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STUDIES ON A BIPHASIC LIPOLYTIC RESPONSE TO CATECHOLAMINES IN ISOLATED FAT CELLS

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Abstract—A biphasic lipolytic response was elicited in isolated fat cells by increasing concentrations of epinephrine, norepinephrine and isoproterenol. The first phase of lipolysis (Lipolysis I) occurred with concentrations of the catecholamines from $3{\cdot}3 \times 10^{-8}$ M to 10^{-5} M and is thought to be equivalent to the lipolytic response often reported in the literature. It was inhibited competitively by the beta-adrenergic blocking agents and noncompetitively by an alpha-adrenergic blocking agent. The second phase of lipolysis (Lipolysis II) occurred with concentrations of catecholamines from 10^{-5} M to 10^{-3} M and was not inhibited by beta-adrenergic blocking agents, but was noncompetitively inhibited by an alpha-adrenergic blocking agent. Both phases of lipolysis appear to be the result of a stimulation of a triglyceride lipase.

IT HAS been repeatedly demonstrated that the catecholamines and other hormones can stimulate the rate of lipolysis in isolated adipose tissues.[1–3] Evidence is accumulating that the lipolytic activity of these agents is the result of the stimulation of the adenyl cyclase enzyme system and the subsequent increased production of cyclic 3′, 5′-adenosine monophosphate (cyclic AMP) in the tissue.[4,5] This cyclic nucleotide presumably stimulates the conversion of an inactive lipase to an active lipase.

The development of the isolated fat cell preparation[6,7] has provided a test system which is very sensitive to the lipolytic activity of the catecholamines. Using this preparation, several authors[8,9] have shown that the rate of lipolysis increases with increasing concentrations of the catecholamines until a maximal response is reached. A further increase in catecholamine concentration produces a decrease in the lipolytic rate. This relationship between concentrations of the catecholamines and lipolytic response has been confirmed by this report. However, it has also been found that an increase in the concentration of these amines to even higher levels results in a second concentration-dependent increase in lipolysis. The properties of this second lipolytic response are the subject of this report.

MATERIALS AND METHODS

Fed, male Holtzman rats weighing 120–200 g were stunned by a blow to the head and killed by exsanguination. The fat pads were removed and isolated fat cells were prepared by the method of Lech and Calvert.[7] Aliquots of the fat cells were placed in polyethylene flasks containing Krebs–Ringer bicarbonate buffer (pH 7·4) with 4% bovine serum albumin and the appropriate drugs. Incubations were carried out at

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37° with gentle shaking in an atmosphere of 95% O_2–5% CO_2 for 60 min. The final volume was 3·0 ml.

The reaction was terminated by adding an aliquot of the cells and medium to 5% trichloroacetic acid, and the rate of lipolysis was determined by measuring the production of glycerol or free fatty acids (FFAs). Glycerol was determined by the method of Korn[10] and FFAs by the method of Dole.[11] Appropriate blank values were obtained for all drugs used. The protein content of the fat cells was determined as described by Lech and Calvert.[7]

All results are expressed as the mean ± standard error of the mean. Unless otherwise stated, values for P were calculated by using the Student *t*-test for paired comparisons.

Bovine serum albumin (Fraction V) was purchased from Sigma Chemical Co. (St. Louis, Mo.). The levo-isomer of INPEA [1,4(-nitrophenyl)-l-hydroxy-2-isopropyl aminoethane] was the kind gift of Dr. Walter Murmann of Selvi and Co. (Milan, Italy), and the *dl*-propranolol (l-isopropylamine-3-naphthyloxy-2-propanol) generously supplied by Ayerst Laboratories (New York, N.Y.). The ACTH was purchased from Parke, Davis & Company (Detroit, Mich.) and amounts of the drug are expressed in terms of U.S.P. units. The levo-isomers of epinephrine, norepinephrine and isoproterenol were used throughout this study.

