Effects of insulin and prior exercise on prostaglandin release from perfused rat muscle

Evidence that prostaglandins do not mediate changes in glucose uptake

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1. Prostaglandin generation and its inter-relation to the metabolic effects of insulin and prior exercise were examined in perfused muscle of fed rats. 2. During a 60 min perfusion of the rat hindquarter, a substantial release of the prostaglandins $\text{PGF}_{2\alpha}$, PGE_2 and 6-oxo $\text{PGF}_{1\alpha}$ was observed. Blood cells present in the perfusate released these substances in negligible amounts indicating the prostaglandins were produced by the hindquarter. 3. Addition of insulin to the perfusate increased both glucose uptake and the generation of PGE_2 and 6-oxo $\text{PGF}_{1\alpha}$. 4. At 30 min after intense treadmill exercise, glucose and α -aminoisobutyric acid (AIB) uptake by the hindquarter were increased in the absence of added insulin, but prostaglandin release was not increased. Insulin further increased glucose and AIB uptake; however, in contrast with its effects in non-exercised rats, insulin no longer stimulated prostaglandin generation. 5. Indomethacin (10 μM) added to the perfusate inhibited the release of $\text{PGF}_{2\alpha}$ and PGE_2 by 90% and the release of 6-oxo $\text{PGF}_{1\alpha}$ by 54%. It had no effect on the stimulation of glucose uptake by either insulin or prior exercise. 6. The data indicate that insulin increases prostaglandin synthesis by perfused rat muscle, and that prior exercise blocks this effect. They suggest that under the conditions studied prostaglandins do not mediate the effects of insulin or prior exercise on glucose uptake.

INTRODUCTION

Insulin and exercise acutely modify muscle metabolism. Thus, they stimulate glucose and AIB uptake in skeletal muscle (Holloszy & Narahara, 1965; Berger *et al.*, 1975; Kipnis & Noall, 1958; Narahara & Holloszy, 1974; Zorzano *et al.*, 1985, 1986) and although not always in parallel, they alter muscle protein turnover (Jefferson *et al.*, 1977; Dohm *et al.*, 1980; Booth & Watson, 1985). The mechanisms that mediate these effects of insulin and exercise are unknown.

Several reports have postulated that prostaglandins modulate certain metabolic effects of insulin and exercise. Thus, the stimulatory effect of insulin and exercise on glucose uptake in the human forearm was blocked by injection of the kallikrein-kinin inhibitor trasylo, which, by inhibiting bradykinin action, decreases prostaglandin generation. In addition, infusion of bradykinin into the brachial artery increased glucose uptake, an effect inhibited by indomethacin (Dietze & Wicklmayr, 1977; Wicklmayr *et al.*, 1979), a known inhibitor of cyclo-oxygenase. Other investigations of skeletal muscle have suggested that the prostaglandins $\text{PGF}_{2\alpha}$ and PGE_2 , in part, control protein synthesis and degradation, respectively (Rodemann *et al.*, 1982; Rodemann & Goldberg, 1982; Palmer *et al.*, 1983). In an elegant series of studies it has been shown that intermittent stretching stimulates protein synthesis in skeletal muscle and that indomethacin blocks this effect (Smith *et al.*, 1983; Palmer *et al.*, 1983). Similarly, both

in vitro and *in vivo* indomethacin blocks insulin-stimulated protein synthesis in muscles of fasted animals (Reeds & Palmer, 1983; Reeds *et al.*, 1985). Finally, in the absence of indomethacin mechanical stretching and insulin both caused an increased generation of prostaglandins (Reeds & Palmer, 1983; Reeds *et al.*, 1985; Palmer *et al.*, 1983).

In the present study we have examined the role of prostaglandins in mediating some of the biological effects of insulin and exercise in skeletal muscle using the perfused rat hindquarter preparation. The effects of insulin and prior exercise on prostaglandin generation, glucose and AIB uptake were assessed. In addition, we have determined the effect of indomethacin on glucose uptake. The results indicate that insulin and exercise modify prostaglandin generation. However, they do not support the view that prostaglandin synthesis mediates the effects of exercise and insulin on glucose or AIB uptake.

