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Dietary Chemicals and Brain Function

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Abstract

Phytochemicals in our diet may play a vital role in maintaining the brain's chemical balance by influencing the function of receptors for the major inhibitory neurotransmitter GABA.

The flavonoids apigenin and epigallocatechin gallate, found in chamomile and green tea respectively, influence the way in which GABA receptors are modulated by drugs such as diazepam. Resveratrol, a flavonoid-like polyphenol found in red wine, acts on a subtype of GABA receptors consistent with its action as a cognitive enhancer. Bilobalide from *Ginkgo biloba*, a herb used in cognitive therapy, also influences GABA receptors. α-Thujone, a terpenoid in the alcoholic beverage Absinthe, acts in a similar manner to bilobalide on GABA receptors. (+)-Borneol and other terpenoids from Valerian, a herb used to promote sleep, enhance the effects of GABA. The effects of these phytochemicals on GABA receptors are consistent with the overall actions of the beverages and herbal preparations that contain them, thus providing a rational basis for the use of these beverages and herbal preparations.

These studies provide evidence that chemicals in our diet may influence brain function in a positive way. The chemical nature of these substances may lead to the development of new therapeutic agents for the treatment of anxiety, epilepsy, memory disorders and insomnia.

Keywords: Brain function, chemicals, diet, balance, dosage

THE BRAIN'S CHEMICAL BALANCE

Two simple chemicals, glutamic acid and GABA (Figure 1), are responsible for most of the communication between nerve cells in the brain. Indeed, at a very simple level, brain function may be thought of as a balance between excitation mediated by glutamic acid and inhibition mediated by GABA.

All nerve cells in the brain have receptors for glutamic acid and GABA. Some 40% of nerve cells release glutamic acid as an excitatory neurotransmitter, while a different 40% release GABA as an inhibitory neurotransmitter. The balance between these two chemical transmitters is vital to normal brain function. An excess of excitation over inhibition results in an over excited brain (as in Figure 1) that can be manifested as anxiety, agitation, exhilaration, convulsions and death. On the other hand, an excess of inhibition over excitation can be manifested by depression, anaesthesia, coma and death. The particular manifestations of such imbalances in the brain depend on what neuronal circuitry is involved.

Ethanol is an example of a chemical that acts on both sides of the brain's chemical balance. The CNS depression that results from ingestion of ethanol is due to a reduction in excitation mediated by glutamic acid acting on a subtype of glutamate receptors known as NMDA receptors and to an enhancement of inhibition mediated by GABA acting on $GABA_A$ receptors.

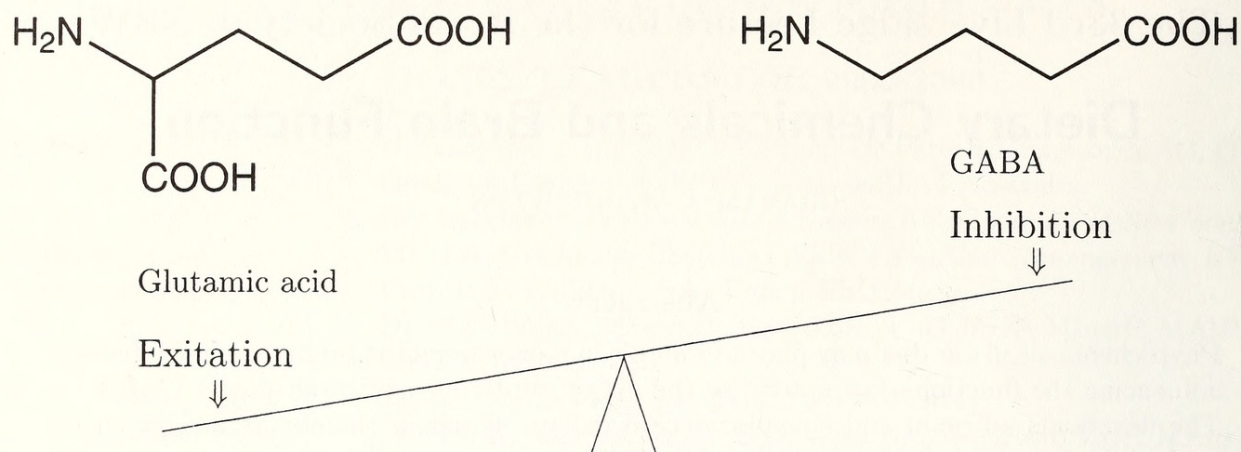Glutamic acid

Exitation
⇓

GABA

Inhibition
⇓

Figure 1: The brain's chemical balance between excitation mediated by the major excitatory neurotransmitter, glutamic acid, and inhibition mediated by the major inhibitory neurotransmitter, GABA.

GABA RECEPTORS

GABA (whose name is derived from the old chemical name, γ-aminobutyric acid) acts on three main types of receptor to influence brain function. $GABA_A$ and $GABA_C$ receptors are fast acting receptors that belong to the group of receptors called ligand-gated ion channels (LGICs) (Chebib and Johnston, 2000). GABA acts as the ligand gating these receptors to open channels specific for chloride ions, allowing these ions to flow rapidly into nerve cells making them more negative and thus harder to excite. $GABA_B$ receptors act more slowly, inducing metabolic changes in nerve cells and belong to the group of receptors called G-protein coupled receptors (GPCRs) (Bowery et al. 1997).

The study of GABA receptors has been revolutionised by the introduction of recombinant receptor technology whereby receptors cloned from human brain are expressed in cells that do not normally express such receptors (Barnard et al. 1998). The recombinant receptors so formed may be studied in relative isolation using standard electrophysiological methodology. Since all GABA receptors are made up of protein subunits, recombinant receptors of known subunit composition may be studied.

