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EFFECT OF POTASSIUM ION, THEOPHYLLINE AND PROPRANOLOL ON THE BIPHASIC LIPOLYTIC RESPONSE TO SOME CATECHOLAMINES*

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Abstract—The biphasic lipolytic dose–response curves to the *levo*-isomers of the catecholamines were investigated to determine what role cyclic AMP played in the mediation of these responses. As has been reported previously, the dose–response curve to *l*-epinephrine was biphasic. The first phase of the response curve has been shown to be equivalent to the lipolytic response reported many times in the literature and was, therefore, considered to be mediated by cyclic AMP. Removal of potassium ion from the incubation medium abolished the first phase of the response (Lipolysis I), but had no effect on the second phase (Lipolysis II). In the absence of potassium ion, lipolytic response to *l*-epinephrine was significantly augmented by the presence of sublipolytic concentrations of theophylline and significantly inhibited by the presence of the beta-adrenergic blocking agent, propranolol. The lipolytic dose–response curve to the *dextro*-isomer of epinephrine was monophasic. Omission of potassium ion from the incubation medium had very little effect on the lipolytic response to this agent. The response to *d*-epinephrine closely resembled the Lipolysis II response to l-epinephrine. The lipolytic activity of *d*-epinephrine was significantly augmented by theophylline and inhibited by propranolol. These data are interpreted to suggest that the second phase of the biphasic dose–response curve to *l*-epinephrine (Lipolysis II) was mediated by cyclic AMP.

WE HAVE recently reported a biphasic lipolytic dose–response curve to increasing concentrations of the *levo*-isomers of epinephrine, norepinephrine and isoproterenol.[1] The first phase of the response (Lipolysis I), which is produced by concentrations of *l*-epinephrine from 10^{-7} to 10^{-5}M, was found to be identical to the lipolytic response to the catecholamines which numerous authors have reported.[2–4] The second phase of the lipolytic response (Lipolysis II), which was produced by concentrations of epinephrine from 10^{-5} to 10^{-3}M, differed in several respects from the hormone-stimulated lipolysis previously reported in the literature.

A large amount of evidence has accumulated to indicate that the lipolytic response to catecholamines and other agents is secondary to the activation of the adenyl cyclase enzyme system and the subsequent increase in tissue levels of cyclic 3′,5′-adenosine monophosphate (cyclic AMP).[5, 6] Because Lipolysis I appears to be identical with the much studied lipolytic response to catecholamines, it is assumed that this phase of lipolysis was the result of increased cyclic AMP production. The purpose of the present work was to investigate the possibility that Lipolysis II was also dependent upon the production of cyclic AMP.

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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. In those experiments in which K^+ was omitted from the incubation medium, it was replaced by an appropriate amount of Na^+. The cells were washed three times with the K^+-free buffer. Incubations were carried out at 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 by the method of Korn.[8] 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]

Phosphodiesterase was assayed in the whole homogenate according to the method of Butcher and Sutherland.[9] Cells were homogenized in all-glass homogenizers in the presence of drugs to be tested. Inorganic phosphate was determined by the method of Fiske and SubbaRow.[10]

All results are expressed as the mean $\pm$ 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 Company (St. Louis, Mo.). The *dl*-propranolol (1-isopropylamine-3-naphthyloxy-2-propanol) was generously supplied by Ayerst Laboratories (New York, N.Y.). The *levo-* and *dextro*-isomers of epinephrine were the kind gift of Sterling-Winthrop Research Institute (Rensselaer, N.Y.).

RESULTS

Figure 1 shows the results of five experiments in which the lipolytic responses to varying concentrations of *l*-epinephrine were measured in the presence and absence of propranolol (10^{-5}M). In the absence of propranolol the dose response to *l*-epinephrine was biphasic, as had been previously reported.[1] The presence of the beta-adrenergic blocking agent abolished the lipolytic response to concentrations of epinephrine from 10^{-7} to 10^{-5}M. The lipolytic responses to concentrations of epinephrine greater than 10^{-5}M were significantly ($P < 0{\cdot}005$) inhibited by the presence of propranolol. The dose–response curve produced by these higher concentrations of *l*-epinephrine was shifted to the right, and the maximum response was reduced.

