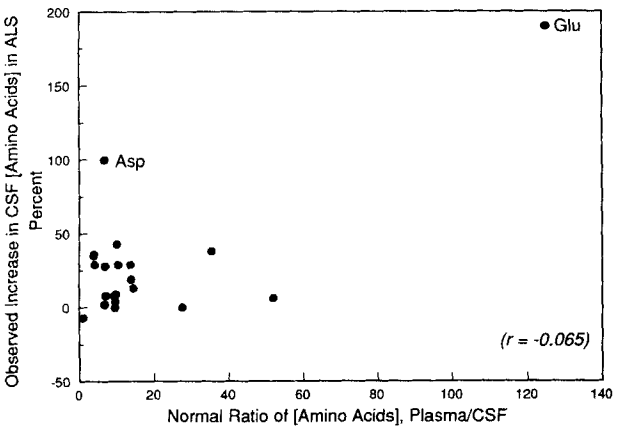

The normal ratios of plasma to CSF concentrations for each amino acid were calculated from published normative data [8] and compared to the corresponding change in amino acid concentration we found in cerebrospinal fluid from ALS patients (an exact ratio for proline could not be calculated because it is found in only trace amounts in CSF). By a computerized residual/outlier analysis (Statistix, Analytical Software), glutamate (Glu) and aspartate (Asp) were determined to be statistically significant outliers ($p < 0.001$). *For the remaining 19 amino acids, there was no significant linear relationship between the normal concentration gradient and percent increase in ALS.*

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References

1. Rothstein JD, Kuncl R, Chaudhry V, et al. Excitatory amino acids in amyotrophic lateral sclerosis: an update. Ann Neurol 1991; 30:224–225
2. Leonardi A, Abbruzzese G, Arata L, et al. Cerebrospinal fluid (CSF) findings in amyotrophic lateral sclerosis. J Neurol 1984; 231:75–78
3. Annunziata P, Volpi N. High levels of C_3c in the cerebrospinal fluid from amyotrophic lateral sclerosis patients. Acta Neurol Scand 1985;72:61–64
4. Conradi S, Kaijser L, Ronnevi LO. Capillary permeability in ALS, determined through transcapillary escape rate of ^{125}I-albumin. Acta Neurol Scand 1978;57:257–261
5. Guiloff RJ, McGregor B, Thompson E, et al. Motor neuron disease with elevated cerebrospinal fluid protein. J Neurol Neurosurg Psychiatry 1980;43:390–396
6. Urca G, Urca R. Neurotoxic effects of excitatory amino acids in the mouse spinal cord: quisqualate and kainate but not N-methyl-D-aspartate induce permanent neural damage. Brain Res 1990;529:7–15
7. Rothstein JD, Kuncl RW. Glutamate transport in amyotrophic lateral sclerosis: pathophysiological implications. Ann Neurol 1991;30:235–236
8. Perry TL, Kreiger C, Hansen S, Eisen A. Amyotrophic lateral sclerosis: amino acid levels in plasma and cerebrospinal fluid. Ann Neurol 1990;28:12–17

The Therapeutic Effect of Bromocriptine on Acute and Chronic Experimental Allergic Encephalomyelitis

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In a recent article in this journal Riskind and collegues demonstrated that treatment with the dopamine receptor antagonist bromocriptine (BCR) results in decreased severity of clinical signs during acute experimental allergic encephalomyelitis (EAE), associated with suppression of the expected rise of plasma prolactin (PRL) levels [1]. Treatment with BCR reduced the incidence and severity of acute EAE both when started 2 days before immunization and when started 1 week after immunization and it was concluded that PRL might have an effect on the effector limb of the immune response during EAE.

We recently performed a series of experiments in which the effects of BCR treatment were investigated in acute EAE as well as in chronic relapsing EAE. To mimic clinical situations more closely, we included groups in which treatment was started after the first occurrence of clinical signs. Furthermore we included chronic relapsing EAE, which closely resembles multiple sclerosis with respect to clinical course [2] and occurrence of pronounced demyelination [3]. In these experiments BCR (5 mg/kg body weight IP) was injected at daily intervals, whereas controls received daily injections with the solvent alone (saline containing 0.01% ethanol and 1.5% tartaric acid IP). The BCR treatment resulted in a sustained decrease of stress-induced PRL plasma levels from 200 to 350 ng/ml to values below 25 ng/ml. These values are comparable to the serum levels in the study of Riskind and associates [1]. Clinical signs of EAE were scored daily by the following standard scoring system: 0, no clinical signs; 1, diminished tonus or paresis of the tail; 2, paresis of the hind limbs; 3, paralysis of the hind limbs; 4, death because of EAE. Statistical analysis of the obtained scores was performed using chi-square tests.

Acute EAE was induced in 30 male Lewis rats by a single injection in the hind foot pad of 50 μl of guinea pig spinal cord homogenate (GPSC): 1 gm of GPSC in 1 ml saline to which 10 mg *Mycobacterium tuberculosis* (H37Ra, Difco, Detroit, MI) in 1 ml Freund's complete adjuvant (Difco) was added. The rats were randomly divided into 3 groups of 10 animals. Group A was injected daily with the solvent alone, starting 3 days before immunization; group B was injected daily with BCR also from 3 days before immunization, and group C received BCR starting on the day of onset of clinical signs. All animals in the three groups developed clinical signs of the disease. However, in group B the severity of the disease was significantly reduced ($p = 0.019$) and the duration of the disease tended to be shorter ($p = 0.067$) as compared to group A. In group C both severity ($p < 0.003$) and duration ($p = 0.024$) of clinical signs were reduced significantly in comparison to Group A.

