# Influence of Prostaglandin $E_1$ on the Adipokinetic Effect of Glucagon in Birds[1] (36598)

FRANCISCO GRANDE AND WILLIAM F. PRIGGE

*Jay Phillips Research Laboratory, Mount Sinai Hospital, Minneapolis, Minnesota 55404; and Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minnesota 55455*

The inhibitory effect of prostaglandin $E_1$ ($PGE_1$) on hormone stimulated lipolysis *in vitro* has been well documented in the adipose tissue of rat (1–3), rabbit (4, 5) and man (6, 7), but no constant inhibitory effect of $PGE_1$ on norepinephrine-stimulated lipolysis has been found in the omental adipose tissue of the dog (8). In this animal, however, $PGE_1$ inhibits the mobilization of free fatty acids (FFA) induced by catecholamines *in vivo* (2, 9–11). On the other hand, administration of $PGE_1$ increases FFA mobilization but does not inhibit the adipokinetic effect of the catecholamines in man (12–15).

In view of the remarkable adipokinetic activity of glucagon in birds (16–20), it is of interest to study the effect of $PGE_1$ on glucagon stimulated lipolysis in these animals. Gascon (21) and Langslow (22) have reported marked inhibition by $PGE_1$ of glucagon-stimulated lipolysis in the adipose tissue and the isolated fat cells of young chicken, but there is no information about the influence of $PGE_1$ on the adipokinetic effect of glucagon in other birds. The results of experiments designed to test *in vivo* and *in vitro*, the influence of $PGE_1$ on the lipolytic effect of glucagon in geese and ducks, are reported here.

*Methods. In vivo* experiments were done with fasting adult male mallard ducks and domestic geese. Experimental conditions and chemical methods for determination of plasma FFA and blood supar (BS) were those described in previous publications (16, 17, 23). In some experiments in ducks, we injected $PGE_1$ first, followed immediately by glucagon. In other experiments, we compared the effects of $PGE_1$ alone and $PGE_1$ injected during the infusion of glucagon in 8 geese; 4 of the animals received $PGE_1$ alone first, and glucagon infusion plus $PGE_1$ 1 week later. The other 4 geese were treated in the reverse order. Similarly, the effect of glucagon alone, and injected during the infusion of $PGE_1$, was tested in another 8 geese. Four of the animals received glucagon alone first, and $PGE_1$ infusion plus glucagon 1 week later. The other 4 geese were treated in the reverse order. All the experiments in geese were done under sodium pentobarbital anesthesia (35 mg/kg, 2/3 iv, 1/3 im).

Experiments *in vitro* were performed with the subcutaneous lateral abdominal pads of adult ducks. Experimental technique and methods of FFA and glycerol analysis were those previously described (18). After 16–18 hr of fasting the ducks were given an intravenous injection of glucose (0.5 g/kg), 1 hr before removal of the fat pads.

Crystalline $PGE_1$, kindly donoted by the Upjohn Company, Kalamazoo, MI, was used. Dilutions in saline were prepared daily, just before the experiments, from a stock solution containing 1.0 mg of $PGE_1$ in 0.1 ml of 96% ethanol plus 0.9 ml of sodium carbonate solution (20 mg of anhydrous $Na_2CO_3$ in 100 ml of saline solution). The stock solution was prepared weekly and kept in the freezer.

Arterial pressure was measured with a mercury manometer connected to a polyethylene catheter inserted into the wing artery. Pulse rate was determined from electrocardiographic tracings.

Standard methods of statistical analysis

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TABLE I. Effect of Prostaglandin $E_1$ ($PGE_1$), Glucagon (G), and Prostaglandin Plus Glucagon on Plasma Free Fatty Acids and Blood Sugar in Male Mallard Ducks.[a]

