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Is There a Relationship Between Sweet Taste and Seizures? Anticonvulsant and Proconvulsant Effects of Non-Nutritive Sweeteners

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Abstract: From a virtual screening campaign, a number of artificial and natural sweeteners were predicted as potential anticonvulsant agents with protective effects in the seizure animal model Maximal Electroshock Seizure (MES) test. In all cases, the predictions were experimentally confirmed in the aforementioned preclinical seizure model. The article reviews and expands previous reports from our group on anticonvulsant activity of those non-nutritive sweeteners, illustrating the potential of virtual screening approaches to propose new medical uses of food additives. This constitutes a particular case of knowledge-based drug repositioning, which may greatly shorten the development time and investment required to introduce novel medications to the pharmaceutical market. We also briefly overview evidence on possible molecular explanations on the anticonvulsant and proconvulsant effects of different non-nutritive sweeteners. Our analysis –based on Swanson's ABC model- suggests that group I metabotropic glutamate receptors and carbonic anhydrase isoform VII (both proposed or validated molecular targets of antiepileptic drugs) might be involved in the anticonvulsant effect of artificial sweeteners. The first hypothesis is in line with recent advances on development of selective modulators of group I metabotropic glutamate receptors as potential antiepileptic agents.

Alan Talevi

Keywords: Drug repositioning, epilepsy, maximal electroshock seizures, non-nutritive sweeteners, pentylenetetrazol, stevia, steviosides, convulsions, Swanson's ABC model, virtual screening.

INTRODUCTION

Drug repositioning refers to finding new therapeutic indications for already known drugs [1]. The potential targets of a drug repositioning campaigns include marketed, discontinued and shelved drugs (the research on new indications for abandoned drugs is often described as *drug rescue*) and yet-to-be-pursued clinical candidates. Drug repositioning has recently raised much interest within the international drug development community, including a number of public programs to promote indication expansion launched by national health authorities in developed countries such as the United States and the United Kingdom [2]. Known drugs have already undergone safety assessment and pharmacokinetic characterization; thus repositioning often allows acceding clinical trials early in the drug discovery process, shortening the development of novel therapeutics and avoiding some phases of clinical development. Most of the known repositioning success stories are related to intelligent exploitation of unforeseen side-effects, being in fact serendipitous discoveries; however, the current trend is to approach drug repositioning through rational and/or high throughput approaches (e.g. cheminformatic and bioinfromatic-guided projects and high throughput screening) [3]. Although drug repositioning often focuses on previously known therapeutics, it may as well present great potential to identify novel therapeutic applications and functionalities of food constituents.

By applying virtual screening we have recently identified the anticonvulsant activity of artificial sweeteners cyclamate, saccharin and acesulfame [4]. Such positive results led us to wonder whether natural sweeteners might also have anticonvulsant effects. Therefore, natural sweeteners from *Stevia rebaudiana* were tested in preclinical seizure models. Stevia is a plant native to South America whose leaves extracts have a long traditional use to sweeten food and beverages in our region, and have also been used for several years in China and Japan and some European countries [5].

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Very recently, stevia was approved as sweetener by the Joint Food and Agriculture Organization World Organization Expert Committee on Food Additives [6]. Ligand-based computational models were applied to predict if stevia constituents and/or their phase I metabolites might have anticonvulsant properties. An aqueous infusion of stevia leaves and isolated steviosides were tested in seizure models (mice, ip) to confirm those predictions, with positive results [7].

Here, we overview and expand on our results on the anticonvulsant effects of artificial and natural sweeteners. We also review evidence and hypothesis on possible molecular explanations to the anticonvulsant and proconvulsant activities of non-nutritive sweeteners. By application of Swamson's ABC model, we propose metabotropic glutamate receptors (mGluR) from group I as molecular targets for the protective effects of the studied compounds and we overview recent research on novel antiepileptic candidates whose action mechanism includes modulation of group I mGluR.

IDENTIFICATION OF ANTICONVULSANT EFFECT OF ARTIFICIAL AND NATURAL NON-NUTRITIVE SWEETENERS THROUGH LIGAND-BASED VIRTUAL SCREENING

Application of a topological discriminant function on the Merck Index 13th database predicted that artificial sweeteners sodium cyclamate, potassium acesulfame and saccharin (Fig. **1**) might have anticonvulsant effect in the Maximal Electroshock Seizure (MES) test (a preclinical seizure model in which the convulsive episode is induced through application of an electric current; the MES test is included in the NIH's Anticonvulsant Screening Program) [8]. The topological function was derived through application of Linear Discriminant Analysis to Dragon 4.0 (Milano Chemometrics, 2003) 0D-2D molecular descriptors and consists in four conformation-independent descriptors:

$$dfMES = 8.110 - 2.206*\mathbf{HVcpx} - 4.277*\mathbf{BIC2} + 0.443*\mathbf{GATS7e} + 1.089*\mathbf{GATS8p} \quad (1)$$

where *dfMES* indicates whether a given compound does or does not elicit protection in the MES test. HVcpx symbolizes the graph vertex complexity index; BIC2 denotes the Bond Information Content (neighborhood symmetry of second order); GATS7e refers to Geary autocorrelation – lag 7, weighted by atomic Sanderson electronegativities, and GATS8p denotes Geary autocorrelation − lag 8, weighted by atomic polarizabilities. The inclusion of conformation-independent descriptors exclusively makes the previous QSAR model capable of screening large virtual repositories efficiently. A detailed interpretation of the discriminant function can be found in literature [9]. Essentially, it has been suggested that the discriminant function reflects the fact that: a) anticonvulsants with protective effects in the MES test tend to possess relatively compact structures with low eccentricities and well differentiated polar and non-polar regions (i.e. non-homogeneous distribution of heteroatoms), thus presenting high Geary autocorrelations of lag 7 and 8 and; b) relatively low complexity and thus low information content. Note that the previous molecular pattern is found in the artificial sweeteners presented in Fig. (**1**).