A series of five experiments was conducted to determine what effect potassium ion had on *l*-epinephrine-stimulated lipolysis (Fig. 2). In the presence of potassium ion (5·0 mM), the biphasic lipolytic response curve was observed. In the same experiments, when potassium ion was omitted from the incubation medium, Lipolysis I was almost abolished, but Lipolysis II was unaffected. Similar results were obtained when *l*-isoproterenol-stimulated lipolysis was examined. In the absence of potassium ion, the dose–response curve to this catecholamine was monophasic (Fig. 3). In terms of drug concentration the response corresponded to Lipolysis II.

Also shown in Fig. 3 are the results of seven experiments conducted in potassium-free buffer measuring the lipolytic response to *l*-isoproterenol in the presence and absence

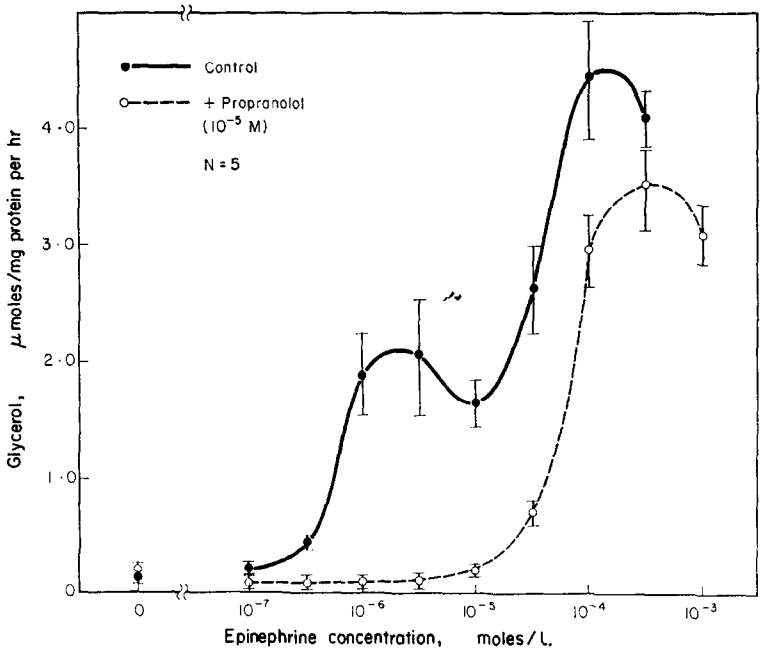

FIG. 1. Lipolytic effects of *l*-epinephrine in the presence and absence of propranolol (10^{-5}M). The buffer contained 5·0 mM potassium ion. Each point is the mean ± S.E.M. of five paired experiments.

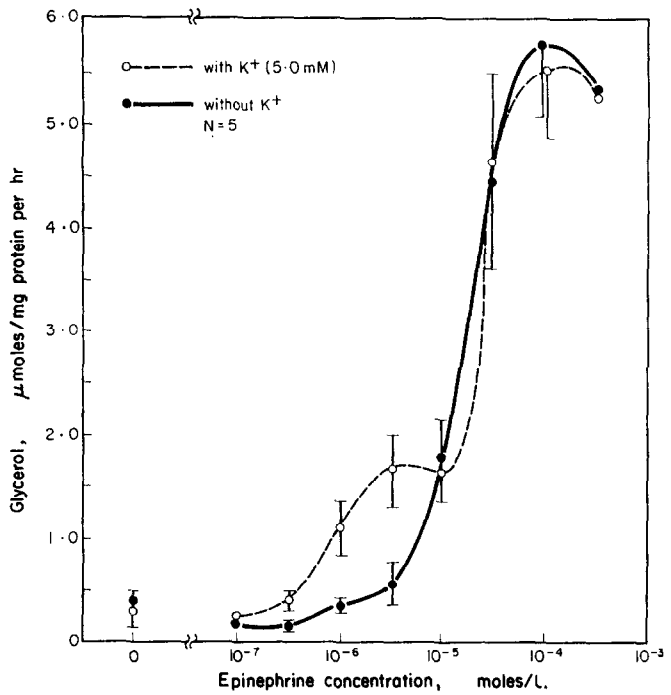

FIG. 2. Lipolytic effects of *l*-epinephrine in the presence and absence of potassium ion (5·0 mM). Each point is the mean ± S.E.M. of five paired experiments.

of theophylline (3×10^{-5}M). This methylxanthine greatly augmented the lipolytic activity of isoproterenol. The response to concentrations of isoproterenol from 10^{-7} through 10^{-5}M were significantly ($P < 0.025$) increased. In the presence of theophylline, a concentration of 3×10^{-6}M isoproterenol produced the maximum lipolytic response, whereas a concentration of 10^{-4}M isoproterenol was required in the absence of the phosphodiesterase inhibitor.