| Injection ($\mu$g/kg) | | No. of | Time after injection (min) | | | | |
|---|---|---|---|---|---|---|---|
| $PGE_1$ | G | ducks | 0 | 5 | 15 | 30 | 60 |
| | | | Free fatty acids (mEq/liter) | | | | |
| 1.0 | — | 6 | 1.72 ± 0.08 | 1.86 ± 0.06 | 1.69 ± 0.09 | 1.67 ± 0.08 | 1.69 ± 0.09 |
| 1.0 | 20.0 | 6 | 1.82 ± 0.12 | 3.56 ± 0.23 | 3.35 ± 0.22 | 2.75 ± 0.16 | 2.15 ± 0.06 |
| 30.0 | — | 6 | 1.58 ± 0.12 | 1.83 ± 0.13 | 1.19 ± 0.11 | 1.22 ± 0.09 | 1.50 ± 0.08 |
| 30.0 | 20.0 | 7 | 1.50 ± 0.15 | 3.07 ± 0.23 | 3.00 ± 0.27 | 2.49 ± 0.19 | 2.00 ± 0.13 |
| — | 20.0 | 12 | 1.47 ± 0.11 | 3.09 ± 0.20 | 2.68 ± 0.14 | 2.04 ± 0.12 | 1.69 ± 0.10 |
| | | | Blood sugar (mg/100 ml) | | | | |
| 1.0 | — | 6 | 124 ± 5.4 | 128 ± 4.4 | 137 ± 6.3 | 142 ± 6.3 | 134 ± 5.6 |
| 1.0 | 20.0 | 6 | 122 ± 3.6 | 147 ± 5.9 | 189 ± 5.7 | 185 ± 4.3 | 130 ± 2.8 |
| 30.0 | — | 6 | 119 ± 3.2 | 133 ± 3.9 | 149 ± 3.6 | 158 ± 3.6 | 156 ± 4.5 |
| 30.0 | 20.0 | 7 | 120 ± 8.2 | 151 ± 9.6 | 188 ± 9.9 | 192 ± 7.1 | 146 ± 5.4 |
| — | 20.0 | 12 | 125 ± 4.0 | 154 ± 3.9 | 203 ± 7.7 | 192 ± 7.4 | 148 ± 6.8 |

[a] Mean and SE.

were used. Unless otherwise stated, the significance of the differences was calculated by the $t$ test for paired variates.

*Results.* The effects of injecting $PGE_1$, glucagon, and $PGE_1$ plus glucagon, on the plasma FFA and BS levels of ducks are presented in Table I. $PGE_1$, at the dose of 1.0 $\mu$g/kg, caused a small, but significant elevation of plasma FFA ($p = .017$), 5 min after the injection. The dose of 30.0 $\mu$g/kg caused an elevation of plasma FFA ($p = .04$) at 5 min followed by a decrease at 15 min, when the mean FFA concentration was 0.39 mEq/liter (SE ± 0.10) lower than before injection ($p = .012$). The elevation of plasma FFA produced by the injection of glucagon was not affected by $PGE_1$ given immediately before. The mean elevations of plasma FFA, 5 min after injecting 20.0 $\mu$g of glucagon/kg, were: 1.62 mEq/liter (SE ± 0.12, $p < .001$), when glucagon was given alone; 1.74 (SE ± 0.20, $p < .001$) and 1.57 (SE ± 0.13, $p < .001$) when glucagon was preceded by $PGE_1$ at the doses of 1.0 and 30.0 $\mu$g/kg, respectively. These three elevations were not statistically different from each other ($p > 0.05$, $t$ test for nonpaired variates).

Both doses of $PGE_1$ caused significant elevations of BS ($p < .01$) except for the 5-min sample after the 1.0 $\mu$g/kg dose. Statistical analysis revealed no influence of $PGE_1$ on the BS changes produced by glucagon injection.

As shown in Fig. 1, intravenous injection of $PGE_1$ (50 $\mu$g/kg) in anesthetized geese produced a sharp drop of arterial pressure and elevations of the pulse rate, plasma FFA, and BS. The mean FFA elevation above preinjection level was 0.31 mEq/liter (SE ± 0.08, $p < .01$) 5 min after injection, and 0.12

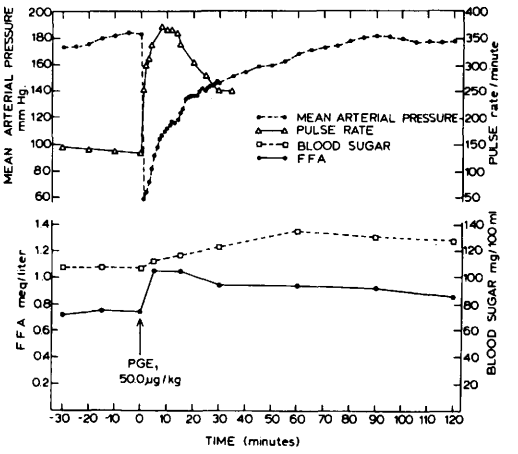


FIG. 1. Effect of a single injection of prostaglandin $E_1$ (50.0 $\mu$g/kg, iv) on mean arterial pressure, pulse rate, blood sugar (BS), and plasma free fatty acids (FFA) in geese. Injection indicated by arrow. Means for 8 geese.

