Effect of Desmethylimpiramine on Hormone-, Theophylline-, and Dibutyryl Cyclic AMP-Induced Lipolysis in Isolated Rat Fat Cells[1] (34761)

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It has been well established that an antidepressant, impiramine, and its analogue, desmethylimpiramine (DMI), block the uptake of norepinephrine (NE) at the adrenergic nerve endings in various tissues and organs, and also potentiate the pharmacodynamic effects of catecholamines (1, 2). Recently, Finger *et al.* (3) found that DMI inhibits NE-induced free fatty acid (FFA) release from the rat adipose tissue *in vitro*. The present study was undertaken to elucidate the biochemical site of the antilipolytic action of DMI in isolated 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 (4, 5) of the technique described by Rodbell (6). The fat tissue was incubated for 1 hr in a 25-ml polyethylene bottle containing 4 ml of Krebs–Ringer–bicarbonate (KRB) buffer (7), 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 solution. Thereafter, an aliquot (0.5 ml) of the isolated fat cell suspension was pipetted into each 25-ml siliconized Erlenmeyer flask containing 3.5 ml of KRB buffer with 3% bovine albumin. After adding the drugs studied, the fat cells were then incubated in a Research Specialties shaker (model 2156) at 37° for 1 hr. At the end of the incubation, an aliquot of the incubation medium was analyzed in duplicate for FFA by the method described by Duncombe (8) and for glycerol by the method described by Korn (9). 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 (10). The data in this study were analyzed statistically employing the t test (11).

Results. The results of the effect of DMI in hormone-, theophylline-, or dibutyryl cyclic AMP-induced lipolysis are summarized in Figs. 1–6. As shown by the previous workers (12–14) and in this laboratory (4, 5, 15), 5.9×10^{-7} M NE, 2×10^{-8} g of ACTH, 1.1×10^{-4} M theophylline and 1×10^{-3} M dibutyryl cyclic AMP increased FFA and glycerol release from the isolated rat fat cells. The lipolytic effect of NE, ACTH, theophylline, and dibutyryl cyclic AMP was blocked significantly or completely by 1×10^{-5} to 10^{-4} M of DMI essentially in proportion to the concentration. As shown in Fig. 7, Lineweaver-Burk double reciprocal plots re-

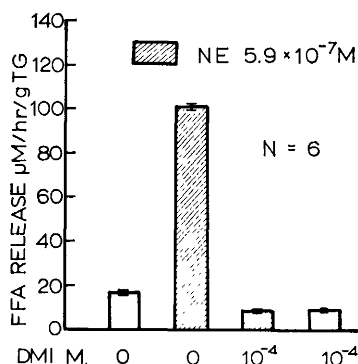

FIG. 1. Effect of desmethylimpiramine (DMI) on norepinephrine (NE)-induced free fatty acid (FFA) release from isolated fat cells. Each bar represents the mean values of FFA release (μmoles/hr/g) of triglyceride (TG). I-shaped bars denote the standard errors of the means.

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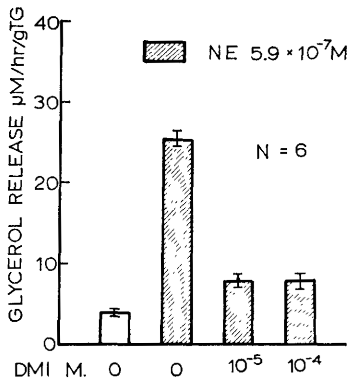

FIG. 2. Effect of desmethylimpiramine on norepinephrine-induced glycerol release from isolated fat cells.

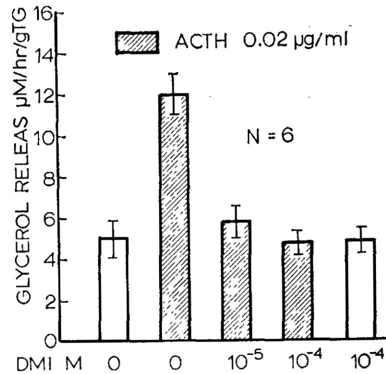

FIG. 4. Effect of desmethylimpiramine on ACTH-induced glycerol release from isolated fat cells.

vealed DMI blocks noncompetitively the lipolytic effect of norepinephrine. K_m of NE for the lipolytic activity and K_i of DMI for the lipolytic activity with NE as antagonist are 2.6×10^{-7} *M* and 1.2×10^{-3} *M*, respectively.

Discussion. Previously, Finger *et al.* (3) demonstrated that DMI inhibits NE-induced FFA release from the rat adipose tissue *in vitro*. The present study shows that DMI blocks not only the lipolytic effect of NE but also that of ACTH, theophylline, and dibutyryl cyclic AMP in isolated rat fat cells.