Subsequent bibliographic revision revealed that the anticonvulsant activity of saccharin has already been reported in 1979 [10]. All three compounds confirmed their protective effects in the MES test (mice, ip) in our laboratory. These results made us wonder if a structural link could be found between known molecular targets of antiepileptic medications and the receptor responsible of triggering the sweet taste sensation (see section The ABC Model and Possible Molecular Explanations to the Anticonvulsant Activity of Sweeteners, later in the text). Results are summarized in Table **1**.

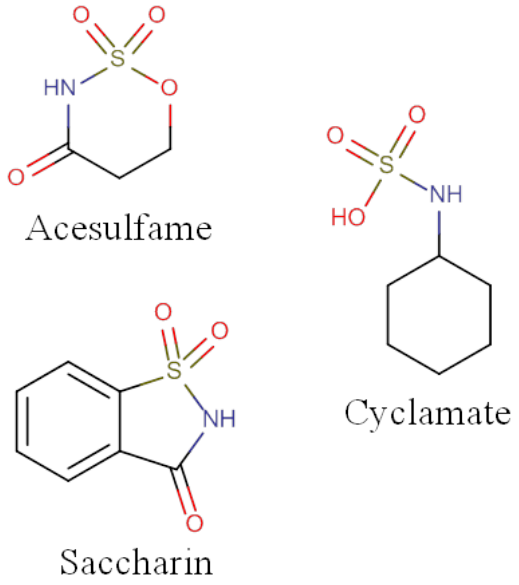


Fig. (1). Molecular structures of the three artificial sweeteners predicted as anticonvulsant agents in a virtual screening campaign on Merck Index 13th.

Table 1. Protective effects of artificial and natural non-nutritive sweeteners in the MES test (mice, ip).

Drug	Doses (mg/kg)	MES Test[a]	
		0.5 h	4 h
Acesulfame	30	0/3	1/3
	100	0/3	1/3
	300	2/3	2/3
Saccharin	30	0/3	1/3
	100	0/3	1/3
	300	0/2	0/2
Cyclamate	30	1/3	1/3
	100	0/3	1/3
	300	0/3	1/3
Stevia aqueous extract	30	0/3	0/3
	100	1/3	3/3
Rebaudioside A	30	0/2	0/2
	100	2/4	2/4
Stevioside	30	0/2	3/4

	100	3/4	1/4

[a]-Number of protected animals/number of tested animals.

To further characterize the anti-seizure profile of cyclamate, saccharin and acesulfame, the protective effect of these drugs (ip, mice) was investigated in the scPTZ test, another preclinical model of epilepsy in which the convulsive episode is elicited through subcutaneous administration of proconvulsant agent pentylenetetrazole (PZT). With this purpose, one hundred and ninety one male Swiss-Webster mice (30 g ± 0.2) were divided in 25 groups according to the substance to be injected. All animals received a daily injection of saline solution (ss, 1 ml/kg i.p.) for 5 days to adjust them to manipulation. Twenty-four hours after the last ss injection, the control animal group (n=21) received one injection of ss, and a second group (n=8), one injection of the vehicle used to dissolve the three compounds to be tested (70% deionized water/ 30% polyethylene glycol 400), 30 min before the administration of PTZ at a convulsive dose of 60 mg/kg (dissolved in saline). The results obtained from this group were used to compare them with the results obtained from the animals pre-treated with the three drugs as follows. Four animal groups were administered with one injection of sodium cyclamate at doses of 3 (n=8), 10 (n=12), 30 (n=11) and 100 (n=8) mg/kg, respectively, 30 min before PTZ. Since cyclamate induced a maximal anticonvulsant effect at the dose of 30 mg/kg, we also tested the effect of this dose of cyclamate at 15, 60 and 120 min before PTZ injection in three additional groups (n=7 in each group). Fourteen groups were administered with one injection of saccharin or potassium acesulfame at different doses, 30 min before PTZ. Two additional groups (n=10 and n=12) were also administered with vehicle and compared in parallel with the groups administered with saccharin or potassium acesulfame, respectively. All substances except when indicated otherwise, were administered i.p. in a volume of 10 ml/kg. Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Dunnett test, significances were assigned at the 0.05 level. All statistical analyses were carried out with GraphPad Prism software™ version 5.0a for Mac.

The dose of PTZ used here in mice induces the following behavioral changes within the 10 min after its administration: myoclonic seizures, defined as a whole-body twitch; clonic seizures, manifested by clonic spasms often followed by stupor or unusual posturing; and tonic seizures consisting of tonic hindlimb extension in at least 90% of the animals [11]. The behavioral changes following PTZ administration were visually evaluated during 30 min. The latency to the first myoclonus, and to the first generalized tonic-clonic seizure were measured, the total number of tonic-clonic seizures, as well as the percentage of animals presenting myoclonic and tonic-clonic seizures were also evaluated. As shown in Fig. (**2A**), in the group injected with ss (control group), as well as in the groups pre-administered with vehicle all animals developed myoclonic seizures. Cyclamate reduced the percentage of animals with seizures in a dose-depending manner, an effect that was significante at 100 mg/kg for myoclonic seizures and 30 mg/kg for tonic-clonic seizures. Fig. (**2B**) shows that 95% of control animals experienced at least one generalized tonic-clonic seizure following PTZ injection, while in the group injected with vehicle, 88% experienced a generalized tonic-clonic seizure to PTZ. Nonetheless, the difference between the percentage of animals presenting seizures in the groups treated with saline or vehicle did not reach statistical significance. Cyclamate at doses of 3, 10 and 100 mg/kg failed to significantly decrease the percentage of animals presenting seizures. However, 30 mg/kg cyclamate inhibited the