METHODS

Animals

Male Sprague–Dawley rats, obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.) were fed Purina rat chow *ad libitum*. To accustom the rats to treadmill exercise, they were run for 5 min at a rate of 36 m/min on 3–4 days during the week prior to the experiment. On the morning of perfusion, the rats

Abbreviations used: PG, prostaglandin; AIB, α -aminoisobutyric acid; IP_3 , inositol trisphosphate.

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(weighing 200–250 g) were assigned to resting and exercising groups. The exercised rats ran on the treadmill at 36 m/min for 50 min (5 × 10 min bouts interspersed with 3–4 min of rest).

Perfusion studies

Non-exercised and exercised rats were anaesthetized by an intraperitoneal injection of pentobarbital (5 mg/100 g body wt.) and prepared for hindquarter perfusion (Ruderman *et al.*, 1971; Goodman *et al.*, 1978). Exercised rats were perfused 30 and 150 min after the completion of the treadmill run and control rats at equivalent times. The hindquarters were perfused with a medium containing Krebs–Henseleit solution, human aged–rejuvenated erythrocytes (Ruderman *et al.*, 1980) added to obtain an haematocrit of 30%, 4% bovine serum albumin (Pentex Corporation, Kankakee, IL, U.S.A.), 6 mM-glucose, 0.15 mM-pyruvate and 1.5–2.0 mM-lactate (originating from the erythrocytes). Porcine insulin (lot no. 615–07J-256), kindly provided by Dr. Ronald Davis (Eli-Lilly Co., Indianapolis, IN, U.S.A.), was added at concentrations of 0, 75, 200 or 20000 μ units/ml of cell free perfusate. The initial volume of perfusate was 150 ml. Immediately before placement in the perfusion apparatus, the rats were killed by an intracardiac injection of pentobarbital. The first 25 ml of perfusate that passed through the hindquarter preparation was discarded, whereupon the medium was recirculated at a flow rate of 12.5 ml/min. In certain experiments, after a 15 min equilibration period, prostaglandin release was determined by changes in concentration during 60 min of perfusion. In another series of experiments, indomethacin was added to the perfusate (10 μ M final concentration) after the 2 min washout, and 15 min later, insulin was added. After another 15 min of

perfusion experimental measurements were begun. At selected time intervals, samples of the perfusate were collected into a final concentration of 10 μ M-indomethacin on ice and centrifuged to remove erythrocytes. The supernatant was aspirated and stored at -20°C for subsequent assay of prostaglandins. Other aliquots of the perfusate were collected for determination of glucose. In a separate series of experiments, 10 μ Ci of [^3H]inulin and 10 μ Ci of [^{14}C]AIB (New England Nuclear) and 0.5 mM of unlabelled AIB were introduced into the reservoir 3 min before the end of the 15 min preperfusion. Samples were taken from the perfusate reservoir at the beginning and at the end of the 30 min perfusion and were placed into 2 vol. of ice-cold 6% (w/v) HClO_4 . At 30 min, samples of the red portion of the gastrocnemius were excised, rapidly washed in saline and frozen in liquid N_2 .

The effect of prior exercise was studied in hindquarters of rats after the completion of 50 min of high-intensity treadmill exercise (Garetto *et al.*, 1984). In addition, hindquarter muscle was studied after contractions induced by electrical stimulation of the sciatic nerves for 10 min in the perfusion cabinet. Stimulation (Grass S-48 stimulator; Grass Instruments, Quincy, MA, U.S.A.) consisted of 500 ms trains at a rate of 1 Hz as described previously (Zorzano *et al.*, 1986). Each train consisted of repeated 6–8 V pulses of 0.1 ms duration delivered at 100 Hz. During the stimulation period, the flow rate of the perfusate was maintained at 25 ml/min to assure adequate oxygenation.

