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STUDIES ON THE INHIBITION OF GLUCOSE METABOLISM IN ISOLATED FAT CELLS BY BETA-ADRENERGIC BLOCKING AGENTS

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Abstract—The effects of the *l*- and *d*-isomers of 1-(4-nitrophenyl)-1-hydroxy-2-isopropyl aminoethane and of propranolol on glucose and pyruvate metabolism were studied in isolated fat cells. At high concentration (10^{-3}M) all three drugs reduced the incorporation of glucose-1-^{14}C, and glucose-6-^{14}C into $^{14}CO_2$ and lipid-^{14}C, both in the presence and absence of insulin. The incorporation of pyruvate-3-^{14}C into $^{14}CO_2$ and lipid-^{14}C was also markedly reduced by these drugs. This effect of beta-adrenergic blocking agents appears to be independent of the blockade of the beta receptor. It is more likely the result of a general depression of cellular metabolism.

A NUMBER of seemingly unrelated agents have the capacity to inhibit hormone-stimulated lipolysis.[1] It now appears that several of these inhibitors act by blocking the hormone-stimulated formation of cyclic 3′,5′-adenosine monophosphate (cyclic-AMP). Two such agents are insulin[2, 3] and the beta-adrenergic blocking drugs.[3–5] Insulin can, of course, also increase the tissue utilization of glucose. Recently, Bewsher *et al.*[6] have reported that several beta-adrenergic blocking drugs can antagonize the insulin-stimulated $^{14}CO_2$ production from glucose-1-^{14}C in the rat epididymal fat pad. Such antagonism could be the result of an interference with the insulin-facilitated entrance of glucose into the cells or the result of some alteration in the intracellular metabolism of glucose. No evidence is available to support either of these possibilities, nor is evidence available to determine if this antagonism is dependent on the ability of beta-adrenergic blockers to inhibit the formation of cyclic-AMP.

The object of the present investigation was to examine the interaction of some beta-adrenergic blocking agents and insulin in the isolated fat cell preparation and to gain some information about the mechanism of the interaction.

METHODS AND MATERIALS

Fed, male, Holtzman rats weighing 120–180 g were stunned and exsanguinated. 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 under 95 per cent O_2–5 per cent

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CO_2 at 37° with gentle shaking. All drugs were added before the beginning of the incubation. The protein content of the fat cells was determined as described by Lech and Calvert.[7]

The effect of insulin (100 μU/ml) on glucose utilization was determined by measuring the incorporation of either glucose-1-^{14}C or glucose-6-^{14}C (0·05 μc, 10^{-3}M) into CO_2 and lipids. After a 90-min incubation, the reaction was terminated by the addition of 1 ml of 2 N H_2SO_4 and the CO_2 was trapped in a hanging well containing a filter-paper wick and 0·8 ml of Hyamine-X100. The contents of the well were transferred to a scintillation vial and counted in Bray's scintillation solution. Lipids were extracted from the incubation mixture by a double extraction according to the method of Dole.[8] The combined heptane phase was washed three times with water and evaporated to dryness in scintillation vials. Bray's scintillation solution was added and all samples were counted in a Packard Tri-Carb liquid scintillation spectrometer. Incorporation of pyruvate-3-^{14}C (10 mM, 0·1 μc) into CO_2 and lipid was measured by the same technique. Results of experiments measuring ^{14}C incorporation into CO_2 and lipids are expressed as a per cent of control.

In experiments measuring lipolysis, the incubation was for 60 min in glucose-free Krebs–Ringer bicarbonate buffer with 4% bovine serum albumin. After the incubation, the glycerol content of an aliquot of the cells and medium was determined by the method of Korn.[9] Results are expressed as micromoles of glycerol per milligram of protein per hour.

All results are expressed as the mean $\pm$ standard error of the mean. Values for P were calculated by using the Student *t*-test.

Bovine serum albumin (Fraction V) was purchased from Sigma Chemical Co. (St. Louis, Mo.). Both the glucose-1-^{14}C and the glucose-6-^{14}C were obtained from Nuclear-Chicago (Desert Plaines, Ill.). The pyruvate-3-^{14}C was supplied by Volk Laboratory (Chicago, Ill.). Bovine insulin (Lot No. 40) was the kind gift of Eli Lilly & Co. (Indianapolis, Ind.), and the *dextro* and *levo* isomers of INPEA [1-(4-nitrophenyl)-1-hydroxy-2-isopropyl aminoethane] were generously supplied by Selvi & Co. (Milano, Italy). Ayerst Laboratory (New York, N.Y.) furnished the propranolol [1-isopropylamine-3-(1-naphthyloxy)-2-propanol].