The most common way to study recombinant GABA receptors is to express them in oocytes from the South African frog, *Xenopus laevis* following injection of either DNA or RNA cloned from human brain and coding for particular GABA receptor protein subunits. These oocytes have the necessary cellular machinery to make the human proteins and assemble them on the surface of the oocytes as functional GABA receptors. The oocytes are approximately one millimetre in diameter and readily penetrated by glass microelectrodes. Using 2-electrode voltage clamp methodology, the effects of chemicals on the function of these GABA receptors may be assessed in a convenient quantitative manner. For example, using recombinant receptor technology, the effects of anti-anxiety agents such as diazepam (Valium) on GABA receptors can be easily shown to be restricted to a specific sub-group of $GABA_A$ receptors. The technology is not restricted to the study of pure chemicals – relatively crude mixtures of chemicals can be studied, for example to follow the purification of chemicals acting on GABA receptors from extracts of herbal products.

FLAVONOIDS AND TERPENOIDS

Flavonoids are polyphenolic chemicals widely distributed in the plant kingdom particularly in flowering plants. Flavonoids are responsible for many of the brilliant colours of fruits

and vegetables and are important constituents of red wine, green tea and many herbal preparations (Aherne and O'Brien, 2002). Chemically, flavonoids are C15 compounds based on the chromane ring structure. Flavonoids have been studied extensively as anti-oxidants and oestrogens (Collins-Burow et al. 2000). Many of them show anti-cancer and anti-viral properties (Le Marchand, 2002).

There is an extensive literature on the effects of flavonoids on GABA receptors (for a recent review see Marder and Paladini (2002), dating from the discovery of some plant derived isoflavans in bovine urine that inhibited the binding of diazepam to brain membranes (Luk et al. 1983). In the present context of actions on GABA receptors, the following flavonoids are of interest: the flavone apigenin; the isoflavone genistein; the flavanone naringenin; and the fla-

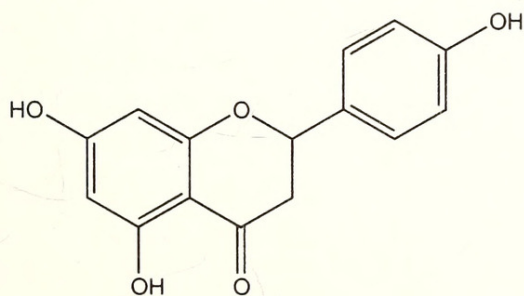van, epigallocatechin gallate (Figure 2).

Terpenoids are also widespread in plants, especially in what are known as essential oils that can be extracted from plants and have a wide range of uses from perfume constituents to paint thinners. Terpenoids are oxygenated products formally derived from C5 isoprene units and are classified by the number of C5 units in their structure. Thus monoterpenoids have 2xC5 units, sesquiterpenoids 3xC5 units, diterpenoids 4xC5 units and triterpenoids 6xC5 units. In the present context of actions on GABA receptors, the following terpenoids are of interest: α-thujone, (+)-borneol, bilobalide and picrotoxinin (Figure 3). Picrotoxinin is widely used experimentally as a non-competitive antagonist of $GABA_A$ and $GABA_C$ receptors, however, its convulsant action restricts its therapeutic use (Chebib and Johnston, 2000).
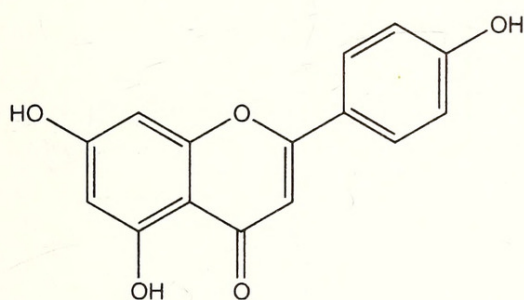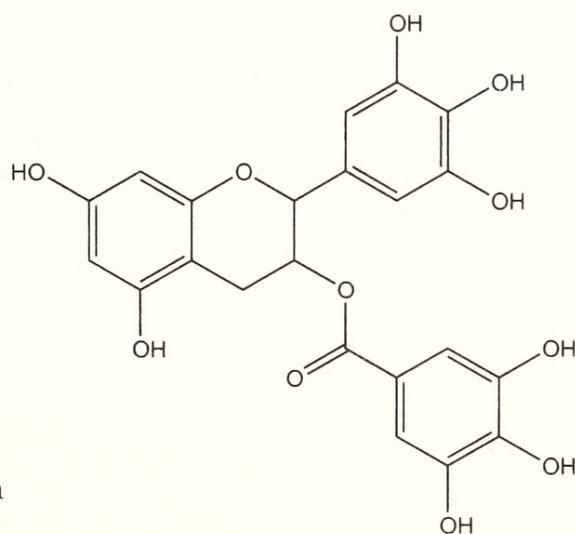

Apigenin, a flavone found in chamomile tea and related beverages.
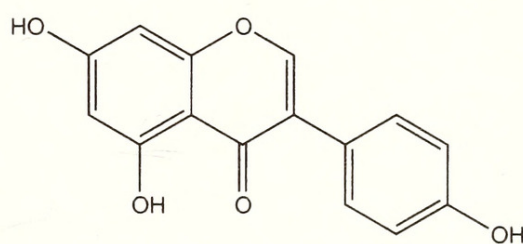
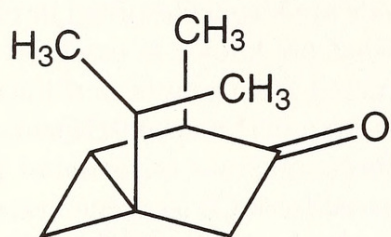**Genistein**, an isoflavone found in soy products, including tofu.
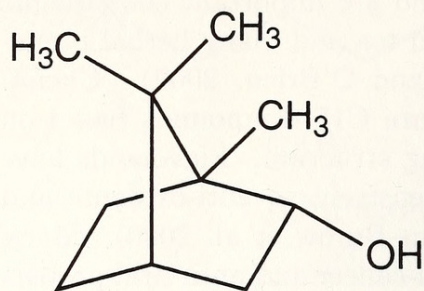
Naringenin, a flavone found in grapefruit.

