Blockade of Norepinephrine-Induced Lipolysis by Isomers of *dl*-1-(Nitrophenyl)-1-Hydroxy-2-Isopropylaminoethane

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SUMMARY

The ability of three new beta adrenergic blocking agents, *dl*-1-(2-nitrophenyl)-1-hydroxy-2-isopropylaminoethane (2-INPEA), *dl*-1-(3-nitrophenyl)-1-hydroxy-2-isopropylaminoethane (3-INPEA), and *dl*-1-(4-nitrophenyl)-1-hydroxy-2-isopropylaminoethane (4-INPEA) to antagonize norepinephrine-induced lipolysis was investigated utilizing an isolated fat cell preparation. All three isomers were capable of blocking norepinephrine competitively. The pA_{10} values for 2-INPEA, 3-INPEA, and 4-INPEA were determined and concentration-inhibition curves were plotted. 4-INPEA was found to be approximately ten times as potent as 2-INPEA and 3-INPEA, the latter two being equipotent. The results are discussed in relation to the classification of the adrenergic receptor in adipose tissue.

INTRODUCTION

Norepinephrine and epinephrine, the sympathetic neurohormones, have been reported to increase lipolysis in adipose tissue *in vivo* and *in vitro* with an accompanying release of free fatty acids and glycerol (1–9). This action, which is shared by other catecholamines (9, 10) and several polypeptide hormones (11, 12), can be blocked by both alpha and beta adrenergic blocking agents (13–17). Recent evidence with several new beta adrenergic blocking agents has indicated the possibility of the adipose tissue receptor for catecholamines being similar to the beta receptors in other tissues (18–20) although several differences remain to be clarified (17). Norepinephrine, which has a relatively weak beta receptor-stimulating action in most tissues, is twice as potent as epinephrine in stimulating lipolysis in adipose tissue (21, 22). Furthermore, the potent alpha receptor stimulant effects of norepinephrine on the resistance blood vessels can be blocked by alpha but not beta adrenergic blocking agents, whereas the lipolytic activity of norepinephrine can be inhibited by both types of adrenergic blocking drugs (13, 16, 17, 23).

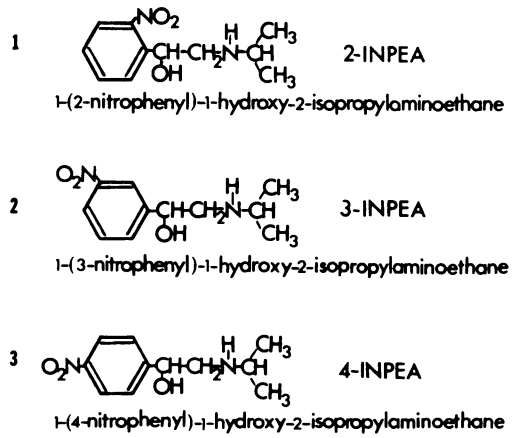

FIG. 1. *Structure of the three isomers of INPEA*

In an attempt to further the understanding of the interaction between norepinephrine and beta blocking agents in adipose tissue, the ability of a series of isomers of *dl*-1-(nitrophenyl-1-hydroxy-2-isopropylaminoethane (INPEA) to antag-

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onize norepinephrine-induced lipolysis was investigated in a preparation of isolated fat cells. These three compounds[2] (Fig. 1) have been shown to possess specific beta adrenergic blocking activity in other tissues (24, 25).

MATERIALS AND METHODS

White, male Holtzman rats, weighing between 180 and 230 g, were fasted for 20 hours and sacrificed by decapitation. The epididymal fat pads were excised immediately and the fat cells were isolated by a modification of the procedure of Rodbell, which has been previously described (26). Aliquots of a suspension of washed fat cells in warm (37°) Krebs-Ringer phosphate buffer at pH 7.4 were transferred to polyethylene incubation vessels containing warm (37°) Krebs-Ringer phosphate buffer at pH 7.4, 5% bovine serum albumin (Armour Pharmaceutical Co., Kankakee, Illinois) and appropriate concentrations of *l*-norepinephrine bitartrate (Winthrop Laboratories, New York, New York). When 2-INPEA, 3-INPEA, or 4-INPEA was used, an appropriate concentration of the blocking agent was allowed to incubate for 3 min at 37° with the fat cells and buffer prior to addition of norepinephrine. The free fatty acids produced under norepinephrdine stimulation were determined in aliquots taken from each incubation vessel prior to and 1 hour after incubation at 37° utilizing the titratometric method of Dole and Meinertz (27). Quantitation of responses was based on adipose cell protein content by a method which has already been described in detail (26).

