Effect of Sepiapterin, 7,8-Dihydrobiopterin, 5,6,7,8-Tetrahydrobiopterin and Xanthopterin on Cholesterol and Phospholipid Content and Phospholipid Biosynthesis *in vitro*

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Summary

Incorporation of fatty acids into phospholipids has been investigated using samples of rat liver tissue homogenate, Krebs-Ringer-phosphate buffer (pH=7.4) containing 0.3% albumin, fatty acid mixture and glycerol. The addition of sepiapterin, 7,8-dihydrobiopterin and 5,6,7,8-tetrahydrobiopterin (5 and 30 pmol/g wet weight) to incubation medium induced a decrease of saturated (stearic acid) and an increase of polyunsaturated (arachidonic acid) fatty acids incorporation into phospholipids. Cholesterol content decreased, but phospholipid content did not change in samples containing sepiapterin, 7,8-dihydrobiopterin and 5,6,7,8-tetrahydrobiopterin. No changes of fatty acid incorporation into phospholipids as well as of the content of cholesterol and phospholipids were observed in samples after the addition of xanthopterin (4 and 20 nmol/g wet weight) to incubation medium for phospholipid biosynthesis *in vitro*. The observations made by incubation with 7,8-dihydrobiopterin, 5,6,7,8-tetrahydrobiopterin and sepiapterin were in opposite to those made earlier employing neopterin and using the same incubation procedure.

Key Words : sepiapterin, 7,8-dihydrobiopterin, 5,6,7,8-tetrahydrobiopterin, xanthopterin, fatty acids, phospholipids, cholesterol

Introduction

Activation of the immune system is followed by the release of specific cytokines such as interferon- γ and tumor necrosis factor- α (1) as well as an increase of kynurenine, neopterin and 5,6,7,8-tetrahydrobiopterin biosynthesis (2,3). It is also found that some types of cellular regulation are associated with changes in membrane fluidity. For example, neoplastic transformation of cells is known to be accompanied by a marked increase in cell surface fluidity. Both, cell cycle regulation and contact inhibition of cells are also known to be linked with membrane fluidity

changes. These changes have been demonstrated to be a function of the membrane cholesterol/phospholipid ratio so that a relatively low ratio is associated with increased fluidity (4), and it also depends on cholesterol content in samples as well as fatty acid composition of phospholipids (5). An increase of cholesterol content in membranes and a decrease of polyunsaturated fatty acid content of membrane phospholipids have been observed in patients with arterial hypertension, myocardial infarction as well as at aging (6-9). Moreover, diseases as arterial hypertension (10-11), cardiomyopathy (12-15), atherosclerosis (16, 17) as well as aging (18) are accompanied with elevated kynurenine and neopterin levels in blood serum.

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Our previous studies have demonstrated that the addition of kynurenine and neopterin to rat homogenized liver samples for phospholipid biosynthesis *in vitro* is followed by a decrease of polyunsaturated fatty acid incorporation into phospholipids as well as an increase of cholesterol content in samples (19, 20). The purpose of this study was to continue these investigations by the examination of the effects of sepiapterin, 7,8-dihydrobiopterin, 5,6,7,8-tetrahydrobiopterin and xanthopterin on phospholipid biosynthesis *in vitro*.

Methods

Homogenized liver tissue of 16 male Wistar rats in Krebs-Ringer-phosphate buffer (pH=7.4) containing 0.3% albumin were used for these experiments (19-21). Samples were incubated at 37°C two times for 4 hours. To investigate the effect of different pteridines, sepiapterin (5 and 30 pmol/g wet weight), 7,8-dihydrobiopterin (5 and 30 pmol/g wet weight), 5,6,7,8-tetrahydrobiopterin (5 and 30 pmol/g wet weight) and xanthopterin (4 and 20 nmol/g wet weight) were added to experimental test tubes before the first incubation. All pteridine derivatives were obtained from Schircks Laboratories, Jona, Switzerland. All the metabolites were diluted with bidistilled water. The same volume of bidistilled water (0.1 ml) without addition of metabolites was added to the control test tubes. After the incubation at 37°C for 4 hours 60 µl of 20% fatty acid solution in chloroform (stearic, palmitic, linoleic, oleic and arachidonic acid in equivalent amounts, Sigma, Munich, Germany) and 40 µl of glycerol were added to all test tubes. Then the test tubes were again incubated at 37°C for 4 hours. After this second incubation 75 ml of chloroform/methanol mixture (2:1) was added to each sample. The samples were mixed and left for 40 min at room temperature. Then the chloroform/methanol extract was filtered and the filtrate was carefully treated according to the method of Folch *et al.* (22). The whole cholesterol concentration was determined by the method of Engelhard and Smirnova (23), the amount of phospholipids by the method of Urbach-Raabe (24). The method of Kates (25) was used to prepare the phospholipid samples for fatty acid examination by gas chromatography. Helium was used as a carrier gas and the sorbent was Chromosorb covered with DEAG. Separated fatty acid methyl esters were monitored at 210°C, using flame ionization detection and an adequate flow rate of helium.

