γ-aminobutyric acid Rich Foods

– Methods for Production and the Effects of Intake

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Abstract

The current widespread interest in healthy lifestyle creates a demand for functional foods. New nutritious food products and supplements are launched on the market and products enriched with γ-aminobutyric acid (GABA) constitutes some of them. γ-aminobutyric acid is a widely present amino acid in plants, animals, fungi and microorganisms. It works as an inhibitory neurotransmitter in the central nervous system and is known for its calming effect. Multiple studies have shown a biologic response in humans after GABA administration including relaxation, inhibition of neuronal damage, prevention of neurological disorder, enhancement of sleep time, anti-hypertensive effect, antioxidant property and more. A development is seen regarding health promoting GABA enriched food products including tea, cheese and sourdough bread. The GABA production is often made using microorganisms, especially fermenting lactic acid bacteria in biosynthetic methods. Useful bacteria strains in fermented foods, such as kimchi and soy sauce, are found by companies as well as in scientific research with a goal to develop new healthy food products. The mechanism for oral GABA to affect human behaviour is not fully established. The ability of the GABA molecule to pass the blood brain barrier is disputed but other metabolic pathways are possible including the gut-brain-axis. In this literature study, several methods for GABA production and enrichment will be presented as well as the biochemical effects of intake of these products on the human body.

Keywords: gamma-aminobutyric acid, GABA, GABA tea, fermentation, functional food, probiotics, lactic acid bacteria

Sammanfattning

Det pågående intresset för en hälsosam livsstil skapar en efterfrågan på hälsosamma livsmedel, så kallade *functional foods*. Nya näringsrika livsmedel och kosttillskott lanseras på marknaden och produkter som är berikade med gammaaminosmörsyra, GABA (γ-*aminobutyric acid*), utgör en del av dem. Gammaaminosmörsyra är en allmänt förekommande aminosyra i växter, djur, svampar och mikroorganismer. Den fungerar som en hämmande signalsubstans i det centrala nervsystemet och är känd för dess lugnande effekt. Ett stort antal studier har visat att intag av GABA kan ge biokemiska effekter i människokroppen, till exempel minskade stressnivåer, inhibering av nervskador, förebyggande av neurologiska skador, förlängd sömntid, förebyggande av hypertoni samt antioxidativ effekt. Livsmedel berikade med GABA, exempelvis te, ost och surdegsbröd ökar i antal. Mikroorganismer, särskilt fermenterande mjölksyrebakterier, används i biosyntetiska metoder tack vare deras förmåga att producera GABA. Livsmedelsföretag och vetenskaplig forskning har upptäckt GABA-producerande bakteriestammar i olika fermenterade livsmedel, såsom kimchi och sojasås, och använder dessa för att utveckla nya hälsosamma produkter. Det är inte fullständigt fastställt om och hur intag av GABA har effekt på människan. GABA-molekylens förmåga att passera blod-hjärn-barriären är omstridd men å andra sidan finns det andra metaboliska vägar, exempelvis tarm-hjärna-axeln, som kan förklara resultaten. Den här litteraturstudien kommer att ta upp metoder som används vid framställning av livsmedel med hög koncentration av GABA. En beskrivning av de biokemiska effekter som kommer av ett intag av GABA-produkter tillkommer.

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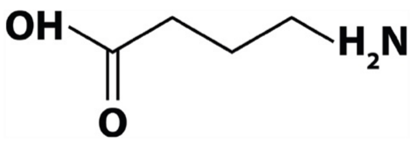
Abbreviations

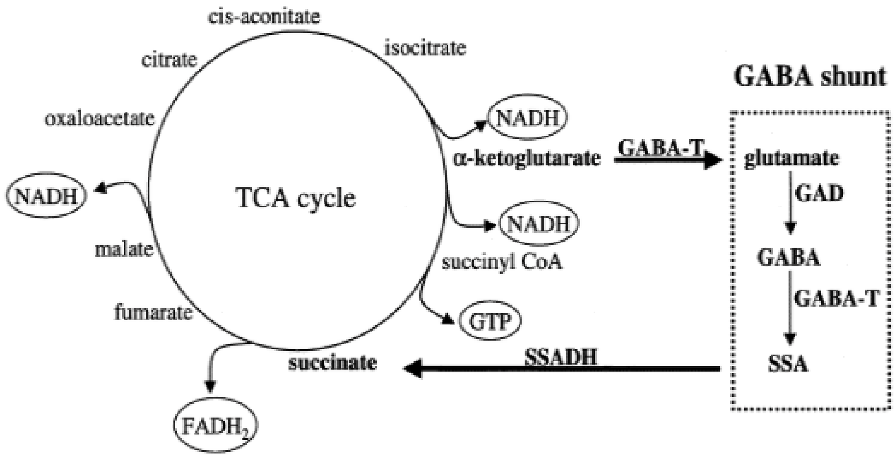
BBB	Blood-brain-barrier
CNS	Central nervous system
GABA	Gamma-aminobutyric acid
GAD	Glutamate decarboxylase
LAB	Lactic acid bacteria
TCA	Tricarboxylic acid cycle