RESULTS

The data obtained in the present investigation demonstrate that the three new beta adrenergic blocking agents effectively inhibited the norepinephrine-induced lipolysis in the isolated fat cells. In the presence of the indicated concentrations of the blocking agent, the concentration-response curves to various doses of norepinephrine are shifted in a parallel fashion to the right without any significant change in the slope (Fig. 2), indicating that the blockade is competitive. The pA_{10} (28) values calculated from these curves were 4.30 for

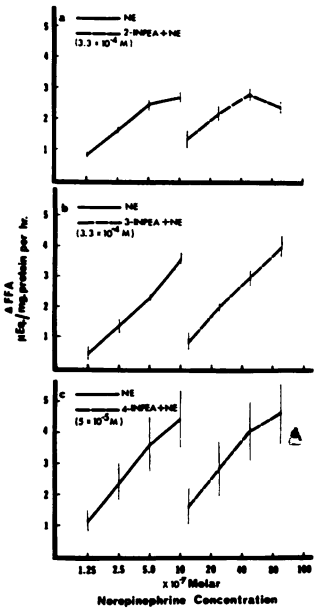

FIG. 2. *Concentration-response curves for l-norepinephrine alone (NE) and in the presence of blocking agents*

l-Norepinephrine in the presence of (a) 2-INPEA (3.33×10^{-4} M) $n = 6$, (b) 3-INPEA (3.33×10^{-4} M) $n = 3$, (c) 4-INPEA (5×10^{-5} M) $n = 4$. Ordinate: change in free fatty acid produced, in microequivalents per milligram of adipose cell protein per hour. Abscissa: molar concentration of *l*-norepinephrine. Each curve is drawn through the means of the indicated number of experiments ± 1 S.E.

4-INPEA and 3.48 for 2-INPEA and 3-INPEA. It can be readily seen from these data that the blocking potency of 4-INPEA is about 10 times greater than that of 2-INPEA and 3-INPEA.

Figure 3 illustrates the linear relationship between concentration of the blocking agents and intensity of blockade of the response to a standard concentration (5×10^{-7} M) of *l*-norepinephrine. Here again, it can be readily seen that 4-INPEA is slightly more than ten times as potent as

[2] These compounds were generously supplied by Dr. Walter Murmann, Selvi & Company, Milan, Italy.

2-INPEA and 3-INPEA. Taken from these curves, the concentrations of 4-INPEA, 2-INPEA, and 3-INPEA needed to reduce the lipolytic response to 5×10^{-7} M of *l*-norepinephrine by 50% was found to be 4×10^{-5} M, 8×10^{-4} M, and 3×10^{-3} M, respectively. Since the concentration inhibition curves for 2-INPEA and 3-INPEA are not significantly different from each other, these two isomers must be considered

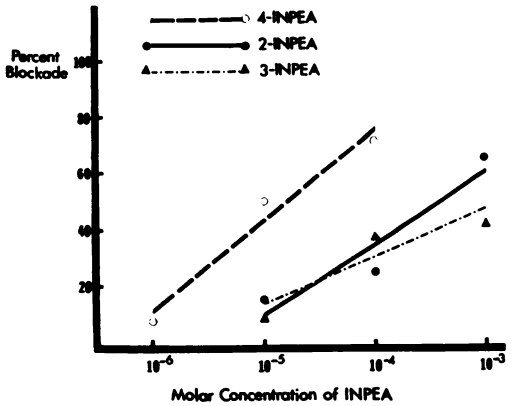

FIG. 3. *Blockade of the lipolytic response to l-norepinephrine (5×10^{-7} M) by 2-INPEA, 3-INPEA, and 4-INPEA*

Ordinate: per cent blockade of control response. Abscissa: molar concentration of blocking agent. Each curve is a regression line drawn through the means of a minimum of two experiments.

equipotent in blocking activity. This observation is consistent with their identical pA_{10} value.

