

# Growth Factors and Lymphokines: Modulators of Cholinergic Neuronal Activity

Rémi Quirion, Dalia M. Araujo, Paul A. Lapchak, David Seto and Jean-Guy Chabot

**ABSTRACT:** It is well known that various markers of the cholinergic synapse are altered in Alzheimer's Disease. Much interest is currently focussing on the evaluation of the possible efficacy of certain growth factors, especially nerve growth factor (NGF), to reduce or reverse cholinergic neuronal losses. Here we report that other growth factors (epidermal growth factor and insulin-like growth factor I) and a lymphokine, interleukin-2, are able to block acetylcholine release in the rat hippocampus. This suggests that while certain growth factors like NGF may have positive effects on the cholinergic neuron, others may act as "negative" factors on this neuronal population.

**RÉSUMÉ:** Facteurs de croissance et lymphokines : des modulateurs de l'activité neuronale cholinergique. Il est bien connu que l'innervation cholinergique est déficiente dans la maladie d'Alzheimer. Récemment, plusieurs équipes ont évalué l'efficacité possible de certains facteurs de croissance (et plus spécialement le facteur de croissance nerveux, NGF) à diminuer ou renverser ces pertes d'activités cholinergiques. Nous nous sommes plus spécialement intéressés aux facteurs de croissance épidermique et insulinique de type I, et à l'interleukine-2. Nous avons observé que ces trois substances bloquent directement la libération de l'acétylcholine dans l'hippocampe de rat. Ceci suggère que certains facteurs de croissance ont probablement des effets négatifs sur l'activité du neurone cholinergique. Il semble donc qu'il serait important de déterminer les modifications possibles du rapport entre les facteurs positifs et "négatifs" dans la maladie d'Alzheimer et des effets sur la survie du neurone cholinergique.

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It is now well recognized and established that while various neurotransmitter systems may be altered in Alzheimer's Disease (AD), the only one to be consistently affected in most, if not all cases, is the cholinergic system.<sup>1-3</sup> Others (*e.g.*, noradrenergic, serotonergic and peptidergic) may be modified in a certain percentage of patients, perhaps due to the existence of sub-types of AD.<sup>2,3</sup>

Thus, a great deal of interest has recently focused on the characterization of the functional organization of the cholinergic synapse.<sup>2,3</sup> Recently obtained information<sup>4,6</sup> suggests new approaches to stimulate cholinergic neurons. For example, the selective blockade of negative muscarinic M<sub>2</sub> autoreceptors (Table 1)<sup>7,12</sup> or the activation of postsynaptic M<sub>1</sub> receptors by highly specific molecules<sup>13</sup> could be more effective than any non-selective cholinergic drugs used thus far in clinical trials of AD (see recent reviews).<sup>1-3,6</sup> This could be of great relevance for the design of effective therapeutic approaches toward the treatment of AD.<sup>2,3,6</sup>

An alternative approach is based on the use of trophic factors either to reverse or slow down cholinergic neuronal losses.<sup>14-16</sup> Thus far, most interests have focussed on nerve growth factor (NGF).<sup>14-16</sup>

## NGF and AD

It has clearly been demonstrated that NGF can very effectively protect cholinergic neurons against excitotoxins as well as rescue them following exposure to toxins or axotomy (for reviews<sup>14-16</sup>). Behaviorally, chronic infusions of NGF have been shown to restore cholinergic memory functions in young as well as aged animals.<sup>17,18</sup> However, rather surprisingly, very few studies have reported on the status of NGF-like systems in AD.<sup>14-16</sup> Preliminary data tend to suggest that NGF, its mRNA and receptors are not altered in AD.<sup>14-20</sup> Thus, it is unlikely that losses of cholinergic neurons seen in AD are related to deficits in NGF and/or its receptors. Consequently, other growth factors could be involved in the pathogenesis of AD.

In any case, the possible use of NGF and related molecules in AD is currently generating much interest.<sup>14,15</sup> However, the recent demonstration of the existence of high densities of NGF receptors in striatal areas,<sup>21</sup> and its well known effects of this growth factor at the spinal cord level,<sup>14,16</sup> indicate that this growth factor could have major side effects. Moreover, the possible  $\beta$ -amyloid deposit-promoting effects of NGF,<sup>22</sup> and its action on proliferative neurite outgrowth,<sup>23</sup> suggest that NGF therapy of AD<sup>14-16</sup> is not without risk. The potential NGF-

From the Departments of Psychiatry, and Pharmacology and Therapeutics, Faculty of Medicine, McGill University and Douglas Hospital Research Centre, Verdun, Quebec

