ly up-tunnel and 90° being perpendicular to the plume axis both to the right and left.

10. The light-emitting diode was flashed at the instant the plume was removed and again when the fan blades had stopped to indicate the moment of wind stoppage. The smoke source visualization resulted in the plume's up-tunnel end coming to rest about 30 cm from the down-tunnel field of view, with an average time of 3.7 seconds from plume removal to wind stoppage. The average stoppage time for all experimental trials was 3.7 ± 0.2 seconds with a wind velocity of 70 cm/sec (N = 13). The slight variation in stoppage time created a bias against finding differences in track reversal distance because the presence of a plume in the "nonplume" section and vice versa would have influenced reversal magnitude, blurring the differences between turning in the two areas.
11. Ground velocities up-tunnel were generally lower in the nonplume area (26.3 ± 23.5 cm/sec, N = 13) than they were in the plume area (45.6 ± 26.2 cm/sec, N = 13) in the plume-removal situation or than they were in the nonplume area in the sham-removal situation (40.9 ± 23.8 cm/sec, N = 18), although not significantly (t-test, $P > .05$).
12. Males were allowed to fly up-tunnel through the recording section and then were brought back down-tunnel at near zero airspeed along the windline with the plume present by moving the ground pattern down-tunnel at a velocity greater than the wind velocity. For trials where the plume was removed, the male was allowed to fly into the recording section and the plume was then removed, so that loss of pheromone occurred near the section's up-tunnel end. The ground pattern's velocity down-tunnel was matched exactly to that of the wind so that males keeping station with the stripes would drift down-tunnel at an effective wind velocity near zero.
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Experimental Hepatic Encephalopathy: Changes in the Binding of γ-Aminobutyric Acid

Abstract. *Two populations of receptors for γ-aminobutyric acid, one with low- and the other with high-affinity characteristics, are detectable in frozen, thawed, Triton-treated synaptic membrane preparations from normal brain. It is now reported that membrane preparations from rats with mild galactosamine-induced hepatic encephalopathy show an increase in the number of low- and high-affinity γ-aminobutyric acid binding sites, whereas those from rats with severe encephalopathy show only high-affinity binding sites. Thus, hepatic encephalopathy appears to involve partial degeneration of the γ-aminobutyric acid–containing presynaptic nerve terminals.*

Hepatic encephalopathy (HE) following fulminant hepatic failure (FHF) occurs when overall liver function is grossly subnormal as a consequence of massive necrosis of the liver. Despite the several metabolic abnormalities described in this situation, the mechanism of the central nervous system (CNS) derangement in the onset of coma is still unknown (*1*). A critical review of the studies of this pathology led us to consider that, whatever pathogenetic factors cause the encephalopathy in FHF, little is known about the functional activity of γ-aminobutyric acid (GABA) receptors in the CNS of animals with FHF. In fact, changes in the function of GABA receptors have largely been ignored in considering the mechanism underlying the neurological disturbances of HE.

A suitable animal model of FHF, that closely resembles the human FHF in both histological lesion and metabolic changes, was obtained by injecting rats with the selective hepatotoxin D-galactosamine hydrochloride (*2, 2a*). Approximately 70 percent of rats so injected develop FHF with an encephalopathy characterized by increasing stupor and flaccidity, poor righting reflex, and diminished response to pain; deep coma develops at 3.5 to 4 days. From the mild stage of HE some of the rats recover as a result of liver regeneration (*3*).

Rats with mild and severe HE were evaluated by means of visual evoked potentials (VEP) as described (*3*) so that we could select for binding studies the brains of animals with the same degree of HE. In male Sprague-Dawley rats (100 to 125 g), two cortical electrodes were implanted through the skull in contact with the cortex and permanently fixed with dental cement under ethyl ether anesthesia. Three days after the surgical procedure a control VEP was recorded just before intraperitoneal injection of D-galactosamine hydrochloride (3 g/kg). Subsequently, VEP's were recorded at different times during the development of HE. For the binding studies, rats in mild and severe stages of HE were killed by decapitation and their brains were removed and frozen.