1. Introduction

γ-aminobutyric acid (GABA) is a non-protein amino acid occurring in both eukaryotes and prokaryotes (figure 1). In plants, GABA is a signal molecule with a functional versatility (Li et al. 2021). In vertebrates, GABA is a major inhibiting neurotransmitter in the central nervous system. GABA is often metabolized in the GABA shunt pathway, a bypass of the tricarboxylic acid (TCA) cycle as seen in figure 2. Glutamate derived from the TCA cycle is decarboxylated to GABA and CO_2 in the cytosol in the cell. The reaction is catabolized by the glutamate decarboxylase (GAD) enzyme. Glutamic acid is a non-essential amino acid in plants and animals including foods with a high protein content. Glutamate is the anionic form of glutamic acid formed at pH 7 (Briguglio et al. 2018). GABA can also be synthesized in the polyamine metabolic pathway, a production from polyamines by oxidation of putrescine. (Li et al. 2021)



1: γ-aminobutyric acid (Boonstra et al. 2015)



2: *The GABA shunt (*4 Aminobutyrate Aminotransferase - an overview | ScienceDirect Topics*)*

1.1. GABA in plants

The GABA metabolism in plants is involved in multiple functions, processes and pathways (Gramazio et al. 2020). The functions of GABA in plant development include pollen tube elongation, root growth, fruit ripening and seed germination. The GABA molecule has the ability to be transported both across the plasma membrane and organelle membranes which makes it possible for the molecule to be produced in the mitochondria and then function as a nitrogen source (Li et al. 2021). The role of GABA in seed germination is to activate α-amylase gene expression and promote seed starch degradation. An increase of GABA levels is seen in germination of soybean, oats, millet, wheat, barley and rice (Li et al. 2021). Several types of legume sprouts have shown a higher GABA content compared to their raw beans (Briguglio et al. 2018). The GABA content in plants depends on species, varieties, environmental factors etc. (Gramazio et al. 2020). Examples of edible plants containing high amounts of GABA include spinach, potato, sweet potato, kale, broccoli, mushrooms, nuts, tomato and Chinese teas (Briguglio et al. 2018). It has also been found in soybean, germinated brown rice, kimchi, cabbage pickles, yoghurt and more (Ngo & Vo 2019).

Biotic and abiotic stress in plants makes the GABA levels increase and the high GABA content improves the resistance to stress in plants. Stress often increases the Ca^{2+} concentration which influence Glutamate decarboxylase in the cytosol.
Biotic stress includes pathogens and pests, for example insects or fungus. GABA works as an inhibitory neurotransmitter in the invertebrate nervous system which makes a high GABA content in a plant a possible defence against insects. Abiotic stress includes low temperature, high temperature, drought, flooding, salt, heavy

metal and production of reactive oxygen species (ROS). The high GABA concentration improves photosynthesis, inhibits generation of ROS, activates antioxidant enzymes and regulates stomatal opening in drought stress, which all elevates the stress tolerance in the plant.

1.2. GABA in humans

GABA plays an important role as a major inhibiting neurotransmitter in the central nervous system (CNS) in humans. Activation of neuroreceptor $GABA_A$ favours sleep and prevent anxiety and chronic pain (Möhler 2010). In healthy human females, the GABA concentration in the prefrontal cortex has shown an increase during ovulation (De Bondt et al. 2015). GABA is also a postbiotic produced by the gut microbiota from L-glutamic acid in the gut (Diez-Gutiérrez et al). GABA is produced in a similar way in the CNS and gut microbiota. The enzyme glutamic acid decarboxylase (GAD) catalyses the irreversible decarboxylation of L-glutamate to GABA as seen in figure 3 (Sherwin et al. 2016). Low GABA levels in blood are related to brain diseases and administration of GABA have shown health effects (Yamatsu et al. 2016).

