Increase in Lipoprotein Lipase Activity in Isolated Rat Adipose Tissue by Selenate

Hiroshi UEKI,* Yusuke OHKURA, Toshio MOTOYASHIKI, Nobuaki TOMINAGA, and Tetsuo MORITA

Department of Biochemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Sanzo 1, Gakuen-cho, Fukuyama, Hiroshima 729-02, Japan. Received May 25, 1992

Sodium selenate (selenate), as well as insulin, increased the lipoprotein lipase (LPL) activity in isolated rat fat pads in a time- and dose-dependent manner. The increase effect of selenate was not additive to that of insulin. The action of selenate and insulin was decreased by amiloride and disappeared when Ca^{2+} was omitted from the incubation medium. Loading of a chelator of intracellular Ca^{2+} to the fat pads also greatly inhibited the action of selenate. The maximal increase in inositol 1,4,5-trisphosphate (IP_3) content was observed with a 30-s incubation of the fat pads with selenate. Dibutyl cyclic AMP, 3-isobutyl-1-methylxanthine, carbonyl cyanide *m*-chlorophenylhydrazone, tunicamycin, and monensin all inhibited the increase effect of selenate on the LPL activity to various extents. These results suggest that selenate increases the LPL activity *via* amiloride- and monensin-sensitive processes, involving the Ca^{2+} mobilization linked to a rapid increase in the IP_3 content in fat pads.

Keywords sodium selenate; lipoprotein lipase; calcium; inositol trisphosphate; amiloride; monensin

Introduction

Lipoprotein lipase (LPL, EC 3.1.1.34) is located on the luminal endothelial surface of blood vessels and hydrolyzes triacylglycerides in very low density lipoprotein and chylomicrons to release fatty acids.¹⁾ Several reports show that insulin increases the LPL activity in adipose tissue or cultured adipocytes by a process dependent on or independent of the protein synthesis, and that the effect occurs *via* posttranscriptional and posttranslational mechanisms.²⁾ These mechanisms, however, are not yet well defined. Selenium is a cofactor of selenoenzymes and generally recognized as an essential trace element for mammals, birds, and several bacteria, as determined by their deficiency syndromes such as poor growth and high mortality.³⁾ A daily selenium dietary requirement is estimated to be approximately 60 μg for an adult human,³⁾ while a lethal dose is reportedly 200–400 mg by extrapolation from laboratory animals.³⁾ In a local area of China, an endemic cardiomyopathy known as Keshan disease occurs frequently in children under 15 years old and women of child-bearing age and is assumed to be caused by selenium deficiency.⁴⁾ Recent reports show that sodium selenate (selenate) mimics the insulin effects such as the stimulation of glucose uptake and cyclic AMP phosphodiesterase activity on isolated rat adipocytes and the normalization of plasma glucose levels of streptozotocin-induced diabetic rats.⁵⁾ However, there are few reports on the effects of selenate on synthesis, activation, degradation, and secretion of LPL in adipose tissue.

In this paper, we show that selenate increases the LPL activity in isolated rat fat pads *via* amiloride- and monensin-sensitive processes involving the Ca^{2+} mobilization.

Materials and Methods

Chemicals The sources of chemicals used in this work were as follows: Selenate (Na_2SeO_4), tunicamycin, tetrakis (acetoxymethyl) ester of quin 2 (quin 2-AM), inositol 1,4,5-trisphosphate (IP_3), dibutyl cyclic AMP (Bt_2cAMP), and 3-isobutyl-1-methylxanthine (IBMX) from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan); monensin and A 23187 from Calbiochem Co. (San Diego, CA); insulin (bovine pancreas, 24.4 IU/mg), bovine serum albumin (essentially fatty acid free), amiloride, and carbonyl

cyanide *m*-chlorophenylhydrazone (CCCP) from Sigma Chemical Co. (St. Louis, MO); Intralipos, an emulsion composed of 1.2% yolk lecithin, 2.5% glycerol, and 10% soybean oil, from Green Cross Co. (Osaka); $[9,10\text{-}^3\text{H}(\text{N})]\text{tri olein}$ (991.6 GBq/mmol), $^{45}\text{CaCl}_2$ (2.77 TBq/g calcium), and Aquasol-2 from New England Nuclear (Boston, MA); and IP_3 [^3H] assay system (TRK 1000) from Amersham Japan (Tokyo, Japan). All other chemicals used were of analytical grade.