RESULTS

Utilization of glucose-1-^{14}C. Glucose-1-^{14}C was readily incorporated into CO_2 and lipid by the isolated fat cells, and in all experiments insulin (100 μU/ml) markedly increased the incorporation of ^{14}C into both products. Under control conditions 5 per cent of the added ^{14}C was recovered in CO_2 and lipids. In Fig. 1 are shown results of experiments with the *dextro* and *levo* isomers of the beta-adrenergic blocking agent, INPEA (10^{-3}M). Both isomers of this drug reduced by more than 40 per cent the insulin-stimulated rate of glucose incorporation into CO_2 ($P < 0·05$). However, the basal rate was not reduced by either isomer. In fact, in the absence of insulin, the *d*-isomer appeared to increase the amount of ^{14}C found in CO_2 ($P < 0·05$). In the same experiments the insulin-stimulated rate of glucose-1-^{14}C incorporation into lipid was also reduced by INPEA. The *d*- and *l*-forms of the drug were equally effective in this regard, reducing the effect of insulin by more than 60 per cent. The basal rate of incorporation of ^{14}C into lipid was significantly reduced as well ($P < 0·05$).

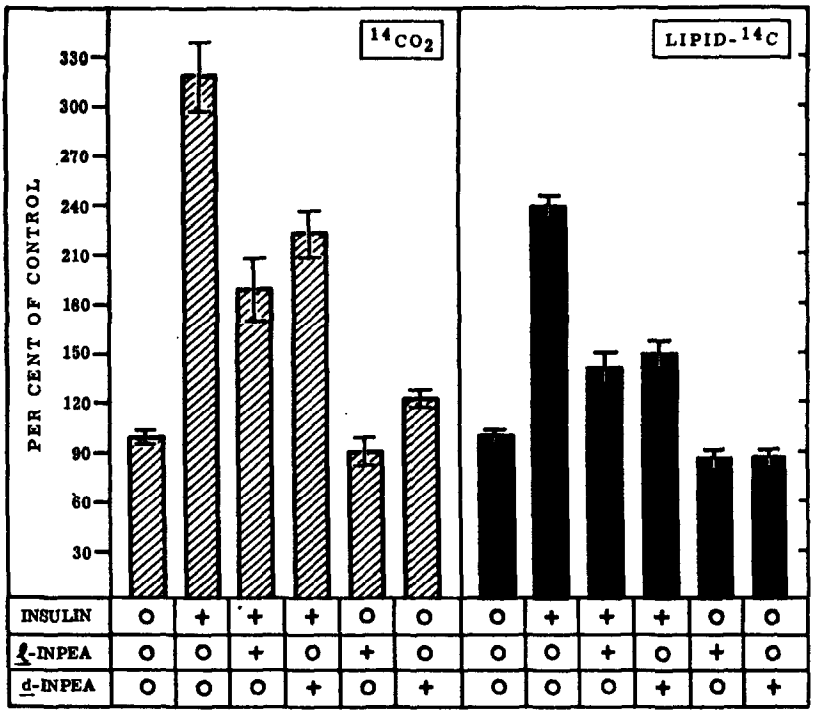

FIG. 1. The effect of *d*- and *l*-INPEA (10^{-3}M) on $^{14}CO_2$ and lipid-^{14}C production from glucose-1-^{14}C. The final concentration of insulin, when present, was 100 μU/ml. Values are means $\pm$S.E.M. of six observations.

Figure 2 shows the results of four experiments using 10^{-4}M *d*- and *l*-INPEA. These drugs at this concentration did not reduce the utilization of glucose-1-^{14}C under any of the conditions tested. In fact, a tendency is seen for these drugs to increase the utilization of glucose-1-^{14}C. However, in no case was this effect significant ($P > 0{\cdot}05$).