(-)-Epigallocatechin Gallate, a flavan found in green tea.
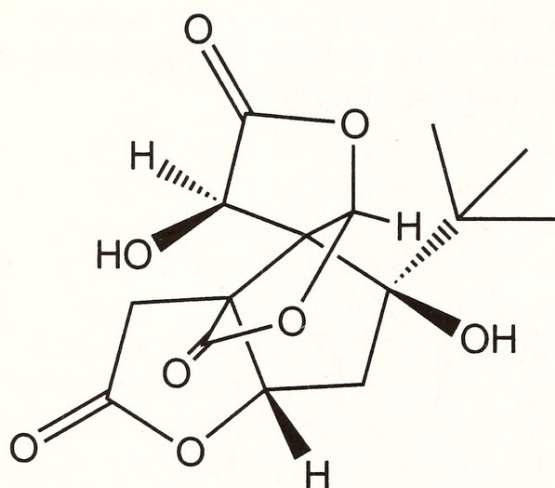
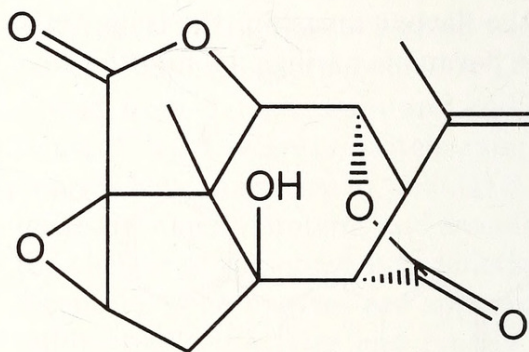Figure 2: Some representative flavonoids

α-**Thujone**, a monoterpene
from *Artemisia absinthium*

(+)-**Borneol**, a monoterpene
from *Valerian officinalis*

Bilobalide, a sesquiterpenoid
from *Ginkgo biloba*

Picrotoxinin, a sesquiterpenoid from
Anamirta cocculus

Figure 3: Some terpenoids that influence GABA receptors

APIGENIN FROM CHAMOMILE TEA

The lead compound for our investigations was apigenin (Figure 2), a flavonoid with a known anti-anxiety action found in chamomile tea. Chamomile tea is used widely to treat anxiety and insomnia. Current therapeutic drugs used for the treatment of anxiety and insomnia such as the benzodiazepines Valium and Serepax act at $GABA_A$ receptors in the brain, increasing chloride flow into neurones, resulting in decreased neural activity. There were divergent reports of the effects of apigenin on $GABA_A$ receptors. Viola et al. (1995) concluded that apigenin is a benzodiazepine agonist, like diazepam. However, Avallone et al. (2000) concluded that apigenin was a benzodiazepine inverse agonist (the exact opposite of diazepam).

Viola et al. (1995) based their conclusion that apigenin is a benzodiazepine agonist on the ability of apigenin to displace the binding of radiolabelled benzodiazepines from rat brain membranes, coupled with benzodiazepine-like effects in a rodent model of anxiety. However, binding studies do not reliably distinguish between agonists, antagonists and inverse agonists. Indeed, on the basis of similar binding studies, Dekermendjian et al. (1999) concluded that apigenin was a benzodiazepine antagonist (that is, it binds to the benzodiazepine site, blocking the binding of benzodiazepine agonists and inverse agonists, without having any effect on GABA responses itself). Avallone et al. (2000) used electrophysiological recordings from rat neurones. This allowed a more direct investigation of the activity of apigenin and showed that apigenin inhibited currents due to GABA, an effect which was blocked by the benzodiazepine antagonist, flumazenil. This fits the profile of a benzodiazepine inverse agonist. However, Avallone et al. (2000) did find some behavioural effects of apigenin which could be consistent with an action as a benzodiazepine agonist.

As part of her PhD studies, Erica Camp-bell in our research group investigated the action of apigenin on recombinant GABA receptors. She used electrophysiological recordings from *Xenopus laevis* oocytes injected with recombinant human RNA for the most common subtype of $GABA_A$ receptor ($\alpha_1\beta_2\gamma_{2L}$) in the brain. The actions of GABA at these receptors are enhanced by a variety of modulators including barbiturates, benzodiazepines, ethanol, and neuroactive steroids.

She showed the action of apigenin on the $GABA_A$ receptor is more complex than suggested by earlier studies. The effects of apigenin were biphasic dependent on the dose used. Moderate doses of apigenin inhibited the actions of GABA, diazepam and the steroid allopregnanalone, whereas low apigenin concentrations enhanced the effects of diazepam only (Figure 4). These effects are unlikely to be due to a simple action at the benzodiazepine site, suggesting a new site on the $GABA_A$ receptor.