Animals Male Wistar rats (200–220 g body weight and 7 weeks old) were fed on a commercial pellet diet *ad libitum* and given free access to water for 1 week and starved for 24 h before experiment.

Determination of LPL Activity Fat pads (3 pieces, 100 mg) were prepared from rat epididymal adipose tissue and incubated with selenate or insulin at 37°C for 90 min in 1 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 5 mM glucose and 2% bovine serum albumin (KRBGA), as described previously.⁶⁾ The fat pads incubated were separated by centrifugation at 100 *g* for 3 min, washed three times with 30 mM Tris–HCl buffer, pH 8.5, at 4°C, homogenized in 1 ml of the same buffer with a Potter–Elvehjem homogenizer, and centrifuged at 1500 *g* for 10 min. The resultant infranatant was used as a crude LPL solution. The LPL activity was determined by a modification of the method of Peterson *et al.*⁷⁾ For preparation of substrate, a solution (20 μl) of the labelled lipid (approximately 370 kBq) was taken to dry under argon on the walls of a plastic tube, dissolved in 1.75 ml of 0.3% Triton X-100 containing 7.5% bovine serum albumin, and sonicated with a Branson sonifier (cell disruptor 200) in ice-cold water. The solution was again sonicated after addition of 1 ml of 1.5 M Tris–HCl buffer, pH 8.5, and 250 μl of intralipos. The emulsion obtained (3 ml) was activated by incubation with 2 ml of heated rat serum at 37°C for 20 min. The enzyme solution (100 μl) was incubated with the activated substrate (50 μl) at 37°C for 60 min. The reaction was terminated by addition of 1 ml of a methanol/chloroform/heptane (141/125/100 by volume) solution.⁸⁾ Oleate released was extracted by addition of 0.1 N KOH. The radioactivity was determined in Aquasol-2 with an Aloka liquid scintillation counter (LSC-700). The LPL activity was calculated from the difference in activity between samples incubated with either activated or nonactivated substrate.⁹⁾ Results are expressed as nmol free fatty acids (FFA) produced/min/mg protein (mU/mg protein). Proteins were determined by the method of Lowry *et al.*¹⁰⁾

Determination of IP_3 Content in Fat Pads The fat pads (2 pieces, 200 mg) were incubated with selenate (1 mM) for 0–120 s in 2 ml of KRBGA. The incubated fat pads were quickly separated, frozen in dry ice-acetone to terminate the reaction, and homogenized in 300 μl of a chilled 12.5% trichloroacetic acid (TCA) for 1 min with a Niton Medical and Scientific homogenizer (Physoctron, NS-310E). After centrifugation at 16000 *g* for 10 min, the infranatant (300 μl) was extracted three times with 4 ml of a chilled H_2O -saturated diethyl ether to remove TCA and adjusted to pH 7.5 with 0.1 M NaHCO_3 .¹¹⁾ The IP_3 content in the solution obtained (5–20 μl) was determined with an IP_3 [^3H] assay system kit, according to the method supplied by the manufacturer (Amersham

International plc).

Determination of Ca^{2+} Release Preparation of $^{45}\text{Ca}^{2+}$ -loaded endoplasmic reticulum (ER) and determination of Ca^{2+} release were carried out essentially according to the method of Delfert *et al.*¹²⁾ Briefly, the fat pads (500 mg/ml) were homogenized in 10 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose and centrifuged. A subcellular fraction enriched in ER (pellets at 105000 *g*, 500–900 μg) was incubated with $^{45}\text{CaCl}_2$ (81.4–133.2 kBq) in 0.5 ml of 50 mM Tris-Pipes buffer, pH 7.0, containing 200 mM sucrose, 100 mM KCl, 20 mM NaN_3 , 2 mM MgCl_2 and ATP, 0.5 mM EGTA, and 0.15 mM CaCl_2 at 30 °C for 20 min. For assay of Ca^{2+} release, samples to test (5 μl) were added to 0.5 ml of the Tris-Pipes buffer containing the ER (500 μg) and $^{45}\text{CaCl}_2$ and incubated at 30 °C for 0–3 min. The incubation was terminated by a rapid filtration of 100 μl of the reaction mixture on a Millipore filter (HA, 0.45 μm) followed by washing of the filter six times with 5 ml of 0.25 M sucrose. The radioactivity in the filters after drying was determined.

All results are expressed as means $\pm$ S.E. of four or five observations. Similar results were obtained with two separate experiments. The data were analyzed by Student's *t*-test for unpaired observations.