Analyses and calculation

Glucose in the perfusate was measured with a YSI model 23A Glucose Analyzer (Yellow Springs Instrument Co.; Yellow Springs, OH, U.S.A.). Levels of the prostaglandins $\text{PGF}_{2\alpha}$, PGE_2 and 6-oxo $\text{PGF}_{1\alpha}$ (the stable metabolite of prostacyclin) in rat hindquarter perfusates were determined by radioimmunoassay as previously described (Jakubowski *et al.*, 1985). In brief, standard curves (10–10000 pg/ml) were constructed in Krebs–Henseleit bovine serum albumin/glucose/pyruvate buffer, pH 7.4, that had been collected from the unused stock on each day of perfusion for this express purpose. All other dilutions (tracer and antibody) were performed with Tris/gelatin buffer (15 mM-Tris/HCl, 140 mM-NaCl, 500 mg of gelatin/l, pH 7.4 at 4°C). Samples or standards (100 μ l) were incubated overnight at 4°C with 100 μ l of [^3H]prostaglandin (4000 c.p.m.) and 100 μ l of antibody. Dextran-coated charcoal was used to separate bound from unbound radioactivity. Following centrifugation, bound radioactivity was decanted into scintillation vials and radioactivity was estimated by liquid-scintillation counting (Packard Instruments). Limit of detection for $\text{PGF}_{2\alpha}$ and PGE_2 was 10 pg/ml; for 6-oxo $\text{PGF}_{1\alpha}$ it was 40 pg/ml. Antibodies to $\text{PGF}_{2\alpha}$ and 6-oxo $\text{PGF}_{1\alpha}$ were a gift from Dr. Lawrence Levine (Brandeis University) and anti- PGE_2 was purchased from Seragen (Boston, MA, U.S.A.). Cross-reactivity of the antisera with other major metabolites of arachidonic acid was 1% or less.

Radiolabelled AIB and inulin were determined in frozen tissue after homogenization in 3 ml of 6% (w/v) HClO_4 using a Polytron tissue homogenizer (Zorzano *et al.*, 1985). Rates of glucose uptake and prostaglandin release by the hindquarter were calculated from changes in their concentration in the perfusate (Goodman *et al.*, 1978). Glucose uptake by erythrocytes over 30 min

Fig. 1. Prostaglandin release by the perfused rat hindquarter

Rats were fed *ad libitum* and had not previously been exercised. Results are means $\pm$ S.E.M. for six to eight observations. After a 2 min washout, samples of perfusate were collected during the next 60 min for determination of $\text{PGF}_{2\alpha}$ (●), PGE_2 (▲) and 6-oxo $\text{PGF}_{1\alpha}$ (■). Insulin was not added to the perfusate.

Fig. 2. Effect of pretreatment of cellular elements of perfusate with aspirin on prostaglandin release by the perfused hindquarter

Results are means $\pm$ S.E.M. for five or six observations. After a 2 min washout, samples of perfusate were collected for the subsequent assay of PGF_{2α} (triangles), 6-oxoPGF_{1α} (squares) and PGE₂ (circles) during perfusions with control cells (filled symbols) or aspirin-pretreated cells (open symbols). All prostaglandin measurements were done in the same experiments. PGF_{2α} results are shown in a separate panel for the sake of clarity. See the legend to Fig. 1 for details.

was negligible. Details concerning prostaglandin release by erythrocytes are given under 'Results'. AIB uptake was calculated by subtracting [¹⁴C]AIB radioactivity in the extracellular space from the total label found in muscle as previously described (Zorzano *et al.*, 1985). Statistical analysis was done by either Student's *t* test or a two-way analysis of variance with further Scheffe test.

RESULTS

Characterization of prostaglandin release by the perfused rat hindquarter

Our initial objective was to assess whether the perfused hindquarter could be used to study prostaglandin generation. Toward this end, the release of specific prostaglandins was initially assessed, based on their concentration in the perfusate. The measurements were performed in samples collected 2 min after the hindquarter was placed in the perfusion cabinet. As shown in Fig. 1, PGF_{2α}, PGE₂ and 6-oxoPGF_{1α} (a stable metabolite of prostacyclin) were continuously released during the 60 min perfusion period, with perhaps a slightly greater rate of release during the second 30 min. The rate of release of 6-oxoPGF_{1α} was substantially greater than that of PGF_{2α} and PGE₂. The presence of PGF_{2α} in the perfusate before addition of the hindquarter suggested that it was initially derived from cells in the perfusate.