$$\text{L-glutamate} + H^+ \xrightarrow{\text{GAD}} \text{GABA} + CO_2$$

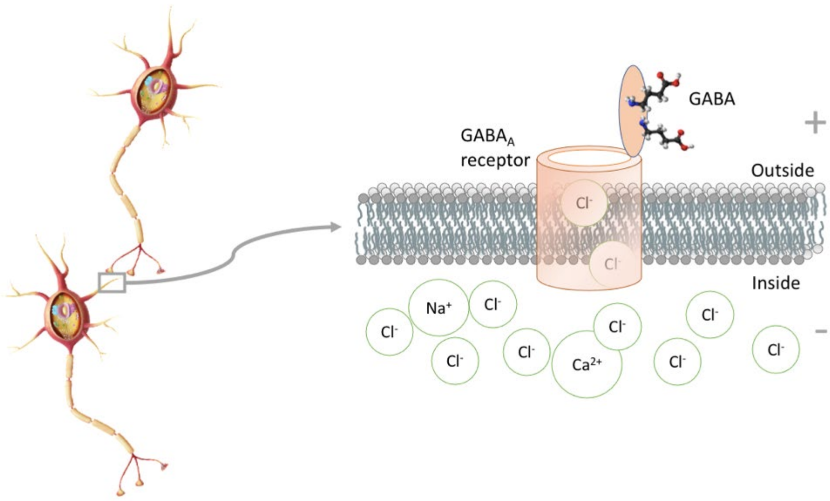
3:Biosynthesis of GABA catalyzed by GAD (Li & Cao 2010)

1.2.1. Neurotransmitters

There are numerous unique neurotransmitters in the human body which can either excite the neuron and have an excitatory effect or inhibit the neuron from sending a signal depending on the receptor. The large group of neurotransmitters is divided into small molecule neurotransmitters and neuropeptides. Small molecule neurotransmitters cover amino acids including glutamate, GABA and glycine, and biogenic amines including dopamine, norepinephrine, epinephrine, serotonin, and histamine, all made from amino acid precursors. Glutamate is the most prevalent neurotransmitter in the human brain and has an excitatory effect. GABA is the second most prevalent neurotransmitter which inhibits or reduces the activity of the nerve cells which gives a relaxing effect. (*Neurotransmitters and receptors (article)*).

1.2.2. GABA receptors

Neurotransmitters are packaged into synaptic vesicles and released into the synaptic cleft before reaching a target receptor by diffusion. The receptors can be ionotropic or metabotropic. Ionotropic receptors are transmembrane ion-channel proteins. Metabotropic membrane receptors initiate metabolic steps and are indirectly linked with ion channels. (Khan Academy 2021). Two classes of GABA membrane-bound receptors with different pharmacological, electrophysiological and biochemical properties have been identified. $GABA_A$ is an ionotropic receptor consisting of GABA-gated chloride channels. When enough GABA is bounded to the extracellular domain of the $GABA_A$ receptor, influx of chloride ions into the cell occurs (figure 4). The influx of negatively charged ions hypopolorize the cell wall and makes a nerve signal less probable. The inhibition is a result of the hypopolarization across the cell wall (Ngo & Vo 2019).



4:GABA binds to the active site on the $GABA_A$ receptor (Agnes Sjöblom)

The $GABA_A$ receptor have multiple allosteric sites, both for direct GABA agonists and antagonists and other non-competitive sites for other ligands. The receptor response can be potentiated or inhibited by multiple ligands including alcohol and phenol derivatives (Hossain et al. 2002). Ethanol creates a relaxing and anti-anxiety effect due to its role as an allosteric activator of the $GABA_A$ receptor which creates a relaxing and anti-anxiety effect. The $GABA_B$ receptor is a G protein-coupled metabotropic receptor which can work as a target of γ-hydroxybutyrate for treating narcolepsy (Ngo & Vo 2019) (Möhler 2010).

1.2.3. Food components affecting the nervous system

Receptors, channels and enzymes in the human brain are affected by components in foods and drinks. Some of the substances in foods that affect the nervous system in humans are neurotransmitters including acetylcholine, the biogenic amines dopamine, serotonin, histamine and the modified amino acids glutamate and GABA. These food components can be naturally present, or derived from controlled or uncontrolled food technology processes. (Briguglio et al. 2018) There are also other components in foods affecting the nervous system. Tobacco contains nicotine which binds to the nicotinic acetylcholine receptors. Alcoholic drinks contain ethanol which potentiates the $GABA_A$ receptor response, inhibit the NMDA receptor response and opens G-protein-coupled inwardly rectifying K^+ channels. Hot chili peppers contain capsaicin which opens warm receptors. Spearmint contains menthol which opens cold receptors. Coffee and tea contain caffeine which stimulate the central nervous system due to increased concentration of cAMP, increased free Ca^{2+} concentration inside nerve cells or antagonism of adenosine actions on purine receptors. All these actions in the brain modulate human consciousness. Many components in food are thought to target the $GABA_A$ receptors in the brain and affect the mood. (Hossain et al. 2002)

Theanine is an amino acid that is found in the highest concentration, 1-2% of dry weight, in tea and contributes to the umami taste. Theanine can cross the blood-brain-barrier, work as a glutamate antagonist and give health benefits including anxiolytic and relaxant effects (Hinton & Johnston 2020)(Yu & Yang 2020).
Caffeine is an alkaloid that constitutes 2–4% w/w of tea and has shown to mildly inhibit $GABA_A$ receptors. The effect of the combination of caffeine and GABA in tea needs further exploration.(Hinton & Johnston 2020)

2. Methods for GABA production

Health benefits of GABA contribute to a development of GABA rich fermented foods and food supplements (Diez-Gutiérrez et al. 2020). To generate positive health effects, a daily intake 10-20mg of GABA is recommended. (Gramazio et al. 2020). The food supplement is broadly available due to its effect on relaxation and sleep enhancement (Boonstra et al. 2015). GABA production is seen in biotechnology research in the food industry. GABA concentration can be increased naturally in foods by use of specific methods.