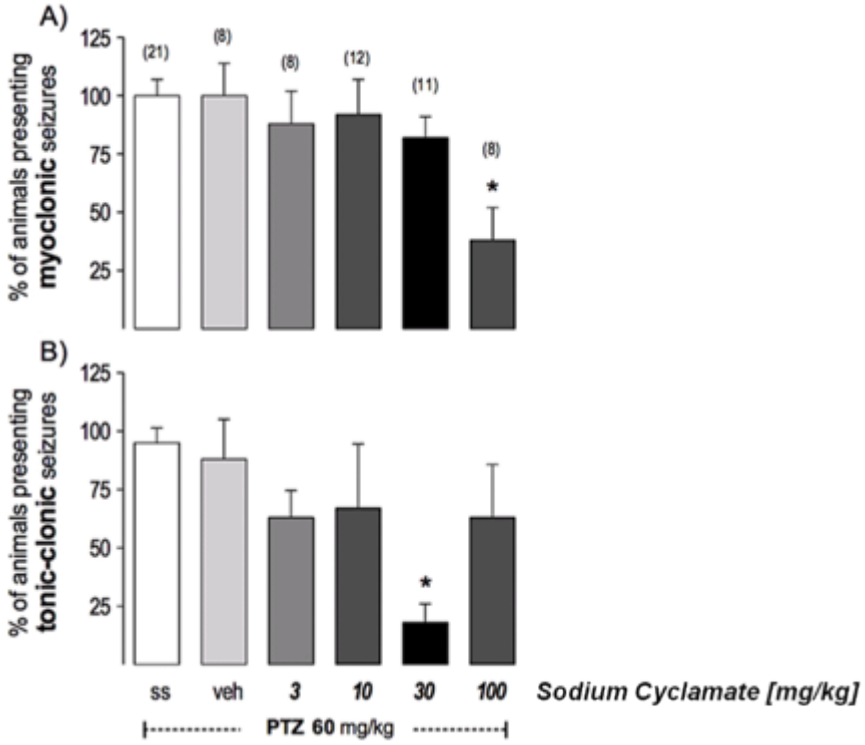


Fig. (2). Changes in the frequency of myoclonic and tonic-clonic episodes according to the administered dose of sodium cyclamate.

generalized tonic-clonic seizures in 9 of the 11 animals (82%) pre-administered 30 min before PTZ. The first myoclonic and tonic-clonic seizures appeared rapidly within 2 min following the injection of PTZ in control animals as shown in the first row of Table **2**. The latency to PTZ-induced seizures slightly increased in the animal group injected with vehicle (second row, Table **2**). Cyclamate at doses of 10 and 30 mg/kg increased the latency to the first myoclonic seizure induced by PTZ. Furthermore, Cyclamate again at the dose of 30 mg/kg, increased the latency to the tonic-clonic seizure in the two animals of the group that presented seizures followed by the injection of PTZ (fifth row, Table **2**).

To determine the time course of the anticonvulsant effect of sodium cyclamate, animals were pre-administered with one injection of 30 mg/kg at 15, 30, 60 and 120 min before PTZ since at that cyclamate dose, the maximal anti-seizure effect of this drug was observed. The latency to the first myoclonic and tonic-clonic seizures were already increased in the animals injected 15 min before PTZ (third row in Table **3**). The increase in the latency of PTZ-induced seizures reached a maximal value when sodium cyclamate was administered 30 min before PTZ (fourth row in Table **3**). Cyclamate administered 1 or 2 h before PTZ did not induce any effect on seizure latency, as shown in the two last rows of Table **3**.

The anticonvulsant effects of saccharin and acesulfame were also tested with a similar approach than the one used to test the effects of cyclamate. Saccharin at 10, 30 and 100 mg/kg did not decrease the percentage of animals presenting myoclonic seizures from the percentage of the control group. On the other hand, doses of 30 and 100 mg/kg failed in decreasing the percentage of animals presenting tonic-clonic seizures; however, only 57% of the animals pre-administered with 10 mg/kg saccharin presented tonic-clonic seizures. In addition, saccharin at a dose of 10 mg/kg was capable of increasing the latency to the first myoclonic seizure and to the first tonic-clonic seizure induced by PTZ, compared with vehicle which by itself increased the latency to seizures. Similarly, potassium acesulfame in a broad range of doses also failed in reducing the percentage of animals presenting myoclonic (data not shown) and tonic-clonic seizures. Interestingly, at a dose of 10 mg/kg it increased the latency to myoclonic and tonic-clonic seizures induced by PTZ, compared to the vehicle. Doses above 100 mg/kg, were needed to increase again the latency to the PTZ-induced seizures.

Table 2. Effect of increasing doses of sodium cyclamate on seizures induced by PTZ.