The separation was controlled by internal methyl ester standard substances (Serva).

Figure 1. Concentrations (mean $\pm$ S.D.) of cholesterol and phospholipids, as well as fatty acid content (in percent: mean $\pm$ S.D.) of phospholipids in controls (open bars) and in samples with 5 pmol/g wet weight (dotted bars) or with 30 pmol/g wet weight (hatched bars) sepiapterin. Samples of rat liver tissue homogenates were used to test for phospholipid biosynthesis *in vitro*. Incubation medium: Krebs-Ringer-phosphate buffer (pH=7.4), fatty acid mixture and glycerol. Samples were incubated twice for 4 h at 37°C.

*The changes are statistically significant ($p < 0.05$).

†The changes are statistically significant ($P < 0.001$).

Statistical analyses were done using Student's *t*-test. *P*-values below 0.05 were considered to indicate significant differences (26).

Results

Fig. 1 shows the changes of phospholipid biosynthesis after addition of sepiapterin (5-30 pmol/g wet weight) to samples. We observed a decrease of stearic acid ($p < 0.05$) and an increase of arachidonic acid ($p < 0.001$) incorporation into phospholipids. Chromatograms of experimental samples showed the appearance of 5,8,11,14-arachidonic acid as well as 8,11,14,17-arachidonic acid, while control samples contained only 5,8,11,14-arachidonic acid. Addition of sepiapterin did not change the phospholipid content in samples, while cholesterol content significantly decreased ($p < 0.001$).

Fig. 2 Points out the deviation of phospholipid biosynthesis after the addition of 7,8-dihydrobiopterin to the incubation medium. We observed the same changes as in the cases with sepiapterin: a decrease of stearic acid ($P < 0.001$) and an increase of two types of arachidonic acid (8,11,14,17-arachidonic acid: $P < 0.001$ and 5,8,11,14-arachidonic acid: $p < 0.001$) content of phospholipids. The cholesterol content in samples containing 7,8-dihydrobiopterin decreased ($P < 0.001$) but the amount of phospholipid remained unchanged.

Fig. 3 shows the changes of phospholipid biosynthesis after addition of 5,6,7,8-tetrahydrobiopterin (5

Figure 2. Concentrations (mean $\pm$ S.D.) of cholesterol and phospholipids, as well as fatty acid content (in percent: mean $\pm$ S.D.) of phospholipids in controls (open bars) and in samples with 5 pmol/g wet weight (dotted bars) or with 30 pmol/g wet weight (hatched bars) 7,8-dihydrobiopterin. Samples of rat liver tissue homogenate were used to test for phospholipid biosynthesis in vitro. Incubation medium: krebs-Ringer-phosphate buffer (pH=7.4), fatty acid mixture and glycerol. Samples were incubated twice for 4 h at 37°C.

*The changes are statistically significant ($p < 0.05$).

†The changes are statistically significant ($p < 0.001$).

Figure 3. Concentrations (mean $\pm$ S.D.) of cholesterol and phospholipids, as well as fatty acid content (in percent: mean $\pm$ S.D.) of phospholipids in controls (open bars) and in samples with 5 pmol/g wet weight (dotted bars) or with 30 pmol/g wet weight (hatched bars) 5,6,7,8-tetrahydrobiopterin. Samples of rat liver tissue homogenate were used to test for phospholipid biosynthesis in vitro. Incubation medium: Krebs-Ringer-phosphate buffer (pH=7.4), fatty acid mixture and glycerol. Samples were incubated twice for 4 h at 37°C.