In potassium ion-free buffer the lipolytic response to *l*-epinephrine was inhibited by propranolol (10^{-5}M). This concentration of the beta-blocking agent shifted the dose–response curve to *l*-epinephrine to the right and depressed the maximum response (Fig. 4).

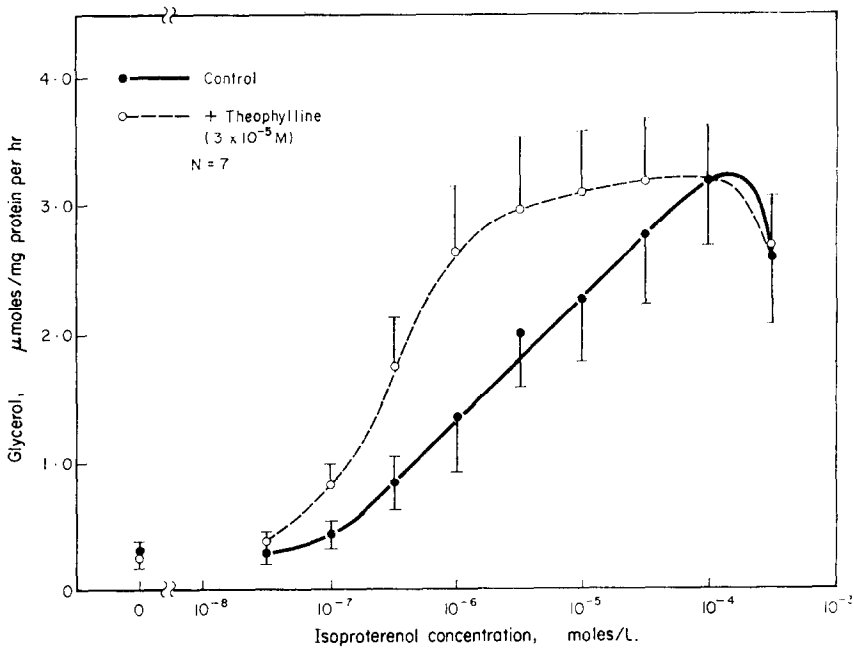

FIG. 3. Lipolytic effects of *l*-isoproterenol in the presence and absence of theophylline (3×10^{-5}M). The buffer contained no potassium ion. Each point is the mean ± S.E.M. of seven paired experiments.

In a series of five experiments the dose–response curves to *l*- and *d*-epinephrine were compared. As before, the dose–response curve to *l*-epinephrine was biphasic. The dose–response curve to *d*-epinephrine, however, was monophasic in the concentrations employed (Fig. 5). The maximum response to the *d*-isomer was significantly ($P < 0.005$) greater than the maximum response in Lipolysis I and significantly ($P < 0.02$) less than the maximum response in Lipolysis II. The response to *d*-epinephrine was similar in the presence or absence of potassium ion (Fig. 6). However, the absence of potassium ion did result in a small but significant ($P < 0.05$) reduction in the response to 5.5×10^{-5}M *d*-epinephrine and increase in the response to 5.5×10^{-4}M *d*-epinephrine.

The lipolytic response to *d*-epinephrine was augmented by the presence of theophylline (Fig. 7). Theophylline at a sublipolytic concentration (3×10^{-5}M)

