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Different Lipolytic Effects of Theophylline and Dibutyryl Cyclic AMP *In Vivo* and *In Vitro* (39183)

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Theophylline and N^6,$O^{2'}$-dibutyryl cAMP (dcAMP) have been shown to stimulate lipolysis in *in vitro* systems of adipose cells, fragments, and fat pads (1-4). These two compounds have been demonstrated to increase intracellular concentrations of cyclic adenosine 3',5'-monophosphate (cAMP), which is an important intermediate in the activation of hormone-sensitive lipase (1-3, 5). Theophylline operates by reducing phosphodiesterase activity (1, 2). dcAMP functions by competing as a substrate for the phosphodiesterase (6) and possibly through its metabolite, N^6-monobutyryl cAMP, which acts similarly to cAMP (7).

Theophylline is also known to stimulate lipolysis *in vivo* (8), presumably by its effect on the degradation of cAMP. Information concerning the *in vivo* effect on lipolysis of dcAMP is limited. dcAMP has been reported to reduce circulating free fatty acid (FFA) levels in the rat (9). The lower FFA levels may result from (a) diminished lipolysis or (b) less FFA available for release from the adipose cell because of increased FFA reutilization with accelerated triglyceride resynthesis. If reduction in FFA levels is due to impaired lipolysis, then there is an important difference in the *in vitro* and *in vivo* responses to dcAMP which represents a notable dissimilarity in the actions of dcAMP and theophylline. If this difference exists, the information will be useful for two reasons. First, by identifying inhibited lipolysis as the basis for low FFA levels in animals given dcAMP, understanding of *in vivo* triglyceride metabolism will be expanded. Second, it will serve to alert other investigators to the possibility of further disparities in the actions of dcAMP and theophylline. To explore the possibility that the difference in lipolytic responses to dcAMP occurs, a comparison of the *in vitro* and *in vivo* effects of theophylline and dcAMP and lipolysis was performed in the young dog. The effects were evaluated during basal and epinephrine-stimulated lipolysis.

Methods and materials. Puppies, 4 to 8 weeks of age and weighing 1.5 to 2.5 kg, were the subjects of this investigation. The animal model was the same as that used in previous studies of lipolysis (10).

The following agents were administered in the *in vivo* studies. dcAMP was infused at a rate of 2 mg/kg/min, theophylline at 3 mg/kg/min, and epinephrine at 0.5 μg/kg/min. Separate venous cannulae were used for each agent.

Periodic arterial blood samples, totaling less than 15 ml, were obtained. Plasma levels of glycerol (11) and FFA (12), the products of triglyceride breakdown, were used as indicators of lipolysis. Plasma glucose (13) and immunoreactive insulin (IRI) (14) were also measured in all animals.

The following series of animal experiments was performed. (a) Theophylline: after a 45-min control period (same in all groups), five animals were given a 15-min infusion of theophylline. (b) dcAMP: Five animals received a 30-min infusion of dcAMP. (c) Epinephrine: Six animals received a 75-min infusion of epinephrine. (d) Epinephrine plus theophylline: Epinephrine was administered for 75 min to six animals. Theophylline was administered in conjunction with the epinephrine after the catecholamine was running for 30 min. (e) Epinephrine plus dcAMP: Six animals were given a 75-min infusion of epinephrine. After the initial 30-min of the catecholamine infusion, an infusion of dcAMP was given in conjunction with the epinephrine.

In vitro experiments were performed on adipose tissue fragments obtained from the posterior portion of the hind limb of puppies similar to those used in the *in vivo* studies. Tissue fragments, weighing 10 to 25 mg, were placed in small plastic flasks containing 2 ml of Krebs-Ringer bicarbonate

buffer, pH 7.4 at 37°. The incubation media contained D-glucose (1 mg/ml) and bovine serum albumin (50 mg/ml). Incubations were continued in a Dubnoff metabolic shaker for 3 hr at 37° with shaking at 60 cycles/min. Incubation media were collected after 1 and 3 hr of incubation for glycerol determination. Triglyceride content was measured on a portion of each adipose tissue fragment obtained prior to incubation. Triglyceride was determined by alkaline hydrolysis and subsequent measurement of glycerol. Lipolysis was expressed as μmole of glycerol/g of tissue triglyceride/2 hr. In addition to the control experiments (no epinephrine, dcAMP, or theophylline), incubations were performed with (a) epinephrine, (b) dcAMP, (c) theophylline, (d) epinephrine plus dcAMP, and (e) epinephrine plus theophylline. Pharmacologic agents used were epinephrine (3 μg/ml), dcAMP (1 μmole/ml), and theophylline (1 μmole/ml buffer). Each of the control and experimental groups consisted of six incubations.