While the inhibitory actions of apigenin at moderate doses were consistent with it acting as a benzodiazepine inverse agonist, the ability of apigenin to enhance the enhancing action of diazepam was novel. At low doses, apigenin had no direct effect on the action of GABA on these recombinant receptors. The presence of diazepam was necessary in order to observe the enhancing effects of apigenin. Thus apigenin appeared to be modulating the action of a modulator, an action not previously described in the pharmacological literature. Apigenin might be described as a second order modulator that influences the modulatory action of diazepam as a first order modulator on the activation of $GABA_A$ receptors.

The second order modulation of $GABA_A$ receptors by apigenin requires the presence of GABA and a first order modulator acting at a benzodiazepine site. The sedative and anxiolytic actions of apigenin observed in rodents (Avallone et al. 2000, Viola et al. 1995) can be interpreted on the basis of apigenin potentiating the action of endogenous benzodiazepine-like agents in the brain. Evidence for the physiologically relevant presence of such agents, termed

endozepines, has come from the discovery of a mutant $GABA_A$ receptor in childhood absence epilepsy and febrile seizures that has diminished sensitivity to benzodiazepines with little other alteration in $GABA_A$ receptor function (Wallace et al. 2001).

Genistein (Figure 2), an isoflavone found in soy products, did not show the biphasic effects of apigenin. Genistein, a structural isomer of apigenin, showed only the $GABA_A$ antagonist action of apigenin. In addition, a dihydro derivative of apigenin, naringenin (Figure 2), a flavanone found in grapefruit juice and other citrus products, also showed only the $GABA_A$ antagonist action of apigenin. Thus, the second order modulatory action of apigenin is structurally specific.
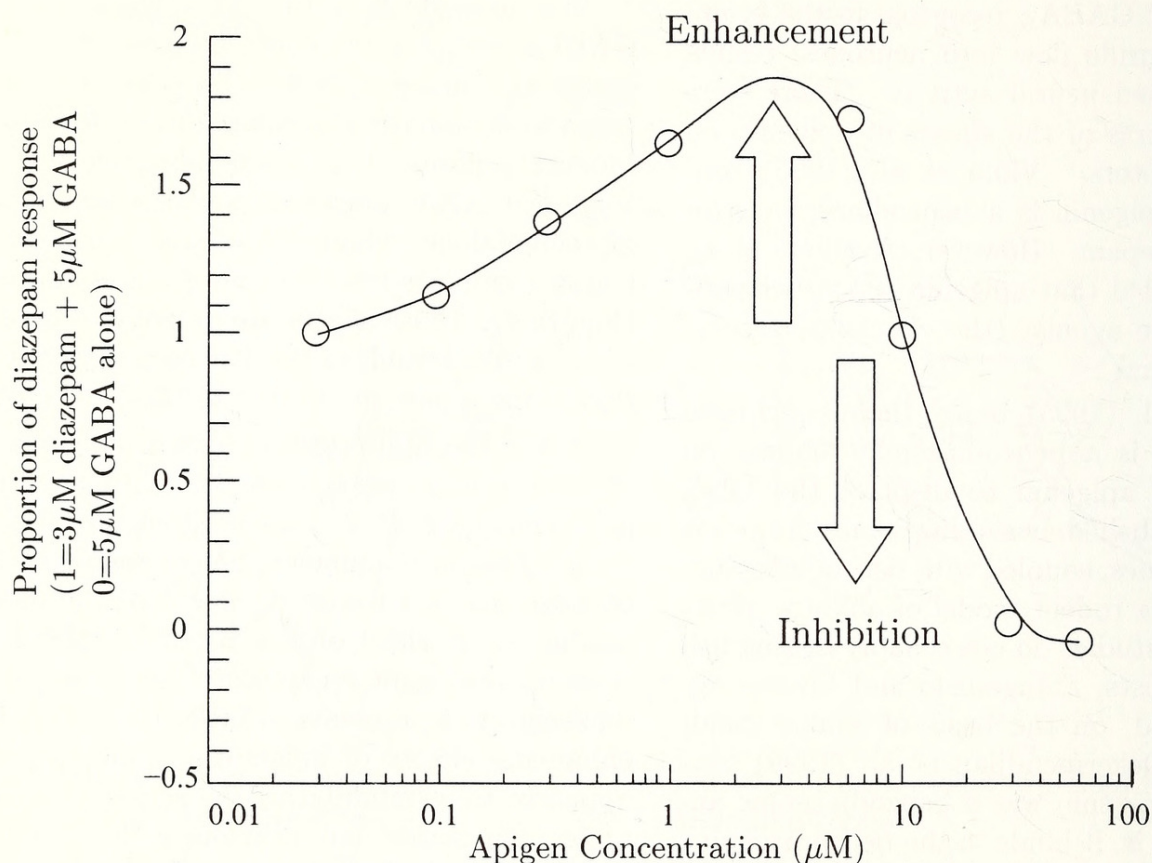

Figure 4: Effects of apigenin on the enhancement by diazepam of the action of GABA on recombinant $GABA_A$ receptors (Campbell et al. 1999).

EPIGALLOCATECHIN GALLATE FROM GREEN TEA

Epigallocatechin gallate (Figure 2, EGCG) is the most abundant flavan in green tea (*Camellia sinensis*). It is found in all teas made from *C. sinensis* but not in many other food products (Arts et al. 2000b). Green tea is known to have many beneficial effects, including prevention of cancer, lowering of blood pressure and lipids, and acting as an antioxidant. EGCG has been shown to contribute to these effects and, in addition, has been shown to have neuroprotective properties.