To evaluate the contribution of the cellular elements of the perfusate, i.e. aged-rejuvenated erythrocytes and platelet and leucocyte contaminants, to the observed release of prostaglandins, rejuvenated erythrocytes were treated with aspirin (0.1 mM for 30 min.), an irreversible inhibitor of cyclo-oxygenase, the day prior to the experiment and then washed. On the experimental day, the release of prostaglandins was compared in hind-

quarters perfused with these cells and cells not treated with aspirin. As shown in Fig. 2, rates of prostaglandin release in the two groups did not differ. In addition, the release of 6-oxoPGF_{1α} was still markedly greater than that of PGF_{2α} and PGE₂. In a further series of experiments, release of prostaglandins into the perfusate was compared in the presence and absence of the hindquarter. As before, in some experiments the blood cells in the perfusate had been previously treated with aspirin. Prostaglandins were released in substantial quantities only when the hindquarter was present; in its absence their release was negligible (results not shown). When the cells were pretreated with aspirin, the concentrations of prostaglandins in the perfusate were slightly lower than in the non-treated cells. *In toto*, these results strongly suggest that the prostaglandins released into the perfusate originated in the hindquarter and not in the blood cells of the perfusion medium.

Effects of insulin on prostaglandin release and glucose uptake

To assess whether insulin affects prostaglandin generation, hindquarters were perfused with different concentrations of insulin and the prostaglandin concentration in the perfusate was determined (Fig. 3). Insulin caused a significant increase in PGE₂ release that was maximal at a concentration of 200 μ units/ml. The release of 6-oxoPGF_{1α} was also increased by insulin; however the difference was only statistically significant at an insulin concentration of 20000 μ units/ml. PGF_{2α} release was increased by 32% at the high insulin concentration; however, the increase was not statistically significant. As expected, stimulation of glucose uptake was observed at both 200 and 20000 μ units of insulin/ml with the lower concentration producing approximately $\frac{1}{3}$ of the maximal effect. Except for 6-oxoPGF_{1α} there was not a parallelism

Fig. 3. Effects of insulin on prostaglandin release and glucose uptake by the perfused rat hindquarter

Results are means $\pm$ S.E.M. for five or six observations. Hindquarters from non-exercised rats fed *ad libitum* were perfused without ($\square$) or with 200 μ units/ml ($\square$) or 20000 μ units/ml of insulin ($\blacksquare$) in the perfusate. After 15 min of equilibration, samples of medium were collected over the subsequent 60 min for determination of prostaglandin release and glucose uptake. The scale for 6-oxoPGF_{1α} is different from that for PGF_{2α} and PGE₂. The symbol * indicates a value significantly different from that of the group perfused without insulin.

Table 1. Effects of insulin and prior exercise on prostaglandin release by the perfused muscle

Results are means $\pm$ S.E.M. of five or six observations. Hindquarters were placed in the perfusion apparatus approx. 30 min after the completion of high-intensity exercise. After a 2 min washout, insulin was added to the perfusate. After 15 min of equilibration, samples of perfusate were collected over the next 60 min for prostaglandin determinations. Hindquarters from non-exercised rats were perfused on the same experimental days. The symbol * indicates a value significantly different from that of the comparable group perfused without added insulin, $P < 0.05$. The symbol † indicates a value significantly different from that of non-exercised rats perfused with the same concentration of insulin, $P < 0.05$.

Insulin (μ units/ml)	Prostaglandin release (pg/min per g)		
	PGF _{2α}	PGE ₂	6-OxoPGF _{1α}
Resting			
0	31 $\pm$ 4	43 $\pm$ 8	193 $\pm$ 19
200	37 $\pm$ 6	71 $\pm$ 10*	245 $\pm$ 25
20000	41 $\pm$ 5	70 $\pm$ 9*	352 $\pm$ 49*
Post-exercise, 30 min			
0	29 $\pm$ 6	41 $\pm$ 11	185 $\pm$ 23
200	32 $\pm$ 6	47 $\pm$ 10	177 $\pm$ 17†
20000	29 $\pm$ 5	35 $\pm$ 9†	197 $\pm$ 32†

between the ability of insulin to stimulate the release of prostaglandins and its effect on glucose uptake.

