Is there a role for uridine and pyrimidine nucleosides in the treatment of vascular dementia?

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Summary

In the 70s, the discovery of a constant loss of acetylcholine (Ach) in the brains of people suffering from dementia led to the development, in order to improve cognitive functions, of drugs that increased Ach levels. The possibility that loss of a given neurotransmitter might be associated with the onset of a specific neurological syndrome led to suggestions that, as had already been found in Parkinson’s disease, replacement therapy might drastically improve the course of the syndrome. We are now aware of the limits of this therapeutic approach.

In this review, we analyse potential factors contributing to the partial failure of Ach replacement therapy, contrasting common beliefs regarding the Ach synapse with the difficulties in restoring its activity through replacement drugs. Considering the search for alternative strategies, in the second part of the review, we overview progress of research into pyrimidine compounds, now emerging as a new modulatory system acting through specific pyrimidino-receptors involved in various steps of cell signalling. Pyrimidine nucleosides might be useful in the chronic treatment of cognitive deficits resulting from vascular dementia.

KEY WORDS: Acetylcholine; Alzheimer’s disease; nucleosides; pyrimidines; vascular dementia.

Introduction

The pyrimidine system (Fig. 1, see over) is a recently discovered modulatory pathway in the central nervous system (CNS) (1-3), which is able to modify synaptic connectivity and modulate long-term phenomena, like sensitization to catecholamine-releasing drugs (4). In particular, while a single injection of pyrimidines does not modify dopamine (DA) release, chronic treatment produces an increase in amphetamine-induced sensitization to DA release and augments DA turnover (5). Inborn deficits of pyrimidine nucleosides lead to development of early cognitive impairment and pervasive developmental disorders in children (6,7). In keeping with this, chronic administration of these nucleosides produces trophic effects in various experimental conditions (3,8), and is able to improve recovery processes taking place after neural damage following metabolic insults (9). Recent literature substantiates the therapeutic potential of long-term treatment with pyrimidine nucleosides, with evidence ranging from experimental models to clinical settings (1,3). This evidence sheds light on the potential usefulness of long-term treatment with these compounds to cure cognitive deficits occurring in vascular dementia (10).

In spite of the variety of triggering mechanisms, the term “vascular dementia” refers to a clearly defined neurological syndrome, in which cognitive decline, including memory loss, is the common feature (11). This cognitive decline is induced by a variety of cerebral vascular disorders, which makes these “dementia syndromes” a major problem for the public health sector and a great challenge for neuropharmacology (12-14). The extensive search for the neurotransmitter(s) responsible for sustaining “dementia syndromes” dates back to the early 70s. Since this time, reduced acetylcholine (Ach) has been a constant finding in the demised brain (15-25). The possibility that loss of a given neurotransmitter might be associated with the onset of a specific neurological syndrome led to suggestions that, as had already been found in Parkinson’s disease, replacement therapy might produce drastic improvements. Based on the belief that the Ach deficit in dementia might be comparable with the DA deficit seen in Parkinson’s disease, an analogous therapeutic approach was tried, aiming to restore brain Ach levels (14).

In keeping with this, various drugs have been employed including receptor agonists, Ach precursors and inhibitors of the degrading enzyme Ach-esterase (14,26).

In spite of expectations, none of these therapeutic approaches provided anything comparable with the Parkinson’s disease therapy. Indeed, looking at their symptomatic effects, the efficacy of all these drugs, including Ach-esterase inhibitors, is seen to be low (14). Despite being aware of the role of Ach in sustaining visuospatial memory, and recognizing its critical modulation of cognition (27-29), we cannot simply assume that this neurotransmitter is the only factor responsible for memory and cognition. Therefore, it seems necessary to look at other neurotransmitters, which, also being involved in cognitive functions, could play a role in the biochemistry of dementia. This applies in particular to the case of vascular dementia, in which there is no...
stereotyped neuropathology and the variety of brain areas involved might imply a constellation of neurotransmitter deficits (22,30,31) sustaining the cognitive symptoms. In degenerative dementia, too, several data associate loss of neurotransmitters other than Ach with the onset and the severity of cognitive decline. These include monoamines noradrenaline (32-35), serotonin (29,36,37) and dopamine (38-40), and the amino acid neurotransmitter glutamate (41-43). All these compounds might be reduced in the demented brain, although less constantly than Ach (19,23,28,30,44).

In this brief overview we focus on potential long-lasting effects of available drugs, which might offer a new perspective on vascular dementia. Our starting point is the cholinergic synapse, since most of the therapeutic trials have targeted this site.

