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SHORT COMMUNICATIONS

Effect of histamine on lipolysis and adenosine 3′,5′-monophosphate levels in canine adipose tissue*

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THE ABILITY of histamine to stimulate adenosine 3′,5′-monophosphate (cyclic AMP) formation has recently been demonstrated in brain and lung slices of various animal species.[1–3] In addition, it has been shown that histamine may be an important lipolytic agent in the dog since glycerol release can be stimulated in perfused inguinal fat pads with doses as low as 2 μg.[4] We have observed that histamine can markedly elevate free fatty acid (FFA) and glycerol levels in isolated dog fat cells; the ED_{50} for histamine-stimulated lipolysis is 8×10^{-7} M compared to 4×10^{-7} M for norepinephrine.[5] The purpose of this series of experiments was to determine whether or not the lipolytic effect of histamine is associated with increased levels of fat cell cyclic AMP and to observe if histamine can stimulate increases in cyclic AMP similar to effects observed with another lipolytic amine, norepinephrine.

Fat cells were prepared as follows: subcutaneous adipose tissue was removed from the inguinal region of male, mongrel dogs weighing 15–30 kg and placed immediately in warm Krebs–bicarbonate buffer containing 2% albumin. The tissue was then sliced and 5-g aliquots were incubated for 1 hr in 5 ml of the buffer containing 15 mg of collagenase. After this the isolated cells were prepared as described by Rodbell.[6] Two ml of a 1:3 suspension of the isolated fat cells was incubated with 4 ml of the Krebs–bicarbonate–albumin buffer system under an atmosphere of 95% O_2 and 5% CO_2 at 37°. The suspension was incubated for 10 min prior to the addition of drugs. If blocking agents were added, the incubations were continued for an additional 15 min before addition of agonists. After removal of 0·3 ml for determination of FFA,[7] the incubation was terminated by shaking the remainder of the suspension in 15 ml of cold acetone. After centrifugation to remove protein precipitate and evaporation to remove acetone, the samples were washed four times in 2 vol. of ether, evaporated to dryness, and redissolved in 0·1 ml of distilled water for determination of cyclic AMP by the protein binding assay of Gilman.[8]

Dry weights of isolated fat cells were estimated by placing 0·5 ml of the washed fat cell suspension onto tared 25-mm Millipore filter papers, removing the fluid phase by vacuum filtration, drying the papers for 24 hr, and weighing the dried papers.

The effect of histamine ($1{\cdot}67 \times 10^{-6}$ M) on cyclic AMP levels and FFA release at different times during a 60-min incubation is shown in Fig. 1. The peak increase in cyclic AMP concentration occurred at 2 min; increases in free fatty acid release were first detected at 5 min with levels rising rapidly after that time during the entire 60-min period. Control levels of cyclic AMP were 2320 $\pm$ 440 (S. E.) pmoles/g (dry wt) and a value of 4000 $\pm$ 500 pmoles/g was achieved in 2 min with a gradual decline in these levels throughout the remainder of the period. FFA rose from a control level of 3·72 $\pm$ 0·60 (S. E.) μEq/g (dry wt) to a high of 33·95 $\pm$ 6·24 μEq/g at 60 min.

A dose–response relationship for histamine stimulation of cyclic AMP accumulation in isolated dog fat cells is illustrated in Fig. 2. The stimulatory effect was dose dependent over a range of histamine concentrations from 2×10^{-7} to 4×10^{-5} M, causing increases in cyclic AMP levels from a control value of 3840 $\pm$ 480 pmoles/g to a maximum of 6100 $\pm$ 560 pmoles/g. Concentrations above 4×10^{-5} M had no additional effect. The ED_{50} value for histamine-induced elevation of cyclic AMP in dog fat cells was approximately 5×10^{-6} M. Although these concentrations are higher than the concentrations of histamine needed to stimulate lipolysis, the dose–effect relationships are generally parallel. The lipolytic effect of histamine is dose dependent over a range of concentrations from 8×10^{-8} to 8×10^{-6} M with an ED_{50} of 8×10^{-7} M.[5]

A comparison of the stimulatory effects of histamine or norepinephrine at a concentration of 8×10^{-6} M is shown in Fig. 3. Both agents promote a significant increase in cyclic AMP levels ($P < 0{\cdot}01$); histamine stimulates a 62 per cent increase as compared to a 96 per cent increase with norepinephrine. In the presence of propranolol (10^{-5} M), an 87 per cent inhibition of the