In a series of seven experiments, propranolol, another beta-adrenergic blocking agent, markedly reduced both the basal and the insulin-stimulated utilization of glucose-1-^{14}C (Fig. 3). Both in the presence and absence of insulin, propranolol (10^{-3}M) reduced the incorporation of glucose-1-^{14}C into CO_2 to 33 per cent of the basal rate ($P < 0{\cdot}05$). Under the same conditions the incorporation of ^{14}C into lipids was reduced to less than 60 per cent of the basal rates ($P < 0{\cdot}05$). At 10^{-4}M, propranolol had no significant effect on any parameter measured ($P > 0{\cdot}05$).

Utilization of glucose-6-^{14}C. The incorporation of glucose-6-^{14}C into CO_2 and lipid was measured in a series of nine experiments. Insulin (100 μU/ml) increased the rate of incorporation of ^{14}C into CO_2 and lipid by 54 and 360 per cent respectively. In the presence of propranolol (10^{-3}M), both the insulin-stimulated and the basal rate of $^{14}CO_2$ production were reduced to less than 21 per cent of the control. The corresponding rates of lipid-^{14}C production were reduced to less than 18 per cent of control (Fig. 4).

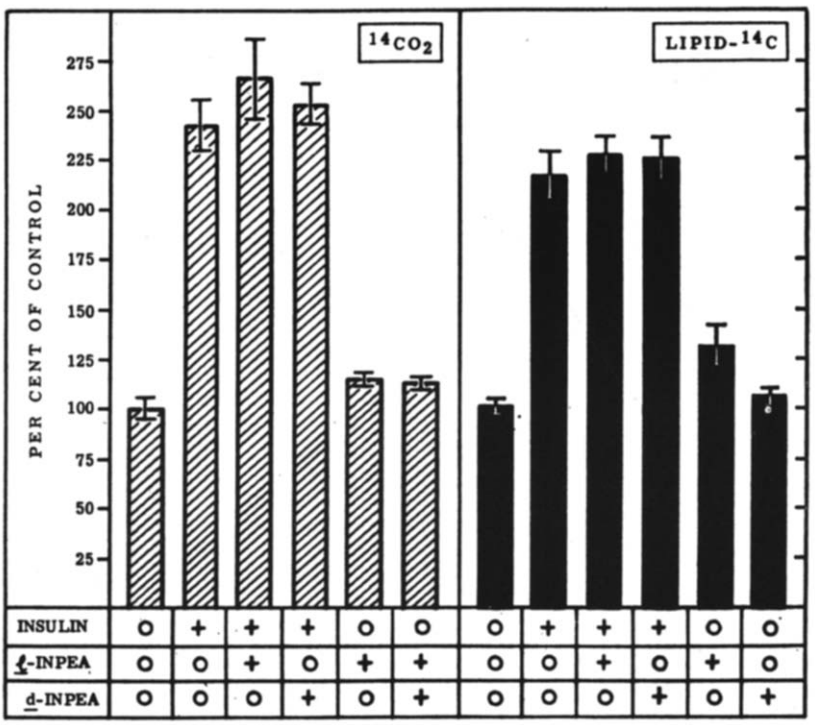

FIG. 2. The effect of *d*- and *l*-INPEA (10^{-4}M) on $^{14}CO_2$ and lipid-^{14}C production from glucose-1-^{14}C. The final concentration of insulin, when present, was 100 μU/ml. Values are means $\pm$S.E.M. of four observations.

Utilization of pyruvate-3-^{14}C. Figure 5 shows the results of six experiments with pyruvate-3-^{14}C. The fat cells readily incorporated pyruvate into lipids and oxidized it to CO_2. The presence of propranolol (10^{-3}M) reduced the amount of ^{14}C found in both CO_2 and lipids. In the same experiments INPEA also reduced the utilization of pyruvate. In the presence of either isomer of INPEA the rates of $^{14}CO_2$ and lipid-^{14}C production were reduced to 80 and 65 per cent of control respectively.

