CMLS, Cell. Mol. Life Sci. 57 (2000) 1874–1879
1420-682X/00/141874-06 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Selenium: an insulin-mimetic

S. R. Stapleton

Department of Chemistry, Western Michigan University, Kalamazoo (Michigan 49008, USA), Fax + 1 616 387 2909, e-mail: stapleton@wmich.edu

Abstract. Insulin or agents that can mimic its action (insulin-mimetics) are necessary to promote the entry of glucose into tissues where the glucose can either be converted into energy or stored for later use. In recent years, selenium has been shown to mediate a number of insulin-like actions both in vivo and in vitro. These insulin-like actions include stimulating glucose uptake and regulating metabolic processes such as glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway. The mechanism by which selenium is capable of mimicking insulin is not clear; however, reports indicate that selenium does activate key proteins involved in the insulin-signal cascade. Various proteins in the insulin-signal cascade have been shown to be necessary for different insulin-regulated events, and presumably data will be forthcoming soon that illustrate this similarly for selenium. This review compares the action of selenium to that of insulin and discusses the available evidence in support of selenium as an insulin-mimetic.

Key words. Selenium; insulin-mimetic; glucose metabolism; diabetes.

Insulin and insulin-mimetics

Insulin, a peptide hormone, is secreted by the β cells in response to a rise in blood glucose levels. Insulin or agents that can mimic its action (insulin-mimetics) are necessary to promote the entry of glucose into tissues where the glucose can either be converted into energy or stored for later use. The lack of critical concentrations of circulating insulin or the ability of insulin to function properly leads to the onset of diabetes mellitus [1].

With increasing numbers of individuals diagnosed with diabetes mellitus, there is a need for not only more mechanistic studies to completely understand insulin action but also to better design and provide therapies that meet the needs of the patient. In recent years, enthusiasm has surfaced for using insulin-mimetic agents to not only help better define the mechanisms of insulin action but also to investigate the action and possible implications of these agents in the design of future treatments of diabetics. Two promising insulin-mimetics that have been studied are vanadate and selenate. Vanadate, a potent tyrosine phosphatase inhibitor, has been reviewed elsewhere [2–4] and will be minimally discussed only in comparison with selenate in this review. Selenate, an essential trace element, serves as an integral component of several enzymes, including formate dehydrogenase, glutathione peroxidase, selenoprotein P and W and the deiodinases (see articles in this multi-author review). In addition to its role in enzyme function, selenate has been touted as an antioxidant [5] and anticancer agent [6 and Schrauzer in this multi-author review]. Biologically, mammals have a limited reservoir of selenate and thus need a regular supply through diet and water [7, 8]. Recently, selenate supplementation has been popularized by its availability either as a nutritional additive or in combination with antioxidant vitamins. If proven successful as an antidiabetic agent, selenate would be an attractive therapy since it is already available for individual use as this oral supplement. This review covers some of the findings that support selenate as an effective insulin-mimetic and potential antidiabetic agent.

Diabetes

Diabetes mellitus is not a single disease but a cluster of disorders that share certain common features, the most familiar of which are elevated levels of blood glucose and a lack of control with regard to glucose

metabolism. High circulating levels of glucose contribute to health risks associated with the disease. Diabetes is a leading cause of kidney failure, blindness and amputations as well as a major risk factor for heart disease, stroke and birth defects [9].

The most common forms of diabetes are type I, insulin-dependent diabetes mellitus (IDDM) and type II, non-insulin-dependent diabetes mellitus (NIDDM). Type I is caused by autoimmune destruction of the pancreatic insulin-producing β cells. Type I diabetics therefore have little to no circulating insulin levels, and the disease is usually managed with injections of insulin. Type II is associated with defects in both insulin action and secretion. A defect in insulin action is characterized by an inability of the cells of the body to respond to the presence of insulin and is often referred to as insulin resistance. Depending on the nature of the disease, type II diabetics can be treated either with insulin or insulin-sensitizing agents. Even though diabetes is a complex disease, treatments typically only target the management of blood glucose levels [9].

