Effect of Dihydroergotamine and Propranolol on Dibutyryl Cyclic 3′,5′-AMP-Induced Lipolysis in Isolated Rat Fat Cells[1] (34169)

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Ellis *et al.* (1) and Mayer *et al.* (2) demonstrated that epinephrine-induced glycogenolysis was blocked by ergotamine in rabbits and dogs, although glucagon-induced glycogenolysis was not influenced by the drug. Recently Northrop and Park (3) postulated that dihydroergotamine (DHE) may inhibit the action of cyclic AMP, thereby interfering with the activation of norepinephrine (NE)–induced glycogenolysis. The majority of the previous workers (4–7) found that ergotamine and DHE reduce the hormone-induced lipolysis in rat adipose tissue and in nonfasting rats. The present study was undertaken (a) to examine the effect of DHE on NE-, ACTH-, theophylline-, and dibutyryl cyclic AMP-induced lipolysis, and also (b) to elucidate the possible site and mode of the action of the drug in isolated rat fat cells.

Methods. Male Holtzman rats (200–220 g) were fasted overnight and were killed by cervical dislocation. The epididymal fat pads were removed, and cut into small pieces with a pair of iridectomy scissors. Fat cells were prepared by a modification (8) of the technique described by Rodbell (9). The fat tissue was incubated for 1 hr in a 25-ml polyethylene bottle containing 4 ml of Krebs–Ringer–bicarbonate (KRB) buffer (10), pH 7.35 (gassed with a mixture of 95% O_2 and 5% CO_2) with 3% bovine albumin (Armour Pharmaceuticals) and 15 mg of collagenase (Worthington Biochem. Co.). The isolated fat cells were then washed thrice with and suspended in the KRB buffer solution. Thereafter, an aliquot (0.5 ml) of the islated fat cell suspension was pipetted into each 25-ml siliconized Erlenmeyer flask containing 3.5 ml of KRB buffer with 3% albumin. The fat cells were then incubated in a Research Specialties shaker (model 2156) at 37.5° for 1 hr after the addition of the drug studied. At the end of the incubation, an aliquot of the incubation medium was analyzed in duplicate for FFA by the method described by Duncombe (11). The triglyceride content of an aliquot (0.5 ml) of isolated fat cell suspension was determined by the method described by Van Handel and Zilversmit (12). The L-norepinephrine bitartrate was obtained from Winthrop Laboratories, N.Y.; ACTH from Mann Research Laboratories, N.Y.; theophylline from Sigma Chemical Co., St. Louis; and N^6, O_2-dibutyryl cyclic 3′,5′-AMP from Boeringer & Sohne, N.Y. Propranolol and dihydroergotamine were generously supplied by Dr. A. Sahagian-Edward, Ayerst Co., N.Y. and by Dr. J. H. Trapold, Sandoz Pharmaceuticals, Hanover, N.J., respectively.

Results. Effect of propranolol on NE-, ACTH-, theophylline-, and dibutyryl cyclic AMP-induced lipolysis. As shown in Fig. 1,

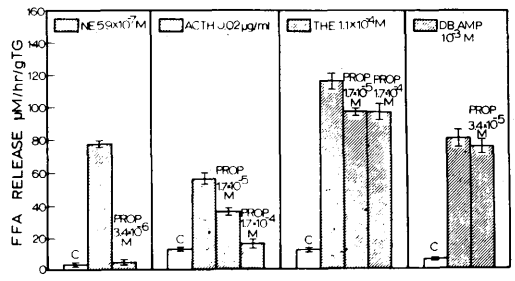

FIG. 1. Effect of propranolol (PROP) on norepinephrine (NE)-, ACTH-theophylline (THE)-, and dibutyryl cyclic AMP(DB-AMP)-induced lipolysis in isolated rat fat cells ($N = 6$). Each bar represents mean ± SEM.

[1] Supported in part by USPHS.