Results

When the fat pads were incubated with selenate (1 mM) or insulin (3 nM) over 150-min periods, those in selenate showed a small increase in LPL activity from the original level. A significant increase by selenate or insulin was observed as compared with the control (Fig. 1). A 90-min incubation of the fat pads with selenate increased the LPL activity in a dose-dependent manner up to 1 mM (data not shown). When the fat pads were incubated with selenate in the presence or absence of insulin (3 nM), no additive effect on the action of insulin was observed by selenate at 0.2 or 1 mM concentrations (data not shown).

To determine whether activation of the insulin receptor and/or Na^+/H^+ exchange system is involved in the increase effect of selenate on LPL activity, the fat pads were incubated with selenate or insulin in the presence of amiloride at the indicated concentrations (Fig. 2). The action of selenate, as well as that of insulin, was decreased with an increase in the concentration of amiloride. Furthermore, when the fat pads were incubated with selenate or insulin in the Na^+ -free medium prepared by using choline ion in place of Na^+ , the action of selenate, in contrast to that of insulin, was well preserved (data not shown).

Next, the requirement of extracellular Ca^{2+} for the

Fig. 1. Time Course of Increase Effects of Selenate and Insulin on LPL Activity in Fat Pads

The fat pads were incubated with selenate (1 mM, ●), insulin (3 nM, ▲), or buffer alone (○) for the indicated periods. Significantly different from the control: a) $p < 0.01$, b) $p < 0.05$.

actions of selenate and insulin was investigated (Fig. 3). Although neither substance increased the LPL activity in Ca^{2+} -free or 0.01 mM Ca^{2+} medium, significant increase effects were observed with insulin and selenate at Ca^{2+} concentrations over 0.1 and 1 mM, respectively. When the fat pads were loaded with quin 2-AM at 100 μM to remove the intracellular Ca^{2+} , the increase effects of selenate and insulin were lowered to 10 and 57%, respectively (Table I). These results suggest that the action of selenate is much more dependent on the intracellular Ca^{2+} pool than is insulin. Insulin may exert its action by stimulating Ca^{2+} entry.

We examined whether changes in the IP_3 content inducing Ca^{2+} mobilization in the fat pads are involved in mechanisms of the selenate action. When the fat pads were incubated with 1 mM selenate, the maximal increase in IP_3 content (488 pmol/g tissue) was observed with a 30-s incubation, and this dropped to the original level (263 pmol/g tissue) with a 60-s incubation (Fig. 4). In investigations of the stimulatory release of Ca^{2+} from the ER, the

Fig. 2. Effect of Amiloride on Increase in LPL Activity in Fat Pads by Selenate or Insulin

The fat pads were incubated with selenate (1 mM, ●), insulin (3 nM, ▲), or buffer alone (○) for 90 min in the presence of amiloride at the indicated concentrations.

Fig. 3. Effect of Extracellular Ca^{2+} on Increase in LPL Activity in Fat Pads by Selenate or Insulin

The fat pads were incubated with selenate (1 mM, ●), insulin (3 nM, ▲), or buffer alone (○) for 90 min in the presence of Ca^{2+} at the indicated concentrations. Significantly different from the control: a) $p < 0.01$.

TABLE I. Effect of Quin 2-AM Loading on Increase in LPL Activity in Fat Pads by Selenate or Insulin

Quin 2-AM (μ M):	Relative LPL activity in fat pads (%)			
	0	20	40	100
Selenate (1 mM)	100	75	40	10
Insulin (3 nM)	100	90	82	57

Quin 2-AM was dissolved in dimethyl sulfoxide at the desired concentrations and 10 μ l was added to the incubation medium. The fat pads (200 mg) were incubated with selenate or insulin in 2 ml of KRBGA containing sodium pyruvate (10 mM) and quin 2-AM (0–100 μ M) at 37°C for 90 min.⁶⁾ Percentage values were calculated from net increase in the LPL activity (1.0 and 1.3 mU/mg protein with selenate and insulin, respectively).

