Nicotinic Acid: Studies on the Mechanism of Its Antilipolytic Action

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SUMMARY

Nicotinic acid *in vitro* is known to depress basal lipolytic rates in adipose tissue and also to inhibit the action of a number of agents which stimulate adipose tissue lipolysis. Three possible mechanisms by which nicotinic acid could exert this antilipolytic effect have been examined. The rat epididymal fat pad was used as an adipose tissue source. *In vitro* nicotinic acid was found to have no effect on fat pad phosphodiesterase activity. Similarly, no direct effect of this drug on lipase activity could be demonstrated. Nicotinic acid *in vitro* was observed to inhibit, by about 50%, the increased lipolytic rates induced in isolated fat pad sections with theophylline. This inhibitory action, however, was antagonized by the further *in vitro* addition of the β-adrenergic blocking agent, nethalide, (pronethalol). Also, this antagonism appeared to be a direct one. It is suggested from these results that nicotinic acid does not exert its antilipolytic action by either increasing the degradation of cyclic 3′,5′-AMP or by inhibiting directly the activity of lipase. It would appear, however, that nicotinic acid acts at a site that is either identical with the receptor for nethalide or is closely associated to it. Thus, it seems possible that nicotinic acid may exert its antilipolytic action by depressing the production of cyclic 3′,5′-AMP.

INTRODUCTION

Nicotinic acid administered *in vivo* to a variety of experimental animals and under a variety of experimental conditions, lowers plasma free fatty acids (FFA) (1–4). For example, this drug lowers plasma FFA in humans (1, 2), dogs (1, 2), and rats (3) and also blocks the rise of plasma FFA produced by the *in vivo* administration of norepinephrine to dogs, without affecting the blood pressure response to this hormone (1, 4). Furthermore, nicotinic acid has been shown to lower the abnormally high plasma levels of FFA observed in alloxan-diabetic rats (5). These effects are presumably due to a direct antilipolytic action of nicotinic acid on adipose tissue (6–9). Nicotinic acid *in vitro* inhibits the stimulation of lipolysis in isolated adipose tissue by catecholamines (6, 7), ACTH (6), growth hormone and glucocorticoids (6), and theophylline (8). The addition of nicotinic acid to isolated fat cell preparations depresses basal rates of glycerol release, even in the presence of glucose (6). In addition, this drug *in vitro* has been observed to reduce the unusually high lipolytic rates seen in adipose tissue removed from alloxan-diabetic rats (9).

Current evidence suggests that many of the known lipolytic and antilipolytic agents may exert their actions at least within the broad framework of regulatory mechanisms which are thought to mediate the lipolytic action of catecholamines (10). A schematic representation of these mechanisms is shown on page 2.

Catecholamines presumably increase intracellular levels of cyclic 3′,5′-AMP (cyclic AMP) by activating an adenyl

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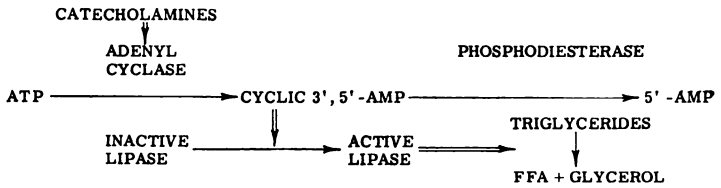

cyclase system, which is probably associated with the cell membrane. Increased cyclic AMP levels are thought to increase the conversion of an inactive lipase to an active lipase. Tissue cyclic AMP levels besides being influenced by the activity of adenyl cyclase, may also be influenced by the activity of the enzyme phosphodiesterase which catalyzes the conversion of cyclic AMP to 5'-AMP. The methyl xanthines, caffeine, and theophylline are well known inhibitors of phosphodiesterase and do in fact markedly stimulate lipolysis as well as potentiate the lipolytic response to catecholamines (11).

With regard to the scheme above, it seemed possible that nicotinic acid could exert its antilipolytic action in one of the following ways:

1. By depressing the production of cyclic AMP through either a direct or indirect action on adenyl cyclase.
2. By stimulating phosphodiesterase and thereby reducing the steady state level of cyclic AMP.
3. By directly inhibiting cyclic AMP-sensitive lipase.

