Minireview

The physiological role of dehydroascorbic acid

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Received 10 June 2002; revised 26 July 2002; accepted 26 July 2002

First published online 15 August 2002

Edited by Guido Tettamanti

Abstract Dehydroascorbic acid (DHA) is abundant in the human diet and also is generated from vitamin C (ascorbic acid, AA) in the lumen of the gastrointestinal tract. DHA is absorbed from the lumen of the small intestine and reduced to AA, which subsequently circulates in the blood. Utilization of AA as an antioxidant and enzyme cofactor causes its oxidation to DHA in extracellular fluid and cells. DHA has an important role in many cell types because it can be used to regenerate AA. Both physiological (e.g. insulin, insulin-like growth factor I, cyclic AMP) and pathological (e.g. oxidative stress, diabetes, sepsis) factors alter the transport and metabolic mechanisms responsible for this DHA recycling.

Key words: Dehydroascorbic acid; Ascorbic acid; Transport; Oxidative stress; Inflammation; Sepsis; Diabetes

1. Introduction

Vitamin C (ascorbic acid, AA) acts as a cofactor in the enzymatic biosynthesis of collagen, carnitine, and catecholamine and peptide neurohormones. AA also prevents injurious effects of oxidants because it reduces reactive oxygen and nitrogen species to stable molecules. Decreased serum vitamin C concentration in humans is associated with neurologic problems and eventually causes scurvy [1]. As AA loses electrons in biosynthetic or antioxidant reactions, it becomes oxidized to the short-lived ascorbyl radical and then to dehydroascorbic acid (DHA). For instance, AA donates reducing equivalents to reactive metabolites of dietary flavonoids (e.g. semiquinone or phenoxy radicals of quercetin), thereby sparing other cellular components from oxidation but incidentally producing DHA [2]. DHA and AA have distinct effects on cell function, as becomes obvious under conditions characterized by oxidative stress (Table 1).

Vitamin C is synthesized de novo from glucose in the livers of most adult mammals except guinea pigs, primates and humans, which depend on dietary sources. Ingesting either AA or DHA raises the serum AA concentration to similar extents in normal human subjects [3,4]. Thus DHA can serve as a dietary source of vitamin C for humans, although it is a poor source for some animals (e.g. Osteogenic Disorder Shionogi rat [5]). Evidently cellular mechanisms of transport and metabolism convert DHA to AA. These mechanisms are important because the human diet normally contains DHA as well as AA [6]. Indeed, the commercial and domestic processing of foods oxidizes AA to DHA. There exists a widely held understanding that dietary AA and DHA possess roughly equivalent bioavailability in humans. It is because both molecules are commonly thought to be bioavailable that the vitamin C content of foods is usually reported as total vitamin C, i.e. the sum of AA and DHA contents. The purpose of this review is to describe the mechanisms by which DHA increases the concentration of AA in cells and extracellular fluid and to discuss their relevance to disease.

2. Transport of AA and DHA

The gastrointestinal tract is the principal site of absorption for AA and DHA. In addition to the amounts ingested, DHA is produced when AA reacts with oxidants in the lumen of the gastrointestinal tract [7]. For instance, oxidation of AA by quercetin metabolites [2] may contribute to the decrease in AA bioavailability caused by quercetin administration in rats [8]. DHA itself is degraded to diketogulonic acid by bicarbonate at alkaline pH in vitro [9]. However, it is unlikely that DHA is exposed to these destabilizing conditions in vivo, because the alkaline secretions from the pancreas and duodenal glands mix with the strongly acidic gastric juice to form a slightly acidic fluid in the lumen of the small intestine.