Insulin and the insulin-mimetic action of selenate on glucose uptake

For any agent to be considered an effective mimetic, it must be comparable to insulin, in its ability to at least regulate glucose uptake. In that regard, some of the early work with both vanadate and selenate showed that these insulin-mimetic compounds were effective in stimulating glucose uptake in both in vitro and in vivo systems [10–15]. As early as 1979, Tolman et al. [10] reported that vanadate exhibited insulin-like properties in rat hepatocytes isolated from normal animals. As with insulin, these investigators observed a threefold stimulation of glycogen synthesis when the hepatocytes were incubated in the presence of vanadium. Vanadate's insulin-like effects on various aspects of glucose homeostasis in other cell types were later described. When added to intact cells, vanadate was reported to stimulate glucose transport and oxidation in adipocytes [11, 12] and skeletal muscle [16], induce glucose transporter (GLUT-1) messenger RNA (mRNA) in NIH3T3 fibroblasts [17] as well as mimic the effect of insulin on glycogen synthase in adipocytes [18, 19]. With culture systems showing such promise, investigators then turned to whole animal systems to investigate the effect of vanadate on glucose homeostasis. Oral administration of vanadate to streptozocin-induced diabetic rats was shown to be effective in markedly reducing blood glucose concentrations [13, 20, 21]. Marked improvement of glucose homeostasis by oral vanadate has also been observed in an animal model that exhibits severe insulin resistance and overt diabetes, the diabetic *ob*/*ob* mouse [22]. These pioneering studies with vanadate set the stage for other elements such as selenate to be considered as insulin mimetics.

A decade after Tolman made the original observations on vanadate as an insulin-mimetic, Osamu Ezaki first showed the effect of selenate as a potent insulin-like agent [14]. In hindsight it may not have been surprising that selenate exhibited insulin-like properties similar to vanadate since the ions share similarities in chemical structure and possible mechanism of action within a cell. In the studies carried out by Ezaki, selenate stimulated glucose transport activity in a dose-dependent manner in isolated rat adipocytes. Maximal transport activity (100 μM for 30 min) was equipotent to 1 nM insulin for 30 min of incubation. Stimulation of glucose transport was observed 2 min after addition of selenate and reached a steady state within 10 min. Like insulin, the observed increase in glucose transport activity by selenate was due to translocation of the glucose transporters (GLUT-1 and GLUT-2) to the membrane surface. This insulin-like effect of selenate on glucose uptake and metabolism was also more recently studied in rat soleus muscle [23]. Exposure of the muscle to selenate markedly stimulated glucose uptake. The dose-dependent uptake in glucose was paralleled by rising aerobic and anaerobic glycolysis.

In 1991, McNeill et al. expanded on Ezaki's original observations and showed that selenium also acted as an insulin-mimetic in vivo [15]. Administration of selenate to streptozotocin-induced diabetic animals improved both food and water intake to near control levels. Weight gain of the animals improved, and within 2 weeks of treatment plasma glucose levels fell significantly and remained low throughout a 7-week experiment. Control animals treated with selenate exhibited lower insulin levels, suggesting that the insulin requirements of the rat were decreased due to the insulin-like effects of the trace element. A blood glucose lowering effect of selenate similar to insulin in streptozotocin-induced diabetic animals was also observed in comparable studies in rats by Berg et al. [24] and in mice by Ghosh et al. [25].

Insulin and the insulin-mimetic action of selenate on glucose metabolism

In addition to glucose uptake, insulin eloquently regulates a variety of other metabolic responses, including facilitating entry of amino acids into cells for the production of cellular protein, increasing precursors for nucleic acid synthesis, transporting critical cellular ions, stimulating Na^+/K^+ ATPase as well as controlling the expression of a number of genes. As recently reviewed by O'Brien and Granner, insulin regulates the transcrip-