These three possibilities have been investigated in the present study.

METHODS

Epididymal fat pads were obtained from male, Wistar strain, rats, maintained on a standard laboratory diet. Body weight at the time of sacrifice ranged from 250 to 300 g. All animals were sacrificed by stunning and exsanguination. In experiments in which adipose tissue from diabetic rats was compared with that of normals, blood, for sugar determination, was collected in heparinized test tubes during exsanguination.

Rats were made diabetic by an injection of alloxan (Eastman Organic Chemicals, Rochester, New York), 40 mg/kg i.v., following a 48-hr fast. All alloxanized animals were maintained on protamine zinc insulin (Eli Lilly and Co., Indianapolis, Indiana), 4 units/day, for at least 2 weeks prior to use. The last injection of insulin was given 72 hr prior to removal of fat pads. Tissue from these animals was used only if plasma glucose, as measured by the glucose oxidase method (12), exceeded 250 mg/100 ml.

Lipolysis in adipose tissue was determined by measuring the rate of glycerol (13) release from sections of whole fat pad incubated in Krebs-Ringer's bicarbonate buffer, pH 7.4, containing 2% bovine albumin (fraction V, Sigma, St. Louis, Missouri). Tissue sections weighed 250–300 mg and were incubated under 95% O_2–5% CO_2 in 4 ml of buffer, at 37°, for 90 min. In experiments measuring glucose utilization, incubations were carried out exactly as above, except that the buffer also contained 100 mg/100 ml glucose-1-^{14}C (specific activity 100 μC/g; Volk, Burbank, California). Labeled CO_2 at the end of these incubations was determined in aliquots of the incubation media by the method of Woeller (14).

Phosphodiesterase activity was measured in the 4000 *g* supernatant (excluding the floating fat cake) of an epididymal fat pad homogenate, by a modification of the method of Nair (15). In this procedure adenylic acid produced during the reaction was treated with a snake venom phosphatase (*Crotalus adamanteus*) and the inorganic phosphate released was measured.

Lipase activity was assayed in either a 12,000 *g* supernatant of an epididymal fat pad homogenate, by the method of Rizack (16) or in a crude 10% homogenate, incubated under 95% N_2–5% CO_2. In the former case, tri-*n*-butyrin (Eastman Kodak, Rochester, New York) was used as the substrate and the rate of glycerol release was measured. In the latter case, epidid-

ymal fat pads were homogenized in Krebs-Ringer's bicarbonate buffer, pH 7.4, containing 5% bovine albumin. Glycerol determinations were made on 1-ml aliquots of the homogenate, immediately before and after a 30-min incubation at 37°. The net production of glycerol over the 30-min period was used as a measure of lipase activity. In this system, glycerol release from endogenous lipid was found to be linear with time up to 50 min.

Protein was determined by the method of Lowry (17).

In vitro additions of one or more of the following agents were made in the various assay systems described: theophylline (Merck, Rahway, New Jersey); isopropylarterenol (isoproterenol) (CalBiochem., Los Angeles, California); nethalide (pronethalol) (alderlin) (Ayerst, New York, New York); nicotinic acid (Nutritional Biochemicals, Cleveland, Ohio).

RESULTS

Effects of Nicotinic Acid on Phosphodiesterase Activity

Table 1 shows the results obtained when phosphodiesterase activity was assayed *in vitro* in the presence and absence of nicotinic acid. Neither 10^{-5} M nor 10^{-4} M nicotinic acid altered significantly the activity of this enzyme. Similar *in vitro* concentrations, however, are known to markedly reduce the lipolytic response of adipose tissue to catecholamines (6, 7), ACTH (6), growth hormone plus dexamethasone (6), and theophylline (8, also see Fig. 4), and also to depress basal lipolytic rates in isolated fat cell preparations (6).

TABLE 1

Effects of nicotinic acid on adipose tissue phosphodiesterase activity

In vitro additions	Final concentration (M)	Phosphodiesterase activity (mμmoles P_i/mg protein/hr)
—	—	162 ± 27[a]
Nicotinic acid	1×10^{-5}	133 ± 6
Nicotinic acid	1×10^{-4}	161 ± 15

[a] Each value represents the means ± S.E.M. of 4 observations.

