When it comes to joint pain, it is mostly the cartilage that is inflamed and chronic headaches often result from neural inflammatory processes. Overcoming this pain will often seem impossible. What is little known: Superhormones, which can regulate the hormonal balance of the body, exist - these are eicosanoids, derivatives of arachidonic acid, an omega-6 fatty acid. What effect do the arachidonic acid derivatives have on the body? How can inflammatory responses be influenced and rheumatic diseases completely cured? You will learn about this and much more, in this article!

Substance Class and Structure of Arachidonic Acid

Arachidonic acid (Latin arachis: “peanut”) belongs to the family of lipids, more specifically, the fatty acids. By definition, fatty acids consist of hydrocarbon chains of different lengths, with a carboxylic acid group (−COOH), which is present physiologically in its dissociated form (−COO⁻). Carboxylic acids with a chain length of four or more carbon atoms are referred to as fatty acids.

The hydrocarbon atoms of the chain are linked by single and double bonds. Each of the carbon atoms that are part of the double bond has only three binding partners and therefore, is not completely saturated with H atoms. These are called unsaturated fatty acids. Arachidonic acid consists of 20 carbon atoms and 4 double bonds (C20:4), which take on the spatial configuration of a trapezoid. These are cis double bonds.
In chemical nomenclature, the terms cis-$\Delta^{5,8,11,14}$-arachidonic acid and eicosatetraenoic acid are used as a synonym for arachidonic acid.

**Occurrence of Arachidonic Acid**

In contrast to the fully saturated arachidonic acid (eicosanoic acid, C20:0), which can be isolated from peanuts, C20:4-arachidonic acid is not found in any plant. Humans can acquire arachidonic acid through animal fats in food, or synthesize it from the essential omega-6 fatty acid linoleic acid through the intermediates γ-linolenic acid and dihomo-γ-linolenic acid. Thus, arachidonic acid is semi-essential.

Physiologically, arachidonic acid is inactive and is present in esterified form, bound to membranes as phospholipids. Eicosanoid biosynthesis begins when a cell is activated by mechanical trauma, ischemia, other physical perturbations, attack by pathogens, or stimuli made by nearby cells, tissues, or pathogens such as chemotactic factors, cytokines, growth factors, and even certain eicosanoids. Activated cells then mobilize the enzyme cPLA$_2$ (cytosolic phospholipase A2), capable of releasing ω-6 and ω-3 fatty acids from membrane storage. These fatty acids are bound in ester linkage to the SN2 position of membrane phospholipids and CPLA$_2$ acts as an esterase to release the fatty acids.

Because of their lipophilic character, fatty acids pass through membranes and cannot be stored in vesicles. Their release is subject to a strict regulatory mechanism of the enzyme, which can only be activated by phosphorylation and simultaneous binding of calcium. Extracellular signaling molecules, such as hormones and cytokines, may cause activation of the enzyme. Release only takes place when necessary!

**Eicosanoids – Derivatives of Arachidonic Acid**

Prostaglandins, thromboxane and leucotrienes are summarized under the group name eicosanoids (Greek eikosi = twenty). They are mediators (messengers) and mediate various effects in the human organism, similar to hormones. Their life span is limited from a few seconds to minutes, which strongly limits the scope of the mediators. However, unlike the substance class of hormones, they are not transported to the periphery via the blood. They act either paracrine on localized neighbouring tissue or autocrine on the producing cell itself.

Eicosanoids are neither synthesized in special glands (like glandular hormones), nor in specialized individual cells (such as growth hormones). All mediators mentioned above are derivatives of polyunsaturated C20 fatty acids, in particular arachidonic acid C20:4 and are therefore referred to as arachidonic acid derivatives.

Other mediators are for example NO (nitric oxide), a strong vasodilator, as well as histamine and bradykinin, which play a significant role during inflammatory reactions.

The three most important substance classes of eicosanoids are: prostaglandins, thromboxane, and leukotrienes. These three substance classes are further subdivided into two groups which result from the respective enzyme involved.

Cyclooxygenase (COX) catalyzes the cyclooxygenase pathway and leads to the prostaglandins and thromboxane. Lipoxygenase (LOX) catalyzes the lipoxygenase pathway, which produces leukotrienes.
Prostaglandins and Thromboxane

The name prostaglandin is derived from the prostate because the substance was isolated for the first time from this gland. Thromboxanes get their name because of the relevance in thrombocytes.