tion of the genes for several key enzymes associated with both carbohydrate and fatty acid metabolism [26]. Glycogen synthase [27], glucokinase [28], phosphoenolpyruvate carboxykinase (PEPCK) [29, 30], fatty acid synthase (FAS) [31, 32] and glucose-6-phosphate dehydrogenase (G6PDH) [33], key enzymes in glycogen synthesis, glycolysis, gluconeogenesis, fatty acid biosynthesis and the pentose phosphate pathway, respectively, have been targets of study with regard to insulin action. Thus, in addition to controlling blood glucose levels diabetics must also concern themselves with the ability to control these other metabolic responses. Any treatment regime taken by a diabetic should also address overall effectiveness on not only glucose uptake and homeostasis but also these other physiological processes. As discussed below, the effect of selenate as an insulin-mimetic has been investigated on several of these metabolic processes.

Several studies have shown both vanadate and selenate to mimic insulin in regard to glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway. Bosch et al. [34] have found that vanadate inhibits the expression of transfected chimeras of PEPCK in both FTO-2B and H4IIE rat hepatoma cells. In adult rat hepatocytes in primary culture, vanadate in the presence of glucose induced L-type pyruvate kinase mRNA [35], and in a streptozocin-induced diabetic rat vanadate was found to increase pancreatic amylase mRNA [36]. In a similar light, oral administration of selenate to diabetic animals partly reversed abnormal liver expression of both glycogenic and gluconeogenic enzymes [37]. In particular, for glycolysis, glucokinase, L-type pyruvate kinase and for gluconeogenesis, PEPCK were restored to near nondiabetic control levels. Regulation by insulin-mimetics of the expression of lipogenic enzymes was also found to be similar to insulin. FAS and G6PDH activity was normalized in the livers of diabetic animals [25] and rat hepatocytes in culture [38] treated with vanadate. This normalization of activity for both FAS and G6PDH was suggested to be due to increases in gene expression, as comparable increases in the level of their respective mRNAs were also found. In these same studies the effect of selenate on the expression of FAS and G6PDH was investigated. Treatment of the diabetic animals or rat hepatocytes in culture with selenate restored the expression of both FAS and G6PDH, demonstrating that selenate was capable of stimulating lipogenesis in the liver [25, 28].

Depressed levels of overall lipid metabolism in diabetics is believed to be a contributing factor to a higher risk of heart disease and stroke. In particular, high plasma lipid levels are known to have a detrimental effect on heart function and may be a cause of heart disease in diabetics. In that light a recent study evaluated cardiac performance in a subset of streptozotocin-induced diabetic rats treated with selenate that had improved glucose tolerance and normalized fed plasma glucose levels [39]. The diabetic untreated rats had left ventricular developed pressure, which was reduced when compared with the values for control rats. Treatment with selenate brought all three parameters to levels which were not different from control, suggesting selenate can improve heart function in diabetics. Plasma lipid levels, triglycerides, cholesterol and free fatty acids were also improved in these treated animals [39]. This lowering of plasma lipid levels by selenate shows another potent medicinal effect of this treatment.

Glycogen metabolism has been another metabolic process shown to be affected by both vanadate and selenate, although some controversy exists for these compounds as both insulin-like and non-insulin-like effects have been observed. Back in 1990, Ezaki found that in rat adipocytes cyclic AMP (cAMP) phosphodiesterase activity was stimulated in the presence of selenate, and the concentration dependence of the curve was biphasic. This enzyme is important in that it breaks down cAMP [14]. cAMP, a second messenger whose concentration is increased in a cell in response to glucagon, stimulates glycogen breakdown and shuts down its synthesis. In another study, however, using rat soleus muscle, glycogen synthesis was not affected by the treatment with selenate even though glucose uptake was positively effected [24]. These results contrast with those found with insulin, since in muscle and other tissue insulin stimulates glycogen synthesis and storage. Thus, the insulin-like effects of selenate in this regard may differ in different tissues.

Similar contradictory results have also been noted with vanadate. In rat adipocytes, vanadate has been shown to increase glycogen synthase, but in skeletal muscle only the peroxides of vanadate and not vanadate itself was shown to have a positive effect [40]. Even though the data appear clear regarding the insulin-like actions of selenate and vanadate on the other metabolic processes, more studies are clearly needed to define their role in glycogen metabolism.