The cholinergic synapse as a target for neurotoxins and therapeutic drugs in dementia

In the last three decades, research efforts focusing on the Ach synapse have sought to reproduce the biochemical deficit through selective neurotoxic lesions of the Ach system, using this model to evaluate the efficacy of Ach-based replacement therapies (14). As shown in Figure 2 and Table I, the Ach synapse possesses various target sites, which are vulnerable to neurotoxic mechanisms and, at the same time, are targeted by currently used therapeutic agents.

Knowledge of the neurotoxicology of the cholinergic system is essential in order to understand the biochemical basis of dementia. This, in turn, is essential both for the creation of experimental models of dementia, and for the search for common mechanisms that might be similar in the action of neurotoxin and in the spontaneous degeneration of the cholinergic system (45).

The empirical use of cholinergic neurotoxins dates back to a time long before the anatomical and chemical definition of central cholinergic pathways. Compared with toxins affecting other neurotransmitters, cholinergic neurotoxins constitute the most widespread component of defense mechanisms produced by living organisms like plants, bacteria and animals (46). This is due to the fact that the cholinergic system is a highly conserved neuronal pathway, whose impairment has dramatic effects on any living organism. In addition to naturally occurring cholinergic neurotoxins,
there exist synthetic compounds that possess a high degree of selectivity. One of these is AF64A, which destroys, specifically, central cholinergic nerve endings. Almost all the steps and components involved in the Ach synapse (shown in Fig. 2) might be targets for Ach neurotoxins (Table II).

Table II - Specific neurotoxins for Ach neurons

<table>
<thead>
<tr>
<th>TOXINS</th>
<th>TARGETS</th>
</tr>
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<tbody>
<tr>
<td>Kainate, Quinolinic acid, Glutamate presynaptic receptors</td>
<td></td>
</tr>
<tr>
<td>Bromo-ACh, mono-/di-/tri-ethylcholine</td>
<td>ChAT</td>
</tr>
<tr>
<td>Hemicolinium-3, AF64-A and analogs</td>
<td>HAcHT</td>
</tr>
<tr>
<td>Vesamicol, Cetiedil, Ach false nts</td>
<td>VAcHT</td>
</tr>
<tr>
<td>Physostigmine, Soman, Sarin</td>
<td>AchE</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>Ach receptors</td>
</tr>
</tbody>
</table>

In this class we should include two main groups of compounds:
1) Pre-synaptic toxins: including bacterial neurotoxins and most spider and snake toxins (erabutoxin, omega cobra toxin and alpha-bungarotoxin, as well as the synthetic compound AF64A). These compounds bind to Ach presynaptic terminals leading to increased Ach release.
2) Post-synaptic toxins: including irreversible antiacetylcholinesterases (organophosphates, carbamates, etc.) and potent Ach receptor agonists.

Interestingly, if one considers the biochemical targets of drugs affecting the cholinergic system, and the steps affected by neurotoxins, it is possible to observe a broad overlap. This is true of Ach receptors (24,45) (Fig. 2), which are stimulated by selective pharmacological agonists (21,47,48), thus providing a specific replacement therapy. The role of these receptors (49) is demonstrated by the cognitive decline and the worsening of memory and cognitive functions occurring in old people treated with receptor antagonists. This observation contributed to the genesis of the Ach hypothesis of dementia.

Another step at which replacement therapy is employed is the Ach precursor choline, which is selectively taken up by Ach nerve endings through a high-affinity vesicular transporter (VChT). After being released, Ach binds either to pre- or post-synaptic receptors (Ach-R), thereby exerting its ionotropic and metabotropic activities. Sudden degradation of Ach by the enzyme Ach-esterase (AchE) suppresses the short activity of the neurotransmitter. The enzymatic products are acetate and choline. The latter is taken back up into the Ach terminals by a specific Ach transporter (HAcHT), which is distinct from the one located on the membrane of the vesicles.