Both DHA and AA are absorbed from the lumen of the human intestine by enterocytes, as has been shown by measuring transport activities in luminal (brush border) membrane vesicles [10]. The absorption sites are found along the entire length of the small intestine. In vesicles prepared from the jejunum, Na^+–ascorbate cotransporters take up the ionized form of AA with high affinity ($K_m = 0.2$ mM) while a Na^+-independent process of facilitated diffusion takes up DHA with lower affinity ($K_m = 0.8$ mM). However, the maximal rates of uptake for AA and DHA are similar. Furthermore, glucose inhibits AA but not DHA uptake, which may increase the relative bioavailability of the oxidized form of vitamin C [10].

Human enterocytes contain DHA reductases that convert DHA to AA [11]. These enzymes keep the intracellular concentration of DHA low and thereby maintain a gradient favoring continued uptake of oxidized vitamin C across the

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Abbreviations: AA, ascorbic acid; DHA, dehydroascorbic acid; IFNγ, interferon-γ

0014-5793/02/$22.00
PII: S0014-5793(02)03167-8

Table 1
The roles of DHA and AA in mammalian cells under conditions characterized by oxidative stress

DHA
Competitively inhibits facilitative glucose transporters, hexokinase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase
Oxidizes NADPH and glutathione
Stimulates synthesis of NADPH and glutathione
Increases AA concentration in metabolically competent cells (e.g. astrocytes)
Kills susceptible cells (e.g. neurons)
AA
Increases synthesis of collagen, carnitine, catecholamine and peptide hormones
Terminates free radical chain reactions by scavenging reactive oxygen and nitrogen species
Prevents or reverses oxidation of glutathione and α-tocopherol
Donates electrons through membrane oxidoreductases to extracellular acceptors
Increases cell survival

enterocytes' luminal membrane. Any DHA that escapes reduction in enterocytes and enters the blood may be taken up and reduced to AA by other cell types. If the DHA concentration in blood rises nonetheless, then it is filtered from the plasma at the renal corpuscles and reabsorbed across the luminal membranes of the renal tubules for subsequent reduction [12].

It is not known how AA is transported out of epithelial cells to the blood during intestinal absorption and renal tubular reabsorption of vitamin C. One possibility is that ionized AA (ascorbate) diffuses from the cytosol to the extracellular fluid through volume-sensitive anion channels in the basolateral membrane and then enters the blood plasma through discontinuities in the capillary wall. There are two lines of evidence in support of this hypothetical mechanism. First, epithelial cells swell markedly during transepithelial transport of nutrients; for example, sodium-dependent absorption of glucose or alanine causes a sustained increase in cell volume in enterocytes and renal tubular cells [13–15]. Second, cell swelling has been shown to transiently and reversibly stimulate ascorbate efflux through channels or pores in the plasma membrane of astrocytes [16,17]. However, the molecular identities of the proteins mediating AA transport across the basolateral membranes of intestinal and renal epithelia have yet to be determined.

AA circulates in the blood, predominantly in the form of the ascorbate anion. Many cell types are capable of high-affinity vitamin C uptake through Na^+–ascorbate cotransporters, including vascular smooth muscle and endothelial cells, bone osteoblasts and cerebral astrocytes [16–22]. Subsequent utilization of vitamin C as either an enzyme cofactor or antioxidant causes its oxidation. The DHA thus generated intracellularly may exit cells through facilitative glucose transporters.

Instead of allowing its immediate excretion from the body, numerous cell types can clear DHA from the extracellular fluid. Among those that take up DHA and reduce it to AA are neutrophils, erythrocytes, smooth muscle cells, hepatocytes, astrocytes and osteoblasts [19–21,23–28]. DHA uptake is not mediated by Na^+–ascorbate cotransporters because it is neither dependent on Na^+ nor blocked by an antagonist of Na^+–ascorbate cotransport (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid) [23]. Many cells possess both Na^+–ascorbate cotransporters and the ability to take up and reduce DHA. In contrast, human neutrophils lack Na^+–ascorbate cotransporters but incubation of these myeloid cells with DHA in vitro increases their intracellular AA concentration levels to those found in mature neutrophils in vivo [26].