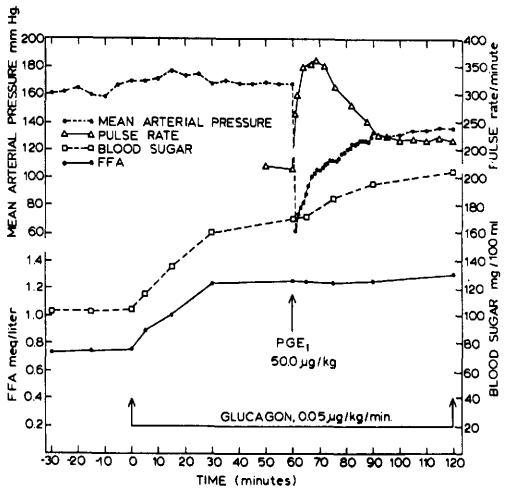


FIG. 2. Effect of a single injection of prostaglandin $E_1$ (50.0 µg/kg, iv), given during the infusion of glucagon (0.05 µg/kg/minute), on mean arterial pressure, pulse rate, blood sugar (BS), and plasma free fatty acids (FFA) in geese. Glucagon infusion and prostaglandin injection indicated by arrows. Means for the same 8 geese as in Fig. 1.

mEq/liter (SE ± 0.04, $p = .02$) 2 hr after injection. The highest BS level occurred 1 hr after injection. BS level was still significantly higher than control 2 hr after injection (21 mg/100 ml, SE ± 2.7 $p < .01$).

The effect of injecting PGE (50 µg/kg) during infusion of glucagon (0.05 µg/kg/min) is shown in Fig. 2. The changes of arterial pressure and pulse rate were similar to those observed after injection of $PGE_1$ alone. Plasma FFA rose after beginning glucagon infusion. Mean elevation above control was 0.52 mEq/liter (SE ± 0.10, $p < .01$) after 30 min of infusion. Plasma FFA level was practically constant between 30 and 120 min of infusion and was not affected by the injection of $PGE_1$. BS rose continuously during glucagon infusion. The mean elevation after 2 hr of infusion (101 mg/100 ml, SE ± 7.1) compares with that observed in 5 geese infused with the same dose of glucagon, without injection of $PGE_1$ (90 mg/100 ml, SE ± 25.6).

Figure 3 shows a comparison of the effects of glucagon (50 µg/kg) when injected during a continuous infusion of $PGE_1$ (0.5 µg/kg/min) and when injected alone in anesthetized geese.

Infusion of $PGE_1$ caused a rapid decrease of arterial pressure that was maintained throughout the infusion period, and an elevation of plasma FFA. The mean FFA elevation at 15 min of infusion was 0.25 mEq/liter (SE ± 0.04, $p < .01$). The injection of glucagon caused a further elevation of plasma FFA. Mean elevation above preinjection level was 0.78 mEq/liter (SE ± 0.10, $p < .01$)