	ε Latency to the First		
Experimental Conditions	**Myoclonic Seizure**	**Tonic-Clonic Seizure**	**Number of Animals**
saline + PTZ	60 ± 4	91 ± 6	21
vehicle + PTZ	132 ± 18*	252 ± 43*	8
3 mg/Kg + PTZ	140 ± 22	172 ± 44	8
10 mg/Kg + PTZ	237 ± 47**	358 ± 83	12
30 mg/Kg + PTZ	233 ± 26**	486 ± 205**	11
100 mg/Kg + PTZ	142 ± 34	186 ± 42	8

ε, in seconds.

Animals were observed for 30 min after i.p. injection of 60 mg/Kg PTZ.

* P <0.05 between the animal group pre-administered with saline and the group with vehicle.

** P <0.01 between the animal group pre-administered with vehicle and the indicated animal group.

Table 3. Time-course of the effects of cyclamate on seizures induced by PTZ.

	ε Latency to the First		
Experimental Conditions	**Myoclonic Seizure**	**Tonic-Clonic Seizure**	**Number of Animals**
saline + PTZ	60 ± 4	91 ± 6	21
vehicle + PTZ	132 ± 18*	252 ± 43*	8
15 min before PTZ	211 ± 36**	403 ± 115**	7
30 min before PTZ	233 ± 26**	486 ± 205**	11
60 min before PTZ	152 ± 29	241 ± 61	7
120 min before PTZ	222 ± 25	350 ± 89	7

ε, in seconds.

Animals were pre-administered i.p. with 30 mg/Kg Sodium Cyclamate at the indicated time before the injection of PTZ at a dose of 60 mg/Kg and observed for 30 min after PTZ.

* P <0.05 between the animal group pre-administered with saline and the group with vehicle.

* * P <0.01 between the animal group pre-administered with vehicle and the indicated animal group.

Based on the previous results, we decided it was worth investigating the protective effects of Stevia natural sweeteners in the MES test. With that purpose, the previously presented discriminant function (equation 1) and a previously reported Sybyl pharmacophore capable of identifying anti-MES compounds [12] were jointly used to predict the potential anti-MES effect of Stevia constituents and their phase I metabolites (Fig. **3**). In order to perform the pharmacophore superposition, Hyperchem's Conformational Search (Hypercube Inc., 2007) module was used with the PM3 optimization method and a RMS gradient of 0.05

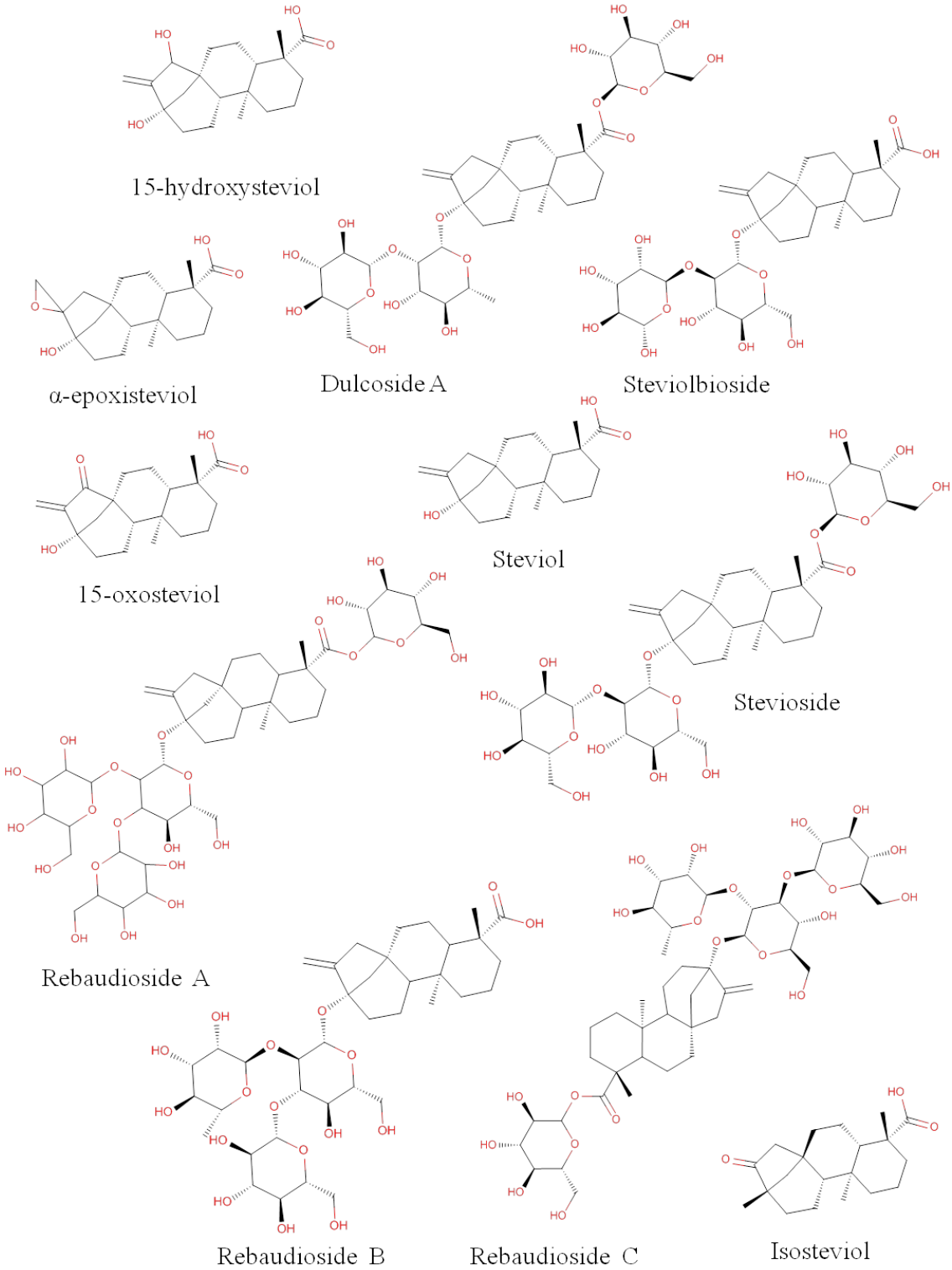


Fig. (3). Stevia constituents screened through our models for potential anticonvulsant activity.

