Creutzfeldt-Jakob infection increases adenylate cyclase activity in specific regions of guinea pig brain

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Creutzfeldt-Jakob disease is a slow, infectious, progressive neurological disorder which results in human dementia. Synaptic membranes from various brain regions of guinea pigs infected with Creutzfeldt-Jakob disease show increased guanyl nucleotide- or 5-hydroxytryptamine-mediated activation of adenylate cyclase. This increased enzyme activity appears due, primarily, to facilitated 'coupling' between the GTP-binding protein which stimulates adenylate cyclase (GN_s) and the catalytic moiety of that enzyme rather than increased sensitivity to 5-hydroxytryptamine. It is possible that this phenomenon is due to direct effects of the Creutzfeldt-Jakob infectious agent, or a pathological product resulting from that agent, upon synaptic membrane adenylate cyclase.

GTP-binding protein *Receptor-effector coupling* *Oncogene product* *Dementia* *Signal transduction*

1. INTRODUCTION

Creutzfeldt-Jakob disease (CJD) is a slow, infectious, progressive neurological disorder which results in human dementia, and may provide a paradigm for more common progressive human dementias such as Alzheimer's disease. CJD is characterized by spongy or membranous changes in affected neuropil, particularly in synaptic regions [1]. Human autopsy material can be used to transmit CJD to rodents [2] and the infectivity is closely associated with a specific, synaptic membrane-associated protein [3,4]. In order to study the possibility that synaptic membrane accumulation of CJD infectious agent, or agent elicited protein, causes clinical and pathological manifestations of CJD, the functional capacity of the neuronal membrane enzyme, adenylate cyclase, was examined.

Note: Various authors have referred to these proteins as N (nucleotide binding protein) and G (GTP binding protein). Here, we refer to these proteins as GN_s or GN_i – GN referring to guanine nucleotide

A variety of hormones or neurotransmitters mediate the activity of adenylate cyclase, however, in neuronal membranes this enzyme can be stimulated or inhibited directly by hydrolysis-resistant guanine nucleotides or F^- and these actions are expressed through separate guanine nucleotide-binding proteins: GN_s (stimulation) and GN_i (inhibition). The coupling of adenylate cyclase refers to the interaction between (or among) neurotransmitter receptor, GN_s or GN_i and the adenylate cyclase catalytic moiety. We have demonstrated previously that neuronal adenylate cyclase coupling might be augmented by treatments which alter cytoskeletal or membrane composition [5,6] and by chronic antidepressant treatment [7]. Thus, we undertook to investigate the possibility that the membrane changes associated with Creutzfeldt-Jakob infection might also effect changes in neuronal adenylate cyclase coupling. Further, recent investigations in rodents infected with scrapie (a spongiform encephalopathy similar to CJD) have detected diminished brain 5-hydroxytryptamine (5HT) levels. In these studies, some of the behavioral aspects of scrapie

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infection were ascribed to 5HT supersensitivity [8,9]. Pursuant to those studies, we chose additionally to examine 5HT-activated adenylate cyclase in brains of normal and CJD-infected guinea pigs.

2. MATERIALS AND METHODS

2.1. *Animals*

We utilized a serially passaged guinea pig model of CJD [10]. Synaptosome-enriched fractions were prepared as described [3] from animals showing typical clinical signs of disease (approx. 20 weeks after i.c. inoculation of young adult guinea pigs). Material from several serial passages was studied (i.e. passages 14–18), and all studies included (i) parietal cerebral cortex, (ii) hippocampus, and (iii) basal ganglia. Regions were rapidly dissected, synaptosomal fractions prepared, and aliquots of prepared membranes stored in liquid nitrogen until assay. Spongy changes are especially apparent in this CJD passaged material in cerebral cortex and basal ganglia [2]. Controls included normal young adult as well as older (2-year-old) guinea pigs of the same strain, and synaptic membrane-enriched fractions were derived identically to those from infected animals.