In bone, the oxidation and regeneration of AA may be an important way for the osteoid-resorbing activity of osteoclasts to stimulate the osteoid-forming activity of osteoblasts [20]. Resorbing osteoclasts are sources of reactive oxygen species that oxidize extracellular AA. As DHA is produced in the extracellular fluid, it may be taken up and reduced to AA by neighboring osteoblasts. The rate of rise in intracellular AA concentration is much faster when osteoblasts are incubated with DHA than with AA [20]. Intracellular AA stimulates the osteoblasts to produce collagenous osteoid that subsequently becomes calcified [29]. Thus DHA may couple bone resorption and formation.

Glucose exerts an effect on the initial rate of DHA uptake that varies between cell types. Some are acutely inhibited by physiological concentrations of glucose (osteoblasts, smooth muscle cells), others are less sensitive (erythrocytes, astrocytes), and DHA uptake across some cell membranes is not detectably changed by glucose (luminal membranes of intestinal enterocytes and renal tubular cells) [10,12,19–21,23–25].

DHA competes with glucose for uptake through several isoforms of the facilitative glucose transporters (e.g. GLUT1, GLUT3 and GLUT4) [19–21,23–26,30]. Agents that enhance the activity of these facilitative glucose transporters also increase the initial rate of DHA uptake, such as colony-stimulating factors in neutrophils [26], insulin and insulin-like growth factor I in osteoblasts [20], and cyclic AMP in astrocytes [21]. It is clear that Na^+–ascorbate cotransporters and facilitative glucose transporters can be regulated independently. For instance, colony-stimulating factors enhance DHA uptake in human neutrophils that lack Na^+–ascorbate cotransporters [26]. Bone-derived osteoblasts provide another intriguing example. In osteoblasts, transforming growth factor-β increases the maximal rate of AA uptake through Na^+–ascorbate cotransporters but does not stimulate DHA uptake [22], while insulin increases the maximal rate of DHA uptake through glucose transporters without changing Na^+–ascorbate cotransport activity [20].

Besides DHA permeation of facilitative glucose transporters, additional DHA uptakes occur through a glucose-insensitive mechanism in human erythrocytes [24] and rat astrocytes [23]. In primary cultures of rat astrocytes, for example, uptake of DHA (5–200 μM) is inhibited only partially by a relatively high concentration of glucose (10 mM) [23]. The

remaining, glucose-insensitive accumulation of intracellular AA from DHA is blocked by phloretin and cytochalasin B, like facilitated glucose transport, but also is inhibited reversibly by sulfinpyrazone, unlike glucose transport.

The epithelial cells of the renal tubules reabsorb DHA after it has been filtered from the plasma at the renal corpuscles. Studies of brush-border membrane vesicles prepared from rat renal cortex, which are representative of the luminal membranes of proximal tubule cells, have shown that AA uptake occurs through Na^+–ascorbate cotransport while DHA uptake occurs by a Na^+-independent process [12]. Since they cannot be inhibited by glucose, it is evident that both AA and DHA uptakes occur independently of glucose transporters in these renal membranes [12].

3. Regeneration of AA from oxidized vitamin C

The dismutation of a pair of ascorbyl radicals, which is catalyzed by NADH-dependent semidehydroascorbate reductase, produces one molecule of AA and one of DHA. DHA can be converted to AA by NADPH-dependent thioredoxin reductase or glutathione-dependent DHA reductase [31]. In contrast to an unsubstantiated opinion published recently [32], these reactions do not produce hydrogen peroxide or other reactive oxygen species while regenerating AA.

The reduction of DHA to AA by intracellular enzymes keeps the cytosolic concentration of DHA low and thus contributes to a gradient favoring DHA uptake across the plasma membrane. DHA may be reduced to AA as it permeates the plasma membrane and at other intracellular locations [24]. Within a few minutes, DHA uptake and reduction can raise the AA concentration in the cytosol of some cell types to millimolar levels that are 10–100 times greater than the AA concentration in extracellular fluids [19,23].