kcal/[Å mol]. At least 250 optimizations were performed for each molecule; optimizations were conducted until convergence (that is, until no new low energy conformers are found). The lowest energy conformer of each drug was then superposed onto the pharmacophore, and the energy difference (conformational strain energy) between the lowest energy conformer and the conformer that approach the most to the pharmacophore conformation was calculated for each molecule. The discriminant function and the pharmacophore have previously been sequentially applied to screen NP libraries, with good results [13]. Both models predicted that steviol and its phase I metabolites would have anticonvulsant activity. Steviosides were predicted as non-anticonvulsants by our discriminant function. Superposition of steviol and its phase I metabolites on the pharmacophore model is presented in Fig. (**4**). The conformational strain energies for steviol and its metabolites were, in all cases, below 5 kcal/mol, suggesting that these compounds can probably fulfill the Sybyl pharmacophore geometrical requisites[14]. Based on the preceding results, we decided to test the effect of an aqueous infusion of stevia leaves in the MES test. Initial results confirmed the anticonvulsant activity of the infusion (Table **1**), which later proved to be dose-dependent, with an estimated ED_{50} of 70.3 mg/kg [47,4 - 104,3] (mice, ip, 4 hours). Isolated stevioside and rebaudioside A also showed protective effects in the preclinical seizure model. It should be noted, however, that important inter-species variation in the biotransformation of steviosides have been described between man and rodents [15], which may lead to very different responses to these compounds in these species (particularly if our models' predictions were true and steviol and their metabolites were the chemical entities with intrinsic anticonvulsant effects). It should be noted that an apparent discrepancy is observed between the prediction of our linear QSAR model and the observed experimental results (steviosides are predicted as non-anticonvulsants, but they actually show anticonvulsant effects in mice). This may emerge from a misprediction of the model or alternativelty, the model might be right and the steviosides may have no intrinsic anticonvulsant activity. In this case, the anticonvulsant activity could be attributed to the aglycone steviol and/or its metabolization products (which are predicted as anticonvulsants). This appears to be consistent with the fact that, at doses of 30 mg/kg (the lower doses tested), stevioside only produces anticonvulsant effects at 4 hours (presumably, after metabolization to steviol has taken place). On the other hand, at higher doses (100 mg/kg) the anticonvulsant effect at 0.5 ours is higher than at 4 hours (it should be taken into consideration that biotransformation frequently follows first order kinetics; therefore, the higher the dose the sooner that the metabolization product might reach effective concentrations). Further research, however, should obviously be conducted in order to test this hypothesis.

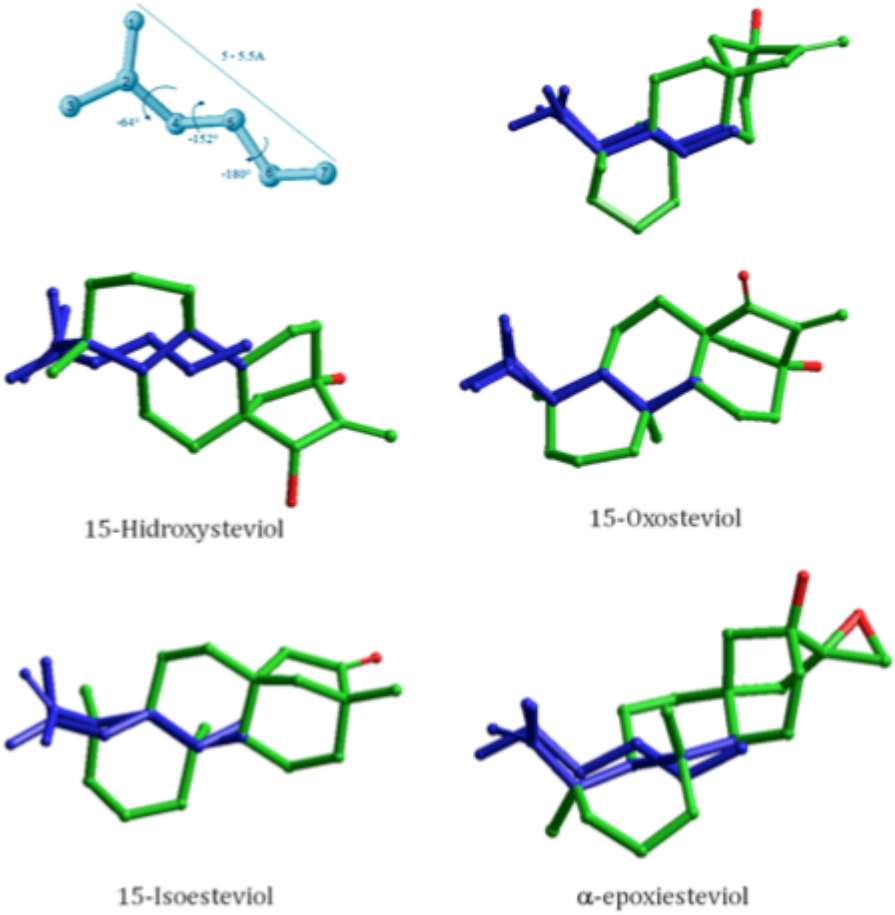
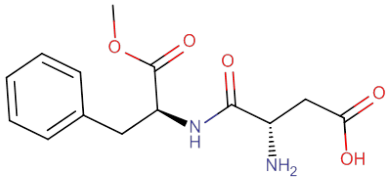


Fig. (4). Superpostion of steviol (upper left) and its phase I metabolites on the Sybyl pharmacophore.

