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Carbonic Anhydrase Activators. The Selective Serotonin Reuptake Inhibitors Fluoxetine, Sertraline and Citalopram Are Strong Activators of Isozymes I and II

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Abstract—The selective serotonin reuptake inhibitors (SSRI) fluoxetine, sertraline and citalopram have been investigated for their ability to activate two carbonic anhydrase (CA) isozymes, hCA I and hCA II, in parallel with two standard activators for which the X-ray structure (in complex with isozyme II) has been resolved: histamine and phenylalanine. All three SSRI activated both isozymes with potencies comparable to that of the standards although the profile was different: for hCA I, best activators were fluoxetine and histamine, with citalopram and sertraline showing weaker activity. For hCA II, the best activators were phenylalanine and citalopram, and the weakest histamine and sertraline, whereas fluoxetine showed an intermediate behavior. These results suggest that SSRI efficacy in major depression complicating Alzheimer's disease may be partly due to their ability to activate CA isozymes and may lead to the development of potent activators for the therapy of diseases associated with significant decreases in brain CA activity.

Introduction

Activation of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) by a multitude of physiologically relevant compounds such as biogenic amines (histamine, serotonin, catecholamines), amino acids or oligopeptides/small proteins among others, has only recently been explained at the molecular level.[1–3] By means of electronic spectroscopy, X-ray crystallography and kinetic measurements, it has been proved that the activator molecule binds within the enzyme active cavity at a site distinct of the inhibitor or substrate binding-sites, participating thereafter in the rate-determining step of the catalytic cycle, that is, the proton transfer processes between the active site and the environment.[1–3]

Except for clarifying basic aspects of the catalytic mechanism of this class of widely spread enzymes over the phylogenetic tree,[4,5] CA activators might also possess pharmacological applications, although this field is largely unexplored for the moment. Thus, recently it has been reported[6] that phenylalanine, a CA activator first investigated by this group,[1,3] when administered to experimental animals produces an important pharmacological enhancement of synaptic efficacy, spatial learning and memory, due to the rapid and efficient increase of bicarbonate concentration in memory-related neural structures.[6] CA activators might thus be useful in the management of conditions in which learning and memory are impaired, such as Alzheimer's disease (AD) or aging, since an increased bicarbonate flux through synaptic $GABA_A$ receptor channels alters postsynaptic neuronal responses to GABA and thus, neuronal responses to different signal inputs.[6] Diverse CA isozymes are highly expressed in the brain, more precisely in neurons,[7] and recent immunocytochemical data showed that within the hippocampus (the structure mediating learning and similar cognitive tasks)[8] all types of principal cells express high amounts of CA.[7] It should be also mentioned that it was previously reported that the levels of several CA isozymes are significantly diminished in the brain of patients affected by AD,[9] a fact strongly supporting the involvement of CAs in cognitive dysfunctions characteristic of this disease.[4,6,9]

The main clinical symptoms of AD include memory decline, thinking impairment, aphasia, impairment of intelectual function, dementia, etc., whereas neuronal loss is the main neuropathological feature underlying the symptoms of this disease.[10] The therapeutical strategies include three classes of agents: (i) disease-modifying

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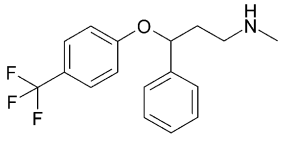
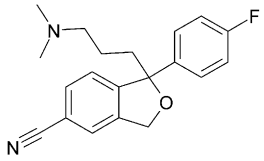
agents such as vitamin E and selegiline; (ii) drugs that compensate for neurotransmitter deficits, such as acetylcholine esterase inhibitors, and (iii) psychotropic agents administered to relieve behavioral symptoms.[11] However, the mechanisms of action of all these drugs are little understood at the moment.

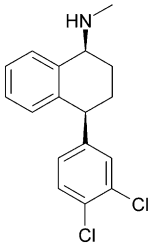
Recently, the well known SSRI antidepressants fluoxetine **1**, sertraline **2** and citalopram **3** were shown to be very effective in patients with Alzheimer's disease who also have major depression,[13] although the mechanism(s) underlying this action too remains to be elucidated.[11,12] Considering the presence of secondary/tertiary amino groups that may act as proton shuttling moieties in enzyme-activator adducts, as well as other favourable structural elements present in the molecules of these three pharmacological agents,[1] we decided to investigate their possible CA activating properties. Here we report the potent activatory properties of compounds **1**–**3** against the most widespread isozymes, hCA I and hCA II, both of which are associated with critical physiological functions in a multitude of tissue.[1-4]