Reductants derived from cell metabolism convert intracellular DHA to AA. The NADPH generated by the pentose phosphate pathway of glucose metabolism is an example. It is likely that redundant mechanisms involving NADPH, glutathione, and perhaps other thiols ensure DHA reduction [21].

Reduction of large amounts of DHA to AA by NADPH- and glutathione-dependent reactions may decrease the intracellular concentrations of NADPH and glutathione markedly [21,33,34]. DHA also inhibits the activities of purified hexokinase, glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase, but this does not occur when sufficiently high concentrations of the enzymes' specific substrates are present [34]. Moreover, DHA indirectly stimulates the pentose phosphate pathway to produce NADPH and subsequently increases intracellular glutathione concentration above basal levels [35]. These time-dependent effects of DHA are reflected in its ability to modulate the resistance of lymphocytes against killing by hydrogen peroxide [35]. Short- (20 min to 2 h) and long-term (24–48 h) DHA pretreatment had opposing effects on cell survival. Preincubation with DHA for 20 min to 2 h enhanced hydrogen peroxide-induced apoptosis. In contrast, pretreatment with DHA for 36 h strongly inhibited cell death [35].

DHA killing of susceptible cell types, such as neurons [36], may be explained by the stress caused by depletion of NADPH and glutathione. But other cell types, such as astrocytes and osteoblasts, recycle DHA abundantly without undergoing acute injury. The explanation for this discrepancy may be that those cells that are most capable of producing reducing equivalents are least susceptible to damage by DHA.

After intracellular DHA becomes reduced to AA, the latter can function as a reductant. Each equivalent of AA in a cell can reduce several equivalents of oxidants if the DHA thereby produced can be recycled in the same cell, as has been demonstrated for human erythrocytes [37]. The AA derived from DHA also provides electrons to plasma membrane oxidoreductases and through them to extracellular oxidants [38]. Furthermore, DHA uptake into cells may be followed by AA efflux. For example, human erythrocytes [27] and HepG2 liver cells [28] take up DHA, reduce it intracellularly and subsequently release AA to the extracellular fluid. This process allows the reducing equivalents derived from cell metabolism to be carried into the extracellular fluid and made available to neighboring cells.

4. Diabetes

An excess of glucose during uncontrolled diabetes may impair DHA uptake into cell types where DHA transport is mediated largely by facilitative glucose transporters (Fig. 1). Hormonal dysregulation may also contribute to local deficiencies in DHA recycling. Normally insulin increases the maximal rate of DHA transport by facilitative glucose transporters in insulin-sensitive cells, thereby raising the intracellular AA concentration [20]. A lack of insulin may impair DHA uptake through facilitative glucose transporters during type I diabetes (Fig. 1). Although insulin-like growth factor I can also activate insulin receptors to stimulate cellular uptake of DHA, it has only one-tenth the potency of insulin [20]. Indeed, the maximal rate of DHA uptake is decreased in lymphoblasts from patients with type I diabetes and nephropathy [39].

Slowing of DHA uptake may lead to impaired regeneration of AA and weakening of antioxidant defences in diabetes,

