Antagonism of Free Fatty Acid Release from Rat Epididymal Fat Tissue by Desmethylimipramine

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Desmethylimipramine (DMI) has been shown to be an antagonist of free fatty acid (FFA) mobilization from rat adipose tissue by both *in vivo* and *in vitro* techniques. Utilizing *in vitro* adipose tissue slices, homogenates, and cell-free preparations, it has been established that (*a*) the *in vitro* antagonism of FFA mobilization occurred whether the mobilization was induced by catecholamines or by other means and (*b*) the addition of DMI to an already activated lipase preparation caused a prompt cessation of lipolytic activity. These results indicate that DMI directly antagonized lipolytic enzymes and that effects on the adrenergic receptor are secondary to the primary inhibition phase. Desmethylimipramine antagonism of catecholamine-induced free fatty acid (FFA) mobilization was also observed to occur *in vivo*.

DURING THE past decade, considerable research effort has been placed on the investigation and development of antagonists of the catecholamine-induced mobilization of free fatty acids (FFA). In addition to providing agents of potential therapeutic usefulness, this type of research can and has served the purpose of elucidating the role of fat mobilization in certain disease states. Among the agents found to antagonize catecholamine-induced mobilization of FFA have been nicotinic acid (1), nethalide (2), the classical α and β adrenergic blocking agents (3), prostaglandins (4), and most recently, analogs of methoxamine (5, 6). The therapeutic value of antagonists of FFA mobilization, however, remains to be proved.

During the course of the authors' studies pertaining to the interaction of catecholamines and certain other phenethylamines with the adipose tissue adrenergic receptor system, desmethylimipramine (DMI) was investigated for its possible effects in modifying the action of catecholamines in this system. Previous work had shown that DMI was capable of enhancing adrenergic effects on certain receptor systems (7, 8), and it was with this in mind that DMI was investigated. Results indicated that DMI was an antagonist of catecholamine-induced mobilization of FFA, *in vitro* (9). This report is concerned with further investigations of the action of DMI on adipose tissue, and, in particular, its locus of action.

EXPERIMENTAL

Materials.—The chemicals used in this study and their sources are as follows: *l*-epinephrine bitartrate (Winthrop Laboratories); *l*-norepinephrine bitartrate (Nutritional Biochemical Corp.); and desmethylimipramine[1].

Methods.—The *in vitro* experiments described in this report utilized nonfasted male, white, Holtzman rats weighing between 200 and 250 Gm. All animals were maintained in their animal quarters at least 1 week prior to their use. The method of sacrifice, preparation, and handling of the epididymal fat tissue and the assay procedures employed have been described previously (9).

In those experiments employing homogenates of adipose tissue, the homogenates were prepared by adding a known weight of epididymal fat tissue to a volume of 0.25 *M* sucrose to make a 1:3 final homogenate, homogenization being accomplished by a motor driven, chilled Tenbroeck glass homogenizer.

Cell-free preparations of lipase were prepared by centrifuging the above-described homogenate at 12,000 × g for 10 min. at 4°, discarding the accumulated fat cake and aspirating off the supernatant phase. It was found that the supernatant phase contained the active lipase with little or no lipolytic activity being associated with either the fat cake or the 12,000 × g sediment in this system.

One milliliter of supernatant was then added to a media containing 2.5 ml. of 20% of 0.06 *M* phosphate buffer, pH 6.8, and 5.0 ml. of distilled water. At zero time, 0.5 ml. of substrate (12.5% emulsion of peanut oil prepared with acacia as the emulsifier) was added, and samples were taken at 0, 20, 40, and 60 min. for assay of FFA by the procedures previously described (9). Assays of the homogenate system were conducted in an identical manner except no exogenous substrate was employed, the endogenous triglycerides of the adipose tissue serving as the substrate in these experiments. It should be pointed out that acacia was found to be the emulsifier of choice primarily because other emulsifiers tried, such as the polysorbates, interacted with the DMI to an extent sufficient to prevent DMI from exerting any effect on the system.

For the *in vivo* experiments described herein, male Holtzman rats weighing between 400 and 600 Gm. were anesthetized with sodium pentobarbital, 35 mg./Kg., i.p., their femoral and carotid arteries cannulated to provide for i.v. administration of drug and the taking of blood samples for FFA analysis,

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respectively. The DMI was administered *via* the femoral vein over a 3-min. period, while norepinephrine (20 mcg./Kg.) was administered rapidly *via* the femoral vein. Blood samples were collected in heparinized tubes and stored over ice until centrifuged to obtain plasma samples. The FFA assays were conducted on 0.5 ml. of plasma obtained in this manner by the modified method of Dole (10, 11).