GABA can be produced actively by using synthetic methods or biosynthetic methods. It can be synthesized through chemical synthesis, enzymatic or whole-cell biocatalysis and microbial fermentation (Briguglio et al. 2018). The biosynthesis consists of the reaction of glutamate to GABA. Biosynthetic methods have a simple reaction procedure, high catalytic efficiency, mild reaction condition and environmental compatibility which may make it more useful compared to chemical synthesis. (Dhakal et al. 2012)

2.1. Biosynthesis in microorganisms

A biosynthesis of GABA is seen in multiple microorganisms including bacteria, fungi and yeast either by the putrescine pathway or the glutamic acid decarboxylase pathway. The putrescine pathway is found in the bacteria *Escherichia coli* and the fungi *Aspergillus oryzae*. The glutamic acid decarboxylase pathway is used by many microorganisms including *Lactobacillus spp*., *Escherichia coli*, *Listeria monocytogenes* and *Aspergillus oryzae* (Diez-Gutiérrez et al. 2020).The biochemical properties of GAD in the microorganism affect the GABA production. It is influenced by several factors including substrate concentration, temperature, initial pH and addition time (Dhakal et al. 2012).

2.1.1. Growth of GABA producing microorganisms

Depending on the microorganism used, environmental factors may be adjusted (Diez-Gutiérrez et al. 2020). The temperature has an effect on cell density, biocatalyst activity and stability, and thermodynamic equilibrium of the reaction. A high GABA yield is usually reached in a fermenting temperature from 25°C to 40°C. The decarboxylation increases pH in the cytosol and environment. For example, H_2SO_4 can be supplemented into the fermenting broth to maintain the optimum pH 5.0 to maximize the GABA production by *Lactobacillus brevis*. Adjustment of the pH of the buffer is also important for inhibition of the GABA-decomposing enzymes (Dhakal et al. 2012).

In fermentation, the degradation of nutrients changes the chemical composition which may enhance preservation, taste and character of a food. Heterofermentative fermentation results in more than one product, for example both lactic acid and ethanol (Olofsson 2012). Different species of fermentative microorganisms show a GABA production (Dhakal et al. 2012). Therefore, traditional fermented foodstuffs with a high glutamate content can be used for isolation of GABA producing microorganisms. (Li & Cao 2010)

Enzyme hydrolysis has been used as a pretreatment in order to achieve a higher GABA level. The proteases alcalase and flavourzyme have been used in combination with lactic acid fermentation to produce GABA-enriched soy milk. The proteolysis releases the L-glutamic acid precursor from the soybean proteins (Le et al. 2021). Cellulase and pectinase were added to date residues before fermentation by *Lactobacillus brevis* (Hasegawa et al. 2018).

2.1.2. Lactic acid bacteria

Lactic acid bacteria can be used to decarboxylate glutamic acid to GABA (Moore et al. 2021). Several LAB fermented foods including cheese, yoghurt, kimchi and sourdough have shown a high GABA content (Dhakal et al. 2012). LAB is used as probiotics and starter cultures in multiple fermented foods including vegetables, yoghurt, cheese, meat, fish and sourdough bread. A development of LAB strains is going on due to their ability to improve taste, texture and other characteristics (Hatti-Kaul et al. 2018).

Lactobacillus is a genus of lactic acid bacteria with an essential role in the human microbiota and food products. Its ability to form lactic acid makes it useful in the production of fermented foods. Numerous strains of *Lactobacillus* have the ability to produce GABA. *Lactobacillus rennini* was used to produce a GABA-rich soy sauce containing 1.0g GABA/100ml (Yamakoshi et al. 2007). The kimchi LAB *Lactobacillus buchneri* produced 251 mM GABA with a 94% conversion rate in the optimum condition in MRS broth with an initial pH of 5.0 at 30 °C for 36 hours (Lahtinen 2012). *Lactobacillus brevis* has been found and isolated from multiple fermented foods (Dhakal et al. 2012). Most LAB species contain one gad gene while heterofermentative LAB *L. brevis* possess two GAD encoding genes in the chromosome and have shown a higher GABA production compared to other *Lactobacillus* strains. The highest GABA production was achieved by *L. brevis* CRL 2013 in MRS-GF broth supplemented with 267 mM MSG with initial pH of 6.5 at 30 °C after growth for 72 h. (Cataldo et al. 2020).