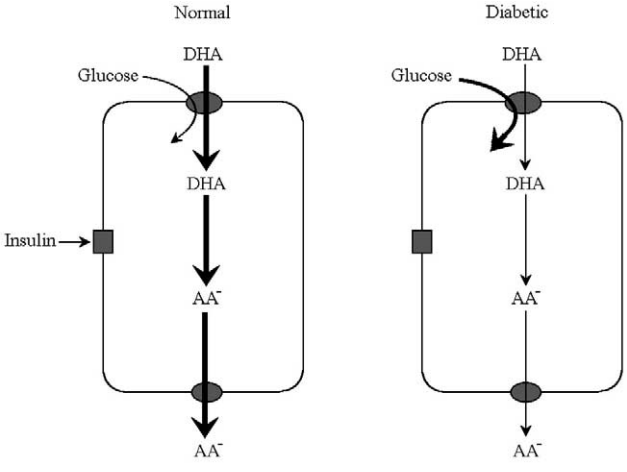

Fig. 1. Insulin stimulates accumulation of AA in osteoblasts exposed to extracellular DHA. The DHA can enter cells through facilitative glucose transporters located in the plasma membrane and then is reduced to AA, accumulating as the ascorbate ion (AA^-). Insulin acts through specific receptor mechanisms to stimulate the activity of facilitative glucose transporters in osteoblasts and other insulin-sensitive cell types. During type I diabetes, a deficiency of insulin and an excess of glucose may inhibit DHA uptake. This may lead to a localized scarcity of AA^- in those osteoblasts that use vitamin C for synthesizing collagen at sites of bone remodelling and thereby contribute to osteopenia.

especially in the presence of hyperglycemia. For instance, the AA concentration is decreased and the DHA concentration is increased in the sciatic nerve of rats made diabetic by streptozotocin [40]. Furthermore, because intracellular AA is required for collagen synthesis by osteoblasts [29], deficient recycling of DHA to AA in this cell type may contribute to the development of osteopenia [20]. Support for this hypothesis comes from the observation that feeding AA to diabetic pregnant rats improves skeletal development in their offspring [41].

Plasma and liver AA concentrations are significantly lower in the Goto-Kakizaki diabetic rat than in controls [42]. NADPH-dependent regeneration of AA from DHA is also suppressed in the liver of this diabetic rat [42]. Glucose-6-phosphate dehydrogenase activity provides NADPH for DHA reduction and a decrease in the activity of this enzyme in diabetic rat liver may account for the subnormal AA concentrations [43].

5. Inflammation, sepsis and ischemia-reperfusion injury

Under pathological conditions characterized by oxidative stress, AA is oxidized by reactive oxygen species at rates that overwhelm the ability of cells to regenerate the vitamin. For example, inflammation in skin during wound healing raises the extracellular concentration of DHA markedly [44]. Similarly, gastritis decreases the AA concentration and increases the DHA concentration in the gastric juice of human patients [45].

Redox cycling of vitamin C may also be abnormal during the inflammatory response to microbial infection, since the rate of AA oxidation is increased in the serum of septic patients [46]. Moreover, findings with an in vitro model indicate that septic conditions decrease recycling of DHA [25]. The model consists of applying bacterial endotoxin (lipopolysaccharide) and the inflammatory cytokine interferon-γ (IFNγ) to primary cultures of astrocytes. Lipopolysaccharide and IFNγ induce nitric oxide synthase isoform 2, increase intracellular levels of reactive oxygen species, and decrease the rate of intracellular AA accumulation from extracellular AA or DHA [25]. The oxidants produced during inflammatory reactions may directly alter the mechanisms by which cells recycle DHA. For instance, prior exposure of astrocytes to peroxyl radicals decreases their subsequent accumulation of intracellular AA from extracellular DHA [23].

Ischemia-reperfusion injury in brain, caused by stroke or trauma, also involves oxidative stress. Interventions that increase cerebral AA concentration may be beneficial. Experiments with transgenic mice lacking one of the most widely distributed Na^+–ascorbate cotransporters (Slc23a1, an ortholog of the rat Svct2 and human hSvct2) indicate that this transporter normally maintains the high AA concentration found in brain [47]. However, because Na^+–ascorbate cotransporters become downregulated when the intracellular AA concentration is high [48], they may not be suitable targets for therapeutic strategies that attempt to raise intracellular AA to supraphysiological levels. Hence the suggestion that DHA be injected as a pro-drug to increase the cerebral AA concentration [49,50]. Radiotracer experiments have shown that blood-borne DHA enters the brain and is converted to AA [49]. Furthermore, intravenous injection of DHA has been shown to improve the neurologic outcome in mice subjected to experimental stroke, although no substantial increase