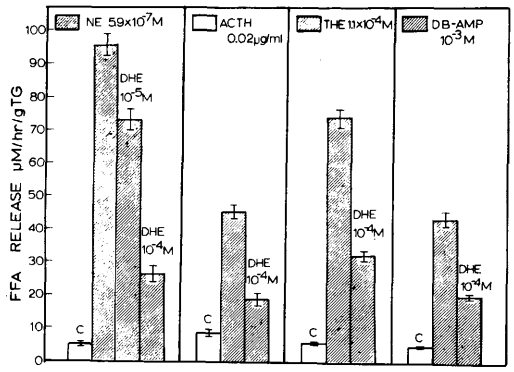

FIG. 2. Effect of dihydroergotamine (DHE) on norepinephrine-, ACTH-theophylline-, and dibutyryl cyclic AMP-induced lipolysis in isolated rat fat cells ($N = 6$).

5.9×10^{-7} *M* of NE, 0.02 µg/ml of ACTH, 1.1×10^{-4} *M* of theophylline and 1×10^{-3} *M* of dibutyryl cyclic AMP increased significantly FFA release from the isolated rat fat cells. These observations were essentially in agreement with those reported from the previous workers (13–15). The lipolytic effect of NE was almost completely blocked by 3.4×10^{-6} *M* of propranolol, whereas that of ACTH was blocked by 1.7×10^{-6} to 10^{-5} *M* of propranolol essentially in proportion to the concentration. On the other hand, the lipolytic effect of theophylline was slightly reduced by 1.7×10^{-4} to 10^{-5} *M* of propranolol. The increase in the concentration of propranolol did not increase its antilipolytic action. In contrast, the lipolytic effect of dibutyryl cyclic AMP was not significantly modified by 3.4×10^{-5} *M* of propranolol.

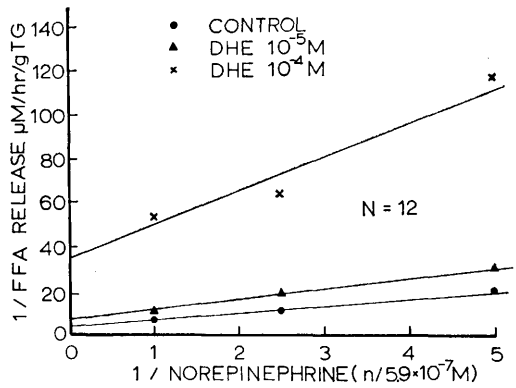

FIG. 3. Noncompetitive antagonism between norepinephrine and dihydroergotamine (DHE).

Effect of dihydroergotamine on NE-, ACTH-, theophylline-, and dibutyryl cyclic AMP-induced lipolysis. Figure 2 shows that the lipolytic action of 5.9×10^{-7} *M* of NE, 0.02 µg/ml of ACTH, 1.1×10^{-4} *M* of theophylline and 1×10^{-3} *M* of dibutyryl cyclic AMP was significantly blocked by 1×10^{-4} to 10^{-5} *M* of DHE. The mode of the inhibition by DHE was evaluated by double reciprocal plots obtained according to the method of Lineweaver and Burk (16) as shown in Figs. 3 and 4. The inhibitory action

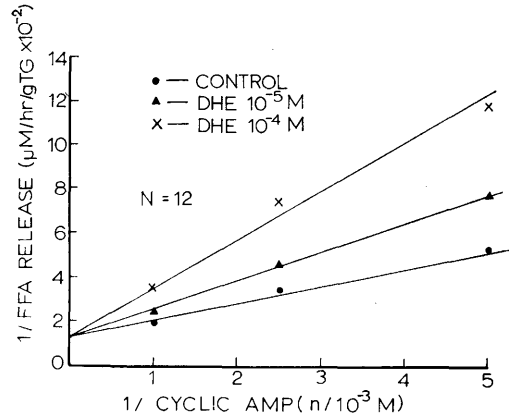

FIG. 4. Competitive antagonism between dibutyryl cyclic AMP and dihydroergotamine (DHE).

of 1×10^{-4} to 10^{-5} *M* of DHE on NE-induced lipolysis was noncompetitive (Fig. 3), whereas that of DHE on dibutyryl cyclic AMP-induced lipolysis was competitive (Fig. 4). The k_m values were 3.1×10^{-7} *M* for NE and 5.6×10^{-4} *M* for dibutyryl cyclic AMP, whereas k_i values for DHE were 2.4×10^{-5} *M* with NE as an agonist and 4.7×10^{-5} *M* with dibutyryl cyclic AMP.