Table I - Targets for neurotoxins at cholinergic neurons

1) Glutamate receptors on cholinergic neurons
2) ChAT (choline acetyltransferase)
3) HAcHT (High-affinity choline transporter)
4) VAcHT (vesicular acetylcholine transporter)
5) AchE (acetylcholine esterase)
6) Presynaptic cholinergic receptors

From the biochemical basis of pharmacology to therapeutics: the limits of Ach-based replacement therapy

Despite the fact that knowledge of the Ach synapse ranges from biochemical mechanisms and neurotoxic...
effects to molecular targets for therapy (25), we still do not possess drugs whose effects are sufficient to provide marked symptomatic relief of cognitive decline (14,24). In particular, neuropharmacology is suffering from the lack of an efficacious replacement therapy. This discrepancy, already mentioned in the introduction to this review, leaves us with several open questions that need to be answered: either our knowledge concerning the loss of specific neurotransmitters is a fallacy, or there exists something that makes it difficult to replace the reduced neurotransmitter. Finally, one should consider the possibility that both explanations might coexist, i.e., that the Ach deficit may not be solely responsible for the syndrome and/or that it is difficult to replace. The first hypothesis leads us to re-consider the role of other neurotransmitters, mentioned above, in sustaining learning and memory, and therefore to consider alternative targets in the therapy of dementia. This might be crucial for vascular dementia, in which the neuropathological pattern is variable and in which distinct pathological (12,13) and biochemical (30) features might co-exist and sustain common cognitive deficits. Even assuming that Ach is constantly implicated, the loss of specific brain nuclei in vascular dementia does not suffice to explain the syndrome. Various cholinergic neurons give rise to topographically organized cortical projections, whose relative role in learning and memory, as well as in cognitive functions in general, is not specifically established. Target areas include the thalamus, septum, habenula, hippocampus and neocortical regions (28,50,51). The biochemical and electrophysiological differences between these cholinergic synapses remain, at present, unknown.

Moving on to the second point, one should consider that progress made in basic pharmacology of therapeutics in neurology has been somehow “spoiled” by the sudden success of L-DOPA replacement therapy in improving the symptoms of Parkinson’s disease. Albeit a wonderful example of a projected therapy, this was in fact a success due to serendipity. In fact, the tonic pattern of DA release is, at least during early stages of the disease, easily mimicked by a replacement therapy. But this does not seem to be true of Ach, possibly due to the lack of a prolonged synaptic efficacy of Ach compared with DA. In particular, Ach release is finely tuned, relying on critical spatial (the synaptic cleft) and temporal (extremely short persistence) patterns. Instead, this is not a critical issue for DA, which has the synaptic properties of a paracrine modulator rather than a classic neurotransmitter (52,53).

Bearing in mind the insufficient symptomatic effects of Ach replacement therapy, one should consider whether available drugs, by acting through alternative pathways, might be able to contribute to improving the natural course of the disease.

The limits of Ach replacement therapy are increased in vascular dementia

This issue becomes more critical when facing vascular dementia, whose wide constellation (at least eight pathological patterns) makes the biochemistry responsible for cognitive decline much less well defined (31). Dementia caused by cerebral vascular diseases results from thromboembolic episodes, hypoxic-ischemic attacks (cardiac arrest or hypotension), and hemorrhagic diseases. In this plethora of disorders affecting a variety of brain regions it is difficult to isolate a common alteration responsible for the cognitive decline (11). It seems, rather, that lesions of different brain areas might concur to produce a deficit of cognitive functions. Even though it has been established that dysfunctions of certain brain vessels in a few brain regions are frequently associated with cognitive symptoms, a common observation is the lack of involvement of any “strategic area” that might fit in with the traditional cholinergic hypothesis of dementia. On this basis, the biochemical specificity may vary substantially, involving different cholinergic nuclei as well as other neurotransmitters, critical for sustaining cognitive functions.

While the biochemical pathways involved in vascular dementia do not appear well defined (11,22,30,54), the neuronal alterations caused by a deficit of blood supply seem, examined at cellular level, to be quite stereotyped (55). Taking this as a starting point the problem is shifted away from the targeting of specific neurotransmitters, and towards the protecting of specific cellular pathways, which are sensitive to a deficit in the oxygen supply. The main cell components suffering from reduced blood supply are the mitochondria and their oxidative metabolic pathways.

The pyrimidines as a novel modulatory system

The pyrimidine nucleosides are among several compounds metabolized at mitochondrial level. These molecules have recently been studied as potentially involved in the regulation of cell physiology, and various pharmacological studies have demonstrated their role in modulating neuronal plasticity (1-3). These latter processes might be crucial in counteracting the progression of cognitive decline resulting from vascular alterations and they should thus be potentiated by long-term therapies. Recently, there has been a growing interest in the modulatory effects obtained by pyrimidine nucleosides. This is based both on the discovery of at least three metabotropic P2 receptors, which are stimulated by pyrimidine nucleosides with a potency at least equal to purine analogs, and on the existence of pyrimidine ionotropic receptors (3). Interestingly, agonists at these receptors have recently been shown to produce a variety of effects, which confer neuroprotection in vascular dementia (54).