ASPARTAME PROCONVULSANT EFFECTS

While all the non-nutritive sweeteners described in the previous section showed anticonvulsant properties, the artificial sweetener aspartame (Fig. **5**), on the contrary, has shown some evidence of proconvulsant activity in animals. Anecdotal cases of possible aspartame-induced seizures have also been reported [15]; according to FDA, the anecdotal complains received by the agency on aspartame-related seizures do not meet most of the criteria for causality [17]. Regarding randomized, double-blind, placebo controlled, crossover studies, Rowan *et al*. [18] and Shaywitz *et al*. [19] did not find evidence on the proconvulsant effect of aspartame at 50 mg/kg administered in divided doses on two days and 34 mg/kg administered for two weeks in a single morning dose, respectively. Camfield *et al*., however, studied 10 children with generalized absence seizures and found a significant increment in the total duration of spike-wave discharge per hour [20]. The later study has been criticized because of the choice of placebo and the length of the baseline period considered [17].



Aspartame

Fig. (5). Molecular structure of aspartame.

Animal studies showed a diversity of results. Though aspartame doses between 1000 and 3000 mg/kg failed to show proconvulsant effects in electroshock induced seizure models [21], kindled animals [21c, 22] and audiogenic seizures in epilepsy-prone animals [23], proconvulsant effect was observed in chemical-induced seizure models (majorly, the scPTZ test) when administering aspartame 1000-2000 mg/kg (po, mice) and 750-1000 mg/kg (po, rats) as a single bolus [21b, 24]. The same effect was not observed, however, when the 1000 mg/kg dose was divided in three 330 mg/kg administered over 120 minutes [21b]. The proconvulsant effect was neither observed in mice and guinea pigs at doses of up to 2000 mg/kg [25], though the proconvulsant effect at 1000 mg/kg in rat was confirmed [25a]. It has been observed that even if aspartame was proconvulsant at the reported doses, these are way above the human daily intake [17] and thus they are unlikely to represent a safety concern. While it should be taken into consideration that the results from animal models suggests high inter-species variability and that an animal dose should not be translated into a human equivalent dose by simple conversion based on body weight [26], dose extrapolation based on body surface area still results in an acute dose of aspartame far above the human daily intake.

Regarding the proposed molecular explanations to the effects of aspartame on the brain, the reader may consult the excellent review from Humphries *et al*. [27]. Essentially, the central effects of aspartame may result from its direct interaction with brain receptors (direct effect) or from the activity of its breakdown products/metabolites (e.g. phenylalanine, aspartic acid, methanol, phenylalanine methyl ester). Regarding its direct effects, aspartame acts as an agonist of glutamate on the NMDA receptor and exposure of rat hippocampal slices to 0.01, 0.1, 1 and 10 mM aspartame potentiated the response of hippocampal CA1 pyramidal cells [28]. In relation to indirect effects, it is thought that they may arise from altered transportation of amino acids such as tyrosine and tryptophan through the blood-brain barrier in present of abnormally high levels of phenylalanine emerging from aspartame breakdown. These and other amino acids compete for a neutral amino acid transporter; what is more, phenylalanine is converted to tyrosine in the liver. Remarkably, the previously mentioned amino acids are precursors of fundamental neurotransmitters such as dopamine and serotonin; thus, their altered flux through the blood-brain barrier might lead to increases or decreases in brain levels of different neurotransmitters [29], with the concomitant altered functioning. It should be highlighted that phenylalanine-induced convulsions may be blocked by valine, which limits the entry of the former into the brain [30].

THE ABC MODEL AND POSSIBLE MOLECULAR EXPLANATIONS TO THE ANTICONVULSANT ACTIVITY OF SWEETENERS

Back in 1980s Swanson and his colleagues hypothesized that, given two scientific facts with no evident and known relationship they might be indirectly linked through a third known fact related to both of them [31]. In fact, the probability of a direct connection between two elements or concepts increases with the number of shared connections. The former principle, which was named Swanson's ABC model, provided the basis for high-throughput literature analysis for the discovery of unsuspected and useful connections between scientific facts. This strategy can be used, for instance, as a tool for drug repositioning [32]. Naturally, it can also be applied to generate other type of valuable associations, as we have done here to construct a hypothesis on possible molecular explanations to the anticonvulsant activity of non-nutritive sweeteners.

After confirming our models predictions, we were puzzled by the fact that, consistently, artificial sweeteners elicited protective effects in seizure models; therefore, it was possible that some biochemical similarity existed between any of the molecular targets of antiepileptic drugs and the receptor which senses the sweet stimuli in humans. Literature search revealed that the sweet taste receptor is a heterodimer formed by two members of a family of proteins named T1R: T1R2 and T1R3 [33]. It was interesting to discover that T1R3 along with another member of the family, T1R1, compose another taste receptor, at least partially responsible for the umami flavor [34], which is present in foods with high glutamate content (the main excitatory neurotransmitter).