RESULTS AND DISCUSSION

Effect of DMI on Catecholamine-Induced FFA Release from Adipose Tissue Slices.—In an earlier study, it was reported that DMI antagonized catecholamine-induced release of FFA from rat epididymal fat tissue (9). Those studies were conducted utilizing a preincubation period in which the DMI was allowed to interact with the tissue for approximately 15 min. prior to the addition of the catecholamine. In the present experiment, it was decided to first allow the catecholamine (epinephrine) to maximally stimulate lipolytic activity in the adipose tissue slices and to add the DMI at a time when FFA release was occurring at a maximal rate. The results of this study are shown in Fig. 1. In these experiments, the adipose tissue slice preparation was divided into two portions. Epinephrine (final concentration 6.5×10^{-6} *M*) was added at zero time to each vessel and samples taken at 15 and 30 min. Immediately after the 30-min. sample, DMI (final concentration 1×10^{-3} *M*) was added to one incubation vessel and distilled water added to the other. Samples were then taken at 45 and 60 min. from both vessels. FFA analyses indicated that DMI caused the rapid cessation of FFA release as is indicated in Fig. 1. The control preparation continued releasing FFA at a constant rate. These results indicated that DMI was capable of antagonizing an already activated lipolytic system suggesting that the site of inhibition may be beyond the activation step, although this does not rule out the possibility that the inhibition is at the site of activation. Recent studies (12, 13) have indicated that the activation of lipolytic activity by the catecholamines involves the catecholamine stimulated conversion of ATP to 3′,5′-cyclic AMP which, in turn, activates the lipolytic enzymes. Thus, in such a sequence of events, several possible sites of inhibition are possible. Attempts were made to stimulate FFA release by the addition of 3′,5′-cyclic AMP to the adipose tissue slices. However, these efforts failed presumably because the cyclic AMP did not enter the adipose tissue slices or, as has been suggested (12), the 3′,5′-cyclic AMP is not sufficiently stable in this system to stimulate effectively lipolytic activity. Further attempts will be made, in this regard, utilizing more stable and lipid soluble analogs of cyclic AMP.

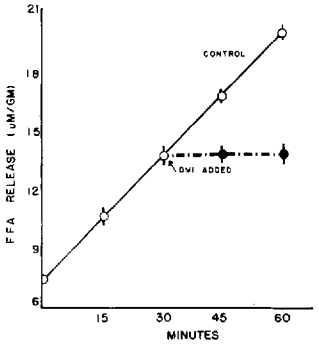

Fig. 1.—Effect of DMI on catecholamine-induced FFA release from adipose tissue slices. Vertical lines indicate standard error of the mean. Each point represents the mean of six determinations.

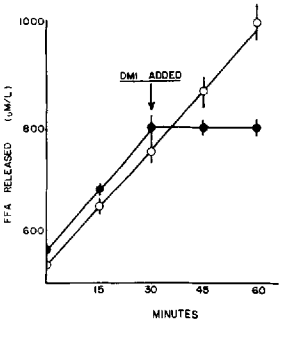

Fig. 2.—Effect of DMI on lipolytic activity of adipose tissue homogenates. Key: ○, control; ●, system to which DMI was added at 30 min. Vertical lines indicate standard error of the mean. Each point represents the mean of six determinations.

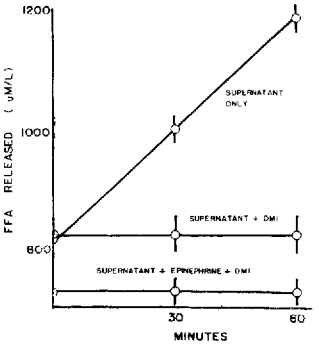

Fig. 3.—Effect of DMI on cell-free lipolytic activity. Vertical lines indicate standard error of the mean. Each point represents the mean of six determinations.

Effect of DMI on Lipolytic Activity of Adipose Tissue Homogenates.—It is characteristic of this type of preparation that the process of homogenization causes the maximal activation of lipolytic activity in the absence of any added catecholamine or other stimulant. Indeed, the addition of epinephrine to this system caused no further release of FFA above that observed in the absence of added catecholamine. Thus, the use of this system allowed the authors to investigate the effects of DMI on lipolytic activity in the absence of any added catecholamine.

In these experiments, a homogenate of adipose tissue was divided into two identical portions. Each incubation mixture was assayed for FFA at 0, 15, and 30 min. After the 30-min. sample was taken, DMI (final concentration 1×10^{-3} *M*) was added to one mixture and distilled water was added to the other. Samples were then taken at 45 and 60 min. from each preparation. The results of these experiments are shown in Fig. 2. It is again apparent that the addition of DMI caused the rapid cessation of FFA release while there was no diminution in the control FFA release. Although inhibition of the activation step is not entirely disproved, these results again suggest that the antagonism of FFA release exhibited by DMI occurred at a site remote from the activation step.

