Influence of Theophylline on the Adipokinetic Effect of Glucagon *in Vivo*[1] (36869)

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Because of its inhibitory effect on phosphodiesterase, the enzyme that inactivates cyclic adenosine 3′,5′-monophosphate (CAMP) in the body (1), theophylline has been used as a tool in the study of the biochemical mechanisms of hormone-induced lipolysis (2–4). Theophylline causes elevation of plasma-free fatty-acid (FFA) concentration in rats (2, 5) and a similar effect has been observed after injection of aminophylline (theophylline-ethylenediamine) in dogs (6) and men (7–9). The adipokinetic effect of glucagon is believed to be mediated by CAMP (3, 4). Glucagon elevates the concentration of CAMP in the adipose tissue and the isolated fat cells of the rat (10, 11) and its lipolytic effect is potentiated *in vitro* by caffeine (10) and by theophylline (12, 13).

In view of the differences between animal species in regard to their responsiveness to the adipokinetic hormones (14–16), we have studied the influence of theophylline on the adipokinetic effect of glucagon in geese, ducks, owls, and rabbits. These animals respond with marked elevations of plasma FFA concentration to the injection of glucagon (16–18) and the lipolytic activity of their adipose tissue is stimulated *in vitro* by the addition of that hormone (16).

Methods. Adult, non-anesthetized, male geese, mallard ducks, and owls, fed and kept as described in previous publications (17–19), were used. The wing veins were used for blood sampling and injections.

Adult male rabbits were anesthetized with sodium pentobarbital[3] (25 mg/kg injected into an ear vein). Polyethylene catheters were inserted into the jugular veins for blood sampling, infusions, and injections. Infusions were made with a syringe pump.[4] All the experiments were made in animals fasting for 16–18 hours.

Solutions of theophylline[5] in saline, and glucagon[6] in glycine buffer (0.02 *M*, pH 9.5 in saline) were prepared daily, just before the experiments. Aminophylline[7] was a commercial preparation containing 500 mg of theophylline-ethylenediamine in 20 ml. Plasma FFA were measured as previously reported (19). Blood sugar (BS) was determined with *o*-toluidine (20).

Results. (*a*) *Effect of theophylline in geese and owls.* As shown in Table I, theophylline given intravenously (two separated doses, each of 50 mg/kg, one hour apart) caused a slight decrease of plasma FFA and a significant elevation of BS. This dose of theophylline was lethal for the geese all of which died during the night following the experiment.

The effect of a single injection of theophylline (50 mg/kg) was tested in 3 owls. No significant change of plasma FFA was noted, but BS rose continuously reaching a level 45% above preinjection one hour after the injection.

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[2] Recipient of a Fellowship from the Juan March Foundation, Madrid, Spain.

[3] Somnopentyl, Pitman-Moore, Inc., Fort Washington, Pa.

[4] Model 600-910, Harvard Apparatus Co., Dover, Mass.

[5] Nutritional Biochemicals Corporation, Cleveland, Ohio.

[6] Crystalline glucagon Lot 258-234B-167-1, kindly donated by the Eli Lilly Co. Laboratories, Indianapolis, Ind.

[7] Elkins Sinn Inc., Cherry Hill, N.J.

TABLE I. Effect of Intravenous Injections of Theophylline (50 mg/kg at 30, and at 90 min) on Plasma-Free Fatty Acids and Blood Sugar in Geese.

	Means ± SE for 6 geese							
	Time (min)							
	0	30	60	90	95	105	120	150
FFA (mEq/liter)	1.02 ±0.14	1.03 ±0.15	0.86 ±0.09	0.74 ±0.09	0.77[a] ±0.10	0.86 ±0.13	0.82 ±0.11	0.75 ±0.11
Blood sugar (mg/100 ml)	111 ±11.4	112 ±12.4	126 ±14.6	127 ±15.6	143[a] ±17.2	162[b] ±12.1	169[b] ±15.3	168[b] ±14.3

[a] Significantly different from means of preinjection values at 0 and 30 min ($0.01 < p < 0.05$).