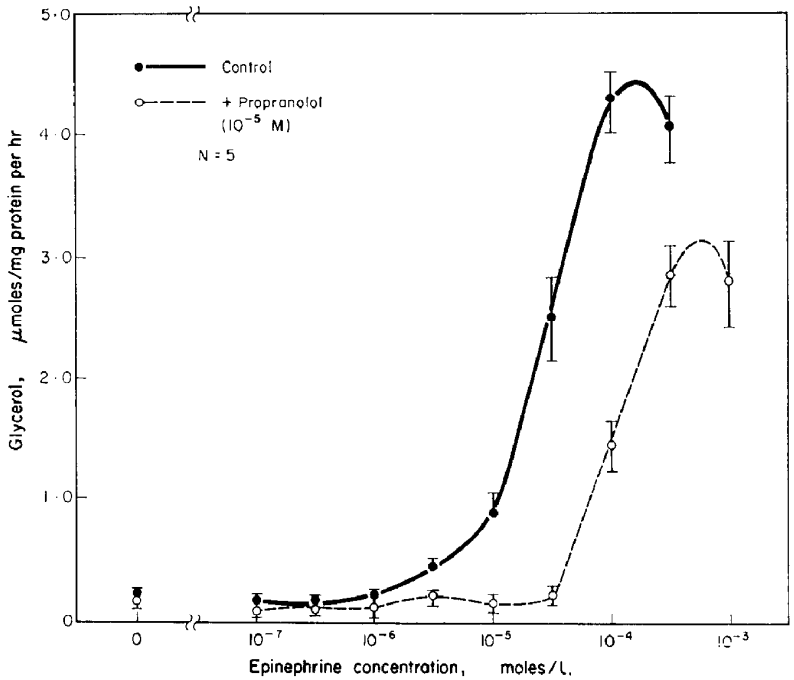

FIG. 4. Lipolytic effects of *l*-epinephrine in the presence and absence of propranolol (10^{-5}M). The buffer contained no potassium ion. Each point is the mean ± S.E.M. of five paired experiments.

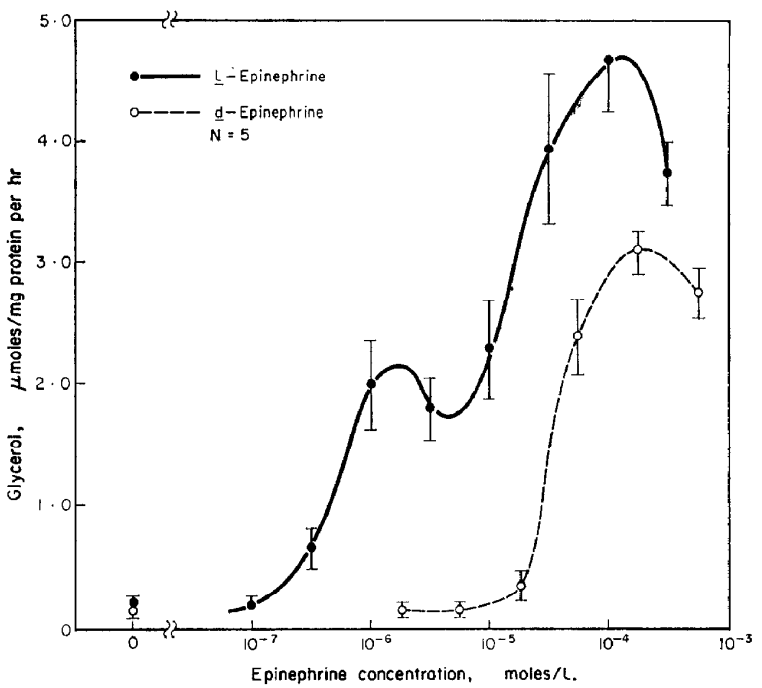

FIG. 5. Lipolytic effects of *l*-epinephrine and *d*-epinephrine. The buffer contained 5·0 mM potassium ion. Each point is the mean ± S.E.M. of five paired experiments.