Biosynthesis

In order to form prostaglandins and thromboxane, the arachidonic acid has to initially enter the smooth endoplasmic reticulum, the site of cyclooxygenase (COX). First, COX causes the formation of endoperoxide, prostaglandin H₂, through cyclization and oxygenation of the ring. Because of this fundamental synthesis, COX is also referred to as PGH₂ synthase. Prostaglandin H₂ is the initial substance for all prostaglandins and thromboxanes. The derivatives of prostaglandin H₂ differ from each other only in the position of the keto group and the hydroxyl group in the original PGH₂.

Prostaglandins (PG)

As a derivative of PGH₂ they contain a cyclopentane ring.

Isomerases and reductases produce prostaglandins E₂ (PGE₂) and F₂α (PGF₂α). Through the activity of prostacyclin synthase, prostaglandin I₂ (PGI₂) is produced.

Thromboxane (TX)

All thromboxanes have an oxane ring (cyclopentane ring and oxygen). Thromboxanes are synthesized in thrombocytes. From PGH₂, thromboxane A₂ (TXA₂) is first formed via thromboxane synthase, resulting in the synthesis of more thromboxanes.

COX-I and COX-II

The enzyme cyclooxygenase exists in two isoforms that perform different tasks. Clinically, this enzyme is very important because it is the target of acetylsalicylic acid (ASA, aspirin).

COX-I is produced continuously (constitutive) and is always active. Important is the presence in the gastrointestinal tract and kidneys since an inhibition of COX-I (for example, aspirin) can have serious side effects.

COX-II is not present ubiquitously, but only in some cells, particularly in leukocytes and macrophages of the immune system. Its activity can be induced by cytokines, growth factors, and endotoxins. COX-II is responsible for three effects which are the reason why COX inhibitors are commonly prescribed: They produce prostaglandins for pain, fever, and inflammation.

Effect of Prostaglandins and Thromboxanes at the Molecular Level

Prostaglandin and thromboxane are hydrophilic, which is why they cannot diffuse freely across membranes. This requires binding to membrane receptors, which are intracellularly linked to a heterotrimeric G protein and trigger a specific ongoing reaction cascade. To this date, five main types of receptor classes are known. Depending on the type of receptor, inhibition or stimulation of adenylate cyclase with a corresponding
decrease or increase in intracellular cAMP concentration occurs.

1. EP receptor binds PGE\(_2\). The subtype EP\(_1\) acts via a cAMP-increase, the subtype EP\(_2\) receptor via an IP\(_3\) mechanism.
2. FP receptor binds PGF\(_2\) via the IP\(_3\) mechanism.
3. IP receptor binds PGI\(_2\) via a cAMP increase.
4. DP receptor binds PGD\(_2\) via a cAMP increase.
5. TP receptor binds TXA\(_2\) via the IP\(_3\) mechanism.

<table>
<thead>
<tr>
<th>Prostaglandin E(_2)</th>
<th>„Main“ prostaglandin</th>
<th>Formation via COX-I protects gastric mucosa by mucus production, formation via COX-II acts on inflammatory process, pain sensitization and temperature increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin F(_{2\alpha})</td>
<td>Stimulation of the stomach mucus production (protective function), vasodilation, bronchoconstriction</td>
<td></td>
</tr>
<tr>
<td>Prostacyclin PGI(_2)</td>
<td>Synthesis in endothelial cells</td>
<td>Inhibition of platelet aggregation, vasodilation</td>
</tr>
<tr>
<td>Thromboxane A(_2)</td>
<td>Synthesis in thrombocytes, opponent of prostacyclin</td>
<td>Promotes platelet aggregation, vasoconstriction</td>
</tr>
</tbody>
</table>

The demand release, production and modulation of eicosanoids are triggered via stressing stimuli on one hand, but also via other mediators such as histamine and bradykinin and gastrointestinal hormones such as gastrin.

The biological action of prostaglandins and other eicosanoids is extremely complex and at this point not fully understood. To recognize a single operating principle is difficult, because the numerous compounds act synergistically or antagonistically in some cases. Not only does the absolute amount of these mediators play an important role, but also the proportion of each mediator in respect to the others.