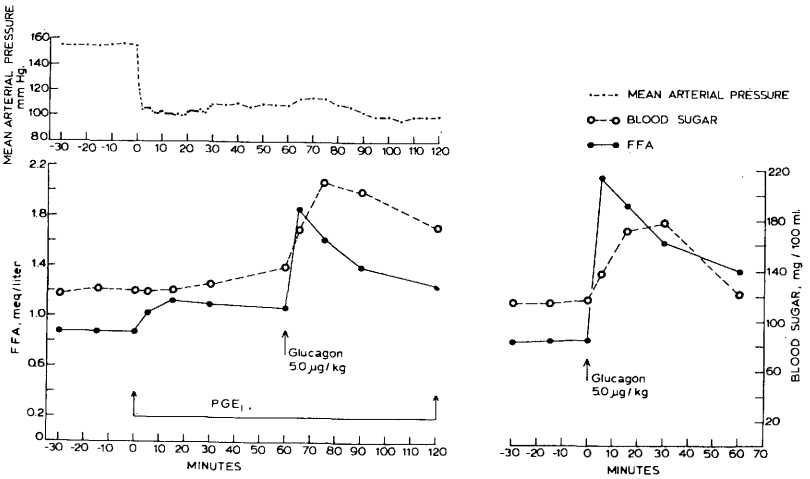


FIG. 3. Comparison of glucagon effects (5.0 µg/kg) when injected during the infusion of prostaglandin $E_1$ (0.5 µg/kg/min) and when injected alone, in anesthetized geese. Prostaglandin infusion and glucagon injection indicated by arrows. Means for 8 geese. The same geese were used for the two tests.

5 min after injection. The same dose of glucagon, injected into the same geese without $PGE_1$ infusion, caused a mean elevation of plasma FFA of 1.29 mEq/liter (SE ± 0.14, $p < .01$) after 5 min. The mean difference between the two elevations (0.51 mEq/liter, SE ± 0.12) was significant ($p < .01$).

There was a slow elevation of BS during the infusion of $PGE_1$. After 60 min of infusion the BS level was 20 mg/100 ml above the mean preinfusion level (SE ± 3.8, $p < .01$). Glucagon injecion caused further elevation of BS with a maximum 15 min after injection which was not significantly different from that observed when glucagon was injected during the infusion of $PGE_1$ ($p > .05$). Pulse rate rose from a mean of 160 beats/min, before $PGE_1$ infusion, to 370 after 10 min of infusion. At the end of infusion the pulse rate was 300 beats/min.

The *in vitro* effect of $PGE_1$ on glucagon-stimulated lipolysis in the adipose tissue of the duck is described in Table II. A constant dose of glucagon (0.5 μg/ml) was used in these experiments. The three levels of $PGE_1$ tested caused a decrease of the effect of glucagon, but only the dose of 50.0 μg/ml caused a significant reduction in the productions of FFA and glycerol.

*Discussion.* The experiments in ducks demonstrate that intravenous injection of $PGE_1$ (up to 30.0 μg/kg), immediately preceding that of glucagon, did not modify the adipokinetic and hyperglycemic effects of this hormone. $PGE_1$ alone, on the other hand, had both adipokinetic and hyperglycemic effects in the duck. These results compare with observations showing that $PGE_1$ increases FFA mobilization, but does not inhibit the adipokinetic effect of catecholamines in man (12–15), and are at variance with those showing that $PGE_1$ inhibits the adipokinetic effect of catecholamines in the dog (2, 9–11).

In ducks, $PGE_1$, at the dose of 30.0 μg/kg, produced an elevation of plasma FFA followed by a decrease. Kupiecki (24) noted a biphasic plasma FFA response to the injection of $PGE_1$ (80.0 μg/kg) in fasted rats, but in this animal, in contrast to the duck, there

TABLE II. Lipolytic Effect[a] of a Constant Dose of Glucagon (0.5 μg/ml) in the Presence of Various Concentrations of Prostaglandin $E_1$.[b]

| | Prostaglandin $E_1$ (μg/ml) | | | |
|---|---|---|---|---|
| | 0 | 0.5 ($1.4 \times 10^{-6}$ M) | 5.0 ($1.4 \times 10^{-5}$ M) | 50.0 ($1.4 \times 10^{-4}$ M) |
| FFA production (μEq/g/hr) | 7.72 ± 1.28 | 5.70 ± 0.92 | 5.86 ± 0.64 | 4.72 ± 0.71 |
| | $p < .01$ | $p < .01$ | $p < .01$ | $p < .01$ |
| Difference[c] | | 2.02 ± 1.02 | 1.86 ± 1.12 | 3.00 ± 0.96 |
| | | $p > .05$ | $p > .05$ | $p = .01$ |
| Glycerol production (μmoles/g/hr) | 3.02 ± 0.54 | 2.54 ± 0.44 | 2.42 ± 0.21 | 1.80 ± 0.24 |
| | $p < .01$ | $p < .01$ | $p < .01$ | $p < .01$ |
| Difference[c] | | 0.48 ± 0.33 | 0.60 ± 0.48 | 1.22 ± 0.48 |
| | | $p > .05$ | $p > .05$ | $p = .025$ |

[a] Glucagon effect calculated by subtracting the values of the corresponding controls treated with the same amounts of prostaglandin, but no glucagon.