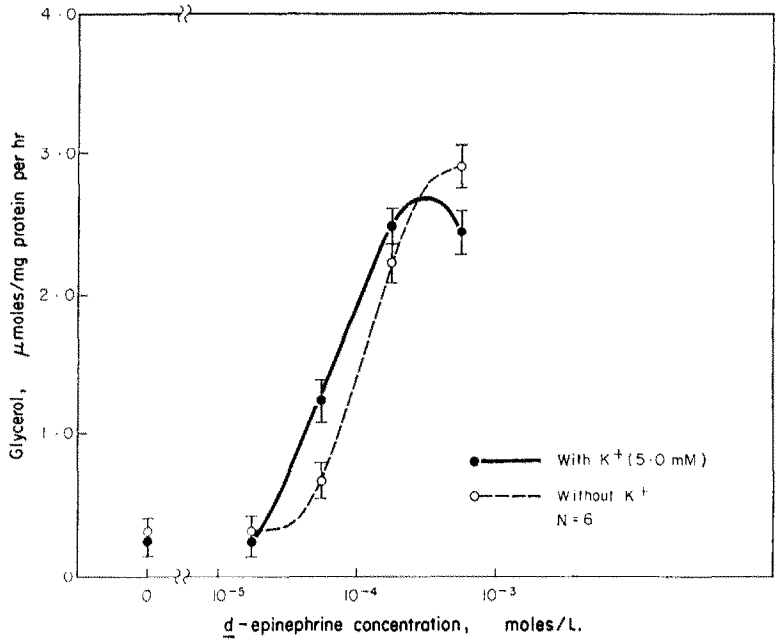

FIG. 6. Lipolytic effects of *d*-epinephrine in the presence and absence of potassium ion (5·0 mM). Each point is the mean ± S.E.M. of six paired experiments.

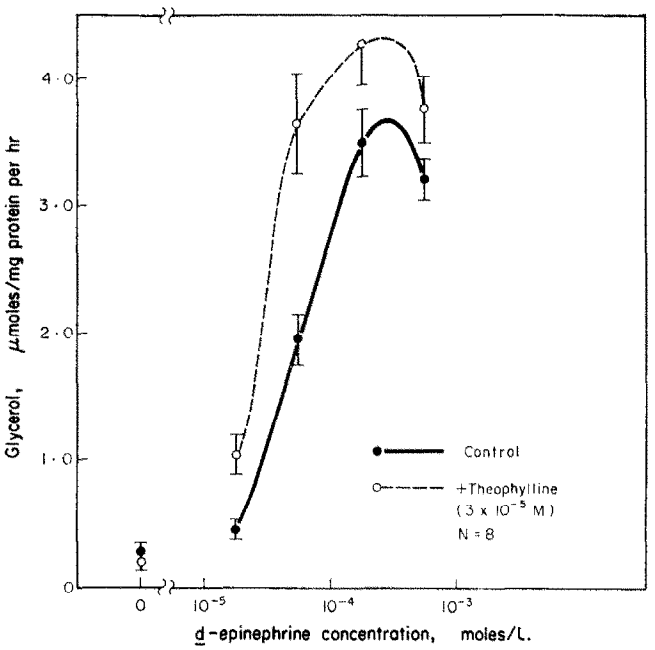

FIG. 7. Lipolytic effects of *d*-epinephrine in the presence and absence of theophylline (3×10^{-5}M). The buffer contained 5·0 mM potassium ion. Each point is the mean ± S.E.M. of eight paired experiments.

significantly ($P < 0.001$) increased the lipolytic response to all concentrations of *d*-epinephrine employed. In another series of experiments propranolol was shown to inhibit the lipolytic response to *d*-epinephrine (Fig. 8). This beta-blocking agent at a concentration of 10^{-6}M inhibited the lipolytic response to all concentrations of catecholamine ($P < 0.01$) with the exception of the lowest concentration employed and produced a shift of the dose–response curve to the right.

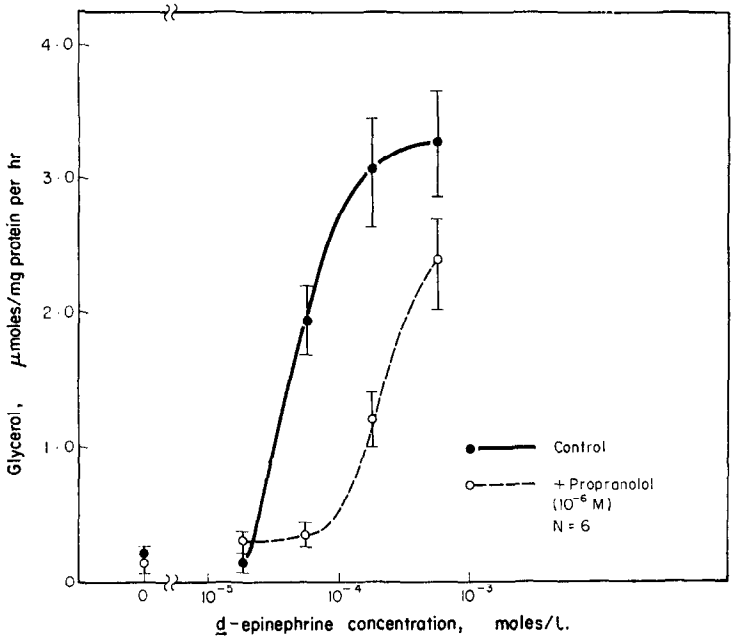

FIG. 8. Lipolytic effects of *d*-epinephrine in the presence and absence of propranolol (10^{-6}M). The buffer contained 5·0 mM potassium ion. Each point is the mean ± S.E.M. of six paired experiments.