Discussion. Since ergotamine and DHE block or reverse the pressor effect of epinephrine, other sympathomimetic amines and sympathetic nerve stimulation, these ergot alkaloids have been considered as alpha-adrenergic blocking agents (17). In spite of this cardiovascular effect the metabolic effects of ergotamine and dihydroergotamine are variable among the investigators. Ellis *et al.* (1), Mayer *et al.* (2), and McElroy and

Spitzer (6) observed that epinephrine-induced glycogenoylsis was blocked by ergotamine in rabbits and dogs. However, Ellis *et al.* (1), Berthet *et al.* (18), and Makman and Sutherland (19) found that ergotamine did not block glucagon-induced glycogenolysis in rabbits, and cat and dog liver homogenates.

Goodman and Knobil (5) showed that ergotamine blocked epinephrine-induced FFA mobilization in nonfasting rats, but not in fasting rats. On the other hand, McElroy and Spitzer (6) showed the similar antilipolytic effect of ergotamine in only two out of eight dogs. Levy and Ramey (4) found that ergotamine blocked FFA release in fasting, adrenalectomized rats, but not in intact rats. Very recently, Booker and Calvert (7) showed that the lipolytic effect of catecholamines was markedly blocked by DHE. The variability of the results of the studies made by the previous workers on the antilipolytic effect of ergotamine and DHE is not clear, but could be due to the difference in the experimental techniques and conditions as well as to the lack of the dose–response relationship.

Sutherland and his associates (20) found that the metabolic and possibly physiological actions of different hormones including NE, ACTH, and glucagon are mediated through the intracellular concentrations of the second messenger, cyclic AMP. The concentrations and thereby actions of cyclic AMP is determined by the activities of adenylcyclase and cyclic nucleotide phosphodiesterase. It appears that cyclic AMP is responsible for the subsequent activations of the hormone-sensitive lipase in adipose tissue. Hence, lipolysis is increased by the increased intracellular cyclic AMP concentrations which may be caused by the enhanced adenylcyclase activity due to catecholamines, ACTH, and glucagon or by the decreased phosphodiesterase activity due to theophylline. Fain (14) and Butcher *et al.* (15) found that propranolol blocks competitively catecholamine-induced lipolysis through its inhibitory action on adenyl cyclase. In the present study, propranolol blocked NE-, and ACTH-induced lipolysis, but did not influence dibutyryl cyclic AMP-induced lypolysis in fat cells. However, DHE inhibited NE-, ACTH-, theophylline-, and dibutyryl cyclic AMP-induced lipolysis. The inhibitory effects of DHE on NE- and dibutyryl cyclic AMP-induced lipolysis were noncompetitive and competitive, respectively. The present study indicates, therefore, that DHE exerts its antilipolytic action beyond the biochemical reaction(s) subsequent production of cyclic AMP, as shown by Peterson *et al.* (21) with phentolamine. However, the precise site of the enzymatic system where DHE acts upon remains uncertain. It could be conceivable, however, that DHE may block NE- and ACTH-induced lipolysis by its inhibitory action on the hormone-sensitive lipase induced by cyclic AMP. On the other hand, DHE may exert its antilipolytic action by its stimulatory action of phosphodiesterase in the similar manner as shown by Krishna *et al.* (22) with nicotinic acid. Butcher and Sutherland (23) found that phosphodiesterase activity should be decreased by 75% or more in order to enhance the cellular concentrations of cyclic AMP, indicating the greatly favorable kinetics of the enzyme in the cells. Recently, Moore *et al.* (24) found that the rate of hydrolysis of dibutyryl cyclic AMP by phosphodiesterase was insignificant compared to that of cyclic AMP. Furthermore, in the present study, the k_i values for DHE were almost similar whether the agonist was NE or dibutyryl cyclic AMP in the fat cells. From these data, it can be concluded that the intracellular concentration of dibutyl cyclic AMP catalyze hormone-sensitive lipase activity without being destroyed by phosphodiesterase in fat cells. The evidence of competitive antagonism between DHE and dibutyryl cyclic AMP indicates that DHE exerts its antilipolytic action by its inhibitory action subsequent to production of cyclic AMP. This is essentially in agreement with the postulate by Northrop and Park (3) with regard to the effect of DHE on NE-induced glycogenolysis. The present observation concerning the noncompetitive antagonism between NE and DHE remains unclear from the present study. This may be partially due to the bidirectional enzymatic pathways of cyclic