RESULTS

Figure 1 shows the lipolytic response to increasing concentrations of epinephrine in the isolated fat cell preparation. The response was concentration-dependent from 10^{-7} M to $3{\cdot}3 \times 10^{-6}$ M epinephrine, while an increase in the concentration to

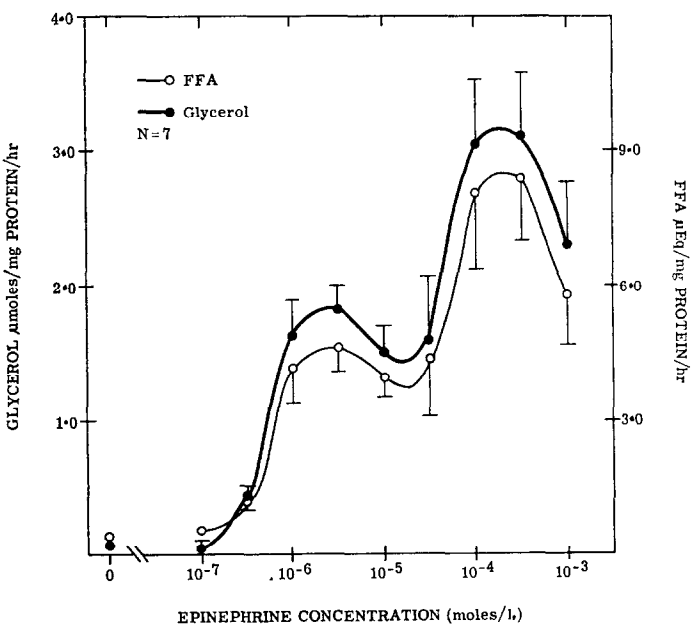

FIG. 1. Lipolytic effect of *l*-epinephrine on isolated fat cells. Each point is the mean ± S.E.M. of seven experiments.

10^{-5} M depressed the rate of lipolysis. A further increase in the concentration of epinephrine from 10^{-5} M to $3{\cdot}3 \times 10^{-4}$ M produced a second concentration-dependent increase in the rate of lipolysis. At 10^{-3} M epinephrine, the lipolytic rate was reduced below the peak rate seen at $3{\cdot}3 \times 10^{-4}$ M. This two-phase lipolytic response was seen if either glycerol or FFA production was used as an indication of the rate of lipolysis. In Fig. 1 are shown the results of seven experiments in which both glycerol and FFA production were measured. Both parameters exhibited a two-phase lipolytic response to increasing concentrations of epinephrine. With the exception of the results at the lowest concentration of epinephrine, the FFA to glycerol ratio was quite constant, varying from 2·55 to 2·77 (theoretical ratio = 3·00).

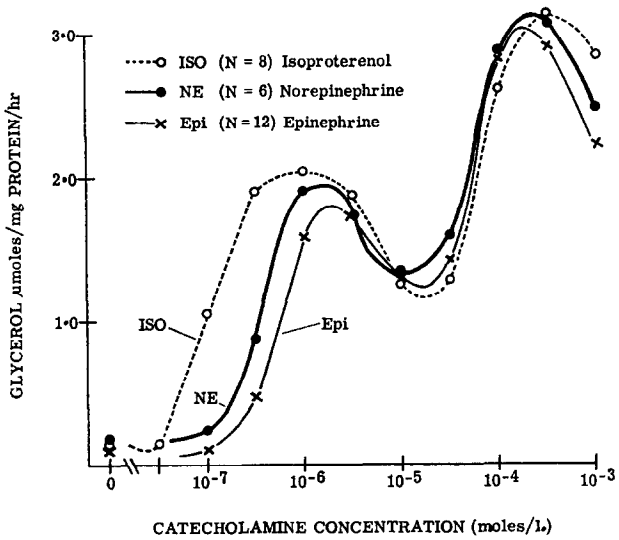

FIG. 2. Lipolytic effect of *l*-isoproterenol, *l*-norepinephrine and *l*-epinephrine on isolated fat cells. Each point is the mean ± S.E.M. of six to twelve experiments.

The biphasic lipolytic response produced by epinephrine was also produced by norepinephrine and isoproterenol (Fig. 2). During the first phase of the lipolytic response, the catecholamines had differing potencies as stimulators of lipolysis. Isoproterenol was the most potent, norepinephrine the next most potent, and epinephrine the least potent of the three. The concentration of isoproterenol which produces a response which was 50 per cent of the first peak was estimated at 1×10^{-7} M. The corresponding concentrations for norepinephrine and epinephrine were 4×10^{-7} M and 5×10^{-7} M respectively. In contrast, all three catecholamines were equipotent in the second phase of the lipolytic response. Assuming that the basal lipolytic rate for this portion of the response curve was equal to the rate seen at 10^{-5} M epinephrine, the concentration of all three agents which produces 50 per cent of the maximum response was 6×10^{-5}M.