Lipolytic activity. The results shown in Table 1 demonstrate that epinephrine

TABLE 1. EFFECTS OF *d* AND *l*-INPEA (10^{-4}M) ON EPINEPHRINE-INDUCED (3×10^{-6}M) LIPOLYSIS

Additions *in vitro*	Glycerol release (μmoles/mg protein/hr)*	P
None (control)	0·11 $\pm$ 0·10	
Epinephrine	1·77 $\pm$ 0·11	< 0·001†
Epinephrine + *l*-INPEA	0·43 $\pm$ 0·05	< 0·001‡
Epinephrine + *d*-INPEA	1·59 $\pm$ 0·05	> 0·05‡
l-INPEA	0·27 $\pm$ 0·07	> 0·05‡
d-INPEA	0·28 $\pm$ 0·09	> 0·05‡

* Values given are means $\pm$S.E.M. for six observations.
† P values are compared to control response.
‡ P value as compared to epinephrine response.

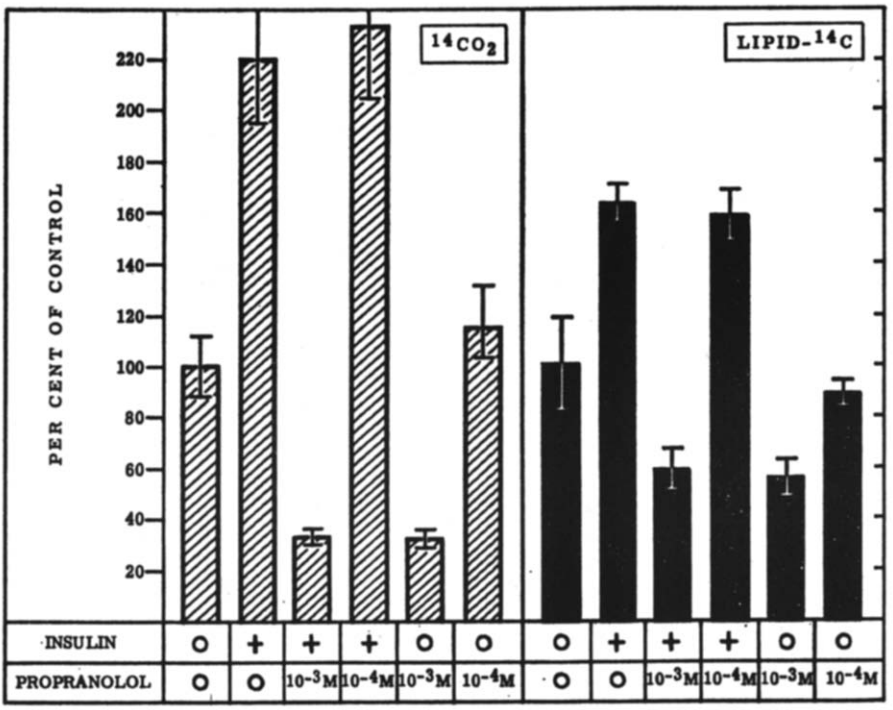

FIG. 3. The effect of propranolol (10^{-3}M and 10^{-4}M) on $^{14}CO_2$ and lipid-^{14}C production from glucose-1-^{14}C. The final concentration of insulin, when present, was 100 μU/ml. Values are means $\pm$S.E.M. of seven observations

(3×10^{-6}M) stimulates the rate of lipolysis as measured by glycerol release. The *levo* isomer of INPEA (10^{-4}M) is able to markedly reduce the lipolytic action of epinephrine ($P < 0{\cdot}001$). In contrast, the *dextro* isomer of INPEA failed to significantly alter the response to epinephrine ($P > 0{\cdot}05$). In the absence of epinephrine, neither isomer of INPEA significantly altered the rate of lipolysis ($P > 0{\cdot}05$).

DISCUSSION

It is well documented that insulin increases the entrance of glucose into adipose tissue cells and thereby increases the utilization of this carbohydrate.[10] Bewsher *et al.*[6] have reported that in the epididymal fat pad of the rat, high concentrations of several beta-adrenergic blocking agents (DCI, pronethalide and propranolol) interfere with the insulin-stimulated utilization of glucose, but had no effect on glucose utilization in the absence of insulin. In the present report, using isolated fat cells from rat epididymal fat pads, it has been found that *d*-INPEA, *l*-INPEA and propranolol inhibit glucose utilization not only in the presence of insulin but also in its absence. In the same preparation the metabolism of pyruvate, which is independent of the action of insulin, was also greatly inhibited by these drugs. From these findings it seems most unlikely that the beta-adrenergic blocking agents are interfering with the insulin-facilitated entry of glucose into the adipose cells. It is more likely that these agents are producing some alteration in intercellular metabolism of glucose.