Insulin and the insulin-mimetic action of selenate on signal transduction

All actions of insulin at the cellular level that were described above are initiated by insulin binding to its plasma membrane receptor. Following insulin binding, the insulin receptor behaves as a classic allosteric enzyme, undergoing both conformational changes and multi-site modification by phosphorylation [41]. Following this ligand-induced autophosphorylation of the receptor, phosphorylation of endogenous substrates

then occurs to mediate the transmission of an insulin signaling pathway [41]. It is clear from studies conducted thus far that under a number of different circumstances a variety of phosphoproteins are activated by insulin [41–43]. The insulin receptor substrate (IRS) family which includes IRS-1, IRS-2 and IRS-3 (p60) are thought to be essential for many of insulin's biological responses [42, 43]. Whereas the insulin-mimetics do not bind the insulin receptor, some studies have reported increased phosphorylation of the receptor and the substrate IRS-1. Increased tyrosine phosphorylation of the insulin receptor's β subunit has been observed when cells in culture have been incubated with vanadate [44]. Similarly, treatment of NIH3T3 HIR 3.5 cells with selenate was found to stimulate phosphorylation of the insulin receptor [45]. In this later study an additional protein of approximately 185-kDa was also phosphorylated in the presence of selenate. Due to the size of the protein and the ability of it to become phosphorylated, it was postulated that the 185-kDa protein corresponded to IRS-I. In a later study, incubation of either primary rat hepatocytes or 3T3-LI adipocytes in culture with selenate caused not only a concentration and time-dependent increase in phosphorylation of the β subunit of the insulin receptor but also IRS-1 as determined through immunoprecipitation studies [46]. This increase in phosphorylation was observed within 1 h of selenate treatment, whereas only minutes of incubation were required for increased phosphorylation by insulin, suggesting that time was needed for selenate to enter the cell and mediate insulin-regulated processes through a post-insulin receptor kinase mechanism. These results are supported by the fact that although selenate is capable of increasing phosphorylation of the insulin receptor, this does not appear to directly increase insulin receptor tyrosine kinase activity [14, 16].

Interestingly, selenate has also been shown to increase phosphorylation of another growth factor receptor. A concentration-dependent increase in tyrosine phosphorylation of the epidermal growth factor receptor (EGFR) was observed in selenate-treated A431 cells [45]. In the presence of epidermal growth factor (EGF), this phosphorylation event induced by selenate was found to be additive. Mercaptoethanol failed to reverse the effects of selenate on EGF-stimulated phosphorylation, suggesting that sulfhydryl group oxidation may not be involved in the mechanism of this action of selenate. In yet another study, a 180-kDa protein that copurified with the insulin receptor increases in phosphorylation when either rat hepatocytes or 3T3-L1 adipocytes in culture were incubated with selenate [46]. Due to its size, copurification with the insulin receptor and response to increased phosphorylation in the presence of selenate, it was suggested that this protein in these cells could correspond to the EGFR. These results indicate that selenate may act to mediate a number of growth factor receptor processes.

Additional downstream targets of the insulin receptor and signal transduction pathway have been identified in recent years. It has been found that activated IRS-1 recognizes and binds to SH2 domains of various signal transduction proteins such as phosphatidylinositol (PI) 3-kinase, SH-PTP-2, Grb 2 and Nck [47]. In a number of independent studies, the critical role of PI 3-kinase in diverse actions of insulin such as stimulation of DNA synthesis, glucose transporter translocation [48], regulation of glycogen synthase [27], glycogen synthase kinase-3 [49], PEPCK [50] and G6PDH expression [33], glut-4-mediated glucose transport [48, 51] and membrane ruffling [52] has been established. In preliminary studies conducted in our laboratory [53], we have been able to show that both vanadate and selenate increase PI 3-kinase activity in rat hepatocytes in culture. One protein that has been identified to lie downstream of PI 3-kinase is pp70 S6 kinase. S6 kinase can play a critical role in initiation of protein synthesis. Selenate has been shown to stimulate not only S6 kinase phosphorylation [14] but also kinase activation [54]. Activation of S6 kinase was reduced when the cells were pretreated with genestein, a potent tyrosine kinase inhibitor, suggesting selenate was activating this protein through an upstream kinase. Rapamycin, an immunosuppressant, was also shown to block S6 kinase activation by selenate without affecting an upstream activator, PI 3-kinase [54].