Reprint requests to: Dr. Rémi Quirion, Douglas Hospital Research Centre, 6875 Blvd. LaSalle, Verdun, Quebec, Canada H4H 1R3

**Table 1: Acute effects of various cholinergic drugs, growth factors and lymphokines on acetylcholine release from rat hippocampal slices *in vitro***

Agent	Concentrations (M)	Acetylcholine Release
M <sub>1</sub> muscarinic antagonists (e.g. pirenzepine)	10 <sup>-7</sup> - 10 <sup>-4</sup>	No effect
M <sub>2</sub> muscarinic antagonists (e.g. AF-DX116)	10 <sup>-7</sup> - 10 <sup>-4</sup>	Stimulation of evoked release
M <sub>2</sub> muscarinic agonists (e.g. oxotremorine)	10 <sup>-7</sup> - 10 <sup>-4</sup>	Inhibition of evoked release
Nicotinic agonists (e.g. nicotine, N-methylcarbamyl-choline)	10 <sup>-7</sup> - 10 <sup>-4</sup>	Stimulation of basal release
NGF	10 <sup>-9</sup> - 10 <sup>-7</sup>	No effect
IGF-1	10 <sup>-9</sup> - 10 <sup>-6</sup>	Inhibition of evoked release
IGF-2	10 <sup>-9</sup> - 10 <sup>-6</sup>	No effect
EGF	10 <sup>-8</sup> - 10 <sup>-6</sup>	Inhibition of evoked release
Interleukin-1 (β)	10 <sup>-9</sup> - 10 <sup>-7</sup>	No effect
Interleukin-2	10 <sup>-10</sup> - 10 <sup>-7</sup>	Potent Inhibition of evoked release
Interleukin-4	10 <sup>-8</sup> - 10 <sup>-7</sup>	No effect
γ-interferon	10 <sup>-8</sup> - 10 <sup>-7</sup>	No effect

Efficacy evaluated on either basal or K<sup>+</sup>(25 mM)-stimulated release. See references<sup>12,26-28</sup> for experimental details. Some of these substances (like NGF) are well known to have effects on cholinergic mechanisms following chronic application.

induced side effects have even brought some to suggest the use of NGF antagonists.<sup>24</sup>

Thus, although the possible use of NGF in AD certainly remains attractive, further investigations in normal and pathological tissues are warranted in order to adequately design future trials for the treatment of AD patients. Additionally, it is now clear that various other growth factors are active in the CNS and should be considered in regard to AD. Consequently, we have studied insulin-like growth factor-1 (IGF-1),<sup>25-27</sup> epidermal growth factor (EGF)<sup>25,27</sup> as well as a lymphokine, interleukin-2.<sup>27-29</sup> Some of our recent data are briefly discussed here.

#### IGF-1 and the Cholinergic Innervation

It is now well established that insulin and IGFs (I and II) are present in the mammalian brain. Moreover, these respective receptor sites are broadly and widely distributed in the CNS (for reviews<sup>16,27,30</sup>). For example, while [<sup>125</sup>I] insulin binding sites are concentrated in the olfactory bulb,<sup>30</sup> [<sup>125</sup>I]IGF-1 binding is localized to the hippocampal formation, cortex, striatum and cerebellum in the rat brain.<sup>26,31</sup> Moreover, the densities of [<sup>125</sup>I]IGF-1 and [<sup>125</sup>I] insulin binding sites do not decrease with age in the rat brain.<sup>32</sup> Up to date, rather limited information is available on the presence and status of IGF-1 and IGF-1 receptors in the normal and pathological aged human brain, although the presence of highly specific IGF-1 binding sites has clearly been demonstrated.<sup>33</sup>

In relation to brain cholinergic innervation, rather scant information is available. Recently, we observed, rather unexpectedly, that IGF-1, but not IGF-2, is able to acutely block acetylcholine release in the rat hippocampal formation, *in vitro* (Table 1).<sup>26</sup> This inhibitory action is observed only in adult tissues, IGF-1 being unable to alter K<sup>+</sup>-stimulated acetylcholine release in 6 and 18 day old rat hippocampus.<sup>26</sup> Electrolytic lesions of the adult hippocampal formation apparently increase the synthesis and release of IGF-1, most likely from glial cells that may be involved in repair mechanisms.<sup>34</sup> Consequently, it is possible that in injured brain tissues, such as in AD, the release of IGF-1 may be enhanced. This may, in turn, result in the inhi-

bition of acetylcholine release from remaining cholinergic neurons. This could eventually exacerbate cognitive deficits associated with cholinergic dysfunctions in AD.<sup>1-3</sup> Thus, IGF-1 can be considered as a "negative" modulator while NGF seems to have positive actions on the cholinergic neuron in the hippocampus. We are currently investigating the integrity of IGF-1 innervation and IGF-1 receptors in AD brains. According to animal data,<sup>34-37</sup> it is possible that IGF-1 systems are hyperactive in injured tissues in human brain. If so, the eventual use of IGF-1 receptor antagonists should be considered in AD, at least in regard to maintaining an enhanced cholinergic function and hence, improved cognitive processes.