[b] Significantly different from mean of preinjection values at 0 and 30 min ($p < 0.01$).

(*b*) *Effect of aminophylline in geese.* The effect of a single dose of aminophylline was tested in 8 geese. The same animals received 2 weeks later a single injection of glucagon and after another 2 weeks they were injected with aminophylline followed by glucagon. The results are reported in Table II.

Aminophylline caused a small, transient elevation of plasma FFA followed by a significant decrease that was maintained until the end of the experiment. Glucagon alone caused a prompt elevation of plasma FFA (0.95 mEq/liter, SE ± 0.10, 5 min after injection). When glucagon was injected immediately after aminophylline the corresponding elevation was 1.07 ± 0.10. These two elevations are not statistically different from each other ($p > 0.05$, t test for paired variates).

Both aminophylline and glucagon caused elevations of BS. The BS levels between 60 and 270 min tended to be higher when glucagon and aminophylline were injected together than when either compound was given separately. However, none of the elevations was significantly greater than the sum of the corresponding elevations caused by aminophylline and by glucagon injected separately ($p > 0.05$, t test for paired variates).

(*c*) *Effect of theophylline in ducks.* Three groups, each of 10 ducks, were used. The first group received theophylline, the second group was injected with glucagon and the third group received both theophylline and glucagon. The results are summarized in Table III.

The first theophylline injection produced significant decrease of plasma FFA. Glucagon alone caused a mean FFA elevation of 1.64 mEq/liter (SE ± 0.15, $p < 0.01$) 5 min after injection. The corresponding elevation when glucagon was given after theophylline was 1.41 mEq/liter (SE ± 0.29, $p < 0.01$). These two elevations were not significantly different from each other ($p > 0.05$, t test for non-paired variates).

Theophylline alone produced elevation of BS in ducks. The BS elevations produced by glucagon plus theophylline were somewhat greater than those produced by glucagon alone, but only the difference between the elevations at 120 min reached statistical significance at the level of $p = 0.05$ (t test for non-paired variates).

(*d*) *Effect of theophylline in rabbits.* The effect of injecting theophylline, glucagon, and theophylline plus glucagon on the plasma FFA and BS of rabbits is shown in Fig. 1.

Theophylline alone caused a marked and sustained elevation of plasma FFA. Glucagon alone caused an elevation of plasma FFA with a peak 15 min after injection (1.10 mEq/liter, SE ± 0.26, $p < 0.01$). When glucagon was injected after theophylline plasma FFA showed an elevation above the level produced by theophylline alone. The FFA elevation (15 min after glucagon injection) was 0.78 mEq/liter (SE ± 0.15, $p < 0.01$).

Both theophylline and glucagon caused elevations of BS. The BS elevations caused by injecting glucagon after theophylline were not significantly different from those pro-

TABLE II. Effect of Aminophylline (40 mg/kg), Glucagon (2.0 μg/kg), and Aminophylline (40 mg/kg) plus Glucagon (2.0 μg/kg) on Plasma-Free Fatty Acids and Blood Sugar in Geese. Means and SE for 8 Geese. The Same 8 Geese Used for the Three Injections. Injections at 30 min.