In addition to the IRS family and the PI 3-kinase pathway, another set of proteins have been identified as a proximal target for several growth factor tyrosine kinases, including the insulin receptor [55–57]. Once activated by phosphorylation, the Shc proteins associate with various downstream effector molecules including Grb 2 and the guanine nucleotide releasing factor, GNRF. Grb 2/SOS interaction plays a role in the insulin activation of Ras since SOS facilitates the exchange of GDP for GTP on Ras proteins [58].

Ras activation is required for initiation of the downstream events leading to mitogen activated protein (MAP) kinase activation. Studies have indicated a reasonable correlation between the activation of MAP kinase and events leading to cellular proliferation [56, 57]. In vitro, MAP kinase phosphorylates transcription factors such as c-Jun and TCF/ELK as well as ATF-2 to restore DNA binding activity [59]. Thus, insulin-mediated MAPK activation may lead in some cases to increased transcription of a gene. Both vanadate and selenate have been shown to activate MAPK [46, 54]. In both hepatocytes in culture and 3T3-L1 adipocytes, selenate caused a marked increase in not only the phosphorylation of MAPK but also its activity as measured by an 'in-gel' kinase assay [46]. This increase in phos-

phorylation and activity was found to be both time and concentration dependent. Similarly, in isolated rat adipocytes, selenate increased MAPK activity approximately twofold, an induction analogous to that of insulin [54]. In the culture systems tested, hepatocytes, 3T3-L1 cells and rat adipocytes, the increase in MAPK observed in the presence of selenate could be reduced in the presence of genestein, suggesting a kinase-mediated activation [46, 54].

Conclusion

The evidence to date clearly supports selenate serving as an effective insulin-mimetic. In whole animal type I (streptozotocin-induced diabetic mice and rats) diabetic models, treatment of the animal with selenate lowers blood glucose levels, similarly to insulin. In addition, selenate has been shown to regulate the fate of this glucose by stimulating glycolysis, fatty acid synthesis and in some cases glycogen synthesis. The increase in the ability of these pathways to shuttle glucose through maybe due in part to the increased expression of key enzymes in these pathways. Increased expression of these important metabolic enzymes is controlled through the insulin signal transduction pathway which selenium does activate. More studies, however, are needed to fully understand selenate's effect on glucose uptake and metabolism. While the evidence provided in this review supports the potential of selenate as an effective insulin-mimetic, other concerns, in particular, potential toxicological considerations of selenate or selenite, need to be fully addressed before it can be safely used in therapy for diabetics.