2.2. *Adenylate cyclase assays*

Membranes were suspended in 20 mM Hepes (pH 7.4) with 5 mM $MgCl_2$, 1 mM DTT and 0.3 mM PMSF and assayed in duplicate for adenylate cyclase as described [5]. Briefly, 20 μg membrane protein was preincubated with 5HT (in 0.02% ascorbate) and/or other agents (as indicated) at 30°C for 20 min. Following this, ATP (500 μM) was added (final incubation volume 100 μl) and the reaction mixtures incubated at 30°C for 10 min. Reactions were stopped by boiling (3 min) and the cAMP produced assayed by protein binding. Some experiments were performed similarly except that the assay was by the method of Salomon [26], and triplicate rather than duplicate determinations were made. These assays were stopped by the addition of 1% SDS rather than by boiling.

3. RESULTS

In all experiments (figs 1,2) there was a clear increase in adenylate cyclase activity in membranes from CJD-infected animals. In membranes from CJD-infected animals, the magnitude of the 5HT response was enhanced (fig.1) without any apparent change in the 5HT sensitivity. Adenylate cyclase activation by 5HT in control membranes was somewhat less sensitive but otherwise comparable to that reported in [11]. Hippocampal membranes from infected animals showed a pattern of increased 5HT-activated adenylate cyclase similar to that observed in the basal ganglia.

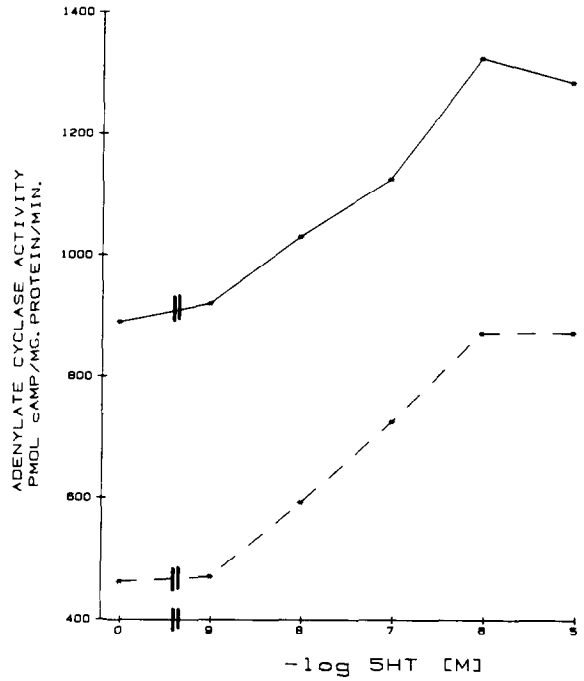

Fig.1. Adenylate cyclase activity in infected and control guinea pig basal ganglia membranes at indicated 5HT concentrations. Guinea pigs were inoculated with a 10% homogenate of CJD passage brain [10] and killed at late clinical stages of the disease. Histological confirmation of the disease was made in parallel cage-mates displaying similar symptomology. Fresh brain (control, – – –; or CJD-infected, ——) synaptosome-enriched fractions from each region were prepared and assayed as described, in the presence of the indicated 5HT concentration plus 5 μM Gpp(NH)p. Values expressed are means of duplicate determinations for one of three similar experiments.

Although the results in fig.1 indicate some increased 5HT responsiveness, they cannot be explained on the basis of increased 5HT sensitivity due to the large increase in overall adenylate

cyclase activity in membranes from CJD-infected animals. Therefore, other aspects of adenylate cyclase activation were examined. Basal adenylate cyclase activity was indistinguishable between membranes prepared from infected animals and controls (fig.2, lanes A). Adenylate cyclase activity in the same preparations was also measured in the presence of $MnSO_4$. Mn^{2+} activates the catalytic moiety of adenylate cyclase, and under these assay conditions, is thought to reflect the activity of the enzyme which is independent of GN_s [5,12]. If membranes were pathologically destroyed in CJD, one would expect to see less adenylate cyclase specific activity under these conditions as compared to controls. In both brain regions, the Mn^{2+}-stimulated catalytic moiety activity of adenylate cyclase in CJD groups was comparable (fig.2, lanes B). We then studied the GN_s-dependent activation of adenylate cyclase by using NaF or the hydrolysis-resistant GTP analog, Gpp(NH)p. In the presence of 5×10^{-6} M Gpp(NH)p (fig.2, lanes C) both basal ganglia and hippocampus membranes showed an activation of adenylate cyclase that was significantly greater than the controls. At a submaximal concentration of Gpp(NH)p (10^{-7} M), adenylate cyclase activities in membranes from infected animals were 85% (basal ganglia) and 40% (hippocampus) greater than controls in the illustrated experiment. The mean and standard deviation for 5 experiments indicated an increase in GppNHp-activated adenylate cyclase of 66 ± 17% (mean ± SD) in CJD-infected basal ganglia and 52 ± 22% in CJD-infected hippocampus compared to controls. In each experiment NaF also elicited increased adenylate cyclase activity in basal ganglia and hippocampus in CJD-infected animals (e.g. lanes D, fig.2). Combined data for all experiments showed an increase in NaF-stimulated adenylate cyclase of 100 ± 20% (basal ganglia) and 56 ± 16% (hippocampus) in membranes from CJD-infected animals as compared to controls.