From this information we hypothesized that T1R3 (the common monomer between sweet and umami taste receptors) might have a structural link with any of the glutamate receptors in the brain that had previously been linked to epilepsy. A pBLAST alignment using human T1R3 sequence as query resulted in several extremely high significant matches with a number of mGluR from several species, including man and rodents. mGluR and the T1Rs

belong to G-protein coupled receptors from family C [35]. Among the mGluR receptors matched we found mGluR1 and mGluR5, which belong to group I mGluR [36], which are linked to activation of phospholipase C, IP_3 generation, release of Ca^{2+} from intracellular stores and PKC activation [37]. Glu stimulation in neural cells results in increased intracellular Ca^{2+} levels *via* differing signaling mechanisms, and it is well known that disregulation of Ca^{2+} is linked to excitoxicosis and neurodegeneration [38]. While mGluR1 activation results in a single and transient rise in intracellular Ca^{2+}, active mGlutR5 cause recurrent Ca^{2+} oscillations [35]. Both group I mGluR and T1R3 present a Ca^{2+} binding site [35, 39, 40]. There are plenty evidence linking group I mGluR and epilepsy. It has been suggested that prolonged activation of these receptors may be related to epileptiform activity in the brain [41]. Their expression and activity is modified in epileptogenesis [42, 43], kindling models of epilepsy [44] and refractory epileptic patients [45]. It has been recently found that low mGluR5 correlates with increased seizure frequency in patients with temporal lobe epilepsy [46]. These and other works have supported the interest in group 1 mGluR as novel potential targets for antiepileptic medication [47-49]. Particular interest has lately been given to allosteric modulators (Fig. **6**), since early mGluR antagonists designed to act as glutamate analogs stabilizing the orthosteric binding site at resting state lead to high polarity candidates with limited brain penetration, while allosteric modulators binding to the transmembrane domains are more likely to gather the typical physicochemical properties of central nervous system drugs [50]. In this sense, the recent work from D'Amore and colleagues results of much interest. These authors showed that both a selective positive modulator of mGluR1 (RO0711401) and mGluR5 (VU0360172) reduce spontaneous absence seizures in WAG/Rij rats [51, 52], with recent reports indicating that RO0711401 produces tolerance to treatment from the second day of a ten-day treatment [53], an effect that was not observed in the for VU0360172.

All the previous information has been condensed in the network illustrated in Fig. (**7**), which on the basis of the ABC model suggests that a possible explanation for the common protective effect of artificial and natural sweeteners might be their allosteric modulation of brain mGluR, following the high sequence similarity and biochemical function between the common monomer in sweet and umami taste receptors and mGluR and the fact that artificial sweetners interact with the sweet receptor to elicit the sweet response. The scientific facts that led us to propose such hypothesis are summarized as follows:

- Several natural and artificial sweeteners present protective effects in different animal models of seizure.
- A substance should interact with the sweet taste receptor to be recognized as sweet-flavored.
- The sweet taste receptor shares a common monomer (T1R3) with the umami taste receptor.
- Umami receptor senses glutamate.
- Glutamate is the main excitatory neurotransmitter.
- T1R3 has a very high sequence similarity to mGluR1 nad mGluR5.
- Sweet receptor, umami receptor and group 1 mGluR belong to G-protein coupled receptors from family C and thus act through the same basic molecular mechanism (activation of phospholipase C, IP_3 generation, release of Ca^{2+} from intracellular stores).
- Umami receptors and group 1 mGluR bind Ca^{2+}.
- Excesive/prolonged Ca^{2+} release leads to neural damage.
- mGluR are known to modulate excitatory activity in the brain.
- mGluR are molecular targets of antiepileptic drugs.
- Group 1 mGluR are subjected to regulated expression in animal models of epilepsy.
- Prolonged activation of mGluR leads to epileptiform activity.
- Group 1 mGluR are upregulated during epileptogenesis.

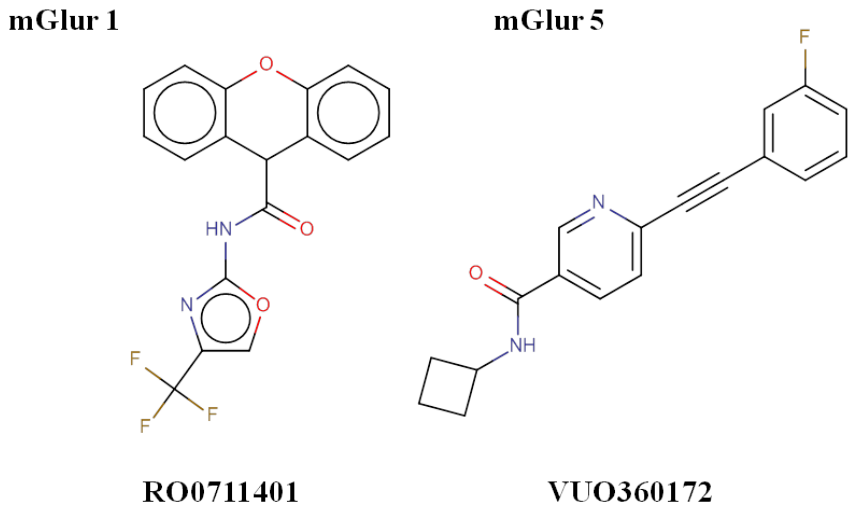


Fig. (6). Selective modulators of group I mGluR with anticonvulsant properties.