1 National Institutes of Health (1999) Conquering Diabetes: A Strategic Plan for the 21st Century, Publication number 99-4398, Washington, DC
2 Shechter Y. (1990) Insulin-mimetic effects of vanadate. Possible implications for future treatment of diabetes. Diabetes **1:** 1–5
3 Goldfine A. B., Simonson D. C., Folli F., Patti M. E. and Kahn C. R. (1995) Metabolic effects of sodium metavanadate in humans with insulin-dependent and noninsulin-dependent diabetes mellitus in vivo and in vitro studies. J. Clin. Endocrinol. Metab. **11:** 3311–3320
4 Orvig C., Thompson K. H., Battell M. and McNeill J. H. (1995) Vanadium compounds as insulin-mimics. Met. Ions Biol. Syst. **31:** 575–594
5 Schrauzer G. N. (1992) Selenium mechanistic aspects of anticarcinogenic action. Biol. Trace Elem. Res. **33:** 51–63
6 Chen H., Pellett L. J., Anderson H. J. and Tappel A. L. (1993) Protection by Vitamin E, selenium and beta carotene against oxiative damage in rat liver slices and homogenate. Free Radic. Biol. Med. **5:** 473–482
7 Bedwal R. S., Nair N., Sharma M. P. and Mathur R. S. (1993) Selenium: its biological perspectives. Med. Hypoth. **41:** 150–159
8 Daniels L. A. (1995) Selenium metabolism and bioavailability. Biol. Trace Elem. Res. **54:** 185–199
9 Diabetes Care (2000) January, Supplement 1, ADA Clincial Practice Recommendations 2000
10 Tolman E. L., Barris E., Burns M., Pansini A. and Partridge R. (1979) Effects of vanadium on glucose metabolism in vitro. Life Sci. **20:** 1159–1164
11 Shechter Y. and Karlish S. J. D. (1980) Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions. Nature **284:** 556–558
12 Dubyak G. R. and Kleinzeller A. (1980) Insulin-mimetic effects of vanadate in isolated rat adipocytes. J. Biol. Chem. **255:** 5306–5312
13 Meyerovitch J., Farfel Z., Sack J. and Schecter Y. (1987) Oral administration of vanadate normalizes blood glucose levels in streptozotocin-treated rats. J. Biol. Chem. **262:** 6658–6662
14 Ezaki O. (1990) The insulin-like effects of selenate in rat adipocytes. J. Biol. Chem. **265:** 1124–1130
15 McNeill J. H., Delgatty H. L. M. and Battell M. L. (1991) Insulin-like effects of sodium selenate in streptozotocin-induced diabetic rats. Diabetes **40:** 1675–1679
16 Claisen T., Anderson T. L., Sturup-Johansen M. and Pitkova O. (1981) The relationship between the transport of glucose and cations across cell membranes in isolated tissues. Biochim. Biophys. Acta **646:** 261–267
17 Mountjoy K. G. and Flier J. S. (1990) Vanadate regulates glucose transporter (Glut-1) expression in NIH3T3 mouse fibroblasts. Endocrinology **126:** 2778–2787
18 Tamura S., Brown T. A., Whipple J. H., Yamaguchi Y. F., Dubler R. E., Cheng K. et al. (1984) A novel mechanism for the insulin-like effects of vanadate on glycogen synthase in rat adipocytes. J. Biol. Chem. **259:** 6650–6656
19 Heyligir C. E., Tahiliani A. G. and McNeill J. H. (1985) Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science **227:** 1474–1476
20 Pederson R. A., Ramanadham S., Buchan A. M. J. and McNeill J. H. (1989) Long-term effects of vanadyl treatment on streptozotocin-induced diabetic rats. Diabetes **38:** 1390–1395
21 Sakurai H., Tsuchiya K., Nukatsuka M., Sofue M. and Kawada J. (1990) Insulin-like effects of vanadyl ion on stretozotocin-induced diabetic rats. J. Endo. **126:** 451–455
22 Brichard S. M., Bailey C. J. and Henquin J. C. (1990) Marked improvement of glucose homeostasis in diabetic ob/ob mice given oral vanadate. Diabetes **39:** 1326–1332
23 Furnsinn C., Englisch R., Ebner K., Nowotny P., Vogle C. and Waldhausl W. (1996) Insulin-like vs. non-insulin stimulation of glucose metabolism by vanadium, tungsten and selenium compounds in rat muscle. Life Sci. **59:** 1989–2000
24 Berg E. A., Wu J. Y., Campbell L., Kagey M. and Stapleton S. R. (1995) Insulin-like effects of vanadate and selenate on the expression of glucose-6-phosphate dehydrogenase and fatty acid synthase in diabetic rats. Biochimie **12:** 919–924
25 Ghosh R., Mukherjee B. and Chatterjee M. (1994) A novel effect of selenium on streptozotocin-induced diabetic mice. Diabetes Res. **25:** 165–171
26 O'Brien R. M. and Granner D. K. (1996) Regulation of gene expression by insulin. Physiol. Rev. **76:** 1109–1161
27 Shepherd P. R., Navé B. T. and Siddle K. (1995) Insulin stimulation of glycogen synthesis and glycogen synthase activity is blocked by wortmannin and rapamycin in 3T3-L1 adipocytes: evidence for the involvement of phosphoinositide-3 kinase and p70 ribosomal protein-S6 kinase. Biochem. J. **305:** 25–28
28 Magnuson M. A., Andreone T. L., Printz R. L., Koch S. and Granner D. K. (1989) Rat glucokinase gene: structure and regulation by insulin. Proc. Natl. Acad Sci. USA **86:** 4838–4842
29 O'Brien R. M., Lucas. P. C., Forest C., Magnuson M. A. and Granner D. K. (1990) Identification of a sequence in the PEPCK gene that mediates a negative effect of insulin on transcription. Science **249:** 533–537
30 Short J. M., Wynshaw-Boris A., Short H. P. and Hanson R. W. (1986) Characterization of the phosphoenolpyruvate carboxykinase (GTP) promoter-regulatory region. J. Biol. Chem. **261:** 9721–9726