	Time (min)										
	0	30	35	45	60	90	120	150	180	210	270
	FFA (mEq/liter)										
Aminophylline	1.19 ±0.08	1.15 ±0.07	1.35[a] ±0.09	1.16 ±0.06	0.99[a] ±0.06	0.87[b] ±0.07	0.78[b] ±0.06	0.73[b] ±0.07	0.75[b] ±0.06	0.77[b] ±0.06	0.82[b] ±0.06
Glucagon	0.98 ±0.07	1.02 ±0.07	1.95[b] ±0.10	1.71[b] ±0.06	1.48[b] ±0.05	1.27 ±0.11	1.06 ±0.12	0.99 ±0.09	0.96 ±0.05	0.91 ±0.03	0.88 ±0.05
Aminophylline + glucagon	0.93 ±0.05	0.95 ±0.04	2.01[b] ±0.11	1.68[b] ±0.10	1.27[b] ±0.07	0.91 ±0.07	0.79 ±0.06	0.74[a] ±0.06	0.70[a] ±0.06	0.69 ±0.07	0.72 ±0.10
	Blood sugar (mg/100 ml)										
Aminophylline	120 ±2.4	120 ±3.9	128[a] ±4.9	133[a] ±6.1	128 ±6.8	127 ±6.6	131 ±6.8	132 ±6.2	134[b] ±4.9	139[b] ±4.2	142[b] ±5.2
Glucagon	123 ±4.2	128 ±4.6	157[b] ±9.3	179[b] ±12.9	158[a] ±14.6	135 ±5.8	135 ±3.9	132 ±3.9	134 ±3.5	134 ±3.3	136 ±5.1
Aminophylline + glucagon	125 ±3.4	132 ±3.6	148[b] ±3.7	174[b] ±7.5	168[b] ±10.6	152[a] ±9.9	150[a] ±9.2	153[b] ±8.7	154[b] ±7.9	156[b] ±7.7	161[b] ±7.3

[a] Significantly different from mean of preinjection values at 0 and 30 min ($0.01 < p < 0.05$).

[b] Significantly different from mean of preinjection values at 0 and 30 min ($p < 0.01$).

TABLE III. Effect of Theophylline, Glucagon, and Theophylline Plus Glucagon on Plasma-Free Fatty Acids and Blood Sugar in Ducks. Means ± SE for 10 Ducks in Each Injection Group.

	Time (min)						
	0	30	60	65	75	90	120
	FFA (mEq/liter)						
Theophylline[a]	1.45 ±0.11	1.22 ±0.15	1.06[e] ±0.12	—	—	1.21[d] ±0.11	1.35[e] ±0.12
Glucagon[b]	1.38 ±0.13	—	1.41 ±0.12	3.05[e] ±0.24	2.62[e] ±0.24	1.98[e] ±0.13	1.61[e] ±0.10
Theophylline + glucagon[c]	1.27 ±0.11	—	0.85[e] ±0.10	2.26[e] ±0.30	2.40[e] ±0.33	2.09[e] ±0.26	1.88[e] ±0.22
	Blood sugar (mg/100 ml)						
Theophylline[a]	130 ±4.8	169[e] ±5.9	180[e] ±7.2	—	—	196[e] ±6.8	200[e] ±9.3
Glucagon[b]	122 ±4.2	—	124 ±4.8	154[e] ±4.6	202[e] ±9.1	191[e] ±9.0	147[d] ±8.3
Theophylline + glucagon[c]	129 ±3.3	—	170[e] ±4.3	190[e] ±7.8	220[e] ±3.7	226[e] ±9.0	217[e] ±10.5

[a] 50.0 mg/kg iv at 0 time and at 60 min.
[b] 20.0 μg/kg iv at 60 min.
[c] 50.0 mg/kg of theophylline at 0 and 60 min plus 20 μg/kg of glucagon at 60 min.
[d] Significantly different from preceding preinjection value ($0.01 < p < 0.05$).
[e] Significantly different from preceding preinjection value ($p < 0.01$).

duced by glucagon alone ($p > 0.05$, t test for non-paired variates).

(*e*) *Effect of glucagon injection during infusion of glucagon.* The effect of injecting glucagon into rabbits whose plasma FFA and BS had been elevated by a continuous infusion of glucagon was tested in three groups of rabbits. The results are shown in Fig. 2. The effects of injecting 20.0 μg/kg of glucagon during glucagon infusion were smaller than those observed when glucagon was injected alone or after injection of theophylline.

The statistical analysis presented in Table IV shows that the effect of glucagon (20.0 μg/kg), when given after injection of theophylline, was greater than that observed when glucagon was injected during an infusion of glucagon producing elevation of plasma FFA comparable to that produced by theophylline.