2.2. GABA rich foods

A demand of GABA rich functional foods is expected (Diez-Gutiérrez et al. 2020). Due to the beneficial health effects of GABA ingestion, the production of fermented GABA-enriched foods is a current trend in the food industry (Le et al. 2021) Foods that have been GABA-enriched include barley, chocolate, cucumber, honey, rice, soybean products, yeast, yoghurt, mung bean and tea (Hinton & Johnston 2020)(Ngo & Vo 2019). The attention for GABA is often due to its health benefits but the amino acid may also contribute to food quality, for example the protein functional properties. GABA presence showed a pH-dependent effect on gelling properties of whey protein gels. (Wang et al. 2019)

Fortification is defined as "the practice of deliberately increasing the content of an essential micronutrient…in a food, so as to improve the nutritional quality of the food supply and to provide a public health benefit with minimal risk to health" and enrichment is defined as "synonymous with fortification and refers to the addition of micronutrients to a food which are lost during processing" by the World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations (FAO) (Allen et al. 2006). There is no addition of GABA in the foods discussed in this literature study. The high levels are a result of a natural internal rise. That may be a reason for using the term GABA-enhanced foods instead of GABA-enriched foods. On the other hand, the term GABA-enriched is used in the literature found.

2.2.1. Tomato

Tomatoes naturally accumulate a high amount of GABA among fresh vegetables. The accumulation seems to be higher in wild genotypes compared to modern cultivars. The reason for this may be the wished umami flavour which is linked to the content of glutamate, a GABA precursor (Gramazio et al. 2020). Tomatoes are produced and consumed in large quantities worldwide and an interest is therefore seen in tomatoes with an enhanced GABA content. Several attempts to develop GABA rich tomatoes have been made. Multiple varieties have been screened and their material identified for future development. *Solanum pennellii* contain approximately 200 mg/100 g FW which is the highest GABA content reported from a tomato wild relative so far. On the other hand, the GABA content rapidly declines during fruit ripening and phenotypes with a different chemical composition might be dysfunctional. (Gramazio et al. 2020)

2.2.2. Cheese

The concentration of GABA in cheese has been measured and GABA-producing bacteria strains in cheese have been found and isolated. In 22 Italian cheeses the GABA concentration varied between 0.26 and 391 mg/kg. 12 lactic acid bacteria strains from Italian cheeses can synthesize GABA. The strains with the highest GABA production during fermentation of reconstituted skimmed milk was *Lactobacillus paracasei* PF6, *Lactobacillus delbrueckii* subsp. bulgaricus PR1, *Lactococcus lactis* PU1, *Lactobacillus plantarum* C48 and *Lactobacillus brevis* PM17 (Siragusa et al. 2007). *Lactobacillus brevis*, *Lactococcus lactis* and *Lactobacillus plantarum* have been isolated from cheese and then used in production of GABA enriched bread (Venturi et al. 2019). *Lactobacillus delbruickii* subsp. *bulgaricus* and *Lactobacillus paracasei* have also been identified as GABA producing bacteria in cheese (Dhakal et al. 2012).

2.2.3. Sourdough bread

Sourdough fermentation improves the flavour, texture, shelf-life, nutrition and overall quality of bread. The activity of lactic acid bacteria and yeast obtain a distinctive process and final product. The metabolism of these microorganisms can also be used to produce a GABA-enriched bread. Eighteen lactobacilli strains were isolated and screened from the five species *L. brevis*, *L. farciminis*, *L. plantarum*, *L. rossiae* and *L. sanfranciscensis* from sourdough. *L. farciminis and L. brevis* were selected for their high GABA-production. Lactobacilli inoculated breads were baked and evaluated. The sourdough breads showed a 350 percent higher GABA-content compared to yeast fermented breads. (Venturi et al. 2019)

2.2.4. Tea

Tea is an important crop and beverage cultivated in China, Taiwan, Japan, India and other countries. The species is called *Camellia sinensis* and includes a large number of varieties and cultivars. It contains numerous components including polyphenol and amino acids. The postharvest processes for tea include several steps including withering, heating, rolling and drying (Hinton & Johnston 2020). The aerobic incubation in the withering makes the tea leaves oxidize. The types of tea are classified according to the oxidation level: unoxidized-green tea, partly oxidized oolong tea, fully oxidized black tea and post-fermented-pu-erh tea. (Lin et al. 2012) The beverage is usually made by an infusion of the leaf of the plant and hot water. The rich taste and health benefits are widely studied. (Yu & Yang 2020)

Amino acids in tea, both proteinaceous and non-proteinaceous, contribute to function, flavour, freshness and health. Tea can supply the human body with eight essential amino acids: lysine, tryptophan, threonine, isoleucine, leucine, methionine, phenylalanine and valine. Treatments of the tea plant during growth or postharvest processes influence the amino acid content. Volatile compounds are synthesized in response to environmental stress. Abiotic stress caused by light, temperature and mechanical damage, and biotic stress caused by living organisms affects the chemical composition and aromatic character. By understanding the biosynthesis and metabolism of the compounds, the function and flavour can be altered. Therefore, regulatory stress-response mechanisms are often wished for in tea production. (Yu & Yang 2020).