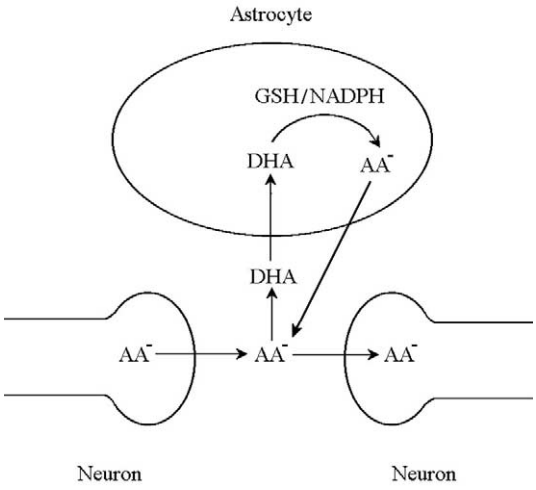

Fig. 2. The DHA–AA shuttle between astrocytes and neurons. AA (ionized as ascorbate, AA^-) exits neurons by exocytosis and other efflux mechanisms. The extracellular AA^- is taken up into adjacent neurons through specific Na^+–AA^- cotransporters located in the plasma membrane. Additionally, AA^- becomes oxidized to DHA and the latter diffuses from the extracellular fluid into astrocytes through Na^+-independent transporters. Intracellular DHA is converted to AA^- by reductases, using reducing equivalents from glutathione (GSH) or NADPH, and then returns to the extracellular fluid. Astrocyte swelling, such as that caused by glutamate, may accelerate this DHA recycling process by activating plasma membrane channels through which AA^- can diffuse from the astrocyte cytosol to the extracellular fluid.

in cerebral AA concentration has been reported [50]. The acute effect of DHA in normal brain may be to inhibit behavioral activation. Infusion of AA oxidase into rat brain converts extracellular AA to DHA and leads to a rapid decline in behavioral activation, with a 50–70% decrease in extracellular AA concentration leading to a near-total inhibition of all recorded behavior [51]. These putative actions of DHA are reminiscent of those of general anesthetics, which also inhibit behavioral activation acutely and confer neuroprotection against cerebral ischemia [52].

Glutathione-dependent DHA reductase has been identified by immunostaining in cerebral endothelial cells, neurons and astrocytes [53]. DHA kills neurons in the absence of astrocytes [36], whereas the latter cells are capable of regenerating AA without ill effect [21,23]. Brain cells produce cyclic AMP in response to neurotransmitters and ischemia and this intracellular messenger stimulates astrocytic uptake of DHA and accumulation of AA [21]. Astrocytes rapidly release the newly formed AA when stimulated appropriately (Fig. 2). For instance, swelling astrocytes with either glutamate or hypotonic medium activates channels or pores in the plasma membrane, through which large quantities of cytosolic AA diffuse to the extracellular fluid [16,17]. Thus astrocytes detoxify the extracellular fluid for neurons by clearing DHA and restoring AA.

6. Conclusions

DHA is ingested in the diet and also formed from oxidative reactions in cells and extracellular fluid. However, vitamin C oxidation is readily reversed by mechanisms that rapidly transport DHA into metabolically competent cells and reduce it there to AA. The resulting AA can be utilized in the same cells or else released to the extracellular fluid. DHA recycling

mechanisms may decrease the amount of AA that humans need to ingest. Pathological conditions that inhibit DHA recycling may decrease AA concentrations and thereby impair AA-dependent enzymatic and antioxidant activities.