Similar analysis indicates that the hyperglycemic effect of glucagon was greater when the hormone was injected after theophylline than when injected during glucagon infusion (0.1 μg/kg/min).

Discussion. The results of our experiments in birds are in striking contrast to the observations in rats, dogs, and men reported in the literature (2, 5–9). No elevation of plasma FFA was observed in birds injected with doses of theophylline or aminophylline greater than those shown to produce elevations of plasma FFA in rats (2) and in man (9).

On the other hand, Hynie *et al.* (2) found elevation of BS in rats only after injection of 180 mg of theophylline/kg, and Ensinck *et al.* (9) found no effect on BS with doses of aminophylline that caused marked elevations of FFA in man. Our results suggest that theophylline is more effective in raising plasma FFA in mammals than in birds but more effective in raising BS in birds than in rats and man.

Methylxantines have been shown to cause release of catecholamines (7, 21–23). Therefore, the elevation of plasma FFA may be the consequence of catecholamine-induced lipol-

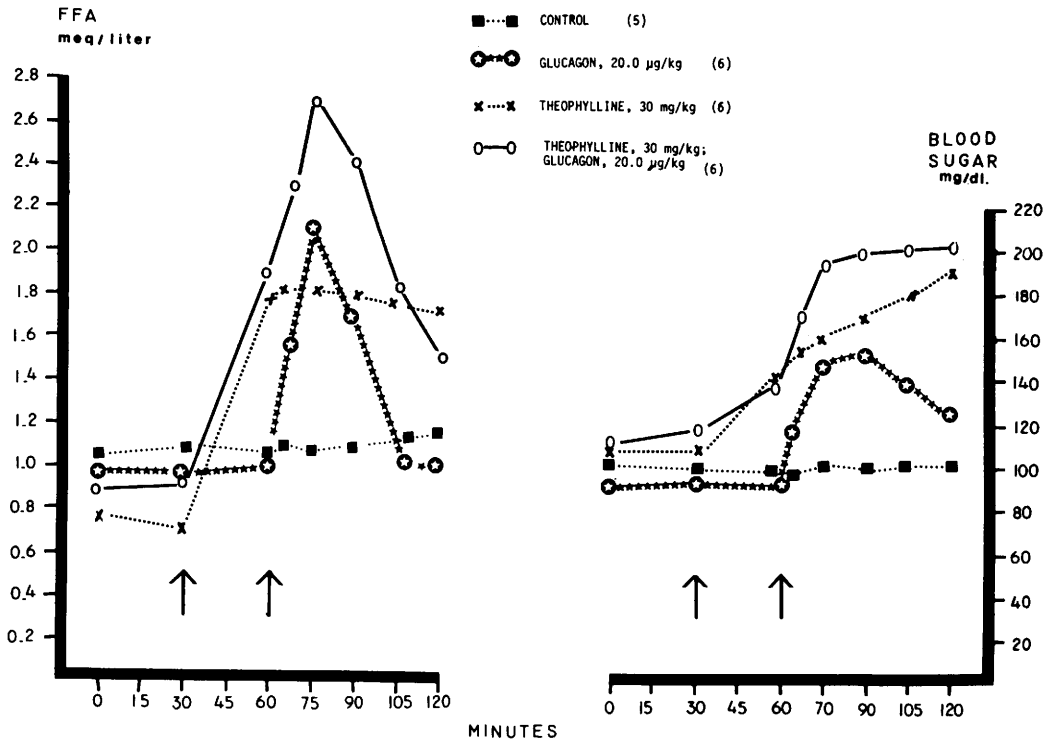

FIG. 1. Effect of glucagon (20.0 µg/kg i.v.), theophylline (30 mg/kg i.v.) and theophylline (30 mg/kg i.v.) plus glucagon (20.0 µg/kg i.v.) on plasma FFA and blood sugar of anesthetized rabbits. Theophylline injected at 30 min. Glucagon injected at 60 min. Rabbits injected with theophylline alone were given an injection of glycine buffer at 60 min. Rabbits injected with glucagon alone were given an injection of saline at 30 min. Control animals received saline at 30 min. and glycine buffer at 60 min. Number of rabbits given in parentheses.