Effect of DMI on Cell-Free Lipolytic Activity.—Figure 3 illustrates the results obtained in studies conducted with the cell-free preparations. In

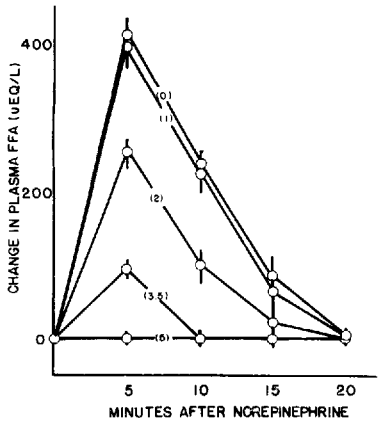

Fig. 4.—Inhibition of catecholamine-induced FFA mobilization, *in vivo*. Figures in parentheses indicate i.v. dose of DMI. Vertical lines indicate standard error of the mean. Each point represents the mean of six determinations.

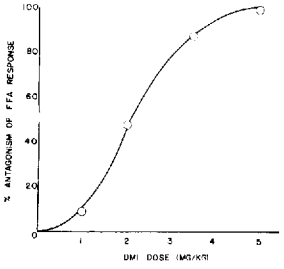

Fig. 5.—Dose-response relationship for inhibition of catecholamine - induced FFA mobilization, *in vivo*, by DMI. Each point represents the mean of six determinations.

these studies, employing a peanut oil emulsion as the substrate, maximal FFA release occurred without the addition of exogenous stimulants. The addition of DMI to yield a final concentration of 1×10^{-3} *M* completely abolished the FFA releasing activity of the preparations. The addition of epinephrine (1×10^{-6} *M*) did not reverse the antagonism as can be seen by the zero slope shown in Fig. 3. These results show that DMI is capable of antagonizing lipolytic activity with or without the addition of catecholamine.

In preliminary experiments, not reported here, the inhibition exhibited by DMI was overcome by the addition of additional enzyme (replacing the water in the media with an equal volume of supernatant enzyme preparation) but not with the addition of large quantities of catecholamine. It would thus appear that the DMI inhibition of lipolytic activity is dependent upon its interaction with enzymic material rather than at an adrenergic receptor site.

In Vivo Effects of DMI.—To ascertain whether DMI possessed the ability to inhibit FFA release *in vivo*, the drug was administered to animals, intravenously, at doses of 1.0, 2.0, 3.5, and 5.0 mg./Kg. Thirty minutes later, following the removal of a control blood sample, norepinephrine (20 mcg./Kg.) was administered *via* rapid i.v. injection. Norepinephrine was chosen for these experiments to minimize any variations in FFA mobilization due to the hyperglycemic responses so characteristic of other catecholamines. Blood samples were then taken at 5-min. intervals for a 20-min. period. The results of these experiments are shown in Fig. 4.

It can be seen in this graph that DMI possessed appreciable *in vivo* activity in antagonizing the norepinephrine-induced release of FFA, significant reductions in FFA mobilization being apparent at DMI doses of 2.0, 3.5, and 5.0 mg./Kg. The dose-response relationship for this inhibition is shown in Fig. 5.

It has been reported by Santi and Fassina (14) that the administration of DMI caused an elevation of plasma FFA, the maximal elevation being observed 150 min. after the administration of 25 mg./Kg. of DMI. These findings were confirmed in this laboratory. The apparent discrepancy in regard to the effects of DMI on plasma FFA can be explained in terms of the doses employed in the two studies and in the time course of events. Utilizing the interperitoneal route of administration, significant elevations of plasma FFA were obtained 150 min. after drug administration at a DMI dose of 25 mg./Kg., but not at a dose of 10 mg./Kg. or lower. Intravenous administration of DMI at doses up to 5.0 mg./Kg. caused no elevation of FFA. Furthermore, the elevations of FFA observed at the higher dose of DMI were rather slow in onset, becoming apparent only after approximately 100 min. following drug administration. DMI failed to elevate FFA, even at 25 mg./Kg., in the reserpinized rat. These results indicated that DMI may mobilize FFA through its central stimulatory activity (adrenergic mechanisms) and these effects may have a different time course than the DMI inhibitory effects on FFA release. These parameters are currently being investigated and will be reported in detail at a later date.

CONCLUSION

The data obtained in this study show that DMI is capable of inhibiting lipolytic activity in adipose tissue slices, homogenates, and cell-free preparations and also exhibits this property *in vivo*. The results suggest that DMI exerts its primary inhibitory effects at a site beyond the adrenergic receptor, probably at the enzymic site although conclusive proof of this postulate is lacking at this time.

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