The lipolytic dose–response curve to theophylline was found to be monophasic in a series of three experiments (Fig. 9). The lack of potassium ion in the medium did not alter the response to any concentration of theophylline.

Phosphodiesterase enzymatic activity was assayed in three experiments. Theophylline, a known inhibitor of this enzyme (10^{-2}M), reduced the activity to 50 per cent of control. In the same experiments epinephrine (10^{-4}M) had no effect on the enzyme activity. The phosphodiesterase activity in the presence of epinephrine was 98 per cent of the control value.

DISCUSSION

It is generally accepted that the lipolytic response to a variety of drugs and hormones is secondary to stimulation of adenyl cyclase and the subsequent increase in tissue concentrations of cyclic AMP.[5, 6] This cyclic nucleotide presumably promotes the conversion of a lipase from an inactive to an active form. We have recently reported a biphasic lipolytic dose–response curve to the catecholamines.[1] It was demonstrated

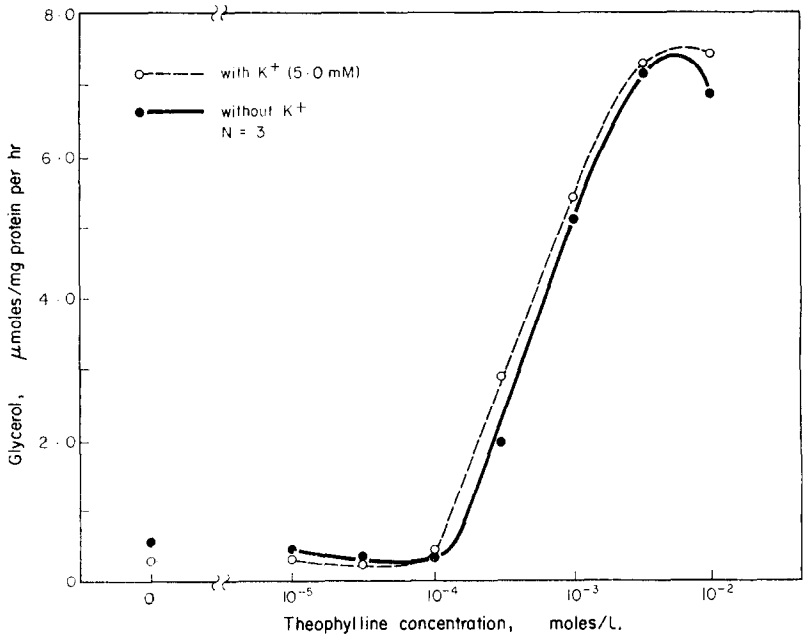

FIG. 9. Lipolytic effects of theophylline in the presence and absence of potassium ion (5·0 mM). Each point is the mean of three paired experiments.

that the first phase of the biphasic response (Lipolysis I) was identical to the much studied hormone-stimulated lipolysis and was, therefore, considered cyclic AMP dependent. Lipolysis II differed in many respects from the classical hormone-stimulated lipolysis and had not previously been studied with regard to its relationship to cyclic AMP. Evidence reported in this communication suggests that the second phase of catecholamine-stimulated lipolysis was also dependent upon the production of cyclic AMP.

The potassium ion has a profound influence on catecholamine-stimulated lipolysis. Several authors have shown that in the absence of this monovalent ion the response to epinephrine was reduced or totally absent.[11–13] In experiments reported here, it was seen that omission of the potassium ion from the incubation medium greatly diminished Lipolysis I. There was, however, no alteration in Lipolysis II. This suggests that some step in Lipolysis I was potassium ion dependent, while all steps in Lipolysis II were potassium ion independent. Potassium ion-free incubation medium thus offers a system for the study of Lipolysis II without any major complications of the presence of Lipolysis I.