31 Moustaid N., Beyer R. S. and Sul H. S. (1994) Identification of an insulin response element in the fatty acid synthase promoter. J. Biol. Chem. **269:** 5629–5634

32 Stapleton S. R., Mitchell D. A., Salati L. M. and Goodridge A. G. (1990) Triiodothyronine stimulates transcription of the fatty acid synthase gene in chick embryo hepatocytes in culture. J. Biol. Chem. **265:** 18442–18446

33 Wagle A., Jivraj S., Garlock G. and Stapleton S. R. (1998) Insulin regulation of glucose-6-phosphate dehydrogenase gene expression is rapamycin sensitive and requires phosphatidylinositol 3-kinase. J. Biol. Chem. **273:** 14968–14974

34 Bosch R., Hatzoglou M., Park E. A. and Hanson R. W. (1990) Vanadate inhibits expression of the gene for phosphoenolpyruvate carboxykinase (GTP) in rat hepatome cells. J. Biol. Chem. **265:** 13677–13682

35 Miralpeix M., Decaux J.-F., Kahn A. and Bartrons R. (1991) Vanadate induction of L type pyruvate kinase mRNA in adult rat hepatocytes in primary culture. Diabetes **40:** 462–467

36 Johnson T. M., Meisler M. H., Bennett M. I. and Willsky G. R. (1990) Vanadate induction of pancreatic amylase mRNA in diabetic rats. Diabetes **39:** 757–762

37 Becker D. J., Reul B., Ozcelikay A. T., Buchet J. P., Henquin J. C. and Brichard S. M. (1996) Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycogenic and gluconeogenic enzymes in diabetic rats. Diabetologia **39:** 3–11

38 Stapleton S. R., Berg E. A., Wu J. Y., Garlock G., Foellmi-Adams L. and Kletzien R. F. (1994) Induction of insulin-regulated genes by insulin-mimetics: evaluation of involvement of the tyrosyl phosphorylation cascade. FASEB J. **8:** 447

39 Battell M. L., Delgatty H. L. M. and McNeill J. H. (1998) Sodium selenate corrects glucose tolerance and heart function in STZ diabetic rats. Mol. Cell. Biochem. **179:** 27–34

40 Sekar N., Li J., He Z., Gefel D. and Schechter Y. (1999) Independent signal-transduction pathways for vanadate and for insulin in the activation of glycogen synthesis and glycogenesis in rat adipocytes. Endocrinology **140:** 1125–1131

41 Kahn C. R., White M. F., Shelson S. E., Backer J. M., Araki E., Cheatham B. et al. (1993) The insulin receptor and its substrate: molecular determinants of early events in insulin action. Recent Progress in Hormone Research **48:** 291–360

42 Goldstein B. J. and White M. F. (1991) Structure of the insulin-receptor substrate IRS-1 defines a unique signal transduction protein. Nature **352:** 73–76