Several areas of metabolism appear to be regulated by the intracellular levels of

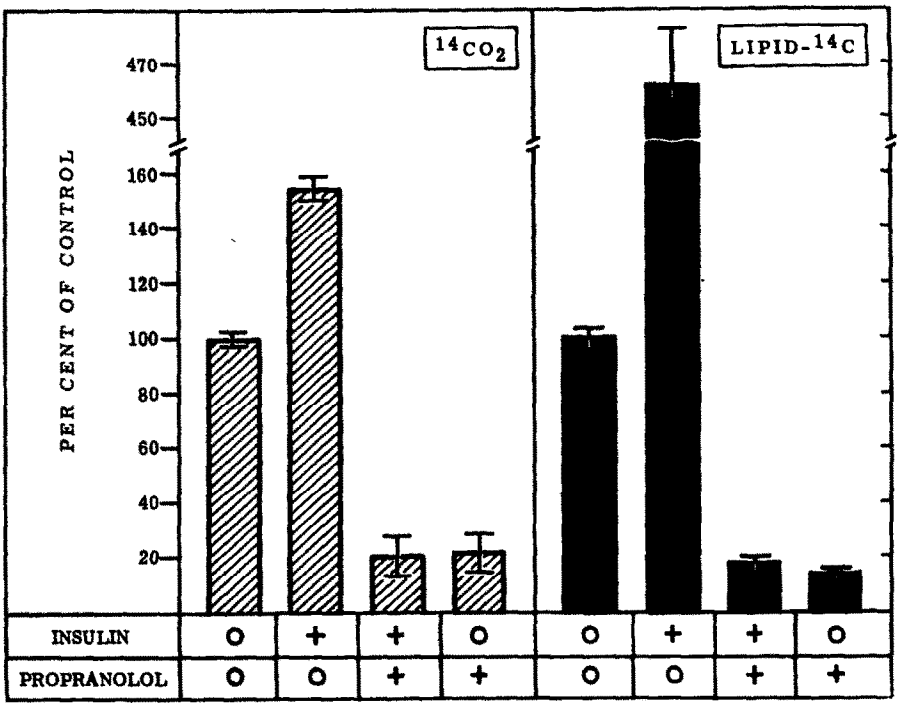

FIG. 4. The effect of propranolol (10^{-3}M) on $^{14}CO_2$ and lipid-^{14}C production from glucose-6-^{14}C. The final concentration of insulin, when present, was 100 μU/ml. Values are means $\pm$S.E.M. of nine observations.

cyclic-AMP. Much evidence is accumulating to suggest that this cyclic nucleotide controls the rate of lipolysis, steroidogenesis, glycogen breakdown and other metabolic processes.[11] The beta-adrenergic blocking agents have been shown to block the epinephrine-stimulated increase in tissue levels of cyclic-AMP presumably by inhibiting adenyl cyclase.[3–5] It is conceivable, therefore, that the beta-adrenergic blocking agents are altering glucose metabolism in the fat cells by blocking adenyl cyclase and subsequently lowering the intracellular level of cyclic-AMP. The experiments using the *dextro* and *levo* isomers of INPEA were designed to test this hypothesis.

The experiments of several authors have shown that pronethalol,[3, 4] propranolol,[5] and DCI[12] inhibit the epinephrine-stimulated production of cyclic-AMP in adipose tissue and thus inhibit epinephrine-stimulated lipolysis. Although neither isomer of INPEA has been shown to inhibit the formation of cyclic-AMP, their effect on epinephrine-stimulated lipolysis has been studied. Experiments reported here (Table 1), confirming the work of Fassina[13] and of Stock and Weatermann,[14] show that *l*-INPEA is a potent inhibitor of epinephrine-stimulated lipolysis while *d*-INPEA is almost without effect in this regard. It seems logical to assume that *l*-INPEA is blocking the activation of adenyl cyclase by epinephrine while *d*-INPEA is unable to do so. In contrast, it has been shown in the results reported here that the *d*- and *l*-isomers of INPEA are equipotent inhibitors of glucose metabolism. In light of these facts, it appears that the ability of these two drugs to interfere with glucose meta-