GABA tea contains at least 150mg GABA/ 100g tea. In the 1980´s Tsushida Tojiro, a Japanese researcher, started to process teas in an anaerobic condition which increased the natural levels (Tsushida et al. 1987). In Japan, GABA tea is called Gabaron and is widely consumed. Due to the health beneficial effect of GABA tea, the drink has been popular in other Asian countries as well. (Lin et al. 2012)

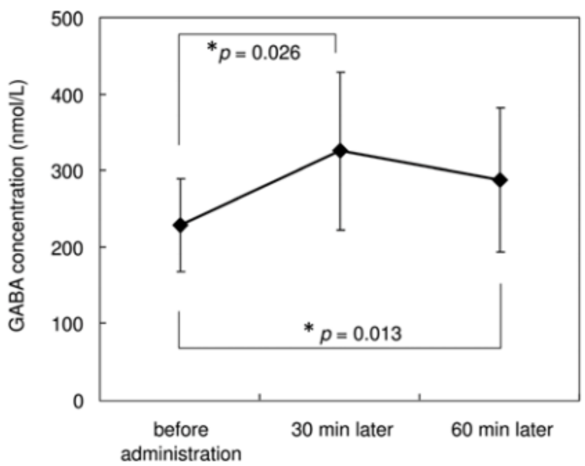
GABA is represented in all types of tea; green tea, white tea, yellow tea, oolong tea, black tea and pu-erh tea (Hinton & Johnston 2020). The GABA content in different tea types was measured and compared. 114 samples of Chinese teas were determined using high-performance liquid chromatography. The result showed that the GABA content in pu-erh teas was lower compared to the other tea types including green, oolong, black and white tea. (Zhao et al. 2011)

When a tea plant is exposed to stress, including mechanical damage, salinity, cold, heat, drought, virus infection or low oxygen, the GABA content increases. An anaerobic postharvest process works as a stress induction and can be used for all types of tea. Nitrogen-, carbon dioxide- and vacuum treatments have been used for GABA accumulation. (Yu & Yang 2020). The GABA content in tea becomes higher in a cycling treatment of anaerobic and aerobic incubation compared to only anaerobic incubation (Sawai et al. 2001). Two active glutamate decarboxylase isoforms have been found in tea (*Camellia Sinensis*) (Mei et al. 2020). Tea leaves exposed to multiple stresses showed that the accumulation of GABA is regulated by dual mechanisms. Combined anoxic stress and mechanical damage activate enzymatic activity of both CsGAD1 and CsGAD2 which increases the accumulation of GABA (Mei et al. 2016).

Several comparisons between the content of bioactive components in ordinary tea and GABA tea have been made. One of them used oolong tea and GABA tea samples from several varieties, seasons and production areas in Taiwan to measure moisture, nitrogen, colour, catechins, free amino acids, reducing sugar, ascorbic acid, caffeine and fat and free fatty acids. The results showed for instance that the contents of total catechin and ascorbic acid were lower in GABA tea. Apart from GABA, the free amino acid contents including theanine does not differ significantly. The content of caffeine and other phenolic compounds are also rather similar between the green tea and GABA tea. (Wang et al. 2006).

3. Effects of GABA intake

Even though both the importance of GABA in the brain and the content of GABA in foods is established, it is contentious if oral GABA has an ability to influence the brain function directly (Diez-Gutiérrez et al. 2020). There is no data on whether the GABA concentration in the human brain is increased by oral GABA (Hinton et al. 2019). Treatment with GABA enriched tea showed an increase of plasma levels of GABA in rats. (Hinton & Johnston 2020). On the other hand, the biochemical effect may be due to other chemical components in the food. However, GABA plasma levels were measured after administration of GABA-solution in humans. The result showed a small increase in GABA concentration from 244 to 329 nmol/L after 30 minutes (figure 5) (Yamatsu et al. 2016).



5: Plasma GABA concentration before, 30 min after, and 60 min after GABA administration in humans (Yamatsu et al. 2016)