[b] Duck adipose tissue *in vitro*; means ± SE for 12 ducks. Spontaneous lipolysis (without addition of glucagon or prostaglandin $E_1$) was 1.59 ± 0.52 μEq of FFA/g/hr and 1.15 ± 0.24 μmoles of glycerol/g/hr.

[c] Difference: Glucagon effect without prostaglandin minus glucagon effect with prostaglandin.

was initially a decrease followed by an elevation of plasma FFA.

In anesthetized geese $PGE_1$ (50.0 μg/kg) had adipokinetic and hyperglycemic effects, but failed to modify the effect of glucagon infusion on plasma FFA. This is clearly shown by comparing Fig. 2 with the effect of glucagon infusion described in a previous publication (23). Furthermore, it appears that the effect of $PGE_1$ on plasma FFA is abolished when that compound is injected during a continuous infusion of glucagon.

Injection of glucagon during infusion of $PGE_1$ caused an elevation of plasma FFA, which was significantly smaller than that observed when the same dose of glucagon was injected to the same geese without $PGE_1$ infusion. Continuous infusion of $PGE_1$, started 1 hr before glucagon injection, therefore, caused significant inhibition of the adipokinetic effect of this hormone. This result compares with the inhibition of the adipokinetic effect of the catecholamines when they are injected during infusion of $PGE_1$, reported by Bergström, Carlson and Oro (10) in dogs.

Our results *in vitro* show that only the largest dose of $PGE_1$ used (50.0 μg/ml) caused significant inhibition of the lipolytic effect of glucagon (0.5 μg/ml). Steinberg *et al.* (2) reported inhibition by much smaller amounts of $PGE_1$ (0.1 μg/ml), of the lipolytic effect of larger glucagon doses (5.0 μg/ml) in rat adipose tissue. Thus, $PGE_1$ appears to be a much weaker inhibitor of the lipolytic effect of glucagon in the duck than in the rat.

It has been reported that $PGE_1$ does not affect lipolysis in the adipose tissue of fasted rats (25, 26). Since our ducks received an injection of glucose 1 hr before removal of the adipose tissue, the small inhibitory effect observed cannot be attributed to fasting. Moreover, Kupiecki (24) observed inhibition of lipolysis by $PGE_1$ in fasted as well as in fed rats. Our *in vitro* results are at variance with those reported by Gascon (21) and by Langslow (22), who found marked inhibition of the lipolytic effect of glucagon by very small doses of $PGE_1$ in the adipose tissue and the isolated fat cells of chickens. These authors used young chickens (25–26 days old), whereas our experiments were made with the adipose tissue of adult ducks (1 year or more). In view of the differences between young and old rats with regard to the lipolytic effect of glucagon (27), the possibility that age modifies the antilipolytic effect of $PGE_1$ must be considered.

$PGE_1$ does not inhibit the lipolytic effect of adenosine 3′,5′-monophosphate, and, therefore, is believed to act on the adenyl cyclase system (28, 29). Some observations suggest that $PGE_1$ behaves as a competitive inhibitor of various hormones (30, 31). Accordingly, the limited ability of $PGE_1$ to inhibit the lipolytic effect of glucagon in adult birds could be explained assuming that the affinity of glucagon for adenyl cyclase, relative to that of $PGE_1$, is higher in the adult avian adipose tissue than in that of mammals.

Both injections and infusion of $PGE_1$ in geese caused a precipitous fall of arterial pressure and elevation of the pulse rate. These circulatory effects compare qualitatively with those reported in man and other mammals (29, 32–34).

*Summary.* The intravenous administration of $PGE_1$ had adipokinetic and hyperglycemic effects in ducks and geese.

No inhibition of the adipokinetic effect of glucagon was demonstrated when $PGE_1$ was injected immediately before glucagon, or during a continuous infusion of glucagon. A significant inhibition of the adipokinetic effect of glucagon in geese was observed, however, when this hormone was injected during a continuous infusion of $PGE_1$.

A significant inhibition of glucagon-stimulated lipolysis *in vitro*, in the adipose tissue of adult ducks, was observed only with the highest dose of $PGE_1$ tested (50.0 μg/ml).

Administration of $PGE_1$ in geese caused a prompt fall of arterial pressure and marked elevation of the pulse rate.

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