Effects of Nicotinic Acid on Lipase Activity

Lipase activity was measured in tissue from diabetic and normal rats. Both tri-*n*-butyrin (Table 2) and endogenous lipid (Table 3) were employed as substrates. Nicotinic acid, again in concentrations which are antilipolytic, was found to have no effect on either the lipase activity in normal adipose tissue or the much higher lipase activity observed in diabetic tissue.

Effect of Nethalide on the "Insulinlike" Action of Nicotinic Acid

Besides its antilipolytic effect, nicotinic acid *in vitro* has been reported to stimulate carbohydrate utilization in fat pads in a manner similar in some respects to that observed for insulin (18). This report includes the observation that nicotinic acid significantly stimulates the production of $^{14}CO_2$ from glucose-1-^{14}C. It has also been observed that an insulin-stimulated production of $^{14}CO_2$ from glucose-1-^{14}C in isolated fat pads is significantly depressed by the β-adrenergic blocking agent neth-

TABLE 2

Effects of nicotinic acid on lipase activity in adipose tissue from normal and diabetic rats

Lipase assays were carried out as described in the text using tri-*n*-butyrin (0.1 M) as a substrate. Each value represents the mean ± S.E.M. of 3 observations.

Fat pad source	*In vitro* additions	Final concentration (M)	Lipase activity (μmoles glycerol/mg protein/hr)
Normal	—	—	0.547 ± 0.013
	Nicotinic acid	1×10^{-5}	0.534 ± 0.018
Diabetic	—	—	1.326 ± 0.017
	Nicotinic acid	1×10^{-5}	1.317 ± 0.026

TABLE 3

Effects of nicotinic acid on lipase activity in adipose tissue from normal and diabetic rats

Lipase assays were carried out as described in the text, using endogenous lipid as the substrate. Each value represents the mean ± S.E.M. of 3 observations.

Fat pad source	*In vitro* additions	Final concentration (M)	Lipase activity (μmoles glycerol/ gram tissue/30 min)
Normal	—	—	2.02 ± 0.10
	Nicotinic acid	2.5×10^{-4}	1.97 ± 0.11
Diabetic	—	—	4.80 ± 0.09
	Nicotinic acid	2.5×10^{-4}	4.86 ± 0.07

alide, which by itself fails to alter basal production (19). That nethalide is also capable of blocking a nicotinic acid-stimulated production of $^{14}CO_2$ from glucose-1-^{14}C in isolated fat pads, is shown in Fig. 1. Nicotinic acid at 5×10^{-4} M increased $^{14}CO_2$ production 70% above control values. While nethalide (10^{-3} M) was found to exert no effect by itself, it completely blocked the effect of nicotinic acid. On the basis of these results it was decided to examine the possibility that nethalide might also antagonize nicotinic acid with respect to its effect on lipolysis.

Effect of Nethalide on the Antipolytic Action of Nicotinic Acid

Theophylline, by virtue of its effect on the enzyme phosphodiesterase, causes very marked increases in the lipolytic rates of

