), the kit for determination of cyclic AMP (Boehringer Mannheim), histamine, trichloracetic acid (TCA), HCl, petroleum ether (Wako Pure Chemical Industries Ltd., Tokyo), chlorpromazine, promethazine (Shionogi...
& Co., Ltd., Osaka), diphenhydramine (Kowa Co., Ltd., Tokyo), and aspirin (Yoshitomi Pharmaceutical Industries, Ltd., Tokyo) were used. Twice-distilled water was used in all experiments.

**Determination of cyclic AMP level**

Male Wistar rats and the experimental procedures to produce inflammation were essentially the same as those described in the preceding report (1). Alkyl-DBAC and histamine were intramuscularly injected in one foot at $10^{-2}$ M and 200 μg/0.1 ml in saline, respectively, and into the other foot was injected 0.1 ml of saline, and such served as control. The rats were sacrificed by micro-waves at scheduled times, and the gastrocnemius muscle of each was immediately excised and frozen in an ethanol-dry ice solution. The muscle (1 g) was homogenized in 20 ml of ice cold 6% TCA aq. and centrifuged at 1,000×g for 10 min at 0°C. To the supernatant (15 ml) was added 1 ml of 1 N HCl aq. and extraction was carried out 5 times with 100 μl of petroleum ether saturated with water. The extract was further evaporated and washed with water. The prepared residue was dissolved in 50 ml of water and used for the determination of cyclic AMP. The concentration of cyclic AMP was determined using the test kit and the radio isotope dilution method as established by Gilman (15) was applied.

**Measurement of effects of some anti-inflammatory agents**

Rats in groups of six were pretreated with the following drugs 1 hr before the injection of alkyl-DBAC: chlorpromazine 20 mg/kg (s.c.), diphenhydramine 20 mg/kg (s.c.), promethazine 20 mg/kg (s.c.), aspirin 150 mg/kg (p.o.), indomethacin 20 mg/kg in gum arabic (p.o.) and saline 1 ml/kg (s.c.) as control. The cyclic AMP level was determined 30 min after the injection of alkyl-DBAC in both inflamed and controlled muscles.

**RESULTS**

The cyclic AMP level was augmented at 15 min and reached its peak (114% of the

![Graph](https://via.placeholder.com/150)

Fig. 1. Changes in the cyclic AMP level in the gastrocnemius muscle of rats in which inflammation had been induced by alkyl-DBAC. Alkyl-DBAC (0.1 ml of $10^{-3}$ M in saline) was injected into one gastrocnemius muscle and the contralateral gastrocnemius muscle injected with saline served as the control. Each point and vertical bars represent mean±S.E. of 5 rats. **, Significantly different from control (p<0.05). Cyclic AMP level in control muscle, Cyclic AMP in inflamed muscle, Cyclic AMP level of inflamed muscle as percentage of control.
control) 30 min after inflammation was induced by alkyl-DBAC, but returned to the control level after 1 hr, as seen in Fig. 1.

A time course similar to that seen with alkyl-DBAC was also observed in muscle in

Fig. 2. Changes in the cyclic AMP level in the gastrocnemius muscle of rats in which inflammation had been induced by histamine. Inflammation was induced by administration of 200 µg/0.1 ml i.m. of histamine. Each point and vertical bars represent mean ± S.E. of 5 rats. *: Application of t-test yields a p value of <0.1. •——•: Cyclic AMP level in control muscle, ○——○: Cyclic AMP level in inflamed muscle, ×——×: Cyclic AMP level of inflamed muscle as percentage of control.

Fig. 3. Effects of drugs on the cyclic AMP level elevated by alkyl-DBAC. Rats were pretreated with these drugs 1 hr before administration of alkyl-DBAC i.m. (0.1 ml of 10^-2 M), and 30 min after the injection of alkyl-DBAC the cyclic AMP level was determined in both inflamed and control gastrocnemius muscle. Each value and vertical bars represent mean ± S.E. of 6 rats. **: Significantly different from control muscle of rats pretreated with saline (p<0.05). +: Significantly different from inflamed muscle of rats pretreated with saline (p<0.05). □: Control muscle, ■: Inflamed muscle by alkyl-DBAC.
which inflammation had been induced by histamine, as illustrated in Fig. 2. The onset and peak here, was also noted at 15 and 30 min, respectively, and the peak cyclic AMP level was 112% of the control. The application of t-test yielded a P value of <0.1 for the change.

The increase in the cyclic AMP level at 30 min was antagonized by pretreatment with diphenhydramine (20 mg/kg, s.c.), chlorpromazine (20 mg/kg, s.c.), promethazine (20 mg/kg, s.c.), aspirin (150 mg/kg, p.o.) and indomethacin (20 mg/kg, p.o.) in the muscle where inflammation had been induced by alkyl-DBAC, and the inhibitory rate ranged from 50 to 60% of the inflamed muscle of the control rats, as shown in Fig. 3. No significant difference was noted between the controlled and the inflamed muscles of rats pretreated with the above mentioned drugs, but the cyclic AMP level in the controlled muscles was significantly reduced with these drugs by 70 to 80%, of that of the controlled muscle pretreated with saline.

**DISCUSSION**

As seen in Figs. 1 and 3, a transient elevation was noted in the cyclic AMP level of the inflamed muscles induced by alkyl-DBAC, and this elevation was antagonized by the pretreatment with anti-histaminics and anti-inflammatory agents known to have suppressing effects on energy metabolism (16–18). As to the role of cyclic AMP in the inflammation induced by alkyl-DBAC, therefore, it seems likely that cyclic AMP may be partly involved in the initiation of the metabolic changes via acceleration of glycolysis or lipolysis.

This elevation of the cyclic AMP level and energy metabolism could not be attributed to a direct initiation by alkyl-DBAC, but, rather, appeared to be a result of certain chemical mediators released by alkyl-DBAC, since activity of succinate oxidation and Na⁺-K⁺-Mg²⁺ ATPase was directly inhibited by alkyl-DBAC, in vitro, although the activity was enhanced in the muscle where inflammation had been induced by alkyl-DBAC (1). The involvement of chemical mediators was also supported by the findings that histamine tended to increase the cyclic AMP level of the muscle (Fig. 2), and alkyl-DBAC-induced elevation of the cyclic AMP level was inhibited by anti-histaminics and anti-inflammatory agents (Fig. 3). In fact, there are reports on histamine and serotonin releasing action (19, 20) by surfactants from mast cells (21, 22), platelets (23) and neutrophiles (24), and such was attributed to lytic action on the membrane (25, 26). Thus, the released chemical mediators would induce an increase in vascular permeability and edema, and elevate the cyclic AMP level as seen in Fig. 2 and as has been demonstrated in other tissues (27, 28).

Cyclic AMP is considered to exert inhibitory effects on energy dependent degranulation of mast cells (5–7, 29) and neutrophiles (11, 12), platelets aggregation (8–10), leukocytes migration and phagocytosis (30, 31), vascular permeability increase (7), edema (13, 14) and granuloma formation (13). The data obtained in the present work suggest that the inflammatory properties of endogenous cyclic AMP do not conflict with those described above, since the doses required for the anti-inflammatory effects of exogenous cyclic AMP (10 mg/kg, i.p.) (7) were relatively higher than that of the endogenous cyclic AMP.

In addition, from the results illustrated in Fig. 3, which show that the cyclic AMP level
in the controlled muscle was decreased by some anti-inflammatory agents, it is plausible that saline itself induced inflammation or anti-inflammatory agents reduced the physiological level of cyclic AMP.

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