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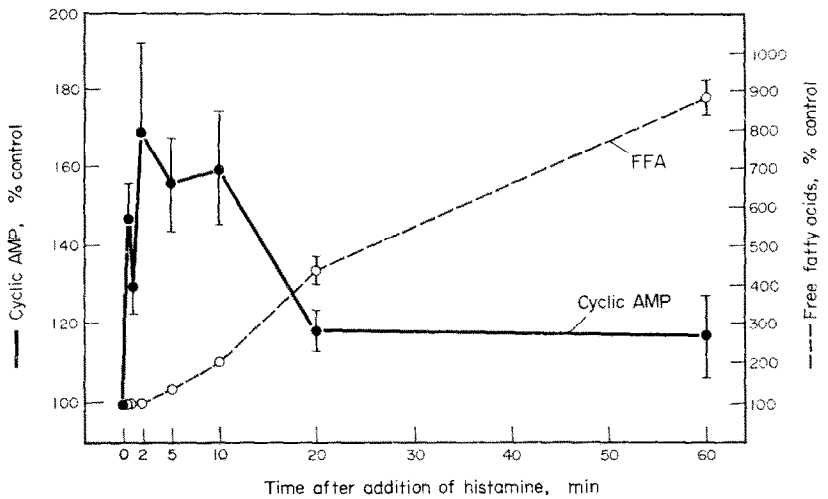

FIG. 1. Effect of histamine ($1{\cdot}7 \times 10^{-6}$ M) on cyclic AMP levels and free fatty acid release at various intervals in isolated dog fat cells. Points and bars represent mean ±S. E. in three experiments.

norepinephrine effect was observed whereas the stimulatory effect of histamine was unaffected. Tripelennamine in concentrations of 10^{-4} and 10^{-3} M produced no inhibition of the norepinephrine effect and a slight inhibition of the histamine effect (mean of 22 per cent inhibition with 10^{-3} M).

Although these concentrations of tripelennamine cause a significant inhibition of histamine-stimulated lipolysis,[5] there is an apparent lack of inhibition of cyclic AMP elevation. This is consistent with the findings of Palmer; in experiments using histamine to elevate cyclic AMP in guinea pig lung slices neither tripelennamine nor chlorpromazine blocked the response.[3] The significance of the failure of the antihistamine to block histamine-stimulated cyclic AMP elevation cannot be determined until other agents are used and the problem is examined more fully.

The effect of theophylline on histamine-induced elevation of fat cell cyclic AMP was also examined in one experiment. These results are shown in Fig. 4. The levels of cyclic AMP after a 5-min incubation increased from 2300 to 4960 pmoles/g with histamine alone ($1{\cdot}67 \times 10^{-5}$ M), to 2820 pmoles/g with theophylline alone (10^{-3} M), and to 25,160 pmoles/g with histamine plus theophylline. These results are consistent with our observations that theophylline can enhance the lipolytic effect of histamine

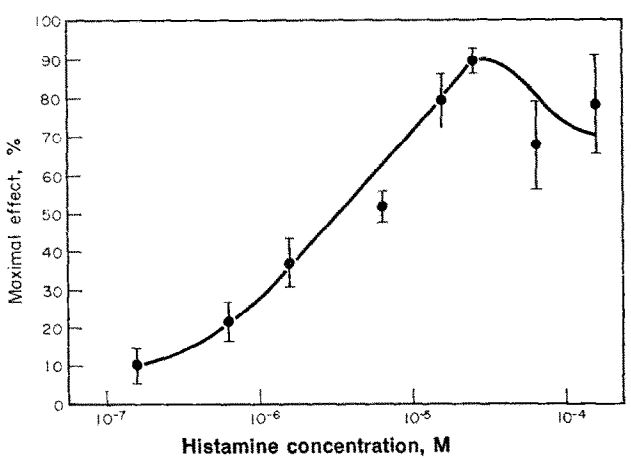

FIG. 2. Dose–response curve for concentrations of histamine (2×10^{-7} to 2×10^{-4} M) stimulating increases in cyclic AMP levels in dog fat cells. Cyclic AMP was measured 2 min after addition of histamine. Points and bars represent mean ±S. E. obtained in three experiments.