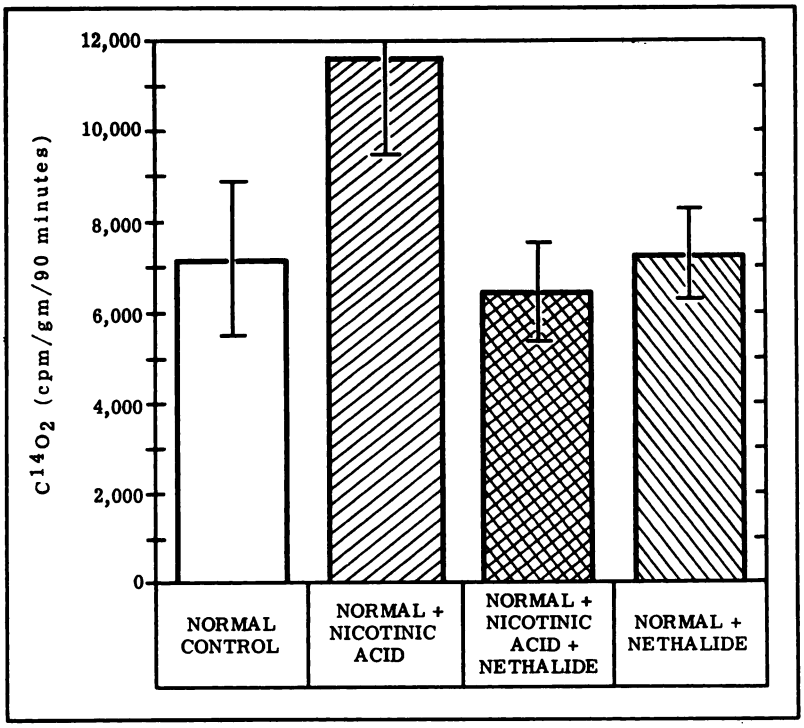

FIG. 1. *Effects of nicotinic acid and nethalide on $^{14}CO_2$ production from glucose-1-^{14}C by rat epididymal fat pad sections*

Each value represents the mean ± S.E.M. of 5 observations.

adipose tissue (11). The lipolytic response of segments of epididymal fat pad to increasing *in vitro* concentrations of theophylline is shown in Fig. 2. Effects of nicotinic acid and nethalide on the maximum lipolytic response which could be obtained with theophylline are summarized in Fig. 3. Nicotinic acid at 10^{-5} and 10^{-4} M depressed the response to 10^{-2} M theophylline by about 50%. While nethalide alone, at similar concentrations, had little, if any effect on theophylline-stimulated lipolytic rates (10^{-5} M nethalide appeared to have a slight stimulatory action), it almost completely blocked the effect produced by nicotinic acid. These results suggest that nethalide is either directly or indirectly antagonizing the antilipolytic action of nicotinic acid. It should be mentioned at this point that the maximum lipolytic response of epididymal fat pads to *in vitro* theophylline has been reported to be approximately three times higher than that obtained when norepinephrine was used as a lipolytic agent; also the maximum response to theophylline alone cannot be further increased by the added presence of the catecholamine (11). This same report demonstrates that at theophylline concentrations which elicit maximum lipolytic rates, tissue phosphodiesterase activity is about 10% of normal while tissue cyclic AMP levels are increased about 5-fold. It is suggested then, that theophylline is capable of increasing tissue cyclic AMP levels to the point where the amount of cyclic AMP-sensitive lipase becomes rate limiting. Therefore, while the further addition of a catecholamine to such a system would tend to increase cyclic AMP concentrations even more, this would not be reflected in increased lipolytic rates. Nethalide at concentrations comparable to those shown in Fig. 3, has been demonstrated to be a weak agonist, as well as a strong antagonist, of the catecholamines with regard to cyclic AMP production in adipose tissue (20). On the basis of this, nethalide would probably not be expected to exert any appreciable effect on the system described in Fig. 3. Thus, while nethalide could be increasing tissue cyclic AMP levels above those