Figure 3 shows the results of six experiments in which the lipolytic responses to varying concentration of norepinephrine were measured in the presence and absence of propranolol (10^{-6} M). This well known blocker of hormone-stimulated lipolysis

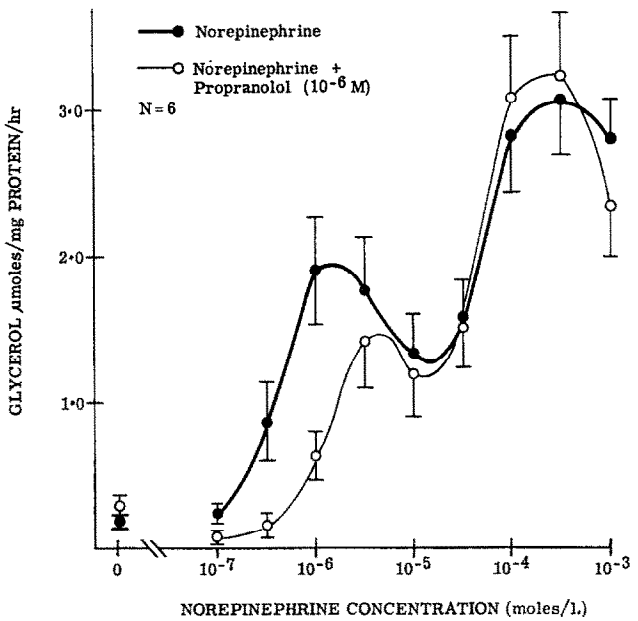

FIG. 3. Inhibitory effect of propranolol (10^{-6} M) on *l*-norepinephrine-induced lipolysis. Each point is the mean ± S.E.M. of six experiments.

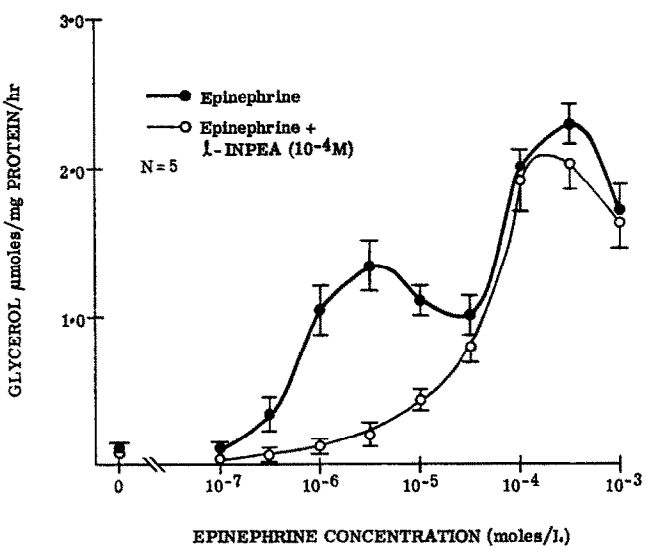

FIG. 4. Inhibitory effects of *l*-INPEA (10^{-4} M) on *l*-epinephrine-induced lipolysis. Each point is the mean ± S.E.M. of six experiments.

produced a parallel shift to the right of the first phase of the response curve. The responses to 10^{-7} M, $3{\cdot}3 \times 10^{-7}$ M, 10^{-6} M and $3{\cdot}3 \times 10^{-6}$ M norepinephrine were significantly ($P < 0{\cdot}05$) reduced by propanolol. The presence of this concentration of the inhibitor failed, however, to alter the response to norepinephrine in the second phase of lipolysis.

In a series of five experiments another beta-adrenergic blocking drug, *l*-INPEA, was investigated in relation to the biphasic lipolytic response to epinephrine. At a concentration of 10^{-4} M, this drug greatly inhibited the first phase response to epinephrine (Fig. 4). A significant ($P < 0{\cdot}05$) reduction was seen in the response to epinephrine in concentrations from 10^{-7} M to 10^{-5} M. The second phase of the response was not inhibited by this concentration of *l*-INPEA.