Fig. 4. Time Course of Increase in IP₃ Content in Fat Pads by Selenate

The fat pads were incubated with 1 mM selenate for the indicated periods and the IP₃ content was determined as described in Materials and Methods.

control, a TCA-soluble fraction from the nontreated fat pads, showed an increase in Ca²⁺ content of the ER (from 8.7 to 9.8 and 10.4 nmol Ca²⁺ bound/mg protein with 1- and 3-min incubation, respectively). No change was observed with incubation of the ER in the Tris-Pipes buffer alone. A 23187, a Ca²⁺ ionophore, (5 μ M), IP₃ (50 μ M), and a TCA-soluble fraction of the selenate-treated fat pads all decreased relative Ca²⁺ content of the ER to the control with 1- and 3-min incubations (Fig. 5). These results suggest that selenate produces a Ca²⁺-mobilizing factor in the fat pads, probably IP₃, and stimulates the Ca²⁺ release from the ER. The fraction from the fat pads treated with selenate in the presence of amiloride or with insulin failed to reduce the relative Ca²⁺ content of the ER.

The fat pads were incubated with selenate or insulin in the presence of chemicals such as IBMX, Bt₂cAMP, an uncoupler of oxidative phosphorylation, and inhibitors of glycosylation of protein (Table II). IBMX, Bt₂cAMP, CCCP, and tunicamycin all inhibited the increase effects of both selenate and insulin to various extents. Monensin inhibited the action of selenate but did not inhibit that of insulin. Thus, selenate appears to increase the LPL activity through a mechanism(s) which partially differs from the action of insulin.

Discussion

We found that selenate, similar to insulin, increased the

Fig. 5. Time Course of Stimulatory Release of Ca²⁺ from ER by TCA-Soluble Fraction of Fat Pads Treated with Selenate or Insulin

The TCA-soluble fraction was prepared from fat pads (200 mg) which were incubated with 1 mM selenate, 1 mM selenate plus 1 mM amiloride, or 15 nM insulin for 30 s. For assay of the release of Ca²⁺, the ⁴⁵Ca²⁺-loaded ER (500 μ g, 8.7 nmol Ca²⁺ bound/mg protein) was incubated with A 23187 (5 μ M, Δ), IP₃ (50 μ M, $\blacktriangle$), or TCA-soluble fractions of fat pads treated with selenate ($\bullet$), selenate plus amiloride ($\circ$), or insulin ($\blacksquare$) for the indicated periods. The buffer alone or TCA-soluble fraction of fat pads treated with neither selenate nor insulin was used as the control. Results are expressed as relative values to the control.

TABLE II. Effects of Chemicals on Increase in LPL Activity in Fat Pads by Selenate or Insulin

	Relative LPL activity in fat pads (%)	
	Selenate (1 mM)	Insulin (3 nM)
None	100	100
IBMX (10 μ M)	45	83
IBMX (100 μ M)	0	10
Bt ₂ cAMP (0.5 mM)	40	88
Bt ₂ cAMP (1 mM)	0	75
Tunicamycin (25 μ g/ml)	75	44
Tunicamycin (50 μ g/ml)	0	0
Monensin (2 μ M)	85	100
Monensin (5 μ M)	25	100
CCCP (10 μ M)	45	40
CCCP (20 μ M)	33	30

All chemicals were added to the incubation medium without preincubation. The fat pads (200 mg) were incubated with selenate or insulin for 90 min. Percentage values were calculated from net increase in the LPL activity (0.9 and 1.1 mU/mg protein with selenate and insulin, respectively).

LPL activity in fat pads in a time- and dose-dependent manner. Because of changes in nutritional state when fat pads were isolated from rats, as reported by Semb and Olivecrona,¹³⁾ the basal LPL activity was progressively decreased over the time course of the incubation of the fat pads in KRBGA, and was also not constant in every experiment. The action of selenate was inhibited by amiloride, an inhibitor of the activation of the Na⁺/H⁺ exchange system and the stimulatory phosphorylation of insulin and growth factor receptors.¹⁴⁾ The action of selenate was well preserved in the Na⁺-free medium. Selenate, therefore, appears to increase the LPL activity in fat pads *via* an amiloride-sensitive process, probably involving insulin or other receptors but not the activation of the Na⁺/H⁺ exchange system.