Unlike basal ganglia and hippocampus, cerebral cortex membranes from CJD-infected animals showed a slight decrease in GppNHp-stimulated adenylate cyclase. The cerebral cortex shows marked vacuolization at end stages of CJD, and it is possible that these pathological changes preclude detection of elevated adenylate cyclase in this region.

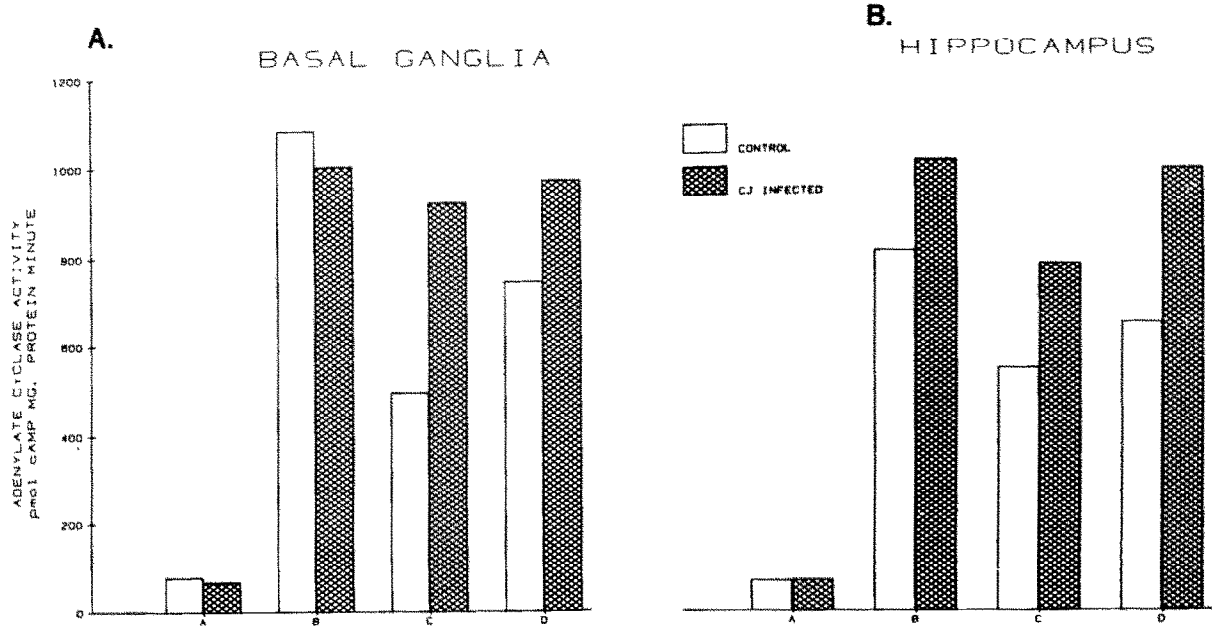

Fig.2. Adenylate cyclase activity in membranes prepared from basal ganglia or hippocampus in CJD-infected and control guinea pigs. Additions to the adenylate cyclase preincubation and assay: (A) H_2O, (B) $MnSO_4$ (10 mM), (C) Gpp(NH)p (5 μM), (D) NaF (20 mM). Points represent the means of duplicate determinations from one of 3–5 similar experiments. Means ± SD of normalized data from all experiments (N = 6–10) are given in the text.