3.1. Health effects

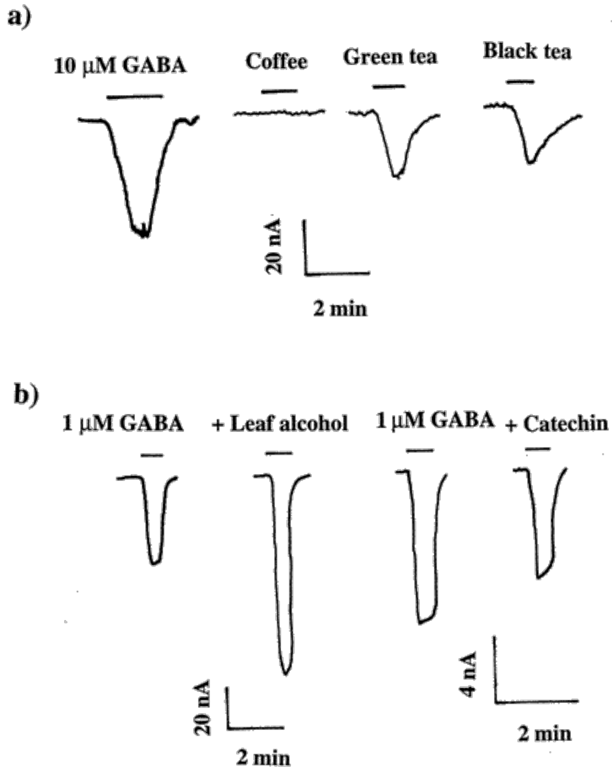
Multiple *in vitro* and *in vivo* studies have reported beneficial effects of the bioactivity and function of GABA (Gramazio et al. 2020). GABA have shown a neuroprotective activity that include inhibition of neuronal damage, neuronal cell death, brain injury and cytodestructive autophagy. Intake of GABA has also shown a prevention of neurological disorders including sleeplessness, somnipathy, anxiety, dementia and depression. An enhancement of sleep time, immunity, memory, cognitive function and relaxation has been seen. Application of several methods for both rats and humans with different GABA-sources including enriched dairy products, enriched rice grains, enriched purple sweet potato-fermented milk by LAB have been used to discover an anti-hypertensive effect. GABA treatments have shown an anti-diabetic effect by an improved glucose tolerance and insulin sensitivity. An anti-cancer effect has been found due to GABA´s ability to inhibit proliferation and metastasis. Numerous studies have shown an antioxidant property of GABA. The compound trapped reactive intermediates during lipid peroxidation, reacted with malondialdehyde, reduced cell death, inhibited production of reactive oxygen species and enhanced antioxidant defence systems. An anti-inflammatory effect has been seen by GABA´s ability to decrease the production of pro-inflammatory mediators. On the other hand, evidence for the effects of oral GABA administration on stress and sleep in humans are very limited (Hepsomali et al. 2020).

3.2. Tea components affecting the $GABA_A$ receptor

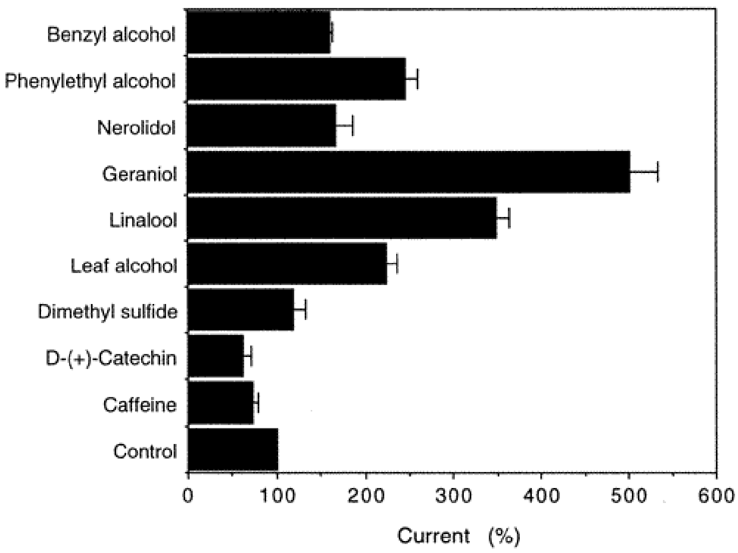
An investigation showed that administration of green GABA tea and oolong GABA tea in mice had an effect on behaviour. After oral administration, a Despair Swimming Test and Tail Suspension Test were used. Levels of lipid peroxidation products and the activity of antioxidant enzymes were determined in the mouse brain in vivo. Antioxidant activity in the teas was shown. (Daglia et al. 2017) The behaviour of the subjects during the test was linked to antidepressive-like behaviour. On the other hand, fear of drowning may differ from experiencing depression as a human.

Both the polyphenol epigallocatechin gallate (EGCG) and L-theanine in tea can cross the blood-brain-barrier and cause a stress reduction (Hinton et al. 2019). Ingestion of a mixture of GABA and L-theanine have shown to increase sleep duration and decrease sleep latency compared to GABA or theanine alone (Kim et al. 2019).