Erica Campbell investigated the actions of EGCG on recombinant GABA receptors (Campbell et al. 1999). She found that it shared the same biphasic action of apigenin, enhancing the action of diazepam at low concentrations and inhibiting at higher concentrations. In both the enhancement and inhibition phases, EGCG was at least 10 times more potent than apigenin.

(+)-Catechin and (-)-epicatechin, the most abundant flavans in nature, being found in many foods (Arts et al. 2000a, Arts et al. 2000b), did not influence recombinant GABA receptors, showing that the basic flavan ring structure is not sufficient for either of the actions of EGCG observed on recombinant GABA$_A$ receptors.

The biphasic actions of apigenin and EGCG emphasise the importance of dose in drug action. Our experiments show that low doses of apigenin and EGCG can enhance the activation of GABA receptors under the right conditions and thus could produce sedation and relief of anxiety. On the other hand, higher doses have the opposite effect and thus are likely to produce stimulation.

The second order modulatory action of apigenin and EGCG might have therapeutic possibilities. Low doses of these substances could reduce the therapeutic dose of diazepam and related benzodiazepines, while higher doses might reduce the effectiveness of such benzodiazepines. The possibilities of interactions between benzodiazepine medication and the consumption of chamomile and green tea need to be considered, particularly as chamomile tea may be used as a home remedy for those conditions for which benzodiazepines are frequently prescribed.

RESVERATROL FROM RED WINE

The relatively low incidence of coronary heart diseases in France, despite intake of a high-fat diet, – the "French Paradox" – has been attributed to the consumption of red wine containing high levels of polyphenolic compounds (Mojzisova and Kuchta 2001, Sun et al. 2002). Resveratrol (3,4',5-trihydroxystilbene, Figure 5) is one of the most interesting polyphenolic compounds found in red wine. It has been shown to have estrogenic (Turner et al. 1999) and neuroprotective effects (Bastianetto et al. 2000).

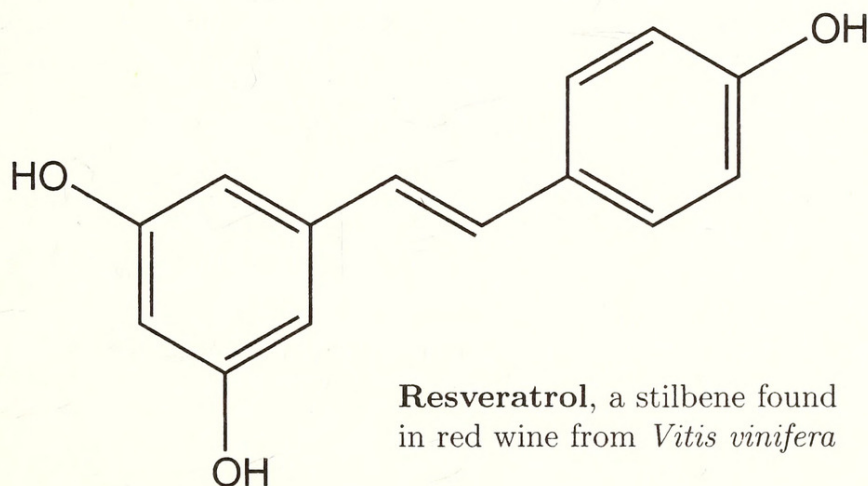**Resveratrol**, a stilbene found in red wine from *Vitis vinifera*

Figure 5: Structure of Resveratrol

In view of the structural similarities between resveratrol and apigenin, we investigated its effects on recombinant GABA receptors expressed in oocytes. To our surprise, resveratrol showed little action on GABA$_A$ receptors but was a GABA$_C$ receptor antagonist (Campbell and Johnston, 2003). Resveratrol non-competitively inhibited the effects of GABA (1 μM) at GABA$_C$ receptors with an IC50 of 72 μM, while having no significant effect at doses up to 100 μM on the effects of GABA (40 μM) at GABA$_A$ receptors. This is the first report of a non-competitive antagonist showing some selectivity for GABA$_C$ over GABA$_A$

receptors, the widely used non-competitive antagonist picrotoxinin being some 30 times more potent at GABA$_A$ than at GABA$_C$ receptors (Chebib and Johnston, 2000).

We have a patent on the use of GABA$_C$ receptor antagonists to enhance cognitive activity and stimulate memory capacity (Johnston et al. 1998). Thus it was interesting to note that resveratrol has also been patented for the treatment of mild cognitive impairment (Wurtman and Lee, 2002) based on its ability to increase the expression of soluble amyloid precursor protein.

Using resveratrol as a lead compound, we are examining structural analogues to see if we can develop more potent and selective compounds acting on GABA$_C$ receptors. Recently we discovered a range of very promising activities, including the ability to enhance GABA$_C$ receptor activity, in a series of compounds synthesized in the 1970s by David Collins and colleagues in Veterinary Physiology at The University of Sydney as antiestrogenic and antifertility agents (Collins et al. 1971).

There is great interest in drugs to treat memory impairment in disorders such as Alzheimer's disease and schizophrenia. The use of such "Smart Drugs" in healthy people to increase their cognitive ability raises a variety of ethical, legal and social issues (Rose 2002).

Resveratrol and related stilbenoids are found in a variety of plants and herbs. Major dietary sources include grapes, wine, peanuts and soy (Burns et al. 2002). These compounds are also found in Itadori tea which has long been used in Japan and China as a traditional remedy for heart disease and stroke. For people who do not wish to consume alcohol, Itadori tea may be a substituent for red wine as a dietary source of resveratrol (Burns et al. 2002). For those who prefer white wine to red, French winemakers have created a Chardonnay, called Paradoxe Blanc, that is enriched in polyphenols and has been shown to be effective in reducing oxidative stress in diabetic rats (Landrault et al. 2003).