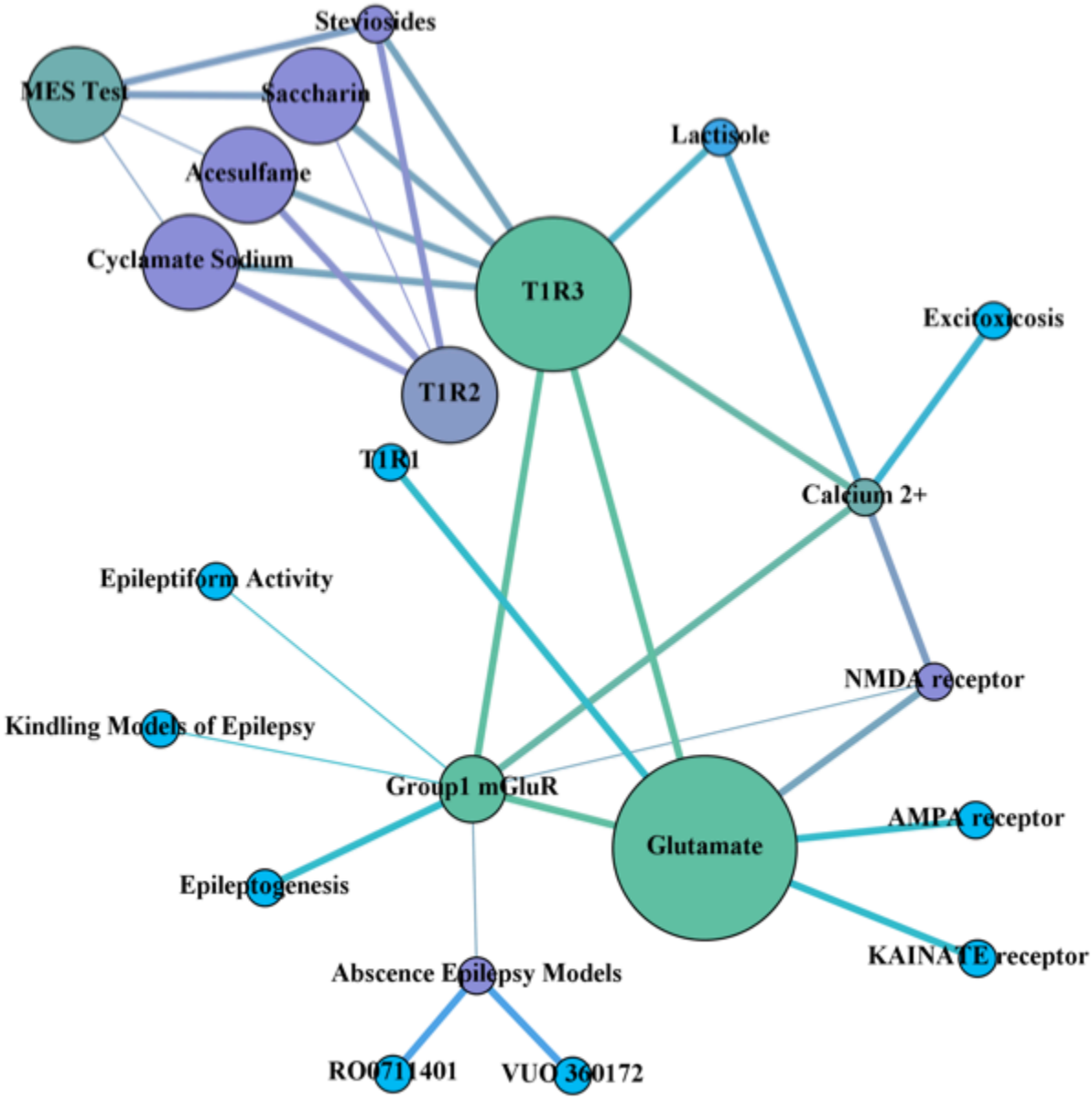


Fig. (7). Network of elements supporting a possible interaction between sweeteners and mGluR.

- Positive allosteric modulators of group I mGluR have shown protective effects in animal models of absence epilepsy.

Noteworthy, an association has recently been described between carbonic anhydrase subtype VII and febrile seizures [54] and high affinity has also been reported between saccharin and that carbonic anhydrase isoform [55], which may indicate a second potential target for non-nutritive sweeteners related to epilepsy. Provided the 3D structures of sweet taste receptor and mGluRs were solved, future docking studies should be recommended to advance in the validation of the previous hypothesis.

CONCLUSION

This review has illustrated the potential of virtual screening approaches in drug repositioning campaigns; in this case, the potentially repositioned candidates are not therapeutic agents (as usually) but food additives, namely non-nutritive sweeteners. We have also exemplified the utility of Swanson's ABC model to build scientific relevant hypothesis, in this case a possible molecular explanation for the consistent protective effects of different sweeteners in animal seizure models. On this basis, we proposed group I mGluR as possible molecular targets explaining the protective effects of cyclamate, saccharin, acesulfame and steviosides, which would likely act as allosteric modulators. This hypothesis supports further studies to confirm if mGluR are indeed molecular targets of non-nutritive sweeteners. Testing the potential anticonvulsant activity of other known ligands of sweet and umami taste receptors (e.g. lactisole) is another possibility that emerges from the current work. Taking into consideration possible inter-species variabilities in drug response, it is convenient to test the anticonvulsant sweeteners in other species apart from mice.

ABBREVIATIONS

ANOVA = Analysis of Variance

MES = Maximal Electroshock

mGluR = Metabotropic glutamate receptors

PTZ = Pentylenetetrazole

scPTZ = Sub-cutaneous Pentylenetetrazole

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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