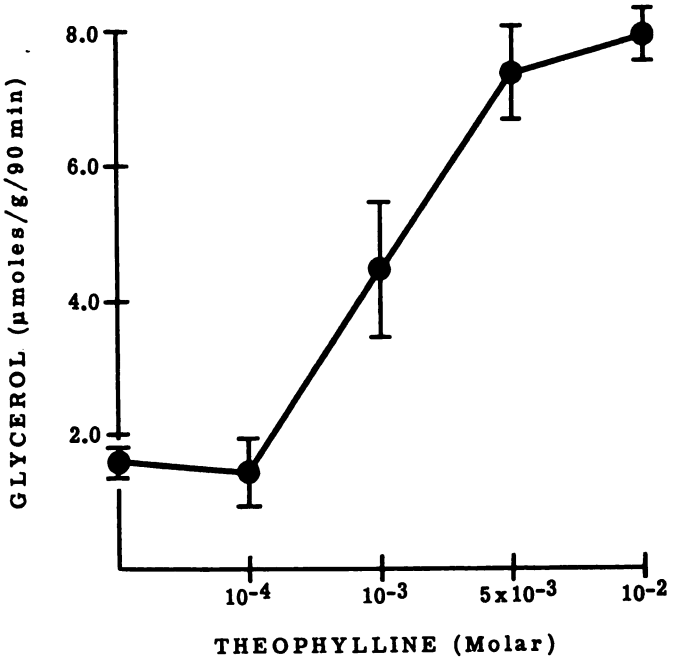

FIG. 2. *Effect of theophylline on the lipolytic activity of rat epididymal fat pad sections*
Each point represents the mean ± S.E.M. of 4 observations.

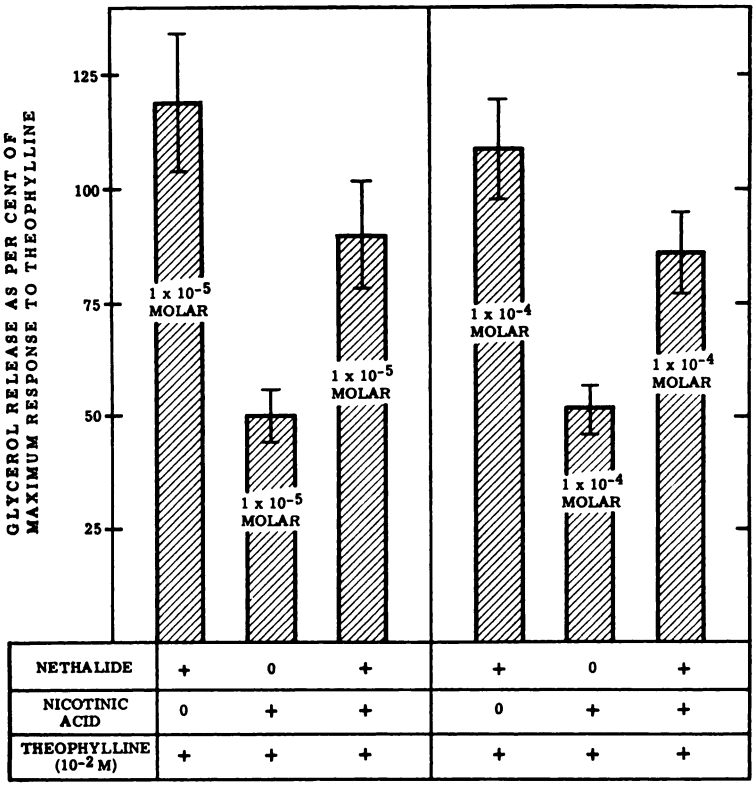

FIG. 3. *Effects of nicotinic acid and nethalide on the maximum lipolytic response of rat epididymal fat pad sections to theophylline*

Each value represents the mean ± S.E.M. of 4 observations.

achieved with theophylline, lipolytic rates would remain unaffected. The nethalide antagonism of the nicotinc acid effect, however, could then be indirect in as much as it could be secondary to the production of this added increment of cyclic AMP.

Theophylline at 10^{-3} M induces a lipolytic response which is in general only about 50% of the maximum response obtained with higher concentrations (see Fig. 2). Therefore, a concomitant stimulation of cyclic AMP production in such a system, might be expected to increase lipolytic rates. It has in fact been observed that the lipolytic response of adipose tissue to low concentrations of theophylline (10^{-4} and 10^{-3} M) can be further stimulated with norepinephrine in concentrations which induce only slight lipolytic activity by themselves (11). In Table 4, are shown the effects of nethalide at various concentrations, on the lipolytic response of fat pads to 10^{-3} M theophylline. In the presence of nethalide at both 10^{-5} M and 10^{-4} M, lipolytic rates in fat pads were found to be over 200% of those obtained with theo-

TABLE 4

Effects of nethalide on the lipolytic response of adipose tissue to theophylline

Theophylline was present at a concentration of 10^{-3} M.