From experiments using the *levo-* and *dextro*-isomers of epinephrine, it was evident that both isomers of epinephrine could produce marked lipolytic responses. The dose–response curve with the *levo*-isomer was biphasic, while, in the concentration employed, the dose–response curve with the *dextro*-isomer was monophasic. The magnitude of this response was significantly larger than that seen in the first phase with *l*-epinephrine. This would indicate that the response to *d*-epinephrine was not analogous to Lipolysis I. Other experiments demonstrated that lack of potassium ion in the

medium did not greatly alter the response to *d*-epinephrine. This too would indicate that the response to the epinephrine was not analogous to Lipolysis I, but rather had characteristics more closely resembling those of Lipolysis II. If this is indeed the case, the use of *d*-epinephrine would provide a convenient system with which to study Lipolysis II.

Study of hormone-stimulated lipolysis in K^+-free medium revealed that the responses to *l*-isoproterenol were augmented by the presence of sublipolytic concentrations of theophylline. This same concentration of theophylline also augmented the lipolytic responses to *d*-epinephrine. An important action of theophylline in adipose tissue is the inhibition of phosphodiesterase,[14, 15] the enzyme responsible for the inactivation of cyclic AMP. Several authors have shown that theophylline will augment the cyclic AMP-mediated lipolytic responses to a number of hormones by retarding the rate of degradation of the cyclic nucleotide.[14, 15] It has also been shown in adipose tissue that the methyl xanthines can augment the hormone-induced increases in cyclic AMP.[6, 16] The present results thus can be interpreted to mean that a similar mechanism is involved in the augmentation of the lipolytic responses reported here. This would suggest that Lipolysis II is cyclic AMP dependent.

In another series of experiments in potassium-free medium, propranolol a beta-adrenergic blocking agent, competitively inhibited the lipolytic response to *l*-epinephrine. Similar results were seen with *d*-epinephrine and propranolol. The beta-adrenergic blocking agents are generally considered to inhibit hormone-stimulated lipolysis by blocking the hormone activation of adenyl cyclase and thereby preventing the increase in tissue level of cyclic AMP.[4, 16] These data demonstrating inhibition of *l*-epinephrine- (K^+-free medium) and *d*-epinephrine-stimulated lipolysis by propranolol were consistent with the suggestion that the Lipolysis II response to the catecholamines is mediated by cyclic AMP.

It has previously been reported that, in potassium-containing medium, propranolol at a concentration of 10^{-6}M competitively inhibited Lipolysis I without altering Lipolysis II.[1] However, in the present report it was shown that inhibition of Lipolysis II can be achieved in K^+ containing medium by using a higher concentration of propranolol. At 10^{-5}M, this agent abolished Lipolysis I while competitively inhibiting Lipolysis II.

The suggestion that Lipolysis II is cyclic AMP dependent has some supporting evidence in the literature. Several authors have reported that the maximum activation of adipose tissue adenyl cyclase occurs with concentrations of catecholamine above 5×10^{-5}M.[16, 17] This is a concentration of catecholamine which produced the Lipolysis II response and which was considerably higher than the concentrations needed to produce the maximum Lipolysis I response. Thus, it can be seen that a concentration of catecholamine which produced Lipolysis II was producing more cyclic AMP than the concentration of catecholamine needed for maximum Lipolysis I was producing.

Although the present data indicate that Lipolysis II is cyclic AMP dependent, they do not suggest how Lipolysis I and Lipolysis II differ. One possibility could be the existence of two different cyclic AMP-stimulated lipase systems which differ in their sensitivity to the cyclic nucleotide. In addition, one of the systems would have to be K^+ dependent. Under these conditions, any agent which increased the intracellular concentration of cyclic AMP would produce a biphasic dose–response curve. This

would appear not to be the case, as the dose–response curve to theophylline was monophasic. Also, the responses to this agent were not altered by the absence of K^+ in the medium. These data are in agreement with those in the literature.[1, 12] In addition, the lipolytic dose–response curves to cyclic AMP and its dibutyryl derivative have been reported to be monophasic.[6] The responses to these lipolytic agents are not reduced but rather are increased by the lack of K^+ media.[18, 19] These results are not consistent with the suggestion that two different cyclic AMP-sensitive systems are present in adipose tissue.

Another possible explanation for Lipolysis II could be that epinephrine at high concentrations inhibited phosphodiesterase. This possibility was tested experimentally and, at concentrations of 10^{-4}M, epinephrine did not alter phosphodiesterase activity. These results rule out the possibility that Lipolysis II was the result of the inhibition of phosphodiesterase.

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