**Aspirin as an Inhibitor of COX**

Aspirin is present in almost every medicine cabinet in households around the world. Whether it is pain in the head and neck area, menstrual problems or a toothache, fever or colds – aspirin is the “superstar” among analgesics and therefore often the first drug of choice when it comes to alleviating the symptoms.

The over-the-counter merchantability of this painkiller in pharmacies is currently much debated by the public. Point of discussion is the responsible management of this drug by patients. In long-term studies, research about patient compliance in everyday life was conducted. They showed that consumers maintained a responsible attitude and were adhering to the information in the package insert for dosage and method of administration.

Aspirin, synthesized by the Leverkusen Company Bayer AG since the beginning of the 20th century, is one of the best and longest researched medicines. The WHO (World Health Organization) placed the drug on its list of essential medicines in 1977.

The active ingredient acetylsalicylic acid is distinguished by its many properties: it acts as an inhibitor of platelet aggregation, as a pain reliever (analgesic), to lower fever (antipyretic) and as an anti-inflammatory.

The mechanism of action is based on an irreversible inhibition of PH\(_3\) synthase, more specifically, of COX-I and COX-II. This results in a decreased formation of inflammatory
prostaglandins and a corresponding mitigation of the symptoms caused by prostaglandins. Since prostaglandin is also involved in the production of the mucus that protects the gastric epithelium, the intake of aspirin in high doses or with prolonged ingestion leads to stomach discomfort, bleeding, heartburn, or vomiting.

In order to keep side effects to a minimum, the pharmaceutical industry has developed COX inhibitors that specifically inhibit COX-II only. These drugs are still in the testing phase.

**Body Reaction: Fever**

Fever is the body’s response to an infectious disease. The cytokines interleukin-1 and TNF-α distributed in the blood migrate to the hypothalamus, where they induce the release of PGE₂. This prostaglandin produces a set point adjustment at the temperature regulation center and a fever occurs.

**Effect of Prostaglandin on Birth and Procreation**

PGE₂ and PGF₂α have an effect on the uterus and trigger uterine contractions during birth. Pharmacologically, this effect can be modulated to imitate contractions for an early induction of labor, for instance, or to inhibit them in order to avoid premature birth.

**Breakdown of Prostaglandin and Thromboxane**

The breakdown of prostaglandins occurs in two steps. In the first and quicker step, widely occurring specific enzymes lead to inactivation of prostaglandins, which are then broken down in the following second (somewhat slower) step in oxidation reactions (e.g., β-oxidation in the liver).

After the half-life of about 30 seconds, thromboxane A₂ breaks down into the inactive but stable TXB₂.

**Leukotrienes**

Unlike prostaglandins and thromboxanes, leukotrienes are formed only in a few types of cells—mainly in macrophages, mast cells and granulocytes. As already described, the polyunsaturated arachidonic acid serves as a starting compound.

Several isoenzymes of lipoxygenases (LOX) exist, of which only 5-lipoxygenase can produce leukotrienes (LT). In contrast to the cyclooxygenases, lipoxygenase is located in the cytoplasm and is mainly found in leukocytes and macrophages.

Intracellular increase in calcium levels activates 5-lipoxygenase, resulting in a release of leukotrienes.

Through the incorporation of molecular oxygen, the 5-lipoxygenase turns arachidonic acid into hydroperoxide. First, 5-hydroperoxy eicosatetraenoic acid (5-HPETEs) is formed, which serves as an unstable precursor of all leukotrienes. The double bonds are then transferred, and a leukotriene A₄ is created, which in turn is the origin of the two most important main groups of leukotrienes: LTB₄ and peptide leukotrienes. LTB₄ is produced by hydrolysis, peptide leukotrienes by the addition of glutathione to the leukotriene A₄ through a thioether.
Effect of Leukotrienes at the Molecular Level

Like the rest of arachidonic acid derivatives, leukotrienes play an important role in the inflammatory process. The precise molecular mechanism of action is largely unknown. It is known that leukotrienes are among the most potent constrictors of the bronchial muscles and therefore play an important role in pathophysiology.

Just like prostaglandins, leukotrienes act on membrane receptors that lead to activation of phospholipase C.