Effect of prior exercise on prostaglandin release and glucose and AIB uptake

The effect of prior exercise on prostaglandin generation and its interaction with insulin was next investigated. Rats were subjected to high-intensity exercise on a treadmill for 50 min and approx. 30 min thereafter their hindquarters were perfused. Non-exercised rats were studied on the same experimental days. As shown in Table 1, prostaglandin release in the absence of insulin was the same in the exercised and non-exercised groups. On the other hand, prior exercise profoundly altered the effect of insulin on prostaglandin release. Thus, whereas 20000 μ units of insulin/ml stimulated the release of both PGE₂ and 6-oxoPGF_{1α} in the control group, it did not do so after exercise.

The effects of prior exercise and insulin on glucose and AIB uptake in these situations are shown in Table 2. In keeping with previous studies, prior exercise by itself increased both glucose uptake by the whole perfused hindquarter and AIB uptake by the red portion of the gastrocnemius. Likewise, addition of a supramaximal concentration of insulin caused the same stimulation of glucose and AIB uptake observed in non-exercised rats. Thus, neither the stimulation of glucose and AIB uptake induced by prior exercise nor the further enhancement of

Table 2. Effect of prior exercise and insulin on glucose uptake by the perfused hindquarter and AIB uptake by red fibres of the gastrocnemius muscle

Results are means $\pm$ S.E.M. for six to eight observations in non-exercised and 30 min post-exercise rats. See details in the legend to Table 1 and the Methods section. The symbol * indicates a value significantly different from that of the non-exercised group at $P < 0.05$.

Insulin (μ units/ml)	Glucose uptake (μ mol/h per g)		AIB uptake (nmol/h per g)	
	Non-exercised	Post-exercise, 30 min	Non-exercised	Post-exercise, 30 min
0	1.6 $\pm$ 0.3	3.6 $\pm$ 0.5*	81 $\pm$ 14	200 $\pm$ 28
20000	10.2 $\pm$ 0.5	12.7 $\pm$ 0.6*	361 $\pm$ 38	452 $\pm$ 32*

Table 3. Effect of indomethacin on prostaglandin release by the perfused rat hindquarter

Values are means $\pm$ S.E.M. for five or six observations per group. Rats were not exercised. After a 2 min washout, indomethacin (10 μ M) was added to the perfusate. Samples of perfusate were collected over a 60 min perfusion in order to determine prostaglandin concentrations. The symbol * indicates a value significantly different ($P < 0.01$) from that of the control group.

	Release (pg/min per g)		Inhibitory effect (%)
	Control	Indomethacin	
PGF _{2α}	50 $\pm$ 5	6 $\pm$ 2*	86
PGE ₂	34 $\pm$ 6	3 $\pm$ 2*	91
6-OxoPGF _{1α}	109 $\pm$ 11	50 $\pm$ 4*	54

their uptake by insulin was paralleled by alterations in prostaglandin generation.

A similar dissociation between prostaglandin release and glucose uptake was observed after muscle contractions induced by electrical stimulation of the sciatic nerves *in situ*. Thus, following 10 min of each stimulation, glucose uptake by the hindquarter was increased 1.8-fold during the next 30 min of perfusion, whereas the release of PGF_{2 α} and 6-oxoPGF_{1 α} was unaltered (results not shown).