†The changes are statistically significant ($p < 0.001$).

and 30 pmol/g wet weight) to samples. We have observed the same changes as in the case with sepiapterin and with 7,8-dihydrobiopterin: a decrease of stearic acid ($p < 0.05$) and an increase of two types of 8,11,14,17-arachidonic acid ($p < 0.001$) and of 5,8,11,14-arachidonic acid ($P < 0.001$) incorporation into phospholipids. The amount of cholesterol decreased in samples, but the content of phospholi-

Figure 4. Concentration (mean $\pm$ S.D.) of cholesterol and phospholipids, as well as fatty acid content (in percent: mean $\pm$ S.D.) of phospholipids in controls (open bars) and in samples with 5 pmol/g wet weight (dotted bars) or with 30 pmol/g wet weight (hatched bars) xanthopterin. Samples of rat liver tissue homogenate were used to test for phospholipid biosynthesis in vitro. Incubation medium: Krebs-Ringer-phosphate buffer (pH=7.4), fatty acid mixture and glycerol. Samples were incubated twice for 4 h at 37°C.

pids was not altered.

Fig. 4 reflects the influence of xanthopterin (4 and 20 nmol/g wet weight) on phospholipid biosynthesis in vitro. Addition of xanthopterin to samples for phospholipid biosynthesis in vitro did neither alter incorporation of fatty acids into phospholipids, nor the content of cholesterol and phospholipid.

Discussion

We have observed a decreased incorporation of saturated fatty acid (stearic acid) and an increase of polyunsaturated fatty acid (arachidonic acid) incorporation into phospholipids 4 hours after the addition of either sepiapterin, 7,8-dihydrobiopterin or 5,6,7,8-tetrahydrobiopterin to the incubation medium for phospholipid biosynthesis in vitro. These deviations of fatty acid incorporation were followed by a decrease of cholesterol but the content of phospholipids did not change in samples. Literature studies show that cell stimulation as well as neoplastic transformation is accompanied by de novo synthesis of phospholipids which is increased not earlier than 6 hours after the stimulation. However the increased incorporation of unsaturated fatty acids was found within the first hours of stimulation (5). Therefore the results received in our present experiments allow us to suggest that 5,6,7,8-tetra-

hydrobiopterin and related compounds like reduced pterin-species, sepiapterin and 7,8-dihydropterin, take part in the stimulation of cell cycle by changing cholesterol/phospholipid ratio and increasing the incorporation of polyunsaturated fatty acids (arachidonic acid especially) into phospholipids.

When comparing the results obtained from this study with our data obtained earlier using neopterin and kynurenine in the same assay system it is exciting to see the inverse relationship between the effects induced by the reduced biopterin derivatives including sepiapterin in relation to neopterin. Whereas 7,8-dihydrobiopterin, 5,6,7,8-tetrahydrobiopterin and sepiapterin supported incorporation of polyunsaturated fatty acids into phospholipids but decreased cholesterol formation, neopterin did the opposite (19,20). From the data it appears that neopterin may interfere with some positive effects which the reduced biopterin derivatives may have on lipid metabolism. The enhanced formation of neopterin during immunopathologies may be involved to induce a shift towards a decrease of polyunsaturated fatty acid content of membrane phospholipids and increase cholesterol content, thus enhancing the risk of, e. g. atherosclerosis and myocardial infarction. Neopterin appears to be the "bad guy" interfering with the protective effects achieved by 7,8-dihydro-biopterin, 5,6,7,8-tetrahydrobiopterin and sepiapterin.

Recently, pteridine derivatives have been shown to be able to interfere with radical-mediated chemical reactions (27, 28). Naturally occurring, fully-oxidized pterin derivatives tend to have a promoting effect on such reactions whereas reduced pterin species tend to react as scavengers. It would be interesting to know whether this behaviour of pterins is important to explain our findings. Further studies are needed to test for eventual divergent effects of 7,8-dihydrobiopterin and biopterin on cholesterol and phospholipid biosynthesis providing a clue whether the configuration of the pterin-(6)-side chain or the oxidation state of the pteridines is more important for the effects seen.

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