<table>
<thead>
<tr>
<th>BLT receptor</th>
<th>Binding of LTB₄</th>
<th>Leukocyte membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>CysLT receptor</td>
<td>Binding of peptide leukotrienes</td>
<td>Membrane of smooth muscle cells</td>
</tr>
</tbody>
</table>

Different leukotrienes can be distinguished: LTA₄, LTB₄, LTC₄, LTD₄ and LTE₄. While LTA₄ is only an intermediate product, the biologically active forms are especially LTB₄ and LTC₄.

Leukotriene B₄ is secreted by macrophages in the event of inflammation in order to attract leukocytes (chemotaxis). LTB₄ increases the vascular permeability, leading to fluid accumulation in the extracellular space and inflammation-associated swelling.

Peptide leukotrienes are involved in the development of bronchial asthma, due to their strong bronchoconstriction effect. Especially LTC₄ and LTD₄ develop an approximately 1,000 times more potent effect than histamine!

Clinical note: The above mentioned context clearly shows why antihistamines can only very weakly counteract manifested bronchial asthma—most importantly, bronchoconstriction is primarily caused by leukotrienes. The most important drugs are still synthetic glucocorticoids, which inhibit phospholipase A₂ and thus—relatively unspecific—the formation of arachidonic acid derivatives, including leukotrienes.

Breakdown of Leukotrienes

The breakdown of leukotrienes takes place in the liver. The oxidation reactions take place in the peroxisomes of hepatocytes and convert leukotrienes in biologically inactive metabolites, which are excreted in the bile.

The Role of Eicosanoids in Allergic Reactions

An allergy occurs when a normally harmless antigen triggers an overreaction of the body’s immune system. This reaction is also called a hypersensitivity reaction (asthma is a Type 1 hypersensitivity). The sensitive organism responds differently than normal (Greek allos = different, Greek ergon = work).

The allergy in a narrower sense of the word is caused by an IgE-mediated release of histamine with immediately occurring symptoms (immediate hypersensitivity). Histamine is synthesized by decarboxylation of the amino acid histidine and stored in granules of the mast cells. A matching antigen contact causes the breakdown of histamine-storing granules (degranulation) and the release of the mediator. Histamine is the most important mediator of the immediate (or Type 1) hypersensitivity reactions.

The effect of histamine is mediated via the specific H₁ receptor. The typical symptoms are itching, increased mucus production, edema (“urtica”), vasodilation of the arterioles and venules as well as bronchoconstriction, which is mainly involved in the pathogenesis of asthma.
Shortly after histamine release, phospholipase A₂ is activated in the mast cells, leading to the above-described release of arachidonic acid from the lipids in the plasma membrane.

Pharmacologically, each level of development of an allergy can be affected, resulting in alleviation of the symptoms and the liberating effect for many allergy sufferers:

- Cromoglycate impedes the release of mediators from mast cells.
- Antihistamines (H₁-blockers) prevent the binding of histamine to the receptor.
- Anti-leukotrienes act antagonistic to leukotrienes, but for some people the first try can be disappointing.
- Cortisone reduces the activity of phospholipase A₂ and inhibits the production of interleukins. The mechanism is not yet understood. Due to this very effective action, it is still the most important treatment of symptomatic asthma.

Review Questions

The answers can be found below the references.

1. What is the chemically correct name of arachidonic acid?
   A. Cis-Δ⁵,⁸,¹¹,¹⁴-arachidonic acid
   B. Cis-Δ¹⁴,¹¹,⁸,⁵-arachidonic acid
   C. Eicosanoic acid
   D. 21:4

2. Which statement about arachidonic acid and its derivatives is incorrect?
   A. Leukotrienes are formed together with thromboxane from the cyclooxygenase pathway.
   B. Leukotrienes are broken down in the peroxisomes of hepatocytes.
   C. Interleukin-1 leads to a fever reaction due to the release of PGE₂.
   D. Arachidonic acid can be derived from peanuts.

3. Which statement is incorrect?
   A. Prostaglandins and thromboxane are formed from an endoperoxide, the prostaglandin H₂.
   B. Allergies are caused by an IgA-mediated histamine release.
   C. LTB₄ increases vascular permeability and leads to inflammation-associated swelling.
   D. Leukotrienes are 100 times more potent constrictors than histamine.

References

Biochemie des Menschen- Das Lehrbuch für das Medizinstudium, 2. korr. Auflage, Thieme Verlag

Biochemie der Ernährung, 2. Auflage, Rehner/Daniel, Spektrum Gustav Fischer Verlag

Correct answers: 1A, 2A, 3B

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