As noted above, ethanol itself enhances the effectiveness of GABA acting on GABA$_A$ receptors and there is evidence that moderate consumption of alcoholic beverages is beneficial to health. However, other substances in these beverages, such as resveratrol, are likely to contribute to the overall beneficial effects. Recently it has been reported by Aoshima and colleagues (Hossain et al. 2002) that the fragrance of whiskey is able to enhance the effectiveness of GABA acting on GABA$_A$ receptors. Several components of the fragrance showed this property, the most potent being ethyl phenylpropionate, which was shown to have an anticonvulsant action in mice on inhalation.

Enhancement of GABA action was also found in extracts of other alcoholic drinks such as wine, sake, brandy and sochu. Hossain et al. (2002) noted "Although these fragrant components are present in alcoholic drinks at low concentrations (extremely small quantities compared with ethanol), they may also modulate the mood or consciousness of the human through the potentiation of the GABA$_A$ receptor response". Aoshima and colleagues have shown that various perfume constituents and aromatherapy agents potentiate GABA$_A$ receptors (Aoshima and Hamamoto 1999, Aoshima et al. 2001).

Clearly there are many interesting and innovative ways to explore the possibilities of influencing cognitive function.

BILOBALIDE FROM GINKGO BILOBA

Extracts of *Ginkgo biloba* leaves are widely employed as herbal medicines to treat symptoms associated with mild-to-moderate dementia, impairment of other cognitive functions associated with ageing and senility and related neurosensory problems (Diamond et al. 2000). A study has indicated that the cognition-enhancing effects of the *Ginkgo* leaf extracts may be partly mediated by bilobalide via GABA receptors (Sasaki et al. 1999b). Enhanced hippocampal pyramidal neuronal excitability has been shown to correlate with learning and memory (Power

et al. 1997). Bilobalide (Figure 3), a sesquiterpenoid isolated from *Ginkgo biloba* leaves, has been shown to enhance this excitability in rat hippocampal slices, an action proposed to involve blockade of GABAergic neurotransmission (Sasaki et al. 1999b).

In collaboration with Sasaki and colleagues, Shelly Huang and Rujee Duke in our research group have shown that bilobalide is a potent antagonist of the action of GABA on recombinant $GABA_A$ and $GABA_C$ receptors (Huang et al. 2003). Bilobalide was only marginally less potent than picrotoxinin in these actions but there were subtle differences between the actions of bilobalide and picrotoxinin. These findings strongly support the proposal by Sasaki and colleagues (Sasaki et al. 1999b) that the observed enhanced neuronal excitability in hippocampal slices was due to its blockade of GABAergic neurotransmission.

Bilobalide and picrotoxinin share common structural features, including a hydrophilic cage and lipophilic side chain. However, bilobalide and picrotoxinin have opposite actions upon systemic administration to animals. Bilobalide is an anticonvulsant (Sasaki et al. 1999a,b) whereas picrotoxinin is a potent convulsant (Jarboe et al. 1968). There are, however, only minor differences in their activities at recombinant $GABA_A$ and $GABA_C$ receptors. Bilobalide has been shown to increase GABA levels in the hippocampus and cerebral cortex of mice (Sasaki et al. 1999a). This increase may override the $GABA_A$ antagonist action of bilobalide that would be expected to produce convulsions and result in the overall anticonvulsant action. Nonetheless, the induction of epileptic seizures by *Ginkgo* extracts has been noted in rare cases (Granger, 2001). The anticonvulsant/convulsant actions of bilobalide need further investigation and may provide vital clues as to the safe use of *Ginkgo* extracts in the treatment of mild cognitive deficits.

Ginkgo leaves were used traditionally in Japan to protect books against harmful worms and insects before the introduction of modern insecticides. Like picrotoxinin, bilobalide is a potent insecticide (Ahn et al. 1997), an action likely to be due to blockade of insect GABA receptors.

THUJONE FROM ABSINTHE

Absinthe was the favoured drink of artists and writers in Paris at the end of the 19th century. The emerald green liqueur made famous by Van Gogh, Toulouse-Lautrec and their colleagues was banned in France and most other countries by 1915 due to its ability to cause convulsions, hallucinations and psychotic disturbances.

The toxic component of absinthe has been identified as the monoterpenoid α-thujone (Figure 3). It is also the active ingredient of wormwood oil and some other herbal medicines and is reported to have antinociceptive, insecticidal, and anthelmintic activity. Hold et al. (2000) showed that α-thujone acted like picrotoxinin as a $GABA_A$ receptor non-competitive antagonist. They showed that α-thujone was rapidly metabolised to less active metabolites and concluded that "α-thujone in absinthe and herbal medicines is a rapid-acting and readily detoxified modulator of the GABA-gated chloride channel".

Matthew Roper in our research group has shown that α-thujone is a non-competitive inhibitor of the action of GABA on recombinant $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ and ρ_1 $GABA_C$ receptors expressed in oocytes. Like picrotoxinin, α-thujone was about 30 times more potent at $GABA_A$ than at $GABA_C$ receptors. Furthermore, site-directed mutagenesis studies showed that mutations in the second membrane-spanning region of the wildtype $GABA_C$ receptors influenced the potency of α-thujone and picrotoxinin in a similar manner indicating that both convulsants interact with the same amino acid residues on the $GABA_C$ receptor.