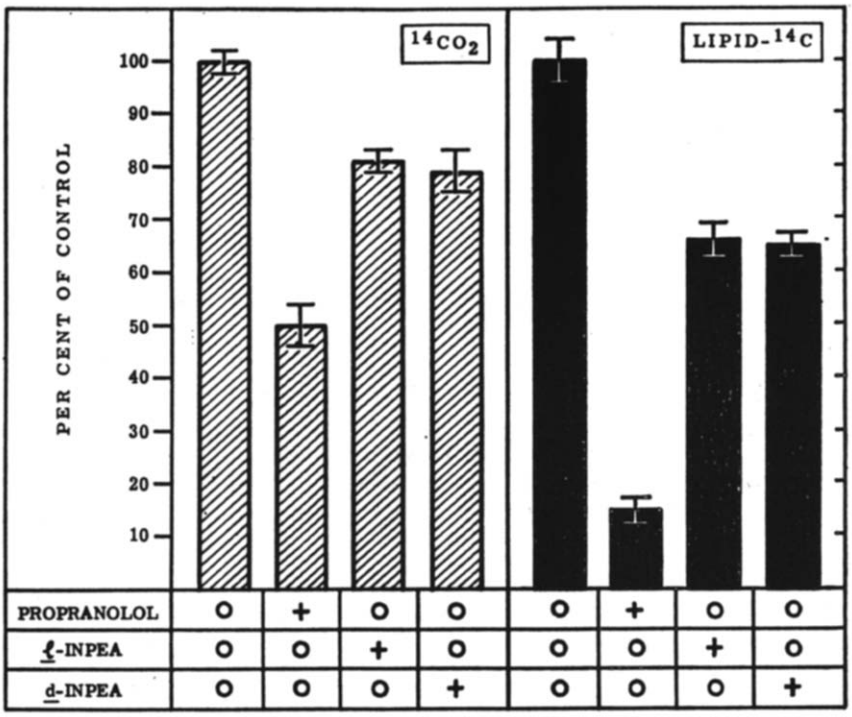

FIG. 5. The effect of propranolol (10^{-3}M) and *d*- and *l*-INPEA (10^{-3}M) on $^{14}CO_2$ and lipid-^{14}C production from pyruvate-3-^{14}C. Values are means ±S.E.M. for six observations.

bolism is independent of their ability to block increases in the tissue levels of cyclic-AMP.

From the available data, it is not possible to show specific reactions in glucose metabolism which are altered by the beta-blocking agents. However, because the production of $^{14}CO_2$ from glucose-1-^{14}C is reduced in the presence of these drugs, it would appear that the activity of the pentose–phosphate shunt is reduced. A reduction in the pentose–phosphate shunt activity could also explain the decreased incorporation of glucose and pyruvate into lipid. A depression of this pathway would lead to a reduction in the availability of NADPH, which is needed for the synthesis of fatty acids and subsequently triglyceride lipid. This does not rule out, however, the possibility that the beta-blocking agents more directly inhibit lipid synthesis. Nor does it indicate that the reduction in the pentose shunt activity or the incorporation of C^{14} into lipids is the result of any specific inhibition. These changes could be the result of a general depression of metabolism.

The fact that the beta-blocking agents are depressing shunt activity cannot explain the reduction in $^{14}CO_2$ from glucose-6-^{14}C or pyruvate-3-^{14}C, as $^{14}CO_2$ is produced from these compounds only in the citric acid cycle. These drugs must, therefore, inhibit another step (or steps) in carbohydrate metabolism.

A possible explanation for the findings reported here and elsewhere[6] is that the beta-adrenergic blocking drugs produce a general depression of metabolism. The nonspecific nature of the inhibition of metabolism produced by these drugs suggests that this is the case. This would also offer an explanation for the findings of Aulich

et al.[15] and Peterson *et al.*[16] These authors found that high concentrations of the beta-blockers, Ko-592[15] and pronethalol,[16] inhibit the lipolytic activity of dibutyryl cyclic-AMP. It is suggested by these authors that the inhibition of this lipolysis was at some site beyond the production of cyclic-AMP. An alternative explanation is that these beta-blocking agents are producing a nonspecific depression of all metabolism of the tissue, including lipolysis.

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