4. DISCUSSION

It is clear from the above studies that the integrity of the adenylate cyclase system of membranes from basal ganglia and hippocampus remains intact in this disease, despite extensive pathological changes at the synaptic level [2,10]. Pathological changes in neurons secondary to CJD infection, and not necessarily entailing a direct or specific effect on neuronal membranes, could lead to the increased adenylate cyclase activation obtained above. In this context, several mechanisms may be considered. For example, disruption of neuronal cytoskeletal integrity, consistent with the focal clearing observed in synaptic processes of CJD-infected animals [12,14,15], may increase adenylate cyclase activity. It has been demonstrated previously by that activation of neuronal adenylate cyclase through GN_s is enhanced as a consequence of treatment with microtubule-disrupting drugs or agents which increase membrane fluidity [5,6,15]. Furthermore, selective damage of 5HT-containing neurons can lead to 5HT-stimulated adenylate cyclase supersensitivity in 5HT-responsive neurons. 5HT supersensitivity (due to reduced 5HT levels) has been invoked to explain some of the behavioral changes observed in scrapie-infected rodents [9,10], and an increased response to 5HT may be present with respect to adenylate cyclase in the CJD-infected animals in these studies. Increased 5HT response observed in basal ganglia, which may be consistent with some depletion of 5HT at presynaptic terminals, is dependent upon low (5×10^{-6}) GppNHp concentrations in the adenylate cyclase assay. However, increased response to 5HT does not alone explain increased adenylate cyclase activation in membranes from CJD-infected animals by NaF or GppHNp. Reserpine treatment [11], chronic electroconvulsive shock and chronic treatment with tricyclic antidepressants [7] can also enhance brain adenylate cyclase. The reserpine effect is similar to that observed in CJD where elevated 5HT responsiveness was found at essentially all concentrations tested and is compatible with enhanced coupling of the GN_s protein to adenylate cyclase, rather than (or in addition to) a specific receptor-mediated enhancement. In summary, this work shows an increased interaction (coupling) of GN_s as the predominant consistent finding in CJD membranes from basal ganglia and hippocampus. Additionally, 5HT supersensitivity in basal ganglia may occur secondary to pathological depletion of 5HT terminals in that region.

Both CJD and scrapie are related infectious agents, and similar specific sialoglycoproteins have been identified in both diseases [4,16,18]; these proteins share common antigenic epitopes [4,15] and are found in subcellular fractions that are highly infectious. These proteins, as well as infectivity and unique fibrils, cosediment with, and are tightly bound to, synaptosome-enriched or synaptic membrane fractions [3,4]. It is possible that insertion of CJD-specific proteins into neuronal membranes directly alters the interaction of GN_s with the adenylate cyclase catalytic moiety. Another possibilty is that a CJD-elicited membrane protein mimics GN_s in the activation of adenylate cyclase. Although at present we have no evidence favoring a direct membrane effect over one secondary to other neuronal pathology (e.g. cytoskeletal changes), it is of interest that recent experiments on *ras* proteins, have indicated that oncogene products (p21-related membrane proteins) can directly bind guanine nucleotides and display GTPase activity [20]. There is some sequence homology between *ras* proteins and adenylate cyclase GN proteins [20,21] and *ras* membrane proteins might substitute for GN_s in the activation of adenylate cyclase [22] associated with transformed growth potentials (this is a matter of some controversy [23]). Although CJD and scrapie are degenerative diseases, there is some evidence that exposure to these agents can also result in altered growth potentials and cell transformation [24,25]. Perhaps a similar protein and similar phenomena account for the observed increase in GN_s activated adenylate cyclase in CJD membranes.

Further investigation of (i) adenylate cyclase response patterns during the long incubation period of CJD (e.g. at early and preclinical stages of infection), (ii) the nature of CJD specific protein(s) interactions with membrane components (i.e. the ability of these proteins to bind guanine nucleotides), and (iii) membrane alterations in CJD-transformed cells should help to clarify the exact target of these 'unconventional' infectious agents and their role in the genesis of encephalopathy.

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