We performed the binding studies with fresh-frozen and frozen Triton X-100–treated membranes from the whole brains of 23 control rats and 23 rats with severe HE, using tris-citrate buffer (*p*H 7.1) and tritiated GABA as described by others and by us (*4, 5*). For studies with the fresh-frozen synaptic preparations, we thawed portions of the frozen homogenates at room temperature and washed them two times with tris-citrate buffer just before the assay, using approximately 300 to 400 μg of proteins per milliliter (*6*). The remaining frozen homogenates were thawed and treated three times with Triton X-100 and extensively washed. The Scatchard plots of six different saturation curves obtained for fresh-frozen membranes from three to four pooled brains revealed the presence of one population of GABA receptors in both the control and comatose rats. However, the mean (±1 standard deviation) of the affinity constants (K_d) of GABA binding to membranes from HE rats (K_d = 90 ± 7 nM) was significantly increased in comparison with that of controls (K_d = 187 ± 14 nM) (Student's t-test, $P < .001$), whereas the maximum binding (B_{max}) was decreased (2.2 ± 0.3 as opposed to 4.4 ± 0.5 pmole per milligram of protein) ($P < .01$), suggesting a reduction of endogenous inhibitors of GABA receptors and a loss of binding sites.

These differences in the GABA bind-

Table 1. Kinetic constants of Na^+-independent [^{3}H]GABA binding in membranes prepared from brains of normal rats, from brains of D-galactosamine–injected rats that did not develop FHF (no HE), and from D-galactosamine–injected rats that developed mild and severe HE. The binding was performed as described in Fig. 1. The reported values represent the means (± 1 standard deviation) of the kinetic components computed from the Scatchard plots obtained from six separate experiments done in triplicate; each experiment was performed on membranes prepared from a pool of three to four brains. Statistical significance was determined by Student's t test.

Rats	Total binding sites	[^{3}H]GABA kinetic components				$\frac{B_{max2}}{B_{max1}}$
		Low affinity		High affinity		
		K_{d1}*	B_{max1}†	K_{d2}*	B_{max2}†	
Controls	7.3	218 ± 15	6.0 ± 0.9	19.6 ± 6	1.3 ± 0.3	0.21
D-Galactosamine treated						
No HE	7.4	240 ± 20	6.0 ± 0.5	18.6 ± 3	1.4 ± 0.2	0.23
Mild HE	10.0	311 ± 6	8.1 ± 0.2‡	24.0 ± 4	1.9 ± 0.2§	0.23
Severe HE	1.9	‖	‖	22.9 ± 4	1.9 ± 0.1§	0

*Measured as nanomolar concentrations. †Measured as picomoles per milligram of protein. ‡$P < .05$. §$P < .01$, compared to controls. ‖ Binding sites undetectable.

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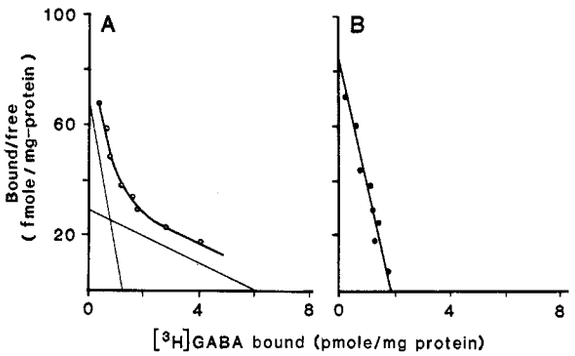

Fig. 1. Scatchard plot analysis of saturation curves from Na^+-independent [^{3}H]GABA binding in crude membranes prepared (*5*) from brains of normal rats and of rats with severe HE. The homogenate, which had been frozen for 18 to 24 hours, was thawed and incubated at 37°C for 30 minutes with 0.05 percent Triton X-100, centrifuged at 48,000*g* (10 minutes), and washed three times with buffer. The homogenate was then frozen again and the complete operation was repeated twice. In the routine [^{3}H]GABA-binding assay a standard portion (200 to 300 μg of protein per milliliter) (*6*) of membranes was incubated at 0° to 4°C for 5 minutes with 5, 10, 20, 30, 60, 80, 120, or 240 n*M* [^{3}H]GABA in the absence or in the presence of 10^{-3}*M* unlabeled GABA. The assay was terminated by centrifugation at 48,000*g* for 10 minutes. The pellet was rapidly rinsed and the tritium was measured by liquid scintillation spectrometry. The Scatchard plots of the data were prepared according to the graphic method described by Rosenthal (*12*) and Feldman (*13*). Each value represents the mean obtained from six Scatchard plots of six separate experiments done in triplicate and performed on membranes from a pool of three to four brains. The means (± standard deviation) of the kinetic constants obtained from the six separate plots are reported in Table 1.