ysis and the failure of theophylline to elevate plasma FFA in birds could be attributed to the limited lipolytic activity of catecholamines in these animals (15, 19, 24). However, Hynie *et al.* (2) have shown that theophylline causes the same elevation of plasma FFA in rats deprived of sympathetic function as in controls and Ensinck *et al.* (9) found that aminophylline produces the same elevation of plasma FFA in subjects without adrenal medullary tissue, as in normal individuals.

Our results in rabbits demonstrate that theophylline produces marked elevations of both plasma FFA and BS in this animal. The effects observed compare qualitatively with those described in the dog (6). The dose of theophylline given to our rabbits (30 mg/kg) was smaller than those necessary to produce elevations of BS in the rat (2, 5, 25). The rise of BS in the rabbits was somewhat greater than that observed by Strubelt (26) in anesthetized rats injected with 60 mg/kg of theophylline. It appears, therefore, that the rabbit is more responsive to the hyperglycemic effect of theophylline than the rat.

No potentiation by theophylline of the adipokinetic effect of glucagon was observed in the experiments with birds. In the rabbit, however, the elevation of plasma FFA produced by glucagon was greater when this hormone was injected after the administration of theophylline than when it was injected during infusion of glucagon causing an elevation of FFA similar to that produced by the injection of theophylline. This observation suggests that theophylline potentiates the adipokinetic effect of glucagon in the rabbit. Such a result compares with the potentiation of the adipokinetic effect of