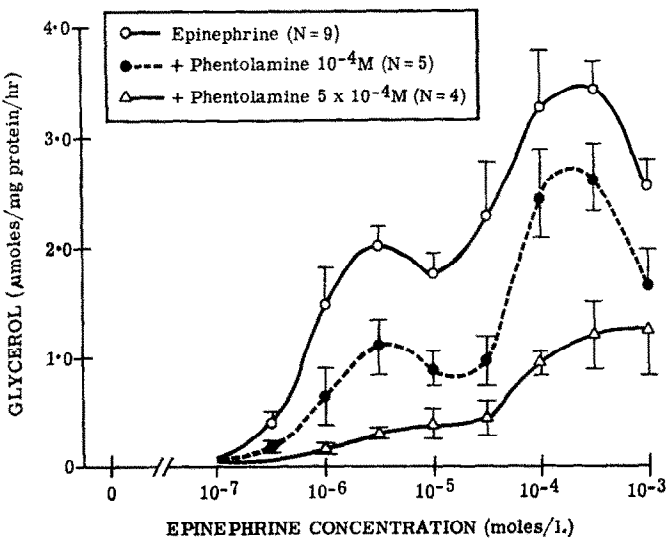

FIG. 5. Inhibitory effects of phentolamine on *l*-epinephrine-induced lipolysis. Each point is the mean ± S.E.M. of four to nine experiments.

The influence of phentolamine, an alpha-adrenergic blocking agent, on the biphasic response to epinephrine was investigated in nine experiments (Fig. 5). At the concentration of 10^{-4} M, this drug produced what appeared to be a noncompetitive inhibition of the first phase of lipolysis. The peak response in the first phase was significantly reduced by phentolamine ($P < 0{\cdot}05$), but the small reduction in the peak response in the second phase was not statistically significant ($P > 0{\cdot}05$). However, a concentration of phentolamine of 5×10^{-4} M did significantly ($P < 0{\cdot}05$) reduce the peak response in the second as well as in the first phase of lipolysis.

In Fig. 6 are shown the results of four experiments in which the lipolytic activity of both epinephrine and ACTH was determined. Although the biphasic response to epinephrine was evident in these experiments, the lipolytic response to ACTH was monophasic. This response corresponded in magnitude to the first phase of the lipolytic response to the catecholamines. The concentration of ACTH producing 50 per cent of the maximal response was calculated to be 0·071 USP units/flask.

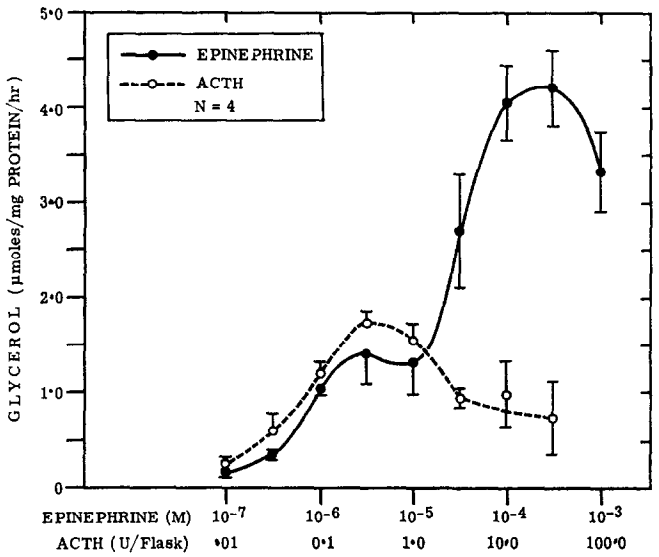

FIG. 6. Lipolytic effect of *l*-epinephrine and ACTH on isolated fat cells. The concentration of ACTH is expressed in terms of USP units per flask. Each point is the mean ± S.E.M. of four experiments.

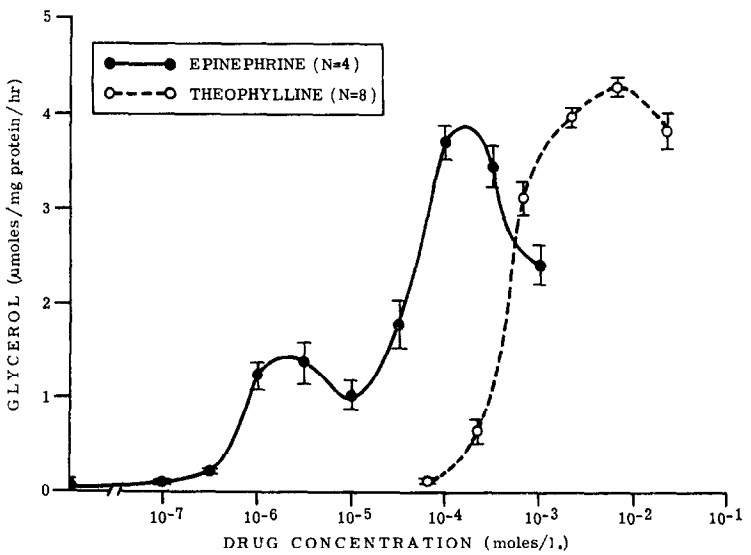

FIG. 7. Lipolytic effect of *l*-epinephrine and theophylline on isolated fat cells. Each point is the mean ± S.E.M. of four or eight experiments.