Acknowledgements: Supported by the Natural Sciences and Engineering Research Council of Canada

References

[1] Richardson, T.I.L., Ball, L. and Rosenfeld, T. (2002) Postgrad. Med. J. 78, 292–294.
[2] Galati, G., Sabzevari, O., Wilson, J.X. and O'Brien, P.J. (2002) Toxicology 177, 91–104.
[3] Linkswiler, H. (1958) J. Nutr. 64, 43–50.
[4] Sabry, J.H., Fisher, K.H. and Dodds, M.L. (1958) J. Nutr. 64, 457–464.
[5] Ogiri, Y., Sun, F., Hayami, S., Fujimura, A., Yamamoto, K., Yaita, M. and Kojo, S. (2002) J. Agric. Food Chem. 50, 227–229.
[6] Vanderslice, J.T. and Higgs, D.J. (1991) Am. J. Clin. Nutr. 54 (Suppl. 6), 1323S–1327S.
[7] Kyrtopoulos, S.A., Pignatelli, B., Karkanias, G., Golematis, B. and Esteve, J. (1991) Carcinogenesis 12, 1371–1376.
[8] Song, J., Kwon, O., Chen, S., Daruwala, R., Eck, P., Park, J.B. and Levine, M. (2002) J. Biol. Chem. 277, 15252–15260.
[9] Koshiishi, I., Mamura, Y. and Imanari, T. (1998) Biochim. Biophys. Acta 379, 257–263.
[10] Malo, C. and Wilson, J.X. (2000) J. Nutr. 130, 63–69.
[11] Buffinton, G.D. and Doe, W.F. (1995) Free Radic. Res. 22, 131–143.
[12] Malo, C. and Wilson, J.X. (2001) FASEB J. 15, A838.
[13] Beck, J.S. and Potts, D.J. (1990) J. Physiol. 425, 369–378.
[14] Breton, S., Marsolais, M., Lapointe, J.Y. and Laprade, R. (1996) J. Am. Soc. Nephrol. 7, 2072–2087.
[15] MacLeod, R.J., Lembessis, P. and Hamilton, J.R. (1992) J. Membr. Biol. 130, 23–31.
[16] Wilson, J.X., Peters, C., Sitar, S.M., Daoust, P. and Gelb, A.W. (2000) Brain Res. 858, 61–66.
[17] Siushansian, R., Dixon, S. and Wilson, J.X. (1996) J. Neurochem. 66, 1227–1233.
[18] Wilson, J.X., Dixon, S.J., Yu, J., Nees, S. and Tyml, K. (1996) Microcirculation 3, 211–221.
[19] Holmes, M.E., Mwanjewe, J., Samson, S.E., Haist, J.V., Wilson, J.X., Dixon, S.J., Karmazyn, M. and Grover, A.K. (2002) Biochem. J. 362, 507–512.
[20] Qutob, S., Dixon, S.J. and Wilson, J.X. (1998) Endocrinology 139, 51–56.
[21] Siushansian, R., Tao, L., Dixon, S.J. and Wilson, J.X. (1997) J. Neurochem. 68, 2378–2385.
[22] Wilson, J.X. and Dixon, S.J. (1995) Am. J. Physiol. 268, E565–E571.
[23] Daskalopoulos, R., Korcok, J., Tao, L. and Wilson, J.X. (2002) Glia 39, 124–132.
[24] Himmelreich, U., Drew, K.N., Serianni, A.S. and Kuchel, P.W. (1998) Biochemistry 37, 7578–7588.
[25] Korcok, J., Wu, F., Tyml, K., Hammond, R.R. and Wilson, J.X. (2002) J. Neurochem. 81, 185–193.
[26] Vera, J.C., Rivas, C.I., Zhang, R.H. and Golde, D.W. (1998) Blood 91, 2536–2546.
[27] Mendiratta, S., Qu, Z.C. and May, J.M. (1998) Free Radic. Biol. Med. 24, 789–797.
[28] Upston, J.M., Karjalainen, A., Bygrave, F.L. and Stocker, R. (1999) Biochem. J. 342, 49–56.
[29] Franceschi, R.T., Wilson, J.X. and Dixon, S.J. (1995) Am. J. Physiol. 268, C1430–C1439.