#### EGF and Cholinergic Innervation

Like IGF-1, EGF potently blocked K<sup>+</sup>-stimulated release of acetylcholine in the rat hippocampus (Table 1).<sup>27</sup> However, it is clear that these two growth factor systems are independent in the CNS, the distribution of EGF-like immunoreactive material and receptor binding is clearly different from that of IGF-1.<sup>16</sup>

Data concerning the presence and localization of EGF and EGF receptor sites in human brain are scarce.<sup>16,38</sup> However, it is known that EGF is a potent mitogenic factor.<sup>39</sup> Thus, a detailed study on the presence of EGF-like molecules and receptors in AD is certainly warranted and could lead to a better understanding of neurite hyperactivity reported in cortical and hippocampal areas in AD. In relation to the cholinergic neuron, work is in progress in order to more precisely define the mechanism by which EGF can acutely block the release of acetylcholine and its possible significance for AD.

#### Interleukin-2 and Cholinergic Innervation

In addition to growth factors, the possible involvement of certain peptides and/or cells derived from the immune system in disorders such as AD is generating great interest (for recent reviews<sup>16,27,40</sup>).

In our laboratory, we have focussed our attention on interleukin-2 (IL-2), a potent cytokine used as an experimental drug for the treatment of certain types of cancer. Our recent data

clearly demonstrate the presence of IL-2 like materials in normal adult rat brain.<sup>28,29</sup> However, concentrations in the normal brain are relatively low, marked increases being observed following various types of lesions.<sup>28,41</sup> It remains to be established if brain IL-2 like immunoreactivity is identical to IL-2 released from T-cells in the periphery. Additionally, it is not known if the IL-2 like immunoreactive material we measured in brain tissue is of neuronal or glial origin, or if it results from infiltration by peripheral IL-2 or by immune cells of certain brain areas.

IL-2 receptor binding proteins are also found in the CNS, especially in the hippocampal formation.<sup>28,29</sup> Other regions also enriched with putative IL-2 receptors are the cerebellum and certain hypothalamic nuclei.<sup>29</sup> Upon injury, the expression of IL-2 binding sites is markedly increased in the hippocampus.<sup>27,28</sup> Preliminary results from our laboratories indicate that IL-2 receptor sites are present in the human hippocampal formation.<sup>42</sup> However, it is still too early to clearly assess if these sites are somewhat altered in AD.

Interestingly, we observed that IL-2 is a very potent, acute blocker of K<sup>+</sup>-stimulated acetylcholine release in the rat hippocampus (Table 1).<sup>28</sup> This effect is tetrodotoxin- and naloxone-sensitive, suggesting the possible involvement of an opioid mechanism. Other lymphokines, including interleukin-1 $\beta$ , interleukin-4 and  $\gamma$ -interferon have no direct acute effect on acetylcholine release in this tissue (Table 1).<sup>27,28</sup> The blockade of acetylcholine release in the hippocampus by low concentrations of IL-2 is of special interest in view of the recent demonstration that this lymphokine also inhibits long-term potentiation, a phenomenon associated with learning and memory.<sup>44</sup> We are now investigating whether the effect of IL-2 is specific to acetylcholine released from the hippocampus, or whether this cytokine can modulate the synthesis and release of other neurotransmitters in various brain regions.

In parallel, we also intend to characterize the distribution of IL-2 and IL-2 receptor sites in normal human brain and to determine whether alterations of this occur in AD. This could lead to new hypotheses on the etiology of this disorder, and suggest possible dysfunctions of the immune system in AD.

### CONCLUSION

It is clear that in addition to various neurotransmitters and modulators, certain growth factors and lymphokines such as IGF-1, EGF and IL-2 can acutely modulate the stimulated release of acetylcholine in the rat hippocampal formation. These substances may act as direct modulators of transmitter release in the brain, in addition to their better known trophic actions. The "negative" effect of those factors on the release of acetylcholine also suggests that it may be important to study possible alterations of ratio between positive (*e.g.*, NGF) and "negative" growth factors in diseases such as AD, instead of focussing exclusively on a given factor. Hopefully, such investigations will provide new avenues toward the treatment of AD.

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