43 White M. F. and Kahn C. R. (1994) The insulin signaling system. J. Biol. Chem. **269:** 1–4

44 Pugazhenthi S. and Khandelwal R. L. (1993) does the insulin-mimetic action of vanadate involve insulin receptor kinase? Mol. Cell. Biochem. **127/128:** 211–218

45 Pillay T. S. and Makgoba M. W. (1992) Enhancement of EGF and insulin-stimulated tyrosine phosphorylation of endogenous subtrates by sodium selenate. FEBS Lett. **308:** 38–42

46 Stapleton S. R., Garlock G., Foellmi-Adams L., Kletzien R. F. (1997) Selenium: potent stimulator of tyrosyl phosphorylation and activator of MAP kinase. Biochem. Biophys. Acta **1355**: 259–269.

47 Van Hoorn, D. J., Myers, M. G., Jr., Backer, J. M., (1994) Direct activation of the phosphatidylinositol-3 kinase by the insulin receptor. J. Biol. Chem. **269**: 29–32

48 Cheatham B., Vlahos C. J., Cheatham L., Wang L., Blenis J. and Kahn C. R. (1994) Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis and glucose transporter translocation. Mol. Cell. Biol. **14:** 4902–4911

49 Cross D. A. E., Alessi D. R., Vandenheede J. R., McDowell H. E., Hundal H. S. and Cohen P. (1994) The inhibition of glycogen synthase kinase-3 by insulin or insulin-like growth factor 1 in the rat skeletel muscle cell line L6 is blocked by wortmannin, but not by rapamycin: evidence that wortmannin blocks activation of the mitogen-activated protein kinase pathway in L6 cells between Ras and Raf. Biochem. J. **303:** 21–26

50 Gabbay R. A., Sutherland C., Gnudi L., Kahn B. B., O'Brien R. M., Granner D. K. and Flier J. S. (1995) Insulin regulation of PEPCK expression does not require activation of the ras/mitogen-activated protein kinase signaling pathway. J. Biol. Chem. **271:** 1890–1897

51 Okada T., Kawanok Y., Sakakibara T., Hazeki O. and Ui M. (1994) Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. J. Biol. Chem. **269:** 3568–3573

52 Kotani, K., Yonezawa, K., Hara, K., Ueda, H., Kitamurea, Y., Sakaue (1994) EMBO J. **13**: 2313–2321

53 Stapleton S. R., Jivraj S. and Wagle A. (1998) Insulin and the insulin-mimetic selenium mediate the regulation of glucose-6-phosphate dehydrogenase gene expression via different signal proteins. FASEB J. **12:** 1404

54 Hei Y.-J., Farahbakhshian S., Chen X., Battell M. L. and McNeill J. H. (1998) Stimulation of MAP kinase and S6 kinase by vanadium and selenium in rat adipocytes. Mol. Cell. Biochem. **178:** 367–375

55 Cox A. D., Garcia A. M., Westwick J. K., Kowalczyk J. J., Lewis M. D., Brenner D. A. et al. (1994) The CAAX peptiomimetic compound B581 specifically blocks farnesylated oncogene ras signaling and transformation. J. Biol. Chem. **269:** 19203–19206

56 Boulton T. G., Nye S. H., Robbins D. J., Ip N. Y., Radziejewska E., Morgenbesser S. D. et al. (1991) Erks-A family of protein-serine threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell **65:** 663–669

57 Cobb M. H. and Goldsmith E. J. (1995) How MAP kinases are regulated. J. Biol. Chem. **270:** 14843–14846

58 Baltensperger K., Kozma L. M., Cherniack A. D., Klarlund J. K., Chawla A., Banerjee U. et al. (1993) Binding of the Ras activator son of sevenless to insulin-receptor substrate-1 signaling complexes. Science **260:** 1950–1954

59 Davis R. J. (1993) Mitogen activated protein kinase signal transduction pathway. J. Biol. Chem. **268:** 14553–14556