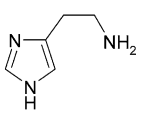
1 Fluoxetine



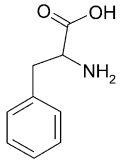
2 Citalopram



3 Sertraline



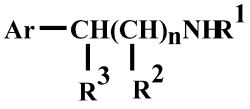
5 Histamine



6 Phenylalanine

Chemistry

In order to act as a CA activator, a compound needs precise steric and electronic factors to be present in its molecule. Most of the efficient activators investigated up to now were shown to possess the general formula **4**.[1–3,14–18]



4

Ar = aromatic/heterocyclic group

$R^1 = R^2 = H, Me$

$R^3 = H, OH, COOH$

n = 1, 2, 3

These derivatives incorporate a bulky aromatic or heterocyclic moiety, and a primary/secondary amino group acting as proton shuttling moiety, these two structural elements being separated by a chain of two–four sp^3 hybridized carbon atoms, possibly substituted as shown above, with compact alkyl (methyl), hydroxy or carboxyl moieties.[1] Indeed, the only X-ray crystal structures of CA-activator adducts (the histamine (**5**)-hCA II adduct,[2] and the phenylalanine (**6**)-hCA II-azide ternary complex[3a]) reported up to now, showed the importance of these structural elements for enhancing the proton transfer processes between the enzyme active site and the environment. Practically, these two simple activators (histamine and phenylalanine) bind at the entrance of the enzyme active site between amino acid residues His 64 (the natural proton shuttle),[1] Gln 92 and Asn 62, having the bulky aromatic/heterocyclic ring (Ar in structure **4**) oriented towards the hydrophobic half of the active site, and the amino groups pointing towards the solvent, and taking part in the transfer of protons between the active site and the environment.[1–3] In fact, using such simple CA activators as lead compounds, a large number of more efficient activators have ultimately been developed by this group.[14–18] Returning to *the* SSRI **1**–**3**, it is obvious that they possess the three structural elements required for acting as a CA activator, that is, a proton shuttling residue (of the methylamino or dimethylamino type), a bulky aromatic/heterocyclic ring, and the aliphatic chain connecting these two moieties.

CA Activation

Activation data against two CA isozymes (hCA I and hCA II) with fluoxetine **1**, citalopram **2** and sertraline **3**, as well as standard CA activators (such as histamine-Hst **5**, and phenylalanine-Phe **6**) are shown in Figures 1 and 2, for the CA-catalyzed CO_2 hydration (the physiological

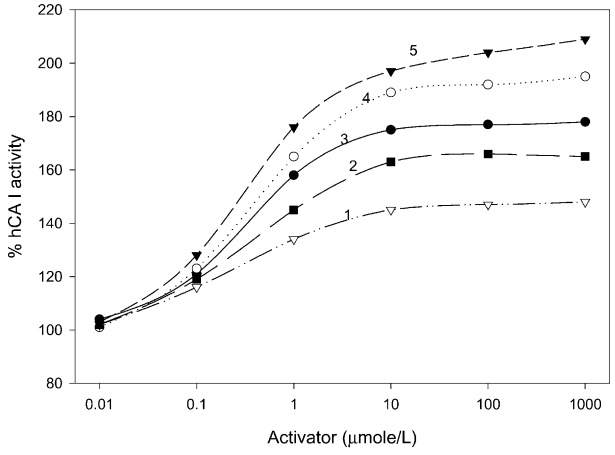


Figure 1. hCA I activation data with compounds **1**–**6**: curve 1-citalopram **2**; curve 2-sertraline **3**; curve 3-phenylalanine **6**; curve 4-histamine **5**; curve 5-fluoxetine **1**. Enzyme activity in the absence of activator has been taken as 100% (errors were in the range of 5% from triplicate experiments).

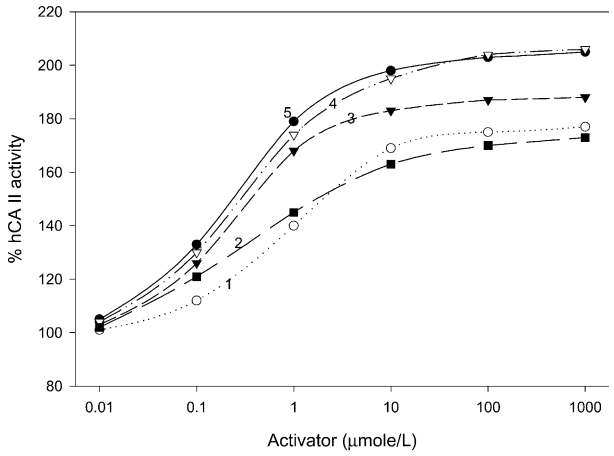


Figure 2. hCA II activation data with compounds **1**–**6**: curve 1-histamine **5**; curve 2-sertraline **3**; curve 3-fluoxetine **1**; curve 4-citalopram **2**; curve 5-phenylalanine **6**. Enzyme activity in the absence of activator has been taken as 100% (errors were in the range of 5% from triplicate experiments).