The direct effect of the three blocking agents on the spontaneous release of free fatty acids was also investigated, and it was observed that none of the three isomers possess significant intrinsic activity in concentrations ranging from 1.25×10^{-5} M to 1×10^{-4} M.

DISCUSSION

The lipolytic effect of norepinephrine, epinephrine, and isoproterenol as well as that of various polypeptide hormones is susceptible to blockade by both alpha and beta adrenergic blocking agents (15, 21). These observations, in addition to the rather diverse list of lipolytic agents with specific effects in other tissues, have led to the idea that adipose tissue receptors may not be highly discriminating to agonists (29).

More recently, however, several investigators have suggested the possibility that the adipose tissue receptors for the lipolytic action of catecholamines are similar to the beta receptors in other tissues. These conclusions are based on the observations that several specific beta adrenergic blocking agents such as dichloroisoproterenol (18), pronethalol, propranolol, and MJ 1999 (19, 20) selectively blocked the lipolytic action of catecholamines. Kö 592, another new beta blocking agent, was found to inhibit the lipolytic action of catecholamines in concentrations which did not block a similar effect of corticotropin (30). Further evidence in support of this theory includes the data from structure-activity relationships in a series of sympathomimetic amines. The structural requirements for beta receptor stimulating activity in most tissues include a catechol nucleus attached to a hydroxyethylalkylamine side chain (31), and omission of the hydroxyl group on either the β-carbon atom of the side chain or on the catechol nucleus greatly decreases the beta stimulant activity of these compounds. The optimum substitution on the side chain nitrogen is the *n*-isopropyl group and the activity is reduced by CH_3 (epinephrine) or H (norepinephrine) substitution in its place. These same structural requirements were necessary for optimal lipolytic activity among arylalkylamines (9, 10). On the other hand, alpha receptor activation does not depend upon the presence of the catechol hydroxyl groups, but is quite dependent upon the *absence* of large alkyl groups on the amino nitrogen (31), as is the case with norepinephrine, phenylephrine, and methoxamine.

We have reported previously that INPEA is a specific beta adrenergic blocking agent (24). The present findings that the lipolytic effect of norepinephrine is blocked competitively by the isomers of INPEA and that the order of blocking potency of these compounds correlates closely with that observed in the myocardium (25) suggests

that some type of common mechanism is shared in stimulating lipolysis and activation of beta adrenergic receptors. The inhibitory effect of INPEA on norepinephrine-induced lipolysis does not appear to be due to a blockade of alpha receptors in adipose tissue, since the alpha receptor stimulant action of norepinephrine on the resistance blood vessels is not blocked by INPEA (32) or other beta adrenergic blocking agents (33, 34). The inhibitory effect of these isomers of INPEA on the lipolytic action of pure alpha receptor stimulants such as phenylephrine or methoxamine cannot be tested adequately since the latter drugs possess a very weak lipoltic activity (9, 35).

A point of departure of the adipose tissue receptor from beta receptors in other tissues appear to be the observation that norepinephrine is approximately twice as potent as epinephrine as a lipolytic agent (21, 22) whereas the potency of norepinephrine is equal to or less than that of epinephrine on other beta receptors (36, 37). It should be pointed out, however, that such potency ratios in different tissues may be influenced by the catecholamine uptake and binding mechanisms associated with sympathetic nerve endings (38) and further experiments will be needed before such a difference can be accepted as meaningful. Another difference is that alpha adrenergic blocking agents, which do not antagonize the effects of beta receptor activation in other tissues (39), have been found to inhibit the lipolytic activity of catecholamines (13, 16, 17, 23), although some doubt has been raised as to the specificity and mechanism of such a blocking action (20).

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