AMP in fat cells, *i.e.*, (a) its degradation by phosphodiesterase, and (b) its stimulatory action on hormone-sensitive lipase activity.

Summary. The effect of dihydroergotamine on lipolysis was studied in isolated rat fat cells. It was found that DHE interferes with lipolytic action of NE, ACTH, theophylline, and dibutyryl cyclic AMP. DHE inhibits noncompetitively the lipolytic action of NE, but does inhibit competitively that of dibutyryl cyclic AMP. It is indicated that DHE inhibits the activation of hormone-sensitive lipase of NE, ACTH, and theophylline at biochemical site(s) subsequent to the increased production of cyclic AMP by these agents in rat adipose tissue.

1. Ellis, S., Anderson, H. L., and Collins, M. C., Proc. Soc. Exptl. Biol. Med. **84**, 383 (1953).

2. Mayer, S., Moran, N. C., and Fain, J. N., J. Pharmacol. Exptl. Therap. **134**, 18 (1961).

3. Northrop, G. and Parks, P. E., J. Pharmacol. Exptl. Therap. **145**, 87 (1964).

4. Levy, A. C. and Ramey, E. R., Proc. Soc. Exptl. Biol. Med. **99**, 637 (1958).

5. Goodman, H. M. and Knobill, E., Proc. Soc. Exptl. Biol. Med. **102**, 493 (1959).

6. McElroy, W. T. and Spitzer, J. J., Am. J. Physiol. **200**, 318 (1961).

7. Booker, W. D. and Calvert, D. N., Arch. Intern. Pharmacodyn. **169**, 117 (1967).

8. Nakano, J., Ishii, T., and Gin, A., Pharmacology **1**, 183 (1968).

9. Rodbell, M., J. Biol. Chem. **239**, 375 (1964).

10. Krebs, H. A. and Henselheit, K., Z. Physiol. Chem. **210**, 33 (1932).

11. Duncombe, W. J., Biochem. J. **88**, 7(1963).

12. Van Handel, E. and Zilversmit, D. B., J. Lab. Clin. Med. **50**, 152 (1957).

13. Vaughan, M., Berger, J. E., and Steinberg, D., J. Biol. Chem. **239**, 401 (1964).

14. Fain, J. N., Ann. N. Y. Acad. Sci. **139**, 879 (1967).

15. Butcher, R. W. and Sutherland, E. W., Ann. N. Y. Acad. Sci. **139**, 879 (1967).

16. Lineweaver, H. and Burk, D., J. Am. Chem. Soc. **76**, 2842 (1934).

17. Goodman, L. S. and Gilman, A., "The Pharmacological Basis of Therapeutics," 3rd ed., pp. 554. Macmillan, New York (1965).

18. Berthet, J., Sutherland, E. W., and Rall, T. W., J. Biol. Chem. **229**, 351 (1957).

19. Makman, M. H. and Sutherland, E. W., Endocrinology **75**, 127 (1964).

20. Sutherland, E. W. and Robison, G. A., Pharmacol. Rev. **18**, 145 (1966).

21. Peterson, M. J., Patterson, C., and Ashmore, J., Life Sci. **7**, 551 (1968).

22. Krishna, G., Weiss, B., Davies, J. I., and Hynie, S., Federation Proc. **24**, 719 (1966).

23. Butcher, R. W. and Sutherland, E. W., J. Biol. Chem. **237**, 1244 (1962).

24. Moore, P. F., Iorio, L. C., and McManus, J. M., J. Pharm. Pharmacol. **20**, 368 (1968).

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