ing between controls and comatose rats were more evident in the Triton X-100–treated membranes, since this treatment removes endogenous inhibitors of high-affinity GABA receptors (*4*, *5*, *7*). As shown in Fig. 1, we detected in the Triton-treated membranes of control brains two receptor components, one ($GABA_1$) with low affinity and one ($GABA_2$) with high affinity. In membranes from the brains of rats with severe HE only the high-affinity GABA receptors were present showing an increase in binding sites in comparison with controls. We confirmed this result by performing the same [^{3}H]GABA binding studies in different brain areas (data not shown).

Because animals with severe HE are in an agonal state, a situation in which cellular mechanisms could already be severely damaged, we selected animals with mild encephalopathy in order to determine whether the changes in GABA receptor binding were typical of developing HE or were only a terminal phenomenon (*8*). Using Triton-treated membranes from the brains of rats with mild HE, we performed a Scatchard analysis of the saturation curves of [^{3}H]GABA binding. This analysis revealed two populations of binding sites, with both the low- and high-affinity GABA receptors being increased in concentration, indicating that a change occurs in the GABA receptors early in the development of this pathological state (Table 1).

Binding studies were also conducted on membranes of the brains of rats that received D-galactosamine but did not develop FHF and consequently did not develop HE. The GABA receptors in these membranes showed no change in comparison with controls, indicating that the D-galactosamine was not directly responsible for the changes in the GABA receptors in the brain (Table 1).

To demonstrate specificity of the changes that occur in HE, we studied the binding of [^{3}H]GABA in Triton-treated membranes from brains of rats in hypoglycemic coma (brought about by tolbutamide, 1 g/kg) and in uremic coma (brought about by bilateral nephrectomy). The Scatchard plot analysis of the data did not reveal any significant difference between the brains of rats in acute hypoglycemic coma (K_{d1} = 300 n*M*, B_{max1} = 6 pmole per milligram of protein, K_{d2} = 16 n*M*, B_{max2} = 1 pmole) or in uremic coma, lasting 4 to 5 days (K_{d1} = 279 n*M*, B_{max1} = 6.7 pmole per milligram of protein, K_{d2} = 21 n*M*, B_{max2} = 1.6 pmole) and the brains of control rats tested in parallel experiments.

Moreover, to define the characteristics of the GABA receptors present in the Triton-treated membranes from brains of rats with severe HE we tested the sensitivity of these receptors to the specific antagonist activity of bicuculline methiodide. Using 20 n*M* [^{3}H]GABA, we found that the specific binding was inhibited by the addition of four increasing concentrations of bicuculline (10^{-8} to 10^{-4}*M*) in a concentration-dependent manner and that the median inhibition concentration (IC_{50}) was 10 μ*M*, whereas in membranes from rats with severe HE the IC_{50} was 0.5 μ*M*, indicating a hypersensitivity of these GABA receptors to the bicuculline inhibition.

These data demonstrate that a progressive change occurs in the GABA receptors of rats developing HE. We have documented by behavioral and electrophysiological (VEP) observations an increased CNS depression in this pathological condition (*3*) and a reduction of glutamic acid decarboxylase in several tested brain areas (*9*). Although other explanations are possible, it seems that the increase in [^{3}H]GABA binding sites in mild HE might be regarded as a supersensitivity phenomenon due to degeneration of GABA nerve terminals in the CNS. A similar increase in GABA binding sites described in localized lesions (that is, of the striatonigral pathway) induced by injections of kainic acid has been described and attributed to a denervation supersensitivity phenomenon (*10*). The disappearance, in the severe stage of HE, of the low-affinity GABA receptors seems to be a further expression of an ongoing degeneration of the GABAergic nerve terminals that may be due to toxins coming from the failing liver (*1*).

At present we do not know the functional consequences in vivo of the selection of high-affinity GABA receptors on the CNS. We can only suggest a remarkable functional inhibitory activity of the remaining GABA receptors, but we cannot rule out, for example, concomitant alterations of other receptor systems to explain the generalized CNS depression in severe HE.