Sutherland and his associates (13, 16, 17) found that various hormones exert their actions by activating adenyl cyclase at the cell membranes, thereby increasing the intracellular concentrations of 3′, 5′-cyclic AMP. This nucleotide then accelerates the activity of hormone-sensitive lipase (14). Sutherland and his collaborators (13, 17, 18) also observed that the tissue concentration of cyclic AMP is determined by the catalytic activity of adenyl cyclase and cyclic nucleotide phosphodiesterase. Hence, the tissue concentration of cyclic AMP can be increased by either an enhanced activity of adenyl cyclase with NE or ACTH (13, 14, 18) or a decreased activity of phosphodiesterase with theophylline (18, 19). The present observation suggests that the antilipolytic effect of DMI is due to its inhibition of the action of cyclic AMP and/or to that of the subsequent enzymatic reaction(s) in fat cells, hence interfering with the activity of hormone-sensitive lipase. An alternative explanation for the antilipolytic action of DMI is that the drug may enhance the activity of phosphodiesterase as imidazole (20).

Allegedly, dibutyryl cyclic AMP can penetrate across the cell membranes more readily than its analogue, cyclic AMP (17, 18). Fur-

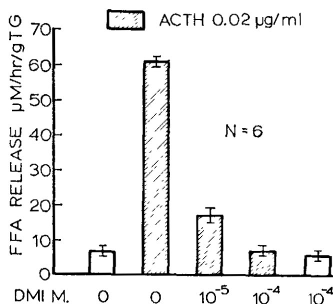

FIG. 3. Effect of desmethylimpiramine on ACTH-induced FFA release from isolated fat cells.

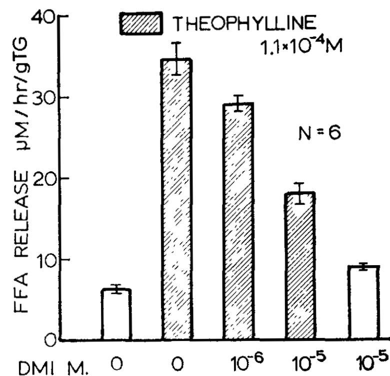

FIG. 5. Effect of desmethylimpiramine on theophylline-induced FFA release from isolated fat cells.

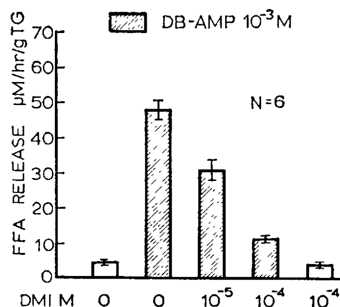

FIG. 6. Effect of desmethylimpiramine on dibutyryl cyclic 3′, 5′-AMP-induced FFA release from isolated fat cells.

thermore, Moore *et al.* (19) found that the rate of hydrolysis of dibutyryl cyclic AMP by phosphodiesterase was insignificant as compared to that of cyclic AMP. This indicates that dibutyryl cyclic AMP is not a substrate or is a very poor substrate for phosphodiesterase. Recently in our laboratory DMI did not cause any significant change in phosphodiesterase activity in rat brain homogenates (unpublished data). Hence, the antilipolytic effect of DMI is not likely due to that of imidazole (20). In contrast, the antilipolytic action of DMI seems to be due to its action on the former mechanism, as seen previously with dihydroergotamine (15). However, the NE-induced lipolysis, K_i for DMI, is much greater than that of dihydroergotamine, indicating a weaker inhibitory effect of DMI.

Summary. The effect of desmethylimpiramine (DMI) on lipolysis was studied in isolated rat fat cells. It was found that DMI interferes with the lipolytic action of NE, ACTH, theophylline, and dibutyryl cyclic AMP. It is suggested that DMI inhibits the activation of hormone-sensitive lipase at biochemical site(s) subsequent to the production of cyclic AMP, which is increased by NE, ACTH, and theophylline in rat adipose tissue.

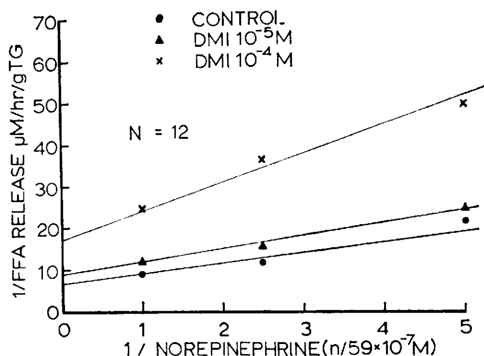

FIG. 7. Noncompetitive antagonism between desmethylimpiramine and norepinephrine.

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