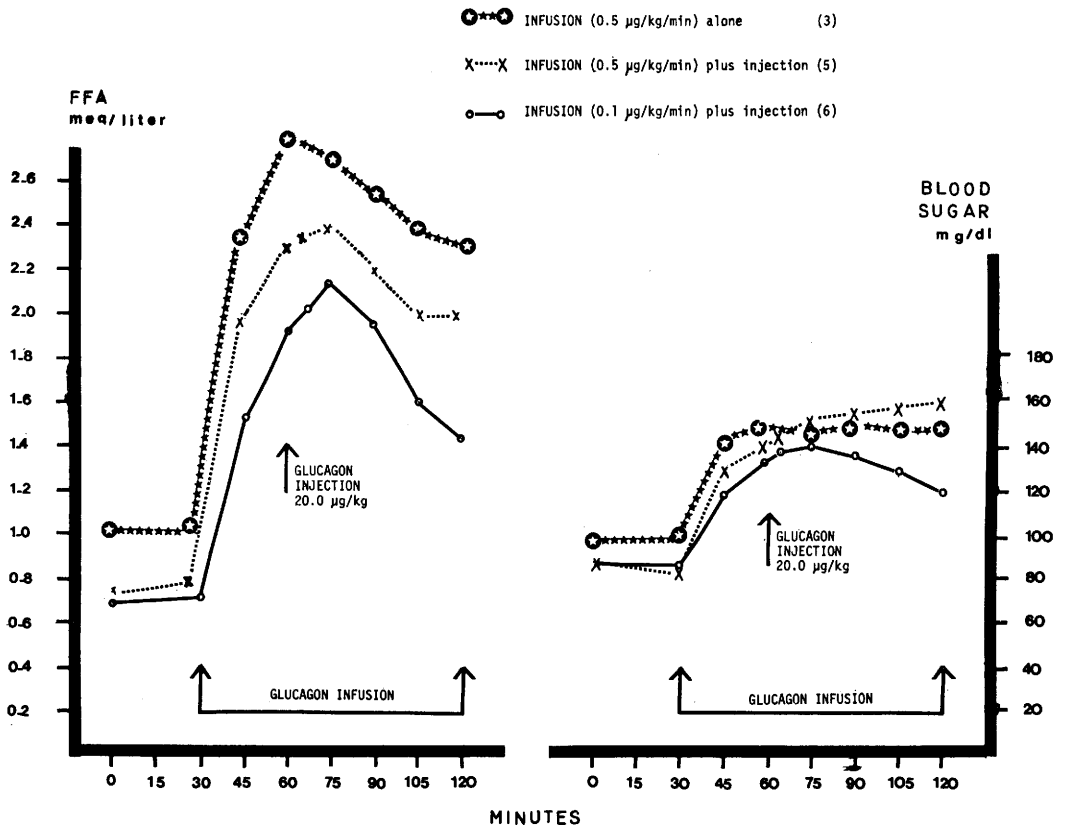

FIG. 2. Effect of glucagon injection (20.0 μg/kg i.v.) on the plasma FFA and blood sugar of anesthetized rabbits receiving a continuous infusion of glucagon. Number of rabbits given in parentheses.

catecholamines in the rat (2, 5) and with the potentiation by methylxantines of the lipolytic effect of glucagon *in vitro*, in the adipose tissue of the rat (10, 12, 13). Our data suggest, therefore, a difference between mammals and birds, with respect to the influence of theophylline upon the adipokinetic effect of glucagon.

Since both the lipolytic and glucogenolytic effects of glucagon are believed to be mediated by CAMP (3, 4), Ensinck *et al.* (9) have discussed possible reasons for the discrepancy between the effects of aminophylline on plasma FFA and BS in man, suggesting that the stable levels of BS reflect concurrent diminution of peripheral glucose utilization and hepatic glucose production. Our experiments show also a discrepancy between those two effects in birds which is, however, opposite to that observed in man. As already noted, theophylline and aminophylline caused elevation of BS but no elevation of plasma FFA in the avian species examined.

We have previously reported that glucagon injection raises plasma FFA concentration in the rabbit (16). The present experiments demonstrate that this animal responds with marked elevations of plasma FFA when glucagon is infused at rates of 0.1 and 0.5 μg/kg/min.

Summary. Injection of theophylline and of aminophylline caused no elevation of plasma FFA, but produced significant elevation of blood sugar concentration in geese, ducks, and owls.

Injection of theophylline caused elevations of both plasma FFA and blood sugar in rabbits.

The hyperglycemic and adipokinetic effects

TABLE IV. Effect of Glucagon Injection (20.0 μg/kg, iv) on Plasma-Free Fatty Acids in Anesthetized Rabbits. Means ± SE for 6 Rabbits per Group.

	FFA (mEq/liter)		
	Before injection	15 Min after injection	Δ
Glucagon injection alone	0.97 ±0.13	2.07 ±0.28	1.10 ±0.22 $p < 0.01$[a]
Glucagon injection plus theophylline[b]	1.91 ±0.21	2.69 ±0.15	0.78 ±0.15 $p < 0.01$[a]
Glucagon injection plus glucagon infusion[c]	1.85 ±0.08	2.10 ±0.13	0.25 ±0.12 $p > 0.05$[a]
Differences:			
Glucagon alone minus glucagon plus theophylline			0.32 ±0.27 $p > 0.05$[d]
Glucagon alone minus glucagon injection plus infusion			0.85 ±0.25 $p < 0.01$[d]
Glucagon plus theophylline minus glucagon injection plus infusion			0.53 ±0.19 $p = 0.019$[d]

[a] *t* test for paired variates.
[b] Theophylline injection, 30 mg/kg, iv 30 min before glucagon injection.
[c] Glucagon infusion, 0.1 μg/kg min. for 90 min started 30 min before glucagon injection.
[d] *t* test for non-paired variates.

of glucagon in birds were not potentiated by the administration of theophylline.

The adipokinetic and hyperglycemic effects of glucagon in rabbits were greater when the hormone was injected after an injection of theophylline, than when it was injected during an infusion of glucagon producing levels of plasma FFA and blood sugar similar to those produced by the administration of theophylline.

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