Effect of indomethacin on prostaglandin release and glucose uptake

The release of prostaglandins from the perfused hindquarter was markedly suppressed by the cyclooxygenase inhibitor indomethacin. Thus, 10 μ M-indomethacin caused a 90% inhibition in the release of PGF_{2 α} and PGE₂ (Table 3) and it inhibited the release of 6-oxoPGF_{1 α} by 54%. Decreases of the same magnitude

occurred in the presence of insulin, and stimulation of prostaglandin generation by insulin was completely blocked by indomethacin (results not shown). To re-examine the possibility that prostaglandins are involved in their ability to stimulate glucose utilization, the effect of indomethacin on exercise or insulin-stimulated glucose uptake was determined. The results shown in Fig. 4 demonstrate that, in non-exercised rats, neither basal nor insulin-stimulated glucose uptake were altered by indomethacin addition. Likewise, indomethacin did not alter glucose uptake in the presence or absence of insulin in rats studied 30 or 150 min post-exercise.

DISCUSSION

The results of the present study strongly suggest that both insulin and exercise can profoundly affect prostaglandin generation in skeletal muscle. Thus, insulin stimulated the generation of several prostaglandins in the perfused hindquarter of fed rats and this effect was completely blocked after intense exercise.

Several tissues are present in the hindquarter preparation; however, skeletal muscle accounts for upwards of 80% of the perfused tissue. The remainder is accounted for by bone, skin and a negligible quantity of adipose tissue (Ruderman *et al.*, 1971). The contribution of these tissues to prostaglandin release by the hindquarter was not ascertained; however it seems likely that the changes in prostaglandin generation caused by insulin or prior exercise are a reflection of events occurring in muscle. In support of this contention, previous studies with incubated muscles have reported rates of release of PGF_{2 α} and PGE₂ of approx. 5 pg/h per mg of muscle (Palmer *et al.*, 1983; Reeds & Palmer, 1983; Turinsky, 1985), values in good agreement with those of the present study (2–2.6 pg/h per mg of muscle). Furthermore, skin and bone do not appear to be insulin-sensitive, as judged by the effect of insulin on 2-deoxyglucose or

Fig. 4. Effects of indomethacin on glucose utilization by the perfused hindquarter of non-exercised and post-exercise rats

Results are means $\pm$ S.E.M. for five to eight observations. Hindquarters of non-exercised rats and rats 30 and 150 min post-exercise (30 min-PE and 150 min-PE) were perfused for an initial 15 min period with (▨) or without (□) 10 μ M-indomethacin. At the end of this time, insulin was added to the perfusate. After an additional 15 min of perfusion, glucose uptake was determined over 30 min. The symbol * indicates a value significantly different from that of the comparable group without insulin at $P < 0.01$. Differences between control and indomethacin-treated groups were insignificant.

AIB uptake by these tissues (Hom *et al.*, 1984; Zorzano *et al.*, 1985). Whether insulin affects prostaglandin generation by skin or bone is not known, however.

Another potential source of prostaglandins in the hindquarter preparation is vascular endothelium. Cultured endothelial cells release abundant quantities of prostaglandins with approx 85% of the total as prostacyclin or its stable metabolite 6-oxoPGF_{1α} (Aanderud *et al.*, 1985). It is unlikely that alterations in the endothelium account for the findings of the present study, as insulin has been shown not to stimulate prostaglandin release by cultured endothelial cells (Aanderud *et al.*, 1985). In addition, PGE₂ and PGF_{2α} were released at a rate similar to that of 6-oxoPGF_{1α} by the hindquarter. *In toto*, these observations strongly suggest that (a) the major component of the prostaglandins released into the perfusate is generated by muscle and (b) insulin-stimulated prostaglandin generation is a consequence of the effect of the hormone on skeletal muscle.

Addition of insulin to the medium at a physiological concentration significantly increased the release of 6-oxoPGF_{1α} and, at a supraphysiological concentration, the release of PGE₂. PGF_{2α} was marginally increased at the high insulin concentration; however, the increase was not statistically significant. Why the dose-response of the three prostaglandins to insulin differed is not clear. In an earlier study Reeds *et al.* (1985) reported that the administration of insulin to fasted rats *in vivo* increased both the release of PGE₂ into the blood and the accumulation of PGE₂ and PGF_{2α} in skeletal muscle. Likewise, they observed that insulin increases PGF_{2α} release by isolated muscle in fasted rabbits (Reeds & Palmer, 1983). Whether our failure to observe a significant increase in PGF_{2α} release relates to the fact we used fed rather than starved rats remains to be determined.