Chronic relapsing EAE was induced in 30 male Lewis rats as described previously [2]. These rats were also divided into

3 groups of 10 animals. Group A was injected with the solvent alone, starting at the onset of the first attack, group B received daily BCR injections also starting at the onset of the first attack, and in group C BCR was started at the onset of the second attack. There was no effect of either BCR regimen on the frequency of clinical signs. In group B there was a tendency toward reduction in severity and duration of the first attack (p values 0.073 and 0.080, respectively); severity of the clinical signs of the second attack was unchanged but its duration was reduced significantly ($p = 0.007$) compared to group A. In group C the severity and duration of clinical signs of the first attack were the same as in Group A. Furthermore, the severity of the second attack did not differ from that in control group A, but the duration of the second attack was significantly reduced in comparison to group A ($p = 0.024$).

This study demonstrates that BCR treatment, although having no effect on the incidence of clinical signs, significantly reduces the duration or severity of subsequent clinical signs in both acute and chronic relapsing EAE. This effect was not only observed when BCR was administered prophylactically (i.e., some days before immunization) but also when BCR was given therapeutically (i.e., after the onset of clinical signs). These findings, in our view, support the conclusion of Riskind and colleagues on the presumed effect of BCR on the effector limb of the immune response. Although the exact mechanism of action of BCR is still unknown and although mechanisms responsible for the occurrence of clinical signs in EAE might differ from those in MS, these findings may have potential as a therapeutic modality in treatment of MS. Our finding that the administration of BCR improved the clinical course in animals with ongoing EAE and the recent findings of Kira [4] that acute relapses in MS may be associated with a rise in serum PRL levels are promising in this respect.

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References

1. Riskind PN, Massacesi L, Doolittle TH, Hauser SL. The role of prolactin in autoimmune demyelination: suppression of experimental allergic encephalomyelitis. Ann Neurol 1991;29:542–547
2. Polman CH, Matthaei I, De Groot CJA, et al. Low-dose cyclosporin A induces relapsing remitting experimental allergic encephalomyelitis in the Lewis rat. J Neuroimmunol 1988; 17:209–216
3. Pender MP, Stanley GP, Young G, Nguyen KB. The neuropathology of chronic relapsing experimental allergic encephalomyelitis induced in Lewis rats by inoculation with whole spinal cord and treatment with cyclosporin A. Acta Neuropathol 1990; 80:172–183
4. Kira J, Harada M, Yamaguchi Y, et al. Hyperprolactinaemia in multiple sclerosis. J Neurol Sci 1991;102:61–66

Reply

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These results generally support our previous observations, which were obtained with female animals. The fact that similar results were obtained with males is of interest, since males typically have lower circulating prolactin levels than females. Bromocriptine might therefore be expected to be less efficacious in males. In contrast to our results, Dijkstra and colleagues did not detect an effect of bromocriptine on the severity of late relapses (although the duration of illness was reduced). This difference may be a consequence of using male, rather than female rats. Alternatively, differences in dose or method of administering bromocriptine (intraperitoneal injection versus continuous-release pellet) may be responsible. In preliminary studies we observed that daily injections were not as effective as continuous-release bromocriptine pellets, presumably because prolactin inhibition was not sustained throughout the day by injections.

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Brain ^{31}P-Magnetic Resonance Spectroscopy in Mitochondrial Cytopathies

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Eleff and colleagues [1] used ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) to test the hypothesis that patients with mitochondrial cytopathies show abnormalities of brain mitochondrial function even in the absence of clinical involvement. In the frontal lobes of 5 patients affected with chronic progressive external ophthalmoplegia (CPEO), mitochondrial encephalopathy with lactic acidosis (MELAS), myoclonus epilepsy with ragged red fibers (MERFF), or Leigh's syndrome, they found decreased phosphocreatine (PCr), increased calculated [ADP] and V/V_{max}, and low phosphorylation potential (PP). They concluded that neither clinical, biochemical, nor molecular diagnosis on peripheral tissues permits prediction of involvement of the brain in certain patients; MRS is thus a valuable tool. Their results were, however, unlike those of the Montreal group [2, 3] who found normal PCr/ATP and Pi/ATP ratios in the brains of 9 patients with MERRF and 1 with Kearns-Sayre syndrome. The latter [3] attributed this discrepancy to poor spectral localization in Eleff and associates' study, which they thought reflected differences in frontal, temporal, and ocular muscle bulk (a tissue with much higher PCr content) rather than true brain metabolism.

We have studied muscle and brain energy metabolism by ^{31}P-MRS in 4 patients affected by CPEO without any clinical brain involvement, and in 3 patients of a family affected with Leber's hereditary optic neuropathy (LHON), in whom a 11778 bp mtDNA mutation was present [4] (Table). Muscle biopsy showed ragged red fibers and cytochrome *c* oxidase–deficient fibers in all CPEO patients, whereas all LHON patients had normal biopsies. Brain ^{31}P-MRS was performed on a 1.5 T GE Signa system according to described methods