The lipolytic responses to epinephrine and to the phosphodiesterase inhibitor, theophylline, were compared in the fat cell preparation (Fig. 7). The biphasic response to epinephrine was again evident but, in the range of concentrations of theophylline that were used, no biphasic response was observed. The peak response to theophylline occurred at $6{\cdot}67 \times 10^{-3}$ M and did not differ significantly ($P > 0{\cdot}95$, group comparison) from the peak response seen in the second phase of the response to epinephrine. Estimation of the concentration of theophylline that produced 50 per cent of the peak response gave a value of 8×10^{-4}M.

DISCUSSION

Many reports have appeared in recent years demonstrating the lipolytic activity of a number of hormones and drugs.[1-3] Prominent among these agents are the catecholamines. The present communication reports the existence of a biphasic lipolytic response to increasing concentrations of epinephrine, norepinephrine and isoproterenol. As an aid in the discussion of this phenomenon, the first phase which was produced by concentrations of catecholamines from $3{\cdot}3 \times 10^{-8}$ to 10^{-5} M will be termed Lipolysis I and the second phase of lipolysis which was produced by concentrations of these drugs from 10^{-5} M to 10^{-3} M will be termed Lipolysis II.

On the basis of the work reported here, it seems likely that Lipolysis I is equivalent to the lipolytic response which numerous authors have reported for the catecholamines. The relative potency of the three agents on Lipolysis I (isoproterenol > norepinephrine > epinephrine) is the same as the relative potency reported by other authors.[8, 12] Also, the concentrations of these drugs which produced 50 per cent of the peak response are in good agreement with published values.[8]

In addition, the blockade of Lipolysis I by alpha- and beta-adrenergic blocking agents was the same as the blockade of the usually reported catecholamine-stimulated lipolysis.[8, 12] In both cases, the beta-adrenergic blocking drugs produced a competitive type of inhibition and the alpha-adrenergic blocking drug, phentolamine, produced a noncompetitive type of inhibition.

Lipolysis II does not have the same characteristics as the previously reported lipolytic response to the catecholamines. All three catecholamines were equipotent stimulators of this lipolytic response and it was not inhibited by the beta-adrenergic blocking agents. It was inhibited though, by phentolamine, an alpha-adrenergic blocking agent. This would be expected if, as suggested by other workers, phentolamine were directly inhibiting the lipase itself.[13, 14]

Due to the fact that the biphasic response was seen in both glycerol and FFA production and that the FFA to glycerol ratio was essentially the same over the entire range of catecholamine concentrations, it seems likely that Lipolysis I and Lipolysis II represent the complete hydrolysis of triglyceride. As pointed out by Vaughan and Steinberg,[15] the hydrolysis of triglyceride to diglyceride is the rate-limiting step in the production of glycerol and FFA from adipose tissue triglyceride. If, during high rates of lipolysis, some step distal to the triglyceride lipase (i.e. mono- or diglyceride lipase) became rate limiting, the FFA to glycerol ratio would increase . Since this was not the case, the rate-limiting step in both phases of lipolysis would appear to be the hydrolysis of triglyceride to diglyceride. Therefore, even in Lipolysis II, the stimulating action of the catecholamines must be the result of an increased activity of a

triglyceride lipase. No evidence is currently available to suggest whether this triglyceride lipase is the same as or different from the so-called hormone-sensitive lipase.

Several possible explanations for the biphasic lipolytic response to the catecholamines are available. However, there is no evidence to support any one theory over another. Pure speculation allows us to acknowledge such possibilities as the existence of two adenyl cyclase systems with differing affinities for the epinephrine molecule, or two cyclic AMP-sensitive triglyceride lipases with differing affinities for the cyclic nucleotide. Obviously other possibilities exist. The biphasic response was not, however, a property of all agents which stimulate lipolysis. Increasing concentrations of ACTH produced only a single-phase lipolytic response which seems to correspond to Lipolysis I. The response to increasing concentrations of theophylline was also a single phase. The maximal response to theophylline, however, corresponded to the maximal response to the catecholamine in Lipolysis II.

Work is currently being conducted to explain more fully the biphasic lipolytic responses to the catceholamines.

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