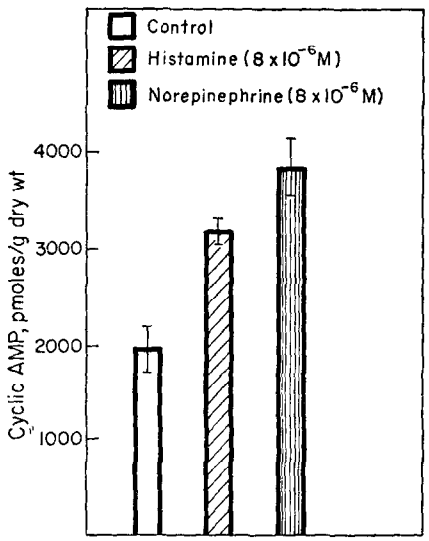

FIG. 3. Comparison of the stimulatory effect of a moderate concentration of histamine or norepinephrine on cyclic AMP levels in dog fat cells. All cyclic AMP measurements were made 2 min after addition of drugs. Bars represent mean ±S. E. of three experiments.

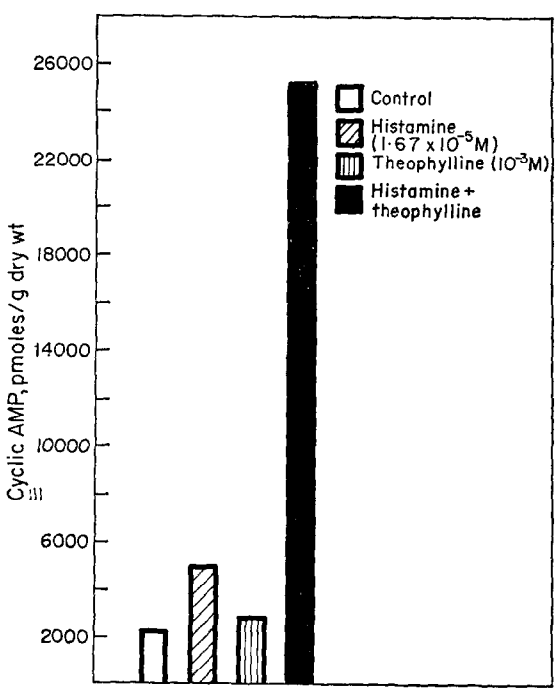

FIG. 4. Effect of theophylline on histamine-induced elevation of fat cell cyclic AMP. Cyclic AMP was measured 5 min after addition of drugs. Bars represent two observations in a single experiment.

(2×10^{-7} M) greater than 10-fold. The effects of theophylline on histamine-induced elevation of cyclic AMP are nearly identical to effects observed when theophylline is added to a similar concentration of epinephrine in isolated rat fat cells.[9]

The observation that histamine elevates cyclic AMP levels in a dose-dependent fashion in isolated dog fat cells, coupled with the observation that cyclic AMP levels rise rapidly before a rise in free fatty acids is detectable, suggests that the lipolytic effect of histamine in the dog is mediated by increases in the levels of cyclic AMP. The ability of histamine to stimulate cyclic AMP accumulation is similar to that of norepinephrine which suggests that histamine may be an important lipolytic agent in the dog and possibly in other species.

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REFERENCES

1. M. CHASIN, I. RIVKIN, F. MAMRAK, S. G. SAMANIEGO and S. M. HESS, *J. biol. Chem.* **246,** 3037 (1971).
2. J. FORN and G. KRISHNA, *Pharmacology* **5,** 193 (1971).
3. G. C. PALMER, *Biochim. biophys. Acta* **252,** 561 (1971).
4. B. B. FREDHOLM, H. C. MENG, B. OBERG and S. ROSELL, *Life Sci.* **7,** 1209 (1968).
5. V. R. GRUND and D. B. HUNNINGHAKE, *Abstracts, Fifth Int. Congress Pharmacology,* p. 90. San Francisco (1972).
6. M. RODBELL, *J. biol. Chem.* **239,** 375 (1964).
7. K. ITAYA and M. UI, *J. Lipid Res.* **6,** 16 (1965).
8. A. GILMAN, *Proc. natn Acad. Sci. U.S.A.* **67,** 305 (1970).
9. R. W. BUTCHER, C. E. BAIRD and E. W. SUTHERLAND, *J. biol. Chem.* **243,** 1705 (1968).

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