Statistical analyses were performed with Student's *t* test. Differences were considered significant when P was less than 0.05.

Results. In vivo experiments: Glycerol and free fatty acids. In all animals studied, control glycerol concentration was 0.59 ± 0.03 μmole/ml, and FFA was 0.45 ± 0.02 μmole/ml (mean ± SE).

When given alone, theophylline produced a marked increase in both mean plasma glycerol ($P < 0.005$) and FFA ($P < 0.005$) (Fig. 1). With dcAMP administration (Fig. 1) glycerol fell from 106 to 94% mean control, and FFA fell from 107 to 74% mean control; however, only the change in FFA was significant ($P < 0.01$).

In animals receiving epinephrine alone, plasma glycerol and FFA levels rose sharply, reaching a plateau within 30 min (Fig. 2). In puppies who received epinephrine and subsequently received dcAMP or theophylline, initial glycerol and FFA responses were slightly smaller than those given only epinephrine, but the differences were not significant. When theophylline was added, circulating glycerol and FFA rose but the resulting levels were not significantly different from those found in puppies given only epinephrine. On the other hand,

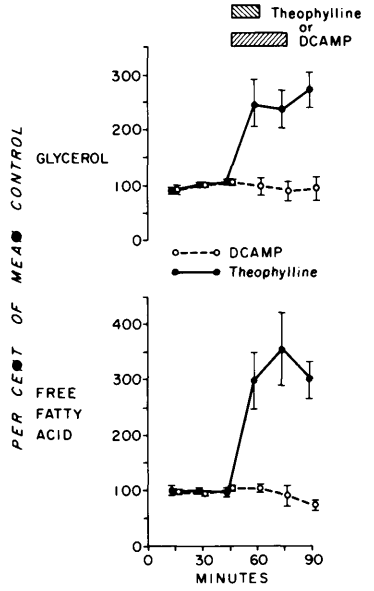

FIG. 1. Points represent means; vertical bars indicate 95% confidence limits.

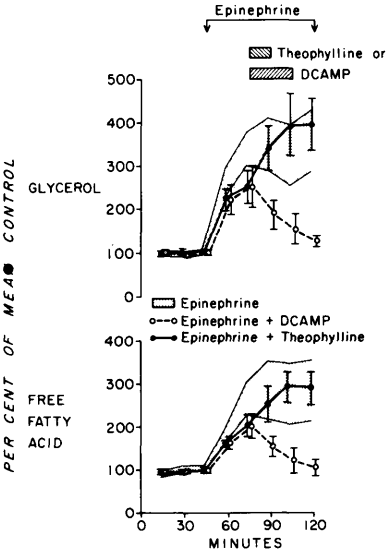

FIG. 2. Points represent means; vertical bars indicate 95% confidence limits. Stippled area represents 95% confidence limits for glycerol and FFA during the infusion of epinephrine only.

dcAMP administration to animals receiving epinephrine produced a sharp fall in plasma glycerol ($P < 0.005$) and FFA ($P < 0.005$). The drops in glycerol and FFA from the epinephrine stimulated elevations resulted in a 120-min concentration significantly lower than those found in puppies receiving

epinephrine alone ($P < 0.025$) and puppies given epinephrine plus theophylline ($P < 0.01$).

Glucose and insulin. Circulating levels of glucose rose in animals receiving theophylline and animals receiving dcAMP (Fig. 3). The increase with dcAMP was greater than with theophylline. Both theophylline and dcAMP augmented the increases in plasma glucose produced by epinephrine, but the rise with dcAMP was greater (Fig. 4). Note that the difference in glucose responses produced by theophylline and dcAMP occurred in conjunction with the differences in glycerol and FFA produced by the two agents.