Additions	Number of observations	Glycerol release as percent of response to theophylline alone
10^{-5} M Nethalide	2	203–231
10^{-4} M Nethalide	2	208–214
10^{-3} M Nethalide	4	42 ± 5.0

phylline alone. Nethalide at 10^{-3} M, however, was found to depress lipolytic rates by 58%.

In Fig. 4, the effects of nicotinic acid and nethalide on the maximum lipolytic response to theophylline (10^{-2} M), are again shown. In this experiment, however, nethalide at a concentration of 5×10^{-4} M depressed this response by about 25%. While the percent inhibition produced by nicotinic acid (2×10^{-5} M), was over twice that produced by nethalide, the addition of both nethalide (5×10^{-4} M) and nicotinic acid (2×10^{-5} M) to this system produced an inhibition which was not significantly different from that obtained with nethalide alone.

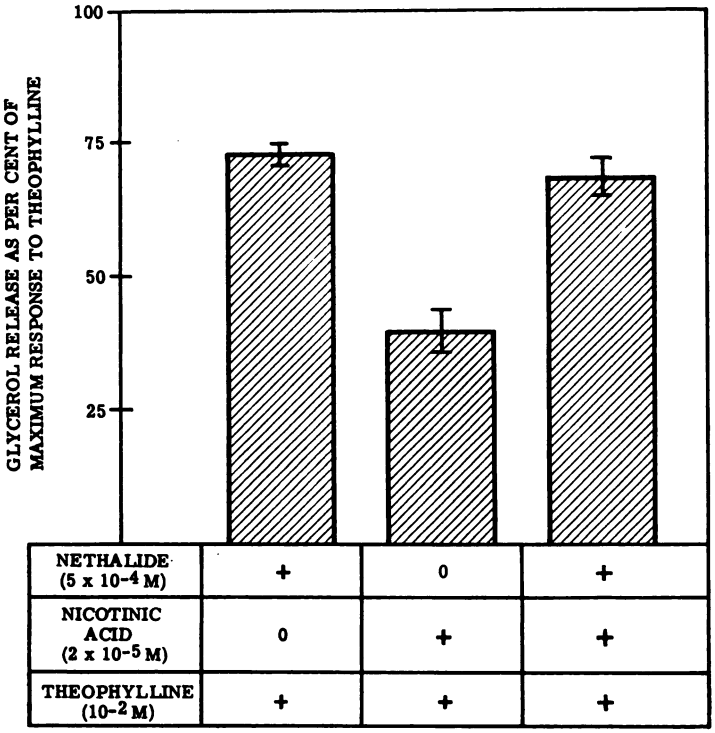

FIG. 4. *Effects of nicotinic acid and nethalide on the maximum lipolytic response of rat epididymal fat pad sections to theophylline*

Each value represents the mean ± S.E.M. of 3 observations.

DISCUSSION

With regard to what is currently known about the mechanisms involved in the regulation of lipolysis in adipose tissue, it seemed possible that nicotinic acid could exert its antilipolytic acid by: (a) decreasing cyclic AMP production, (b) increasing cyclic AMP degradation, or by (c) depressing directly the activity of the cyclic AMP-sensitive lipase. The last two possibilities would appear doubtful, since in this study no direct effect of nicotinic on either phosphodiesterase activity or lipase activity could be demonstrated. It should be mentioned, however, that although *in vitro* nicotinic acid has been observed to depress basal lipolytic rates in isolated fat cell preparations, the degree of inhibition produced is much greater when nicotinic acid is used to antagonize the action of lipolytic agents such as epinephrine and ACTH (6). Furthermore, and in contrast to the observations made with isolated fat cells, basal lipolytic rates in whole sections of epididymal fat pad removed from normal rats, are unaltered by *in vitro* nicotinic acid (6). This drug, however, depresses the much higher lipolytic rates observed in fat pad

sections from diabetic rats (9). On the basis of these observations and since as is shown in this study, lipase activity is much higher in diabetic adipose tissue than in normal, it seemed possible that an effect of nicotinic acid on lipase might be demonstrable only in a system in which increased amounts of active lipase are present. However, in the one such system studied this was found not to be the case. While lipase activity in diabetic fat pads was found to be over twice that found in normal tissue, it was not altered by the *in vitro* addition of nicotinic acid.