Draznin *et al.*¹⁵⁾ reported the possibility that intracellular Ca^{2+} is an essential component of the intracellular mechanism of insulin action in adipocytes. Preincubation of adipocytes with quin 2-AM,¹⁶⁾ a chelator of intracellular Ca^{2+} , inhibited the effects of insulin such as the stimulations of glucose transport and oxidation and the suppression of the hormone-dependent lipolysis.¹⁷⁾ The increasing effect of selenate on the LPL activity in fat pads was inhibited by quin 2-AM at low concentrations, suggesting that the action of selenate is dependent on the intracellular concentration of Ca^{2+} . IP_3 is known to be a mediator of the intracellular Ca^{2+} mobilization in response to various extracellular stimulations.¹⁸⁾ Delfert *et al.*¹²⁾ reported that IP_3 caused the release of Ca^{2+} from the ER of isolated rat adipocytes but not from the plasma membrane. A rapid increase in the IP_3 content in the fat pads was observed with selenate. Amiloride inhibited the production of a Ca^{2+} -mobilizing factor by selenate, suggesting that the factor, probably IP_3 , is produced by selenate *via* an amiloride-sensitive process. The possibility is reported that epidermal growth factor produces IP_3 through mechanisms involving a pertussis toxin-sensitive G protein¹⁹⁾ and the activation of a specific type of phospholipase C, PLC_γ .²⁰⁾ Whether selenate increases IP_3 through similar mechanisms is unknown. Increased IP_3 may interact with Ca^{2+} stores in the fat pads to stimulate the release of Ca^{2+} and consequently cause a rise in the intracellular concentration of Ca^{2+} . Slack *et al.*²¹⁾ reported that the IP_3 -induced effects of sea urchin eggs were dependent on extracellular Ca^{2+} , and that there were two distinct responses in membrane potential generated by microinjection of IP_3 : an extracellular Ca^{2+} -independent depolarization and extracellular Ca^{2+} -dependent prolonged hyperpolarization which is presumably associated with the elevation of the fertilization envelope. When thio- IP_3 was microinjected into the Ca^{2+} -depleted hepatocytes, the stimulatory Ca^{2+} entry into the cells was observed by readdition of Ca^{2+} to the medium, suggesting that IP_3 coordinates the intracellular Ca^{2+} mobilization and Ca^{2+} entry.²²⁾ In the action of selenate, the extracellular Ca^{2+} may be required to prolong cellular responses after the initial pulse of Ca^{2+} mobilization stimulated by selenate. Although the insulin-stimulatory release of Ca^{2+} from the intracellular pool *via* IP_3 was not found, insulin, in contrast to selenate, shows an increase in LPL activity at 0.1 mM concentration of extracellular Ca^{2+} , and this increase effect was lessened by the loading of quin 2-AM at a rather high concentration. These results show the possibility that insulin stimulates Ca^{2+} entry by mechanisms independent of IP_3 ; further details, however, are still unknown.

Bt_2cAMP , causing a decrease in the LPL activity in cultured avian adipocytes,²³⁾ and IBMX, a potent inhibitor of the phosphodiesterase activity in adipocytes,²⁴⁾ inhibited the increase effect of selenate on the LPL activity, probably by increasing the intracellular concentration of cyclic AMP. Two inhibitions may occur by a modification of the full active enzyme to a less active one and/or by its accelerated intracellular degradation, as suggested by the inhibition of insulin-increased LPL activity by adrenaline.²⁵⁾

Tunicamycin, monensin, and CCCP all inhibited the action of selenate to various extents, while that of insulin

was not inhibited by monensin at tested concentrations. Tunicamycin, an inhibitor of the first step of N-linked glycosylation of proteins,²⁶⁾ decreases cellular LPL activity by inhibiting the incorporation of sugars into the enzyme in cultured adipocytes.²⁷⁾ Monensin, an Na^+ ionophore, inhibits the incorporation of terminal sugars into secretory glycoproteins and the transport of proteins from medial- to trans-Golgi cisternae.²⁸⁾ CCCP also is an inhibitor of energy-dependent transfer of proteins from the ER to the Golgi.²⁹⁾ When cultured adipocytes were incubated in the presence of monensin or CCCP, an inactive precursor form of LPL was detected in the ER, suggesting that the intracellular activation of the enzyme occurs before the enzyme reaches the trans-Golgi cisternae and that monensin and CCCP both inhibit transport of the enzyme from the ER to the trans-Golgi cisternae.²⁹⁾ Selenate had no effect on the incorporation of [^3H]leucine into proteins of the fat pads with a 90-min incubation, suggesting that the action of selenate is not due to the increase in protein synthesis (data not shown). Selenate may, in part, stimulate the trimming and processing of the oligosaccharide chains of LPL, which are required for the conversion of the less active form to the fully active one.

In conclusion, selenate appears to increase LPL activity through mechanisms involving Ca^{2+} mobilization and stimulatory glycosylation of the enzyme for the processing.

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