IRI levels rose with both dcAMP and theophylline (Fig. 3). However, the IRI increase with theophylline was greater, the period of increase was shorter, and the onset earlier than with dcAMP. It is of importance that the initial large IRI increase took place at the same time that lipolysis was being stimulated by theophylline; the IRI elevation produced by dcAMP was smaller and delayed and was associated with no rise in glycerol or FFA. IRI in animals given epinephrine and dcAMP remained at baseline

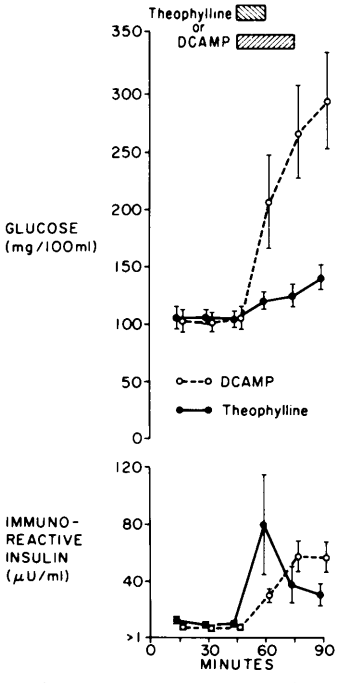

FIG. 3. Points represent means; vertical bars indicate 95% confidence limits.

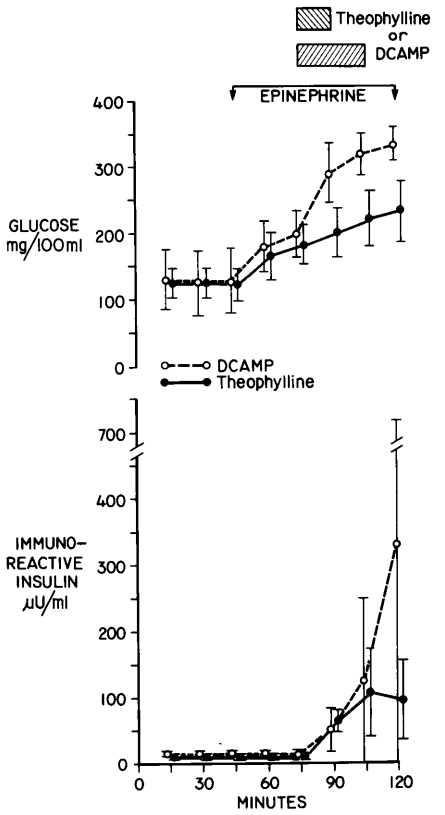

FIG. 4. Points represent means; vertical bars indicate 95% confidence limits.

levels with epinephrine and rose with the addition of dcAMP. In the others given epinephrine and theophylline, no change was noted with epinephrine and increases were found with theophylline. Thus, although there were similar IRI elevations when either theophylline or dcAMP was infused in animals already receiving epinephrine, the glycerol and FFA responses were markedly different.

In vitro experiments: Glycerol. As shown in Table I, glycerol release was produced by epinephrine, dcAMP, and theophylline. The responses to dcAMP and theophylline were similar. Glycerol release with epinephrine plus theophylline was as great as, or greater than, it was with either epinephrine, theophylline, or dcAMP. However, the response observed in incubations with epinephrine plus dcAMP was the largest of all, being significantly greater than that with either epinephrine ($P < 0.025$) or dcAMP ($P < 0.05$) alone. Thus, the inhibitory effect of

TABLE I. ADIPOSE TISSUE INCUBATIONS: GLYCEROL RELEASE (μmole GLYCEROL/g ADIPOSE TISSUE TRIGLYCERIDE/2 hr)[a]

Experiment number	Control	Epinephrine	dcAMP	Theophylline	Epinephrine + dcAMP	Epinephrine + theophylline
1	3.75	8.52	21.19	15.96	16.53	13.89
2	6.73	13.57	8.48	9.55	17.96	23.45
3	5.61	13.92	11.12	8.02	14.65	11.48
4	6.80	13.26	16.45	12.44	25.16	16.11
5	4.01	15.72	13.53	14.15	26.00	18.54
6	5.42	15.70	12.06	17.90	27.60	15.13
Mean ± SE	5.39 ± 0.52	13.45 ± 1.07	13.81 ± 1.82	13.00 ± 1.54	21.32 ± 2.27	16.43 ± 1.69

[a] Glycerol release with all agents, singly or in combination, was significantly greater than it was in the control group ($P < 0.025$). The responses to epinephrine, dcAMP, theophylline and epinephrine plus theophylline were not significantly different ($P > 0.05$). However, glycerol release with epinephrine plus dcAMP was significantly greater than with either epinephrine ($P < 0.025$) or dcAMP ($P < 0.05$) alone.

dcAMP on lipolysis *in vivo* was not found in these *in vitro* studies.