reaction).[19] Very similar results have also been obtained for the esterase activity of these enzymes, when working with 4-nitrophenyl acetate as substrate (data not shown).[20]

As seen from Figure 1, the five investigated compounds dose-dependently activate isozyme hCA I, in the concentration range of 0.01 μM–1 mM, for the physiological reaction catalyzed by it, that is, CO_2 hydration to bicarbonate. Notable activatory effects start to be observed at around 1 μM concentration of activator present in the reaction medium, both for the two standards investigated (histamine **5** and phenylalanine **6**) as well as for the newly investigated derivatives **1**–**3**. Thus, histamine **5**, one of the best hCA I activators investigated in detail,[2] activates this isozyme with 165% at 1 μM, wheras increasing its concentration leads to a maximal activatory effect of around 185% (at 1 mM activator present in the assay system). Phenylalanine **6** is slightly less effective than histamine, arriving at a maximal activation of 160% for hCA I. To our surprise, the best hCA I activator in this series of derivatives was fluoxetine **1**, which was more effective than histamine, arriving at an activation of about 175% at 1 μM, and a maximal effect of 210% at 1 mM concentration. Sertraline **3** and citalopram **2** were much weaker hCA I activators as compared to fluoxetine **1**, their activity being also weaker than that of phenylalanine. Thus, at 1 μM they achieved an activation of 134% (citalopram) and 145% (sertraline), respectively, whereas the maximal effect reached a plateau of around 148% for citalopram and 165% for sertraline.

For isozyme hCA II, again the dose–dependence curves showed all these compounds to behave as effective activators (Fig. 2). Furthermore, the activation profile of this isozyme is very different from that of hCA I, a fact well-known for activators such as histamine or phenylalanine.[1–3] Thus, histamine is a much weaker hCA II activator (as compared to its behavior against isozyme I), whereas phenylalanine on the contrary, is a very efficient hCA II activator and a rather inefficient isozyme I activator. Indeed, as seen from curves 1 and 5 of Figure 2, the maximal activatory effect of histamine is of 175%, whereas that of phenylalanine of around 210% againts hCA II. Sertraline **3** had a very similar behavior with histamine, the curves of the two compounds being hardly distinguishable (curves 1 and 2 in Fig. 2). Citalopram, the weakest hCA I activator in this series, behaved on the other hand as a very potent hCA II activator, its behavior being quite similar with that of phenylalanine (curves 4 and 5 of Fig. 2). Fluoxetine showed an intermediate behavior between that of the weak activators histamine and sertraline, and the one of the strong activators citalopram and phenylalanine, activating hCA II with around 170% at 1 μM, and a maximal effect of around 190% at 1 mM concentration. In patients with depression fluoxetine achieves steady-state plasma concentrations around 1 μM, even higher its active metabolite norfluoxetine.[21] In rodents fluoxetine and norfluoxetine concentrate markedly in brain tissue, reaching concentrations several times those in plasma, like the other SSRI agents.[22]

Conclusions

The SSRI fluoxetine, sertraline and citalopram possess structural elements typical of amine type CA activators, and have been investigated for their ability to activate isozymes hCA I and hCA II. For comparison, two standard CA activators for which the X-ray structure in complex with hCA II has been resolved (histamine and phenylalanine) were also included in the assay. All three drugs dose-dependently activated both isozymes with potencies comparable to that of the standards, but the profile was different: for hCA I, best activators were fluoxetine and histamine, with citalopram and sertraline showing weaker such properties. For hCA II, the best activators were phenylalanine and citalopram, and the weakest histamine and sertraline, whereas fluoxetine showed an intermediate behavior. It is probable that similarly with the standards histamine and phenylalanine, the compounds investigated here take part in the CA catalytic cycle, favoring the rate-determining step, the proton transfer reactions between the active site and the reaction medium. In fact all these activators possess primary, secondary or tertiary amino groups that may assist such processes, allowing thus for supplementary pathways of proton release between the active site cavity and the environment. This suggest that the efficacy of the SSRI fluoxetine, sertraline and citalopram in patients with AD who also have major depression may be due, at least in part, to their CA activating properties. These drugs may therefore constitute leads for the development of more potent CA activators for use in therapeutics in need of activation of this enzyme.

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