It appears much more likely that nicotinic acid is exerting its action at a site which is at least closely associated with the receptor for the β-adrenergic blocking agent nethalide. The β-adrenergic blocking agents, in general, have been found to be weak agonists as well as strong antagonists of catecholamine action on lipolysis in adipose tissue (21, 22). This observation taken together with the finding (20) that nethalide is also a weak agonist and strong antagonist of catecholamine stimulation of cyclic AMP production, strongly suggests that nethalide exerts its action via the adenyl cyclase system.

Nethalide at 10^{-5} and 10^{-4} M was observed to increase the lipolytic response of adipose tissue to relatively low concentrations (10^{-3} M) of theophylline. This finding supports the observation that nethalide, at least in the above concentrations, has a stimulatory effect on cyclic AMP production. At higher concentrations (5×10^{-4} and 10^{-3} M) however, nethalide was found to depress the lipolytic response to theophylline. It was not possible during the course of this study to demonstrate any direct effect of nethalide, at 5×10^{-4} M and 10^{-3} M, on either lipase activity or phosphodiesterase activity. If it can be assumed that nethalide acts exclusively via the adenyl cyclase system, then it might be concluded from this latter observation that nethalide in relatively high concentrations depresses cyclic AMP production.

While nethalide in the lower concentration range (10^{-5} and 10^{-4} M), had relatively little effect on the maximum lipolytic response of adipose tissue to theophylline, it almost completely blocked the inhibition produced in this system by nicotinic acid. Although these results demonstrate an antagonism between nicotinic acid and nethalide, it would not be reasonable, on the basis of these results alone, to suggest that this antagonism is a direct one. Since in the above system, theophylline has probably caused complete lipase activation, nethalide, in the concentrations used, would not be expected to appreciably alter lipolytic rates. It might, however, be expected to increase intracellular cyclic AMP levels above those which are obtained with theophylline alone. This latter situation would then provide a possible indirect mechanism by which nethalide could antagonize the antilipolytic effect of nicotinic acid. The subsequent observation, however, that nethalide at a concentration (5×10^{-4} M) which itself depressed the maximum lipolytic response of adipose tissue to theophylline, still blocked the much greater depression produced by nicotinic acid, strongly suggests that these two agents act on either the same receptor or at receptors closely enough associated so that a direct antagonism can take place. If these two agents were acting at completely independent sites to depress lipolysis, one should expect at least an additive effect when both are present in this system.

Since the receptor for nethalide is most likely the adenyl cyclase system, it seems reasonable to speculate that nicotinic acid may exert its antilipolytic action directly on this system, to depress cyclic AMP production. Nethalide, however, was also found to antagonize the action of nicotinic acid in stimulating glucose utilization, and there is no evidence that this effect of nicotinic acid is mediated through the adenyl cyclase system. It is possible, however, that this effect might be at least partially mediated through an alteration of cell membrane permeability. This possibility finds some support in the observations that nicotinic acid administration to both normal (23–25) and diabetic (26, 27) subjects leads to significant depressions in blood sugar. Since a likely location for the

adenyl cyclase system is the cell membrane (28), it is, therefore, possible that nicotinic acid and nethalide may have cell surface receptors which are closely associated. It would be conceivable then, that a nicotinic acid interaction with its receptor could lead to abnormal functioning of the adenyl cyclase system. Nethalide on the other hand, could block this interaction if, in reacting with the adenyl cyclase system, it overlapped the receptor for nicotinic acid. There is recent evidence suggesting that a somewhat similar type of antagonism might occur between the β-adrenergic blocking agents and insulin (16).

In conclusion, our results indicate that nicotinic acid does not exert its antilipolytic action either by increasing the degradation of cyclic AMP or by depressing directly the activity of the cyclic AMP-sensitive lipase. The antilipolytic action of nicotinic acid, however, does appear to be directly antagonized by the β-adrenergic blocking agent, nethalide. Since the most likely receptor for nethalide is the adenyl cyclase system, it seems reasonable to propose that nicotinic acid may exert its antilipolytic action by depressing the production of cyclic AMP.

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