Pyrimidine nucleosides include a class of endogenous compounds that are synthesized from orotic acid (7). Among them, we find cytidine, uridine, or the metabolic product of their metabolism cytidine diphosphocholine (CDP) (56). As shown in figure 1, pyrimidine nucleosides possess a neuronal metabolism (57). They are interconverted by ubiquitous enzymatic pathways which are responsible for producing phosphorylated nucleotides (CTP, UTP), for converting one nucleoside into the other, for degrading the nucleosides to their corresponding base, or for binding them in complex molecules that are precursors of membrane synthesis, like CDP (2). Unlike purine nucleosides, pyrimidines can be absorbed by the oral route and cannot be recycled from their base (3). Pyrimidines are kept at low concentrations in the blood and cerebrospinal fluid, as proven by the fast clearance of a high dose of uridine given i.v.. This is
Pyrimidines in vascular dementia

thanks to the existence of mechanisms finely tuning pyrimidine plasma levels.

In their elegant review, Connolly and Duley (3) recently summarized the renewed interest in uridine and cytidine that is based on data showing the effects of these nucleosides on cell physiology, including energy metabolism and the activation of specific newly discovered pyrimidine receptors (2). Their potential applications relate to metabolic disorders involving enzymatic deficits located upstream in the biochemical pathways synthesizing these compounds. However, based on their mechanisms of action, pyrimidine nucleosides have been put forward as possible candidates to improve the pharmacological treatment of circulatory disorders involving the CNS (3). We analyze whether there is a role for these compounds in the long-term therapy of cognitive disorders resulting from vascular insults and possibly in degenerative dementia.

Effects of uridine, cytidine and related pyrimidine nucleosides

Apart from their physiological role in cell metabolism, uridine and related metabolites are known to bind to specific receptors located on plasma membrane, the so-called “pyrimidino-receptors” (2). On this basis it has been suggested that pyrimidines, as shown for adenosine and other purines, might, when released from pyrimidine storing cells, act as modulators of neural functions. This hypothesis has been confirmed in a glial cell line showing a release of uridinetriphosphate (UTP) which, in turn, can be metabolized by either glial or neuronal cells possessing ectonucleotidases (2) (Fig. 1). In keeping with a significant effect during a deficit of vascular supply, pyrimidine compounds maintain brain metabolism during ischemia and severe hypoglycemia showing that the activity of the human brain largely relies not only on glucose, but also on the supply of circulating pyrimidines (3).

This emerging role of pyrimidines as neuromodulators is supported by data obtained in neuropharmacological studies. These findings are crucial in substantiating a role for these molecules in cerebral metabolic diseases including vascular disorders.

In keeping with this, blockade of pyrimidine metabolism is implicated in the onset of pervasive developmental disorders, whereas specific genetic disorders of pyrimidine metabolism can be manifest in children with mental retardation.

These data, together with long-term clinical studies on cerebral vascular dementia (see later), provide striking symptomatic improvement, possessing a trophic efficacy in the treatment of dementia (9).

Concluding remarks

At present, we do not possess any drug readily able to revert cognitive impairment due to vascular deficits and/or degenerative processes. Replacement therapy designed to improve Ach transmission remains, in spite of the availability of several drugs, a remedy of little efficacy. The literature is currently pointing to a novel class of neuromodulators, the emerging pyrimidine compounds, as potential therapeutic agents in CNS disorders. These compounds include uridine, cytidine, CDP and other naturally interconvertible molecules (2,56). The relationship between pyrimidine nucleosides and neurotransmission remains at present very complex and fascinating. As occurred with the purines, recent data suggest the presence in the CNS of a newly-discovered signaling pathway, which produces long-term effects involving
synaptic plasticity, trophic activity and neuroprotection. This evidence converges with data showing mental retardation in humans with inborn pyrimidine metabolism defects and with long-term clinical studies showing an effect of pyrimidines in vascular dementia. Experimental studies specifically aimed at defining the potential mechanisms of pyrimidine neurotransmission will better elucidate the biochemical basis of these effects in order to optimize the therapeutic use of pyrimidine nucleosides, as in the therapy of neurological deficits in which recovery processes play a key role. For instance, compensatory mechanisms are crucial in contrasting the stepwise deterioration of cognitive symptoms occurring in vascular dementia. Finally it should be considered that abnormal deposition of beta-amyloid, which characterizes Alzheimer's disease, leads to specific vascular endothelial damage (64), turning Alzheimer's disease into a microvascular disorder (65). This is more than a general hypothesis and it renders the pathogenesis of vascular and degenerative dementia partially convergent, calling for common therapeutic strategies.

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