Many plant-derived essential oils, such as wormwood, have been known for over a century to have convulsant properties. Burkhard et al. (1999) reported on case studies of plant-related toxic seizures related to use of these oils

for therapeutic purposes. They noted that "the literature shows essential oils of 11 plants to be powerful convulsants (eucalyptus, fennel, hyssop, pennyroyal, rosemary, sage, savin, tansy, thuja, turpentine, and wormwood) due to their content of highly reactive monoterpene ketones, such as camphor, pinocamphone, thujone, cineole, pulegone, sabinyl acetate, and fenchone." They went on to state "Nowadays the wide use of these compounds in certain unconventional medicines makes this severe complication again possible".

Absinthe is now available in a less potent form that contains less than 10 parts per million of α-thujone, whereas traditional absinthe contained more than 250 parts per million.

BORNEOL FROM VALERIAN

Valerian (*Valeriana officinalis*) is a medicinal plant used widely throughout the world. Extracts of the dried underground parts of the plant are used to relieve anxiety, restlessness and nervous sleep disorders. There is evidence of its use by the ancient Greeks, Romans and Chinese for healing purposes. Early herbalists and physicians such as Hippocrates, Galen and Culpeper noted the sedative and digestive properties of valerian, advocating its use as a muscle relaxant, diuretic, expectorant and wound healer (Plushner, 2000). Today there are over 400 commercially available products containing valerian in Germany, more than 80 in the United Kingdom and more than 30 available in Australia (Houghton, 1999; Shohet et al. 2001).

Valerian extracts may be considered to be a "herbal Valium", given that they have a benzodiazepine-like action reducing the latency of sleep onset and increasing the depth of sleep and the perception of well-being. These extracts contain a large number of constituents, many of which are thought to be active at GABA receptors. Compounds that have been identified include acids (valerenic acid and isovalerenic acid), ketones (valeranone), alcohols (valerenol, maaliol), aldehydes (valerenal) and valepotriates (valtrate, isovaltrate). Valerian extracts

also contain various flavonoids, alkaloids, tannins and amino acids.

Renee Granger in our research group obtained 2 kg of the dried underground parts of *Valeriana officinalis* from Newton's Herbal Pharmacy in Sydney. She extracted this with hexane, ethyl acetate, methanol and methanol:water (1:5), and fractionated the extracts using silica gel chromatography. This procedure produced more than 450 fractions, which were assessed using thin layer chromatography and functional studies on recombinant receptors many fractions influenced GABA action on $GABA_A$ and $GABA_C$ receptors.

Dried valerian root normally contains 0.3–0.8% volatile oil, including borneol, valerenic acid, valeranone and kessyl glycol . These essential oil fractions proved very difficult to purify, so pure compounds were purchased and tested on recombinant receptors. This produced a very surprising result.

(+)-Borneol, the natural enantiomer found in Valerian, produced a 12 fold enhancement of the action of $10\,\mu M$ GABA on recombinant $GABA_A$ receptors under conditions whereby diazepam would give at best a 2 fold enhancement (Figure 6; Granger et al. 2002). While relatively high concentrations of (+)-borneol were needed (EC50 $400\,\mu M$), this degree of enhancement is unprecedented.

Under these conditions, (-)-borneol produced a 4 fold enhancement (EC50 $450\,\mu M$), isoborneol a 7 fold enhancement (EC50 $680\,\mu M$), while the structurally related monoterpenes camphor, bornyl acetate and *p*-cymene each produced an approximately 3x potentiation (with EC50's around $300\,\mu M$).

While many general anaesthetics, barbiturates and benzodiazepine are known to produce up to 2 fold enhancement of the response of $GABA_A$ receptors to GABA (Belelli et al. 1999b), the general anaesthetic etomidate is known to cause much larger enhancements, particularly at mutated GABA receptors, e.g. etomidate produces a 10 fold enhancement of GABA responses at $GABA_C$ receptors where the wild type isoleucine residue at position 307

had been mutated to a serine (Belelli et al. 1999a).

(+)-Borneol represents an intriguing structural lead for the development of a new class of therapeutic agents acting on GABA receptors. Purified (+)-borneol has been used for medicinal purposes in China and Japan (Hattori, 2000).