The effect of tea components on the $GABA_A$ receptor has been studied. Both caffeine and catechin have shown an inhibition of the $GABA_A$ receptor (figure 7). Fragrant higher alcohols potentiated the response (figure 6). (Hossain et al. 2002)



6:(a) The electric responses shows that the teas contained GABA while coffee did not. (b) Leaf alcohol potentiated the receptor response while catechin inhibited the response. (Hossain et al. 2002)



7: Effect of tea components at 1 mM on the response of GABAA receptors elicited by 1 μM GABA. (Hossain et al. 2002)

3.3. Blood-brain-barrier

The blood-brain-barrier is the semipermeable border between the circulating blood and the extracellular fluid of the central nervous system. It constitutes neighbouring capillary endothelial cells and is highly selective with which molecules that may cross the barrier. Factors which may prevent the entry of solutes include size, charge and solubility (Jung et al. 2019). The types of passages include passive diffusion, active transport and selective transport. The BBB both protects the brain from toxins and enables essential passage. (Boonstra et al. 2015)

It is disputed whether transport of GABA from blood across the BBB is possible either through diffusion or active transport and if an effect on GABA receptors in the brain would occur after GABA ingestion. The mechanisms for BBB permeability are difficult to identify (Jung et al. 2019). An ability for GABA to cross the blood-brain-barrier via the transporter GAT2/BGT-1 has been shown in mice (Takanaga et al. 2001). Due to the limited amounts of methods to measure the GABA levels in the human brain, no studies have been made with GABA´s BBB permeability in the human brain (Boonstra et al. 2015). However, there are other pathways that may be used.

3.4. Gut-brain-axis

The occurrence of GABA in the gut microbiota may have an influence on the human brain via the gut-brain-axis (Hinton & Johnston 2020). The central nervous system, the enteric nervous system and the gastrointestinal tract have bidirectional interactions. Therefore, brain neurotransmitter systems, stress- and pain-modulation systems and development of emotional behaviour are all influenced by the gut microbiota. Multiple mechanisms may be involved in the gut-brain-axis including endocrine and neurocrine pathways. (Mayer et al. 2015) The vagus nerve plays a crucial role representing the main afferent pathway from the gut to the brain. The role includes mediating behavioural effects of probiotics. Studies have shown that the gut microbiota regulates neurogenesis, neurotransmission and the inflammatory status of the brain. (Sherwin et al. 2016)

Human intestinally derived bacteria strains have shown ability to produce GABA. Bacteria growth and conversion of monosodium glutamate to GABA were measured after growth in medium containing monosodium glutamate. Four *Bifidobacterium* strains produced GABA. However, *Lactobacillus brevis* were the most efficient GABA producer. (Barrett et al. 2012). *Bifidobacterium* is genus of beneficial gut bacteria not classified as LAB but considered to be a probiotic microorganism and is therefore used in food products (*Bifidobacterium - an* *overview | ScienceDirect Topics*). Several *Bifidobacterium* strains are GABA producing (Diez-Gutiérrez et al. 2020).

The bacterial genus *Bacteroides* is the largest group of GABA producers in the gut (Pikusaeva et al. 2017). Due to the distribution of GABA within the enteric nervous system, it may cause a biological effect via the vagus nerve (Hinton et al. 2019). In a study, mice showed antidepressant-like and less anxious behaviour after administration of *Lactobacillus rhamnosus* compared to control mice. In mice that underwent vagotomy, none of these effects were present (Bravo et al. 2011). Therefore, it appears that due to the vagus nerve, the gut microbiota can regulate GABAergic neurotransmission indirectly (Sherwin et al. 2016). GABA-producing lactic bacillus bacteria (LAB) have shown ability to survive and produce GABA under simulated gastrointestinal conditions. These bacteria strains may be used as probiotics and for their ability to produce GABA *in situ* (Li & Cao 2010). Modulation of the GABAergic transmitter system may be involved in the beneficial behavioural effects of *Lactobacillus*. (Sherwin et al. 2016)

4. Discussion and conclusion

Production of GABA has been made in several ways. Specific microorganisms are tested and chosen to produce fermented GABA-enriched food using biosynthetic methods with a simple reaction procedure. GABA in functional foods can be consumed both for the neurological and probiotic effect. In addition, GABA enrichment affects the chemical composition in food which gives opportunity to consider the ability of GABA to enhance physical properties and taste. More research is needed considering abilities to increase GABA production in foods and ways to maximise bioavailability. Due to the widespread presence and known health effects, the GABA levels in foods may deserve more attention from producers and consumers. The GABA content in foods may become a quality factor in a nutrient perspective, equally important as vitamins, minerals, probiotics etc.

Multiple studies have shown a health effect in humans after administration of GABA from different sources including several food products. Inhibition of neuronal damage, prevention of neurological disorder, enhancement of sleep time, anti-hypertensive effect, an antioxidant property and more have been seen. Therefore, the pharmaceutical properties of GABA may be used as prevention and treatment of various diseases. However, the mechanism is disputed. More research is needed regarding the ability for the GABA molecule to pass the BBB, use the gut-brain-axis and make biological effects through other metabolic pathways. Additionally, GABA sources including tea and fermented foods contain numerous compounds which may influence the effect of GABA intake. The biochemical effect of a combination of GABA and other substances may be studied further.

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