[30] Rumsey, S.C., Kwon, O., Xu, G.W., Burant, C.F., Simpson, I. and Levine, M. (1997) J. Biol. Chem. 272, 18982–18989.
[31] May, J.M. (2002) Methods Enzymol. 347, 327–332.
[32] Jamison, J.M., Gilloteaux, J., Taper, H.S., Calderon, P.B. and Summers, J.L. (2002) Biochem. Pharmacol. 63, 1773–1783.
[33] May, J.M., Qu, Z. and Morrow, J.D. (2001) Biochim. Biophys. Acta 1528, 159–166.
[34] Fiorani, M., De Sanctis, R., Scarlatti, F. and Stocchi, V. (1998) Arch. Biochem. Biophys. 356, 159–166.
[35] Puskas, F., Gergely Jr., P., Banki, K. and Perl, A. (2000) FASEB J. 14, 1352–1361.
[36] Song, J.H., Shin, S.H. and Ross, G.M. (2001) Brain Res. 895, 66–72.
[37] Mehlhorn, R.J. (1991) J. Biol. Chem. 266, 2724–2731.
[38] Jones, W., Li, X., Qu, Z., Perriott, L., Whitesell, R.R. and May, J.M. (2002) Free Radic. Biol. Med. 33, 83–93.
[39] Ng, L.L., Ngkeekwong, F.C., Quinn, P.A. and Davies, J.E. (1998) Diabetologia 41, 435–442.
[40] Obrosova, I.G., Fathallah, L. and Stevens, M.J. (2001) Exp. Neurol. 172, 211–219.
[41] Braddock, R., Siman, C.M., Hamilton, K., Garland, H.O. and Sibley, C.P. (2002) Pediatr. Res. 51, 647–652.
[42] Kashiba, M., Oka, J., Ichikawa, R., Kageyama, A., Inayama, T., Kageyama, H., Ishikawa, T., Nishikimi, M., Inoue, M. and Inoue, S. (2000) Biochem. J. 351, 313–318.
[43] Bode, A.M., Yavarow, C.R., Fry, D.A. and Vargas, T. (1993) Biochem. Biophys. Res. Commun. 191, 1347–1353.
[44] Kim, M., Otsuka, M., Yu, R., Kurata, T. and Arakawa, N. (1994) Int. J. Vitam. Nutr. Res. 64, 56–59.
[45] Rathbone, B.J., Johnson, A.W., Wyatt, J.I., Kelleher, J., Heatley, R.V. and Losowsky, M.S. (1989) Clin. Sci. 76, 237–241.
[46] Galley, H.F., Davies, M.J. and Webster, N.R. (1996) Free Radic. Biol. Med. 20, 139–143.
[47] Sotiriou, S., Gispert, S., Cheng, J., Wang, Y., Chen, A., Hoogstraten-Miller, S., Miller, G.F., Kwon, O., Levine, M., Guttentag, S.H. and Nussbaum, R.L. (2002) Nat. Med. 8, 514–517.
[48] Wilson, J.X., Jaworski, E.M., Kulaga, A. and Dixon, S.J. (1990) Neurochem. Res. 15, 1037–1043.
[49] Agus, D.B., Gambhir, S.S., Pardridge, W.M., Spielholz, C., Baselga, J., Vera, J.C. and Golde, D.W. (1997) J. Clin. Invest. 100, 2842–2848.
[50] Huang, J., Agus, D.B., Winfree, C.J., Kiss, S., Mack, W.J., McTaggart, R.A., Choudhri, T.F., Kim, L.J., Mocco, J., Pinsky, D.J., Fox, W.D., Israel, R.J., Boyd, T.A., Golde, D.W. and Connolly Jr., E.S. (2001) Proc. Natl. Acad. Sci. USA 98, 11720–11724.
[51] Rebec, G.V. and Wang, Z. (2001) J. Neurosci. 21, 668–675.
[52] Wilson, J.X. and Gelb, A.W. (2002) J. Neurosurg. Anesth. 14, 66–79.
[53] Fornai, F., Piaggi, S., Gesi, M., Saviozzi, M., Lenzi, P., Paparelli, A. and Casini, A.F. (2001) Neuroscience 104, 15–31.