Borneol is a common constituent of the essential oil component of many plants and thus a component of many herbal preparations. On the basis of our studies, (+)-borneol would be expected to have antianxiety, anticonvulsant and sedative properties.
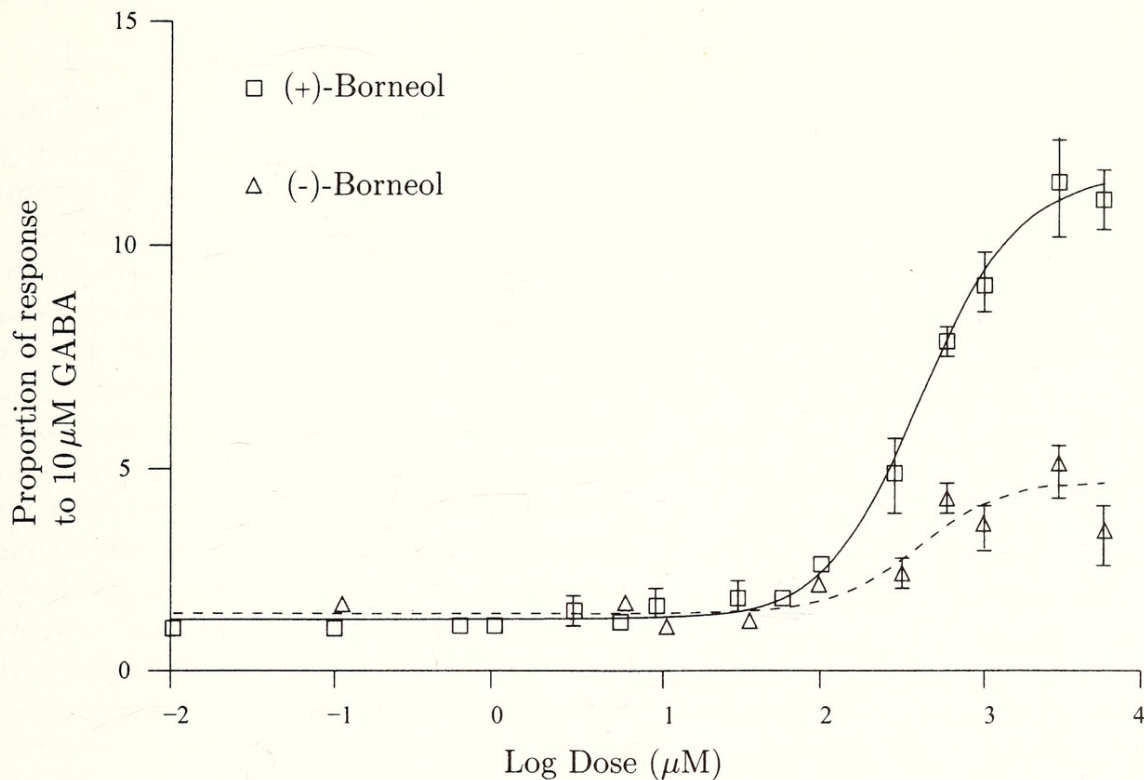
Figure 6: Dose-response curve for the potentiation of the response to $10\,\mu$M GABA by (+)- and (-)-borneol at recombinant GABA$_A$ receptors (Granger et al. 2002).

NATURAL VERSUS SYNTHETIC

Natural products derived from plants provided the first medicines. These were complex mixtures of chemicals. The active principles in these mixtures were isolated and developed as single chemical entities to use as drugs and from which to develop new therapeutic agents. The development of aspirin from the salicylates found in Willow bark is a classic example of this. Natural products continue to be an important source of new drugs. There are sophisticated laboratories in many countries, including

Australia, using high throughput technology to screen extracts of natural products for desired biological activities.

Herbal preparations are by their nature mixtures of chemicals. It is a basic tenet of herbal medicines that the whole is more than the sum of the parts. The various chemicals in herbal preparations are considered to act together in a synergistic way to effect treatment of particular disorders. This is in direct contrast to the "magic bullet" approach of single chemical entity drugs designed to hit a particular target in a highly selective manner. Both approaches have

their role in promoting health and well-being.

There is a widespread belief on the part of the general public that natural substances are inherently superior to synthetic substances with regard to efficacy and safety in matters related to human health. This question has been addressed recently by a task force of the International Union of Pure and Applied Chemistry (Topliss et al. 2002). A comparison of the characteristics of natural and synthetic substances used in a variety of therapeutic drugs, herbal medicines, vitamins and nutrients shows a similar range of favorable and unfavorable effects. It was apparent that molecular structure and dose determine the effect of chemicals on human health, not whether they are of natural or synthetic origin.

Natural chemicals such as many flavonoids have been consumed in our diet for countless generations. This suggests that they would be unlikely to have serious adverse effects severe enough to prevent their use as therapeutic agents. However, it is likely that the overall balance of flavonoids and related natural chemicals in our diet is of vital importance, given the examples in this review of such chemicals having opposing actions on GABA receptors. Recombinant receptor technology offers the means to assess the overall effects of complex mixtures of chemicals on the functioning of key receptors. Such technology is expected to play an increasingly important role in the quality control of herbal preparations and "functional foods".

Herbal preparations can have significant interactions with therapeutic drugs, for example by altering the metabolism of these drugs and thus influencing their potency and duration of action (Izzo and Ernst 2001). It is important that health care professionals ask their patients about their use of herbal products and consider the possibility of herb-drug interactions. Food-drug interactions are also important (Sorensen 2002) as many naturally occurring substances influence the cytochrome p450 enzymes that play such an important role in drug metabolism. Grapefruit juice is a classic example. It contains substances, including the flavonoid narin-

genin (Figure 2), that inhibit the p450 enzyme CYP3A4 resulting in higher bioavailability of drugs with a high firstpass metabolism (Fuhr 1998). While there may be a place for grapefruit juice as a drug-sparing agent, more research is needed and drugs possibly influenced by the consumption of grapefruit juice should be appropriately labelled.

CONCLUSIONS

GABA receptors in our brain are susceptible to a wide variety of chemicals consumed in the diet. Our GABA receptors exist in a milieu of substances that influences their function, often in opposing ways. The balance between the effects of these substances will determine at any particular point in time how the receptors respond to GABA, their natural neurotransmitter. This review has summarised the effects of some substances found in four beverages (chamomile and green tea, red wine, absinthe) and two herbal preparations (*Gingko* and *Valerian*) that have significant actions on recombinant GABA receptors consistent with the overall actions of the beverages and herbal preparations. The chemical nature of these substances may lead to the development of new therapeutic agents for the treatment of anxiety, epilepsy, memory disorders, and insomnia. Does the concept of dietary substances influencing brain function indicate that we have entered an era of neuraceuticals?

These studies provide a chemical basis for some of the effects that these beverages and herbal preparations have on brain function, and may lead to rational improvements in their quality control and use, especially in combination with other agents known to influence GABA receptors, such as alcohol, anaesthetics and benzodiazepines.

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