The major factor controlling the synthesis of prostaglandins is thought to be the availability of free arachidonic acid. Whether insulin affects the generation of arachidonic acid by activating phospholipase A₂ and/or by another mechanism is not known. Insulin has been shown to have acute effects on phospholipid turnover in rat adipose tissue both *in vivo* and *in vitro* (Farese *et al.*, 1982, 1983, 1984a; Pennington & Martin, 1985) and in cultured BC3H1 myocytes (Farese *et al.*, 1984b, 1985). In the latter it has also been shown to cause an increase in diacylglycerol (Farese *et al.*, 1984b, 1985); however, this is not associated with an increase in inositol trisphosphate (IP₃), suggesting it might be due to diacylglycerol synthesis rather than to activation of phospholipase C.

Perhaps the most remarkable finding of the present study was that prior exercise completely ablated the insulin-induced increase in prostaglandin release by the hindquarter. This finding is difficult to explain, for, as noted earlier, it is still unclear how insulin stimulates prostaglandin generation in muscle. Presumably prior exercise diminishes the sensitivity of a phospholipase to insulin or it depletes the phospholipid substrate(s) from which insulin stimulates arachidonic acid release. In line with these possibilities recent reports indicate that electrical stimulation causes IP₃ generation in muscle (Vergara *et al.*, 1985), and that IP₃ might be a key chemical link in mediating calcium release from the sarcoplasmic reticulum during muscle contraction (Ver-

gara *et al.*, 1985; Somlyo *et al.*, 1985). Increased generation of IP₃ is usually the consequence of activation of a phospholipase C specific for phosphatidylinositol bisphosphate. Likewise, Ca²⁺ is a potent activator of phospholipase A₂ (Van den Bosch, 1982). Thus, it is reasonable to suggest that electrical stimulation or exercise activates these phospholipases and at least transiently depletes certain phospholipids. Further experiments are required to determine whether phospholipase A₂ or C are activated by insulin, whether insulin or exercise alter the composition of membrane phospholipids and/or whether the presence of esterified arachidonic acid in certain phospholipid pools is altered. Another puzzling finding that requires explanation is why prior exercise had no effect on the rate of prostaglandin release by the hindquarter in the absence of added insulin. Presumably different mechanisms are involved in the regulation of basal and insulin-stimulated prostaglandin release.

Prostaglandins have been implicated in the stimulation of glucose uptake and metabolism by insulin. Thus, in incubated adipocytes (Olefsky, 1977) and epitrochlearis muscle (Leighton *et al.*, 1985; Nesher *et al.*, 1985) addition of μ molar concentrations of PGE₂ has been shown to enhance the stimulation by insulin of glucose transport and glycolysis. In addition, Wicklmayr *et al.* (1979) have presented evidence that prostaglandins are involved in insulin action on glucose uptake in the human forearm. Our results do not support these findings, since inhibition of PGF_{2α} or PGE₂ production by 90% did not alter insulin-stimulated glucose uptake by the perfused hindquarter. Our data also do not support the hypothesis of Dietze & Wicklmayr (1977) that prostaglandins mediate the increase in glucose uptake by muscle during work. Two lines of evidence lead to this conclusion. First, exercise-stimulated glucose and AIB uptake by the hindquarter without a parallel increase in the prostaglandin production, and second, inhibition of prostaglandin synthesis by indomethacin did not affect the increase in glucose uptake following intense exercise.

In conclusion, the results indicate that insulin and exercise modify prostaglandin generation by perfused rat muscle. They suggest that insulin alters the availability of free arachidonic acid, by a mechanism that is presently unknown, and that by doing so it stimulates prostaglandin synthesis. Immediately after exercise, this effect of insulin is blocked. The biological significance of this alteration in prostaglandin synthesis remains to be established. Finally, the data suggest that prostaglandins do not mediate the effect of insulin or exercise on glucose and AIB uptake in skeletal muscle.

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