Discussion. In the young air-ventilated puppy, lipolysis was stimulated by theophylline and perhaps inhibited by dcAMP. These conclusions are based upon the large increases of plasma glycerol and FFA levels associated with theophylline administration and the small, although significant, fall in FFA with dcAMP. It may be argued that the lack of glycerol and FFA elevation following dcAMP infusion was due to improper dosage. Against this possibility was the glucose mobilization and insulin release affected at the dcAMP dosage used. In addition, dcAMP infused at 1 or 3 mg/kg/min in identically air-ventilated puppies resulted in glycerol, FFA, glucose, and IRI responses similar to those found with 2 mg/kg/min (unpublished data). That dcAMP's small lipolytic effect was due to the dog adipocytes' impermeability to the molecule is implausible because dcAMP was strikingly effective *in vitro* in the current investigation and in the studies of others (1–4).

Because of dcAMP's inconclusive effect on the important glycerol parameter, the responses were compared during epinephrine-stimulated lipolysis. Theophylline given along with epinephrine did not alter the high concentrations of plasma glycerol and FFA resulting from epinephrine alone. However, the levels resulting from dcAMP given with epinephrine were significantly and markedly lower than those produced by epinephrine alone and with epinephrine plus theophylline, conclusively demonstrating the inhibitory effect of dcAMP.

Since both dcAMP and theophylline stimulate lipolysis in dog adipose tissue fragments as they do in many other *in vitro* preparations (1–4), inhibition produced by dcAMP in the intact animal cannot be related to a peculiarity of the dog's adipose tissue. On the other hand, the data obtained from puppies suggests that dcAMP may exert some extra-adipose influence which secondarily interferes with lipolysis. Of the possibilities, the data only allowed consideration of the known inhibitory effect of insulin (15). This hormone's antilipolytic action does not appear to be a major factor for two reasons. First, theophylline alone generated high, early IRI levels while causing lipolysis. In contrast, lower IRI levels followed dcAMP administration, but there was no evidence of lipolysis. Secondly, in the presence of epinephrine, the addition of dcAMP but not theophylline markedly impaired lipolysis in animals although neither dcAMP nor theophylline produced a significant difference in IRI response.

The difference in lipolytic response to dcAMP *in vitro* and in the intact animal needs emphasis. The dissimilarity from the lipolytic actions of theophylline is of importance to investigators interested in triglyceride metabolism. Since it also suggests the possibility of other differences in the *in vivo* and *in vitro* functions of the two agents, it prompts the need for caution whenever using theophylline and dcAMP in studies involving the intact animal.

Summary. Both dcAMP and theophylline are known to promote lipolysis *in vitro* by increasing intracellular cAMP. Although

theophylline stimulates FFA mobilization *in vivo* as well, a report of low circulating FFA levels in the rat given dcAMP suggested that dcAMP may inhibit lipolysis in the intact animal. To explore this possibility, a comparison of the *in vitro* and *in vivo* lipolytic effects of theophylline and dcAMP was made in the young dog.

Circulating glycerol and FFA levels rose following the administration of theophylline. While glycerol and FFA fell slightly in puppies given dcAMP, only the FFA change was significant. Epinephrine infusions given alone produced sustained elevations of glycerol and FFA. When theophylline was given in conjunction with ongoing epinephrine infusions, plasma glycerol and FFA levels remained high. On the other hand, epinephrine-stimulated lipolysis was markedly inhibited by dcAMP, as shown by pronounced falls of glycerol and FFA from the elevated levels found with epinephrine alone.

In vitro studies involving fragments of puppy adipose tissue reveal that epinephrine, theophylline, and dcAMP promoted glycerol release. In contrast to the *in vivo* observations, lipolysis was also stimulated by combinations of both epinephrine and theophylline as well as by epinephrine and dcAMP. Thus, theophylline stimulates lipolysis *in vitro* and *in vivo* in the puppy. In contrast, dcAMP stimulates lipolysis *in vitro* but inhibits this action in the intact animal. This important difference in the two pharmacologic agents suggests the need for caution when using them in *in vivo* studies involving the action of cAMP.

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