From the heuristic point of view, these findings seem to corroborate the concept that the two synaptic GABA receptor populations are distinct entities (*11*) and that in pathological conditions, such as HE, a disappearance of one of the two can occur.

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Systemic Lupus Erythematosus: Presence in Human Serum of an Unusual Acid-Labile Leukocyte Interferon

Abstract. *A previously undescribed species of human leukocyte, or alpha, interferon is present in the serum of many patients with systemic lupus erythematosus. It was shown to be α-interferon by neutralization with specific antiserums, affinity column chromatography, and antiviral activity on bovine cells. However, 23 of 30 interferon samples tested were inactivated by incubation at* p*H 2, a characteristic of human "immune," or gamma, interferon. Multiple samples of interferon from the same patient had similar biological properties, but samples from different patients were not all identical, suggesting that several variants of this species of human α-interferon may exist.*

Human interferons (IFN's) are classified into three groups on the basis of their antigenic properties (*1*). α-Interferon is produced mainly by leukocytes in response to a variety of viral and nonviral stimuli and is stable at *p*H 2. At least 12 distinct species of human α-IFN have been cloned (*2*), and some differ in biological properties (*3*). β-Interferon is synthesized predominantly by fibroblast-like cells and, to a much lesser extent, by leukocytes, and is also acid-stable. Gamma, or "immune," IFN is released by lymphocytes following exposure to mitogens or specific antigens (*4*). γ-Interferon is inactivated by incubation at *p*H 2 and is generally more heat-labile than α- or β-IFN (*4*, *5*). Both α- and γ-IFN may be involved in the regulation of immune responses in vivo.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by a disfiguring skin rash, arthritis, and nephritis. Individuals with active SLE may have high concentrations of circulating immune complexes, antibodies to single- or double-stranded DNA, or antibodies to several small nuclear or cytoplasmic ribonucleoprotein complexes (*6*). Many patients with SLE also have high concentrations of circulating IFN (*7*, *8*), which may contribute to the pathogenesis of the disease. Hooks *et al.* (*8*) concluded that γ-IFN was present in these patients because the IFN was inactivated at *p*H 2, and they hypothesized that circulating autoantibodies or immune complexes stimulated production of γ-IFN by sensitized lymphocytes.

The recent demonstration of distinct classes of α-IFN (*3*) prompted us to reexamine the type of IFN in patients with SLE by using more specific criteria to identify the IFN. Our data show that the IFN present in SLE is of the alpha type. This was determined by neutralization with specific antiserums, affinity column chromatography, and antiviral activity on nonhuman cells. However, unlike reference human leukocyte IFN, the α-IFN found in most IFN-positive SLE patients was inactivated by incubation at *p*H 2 and therefore appears to be a previously undescribed form of human α-IFN.

Serum samples from 138 SLE patients (*9*) were analyzed; 65 patients (47.1 percent) yielded one or more samples that contained significant (8 to 128 I.U./ml) concentrations of IFN. About 60 percent of these samples had IFN titers between 16 and 64 I.U./ml. In contrast, IFN was undetectable (≤ 4 I.U./ml) in serum samples from 22 control (healthy) individuals and from 13 patients with drug-induced SLE (*9*). Of 86 samples obtained during exacerbations of the disease, 45 contained IFN (8 to 128 I.U./ml). Only 25 percent of the samples obtained from patients during disease remission contained IFN. These values are similar to those obtained previously (*7*, *8*).

Standard human leukocyte IFN had equal antiviral activity on human and bovine cells (Table 1). Seventy-nine serum samples from 65 IFN-positive pa-

Table 1. Comparison of IFN from SLE patients with standard human α-, β-, and γ-IFN. Interferon in samples from SLE patients was quantitated by a semimicro method on human (GM 2504) or bovine (MDBK) cell cultures. About 2×10^4 cells were incubated (18 hours at 37°C) with twofold serial dilutions of IFN in 96-well microtiter plates. Virus was then added and incubation was continued for 24 to 36 hours. In experiment 1, vesicular stomatitis virus was used as the challenge virus for all assays. In experiment 2, all assays were performed on GM 2504 cells, and the challenge virus was encephalomyocarditis virus. Virus-induced cytopathic effects were evaluated microscopically, and the IFN titer was defined as the reciprocal of the highest dilution of sample to protect 50 percent of the cells. Standard α-, β-, and γ-IFN were included in each assay. Results were standardized to 023-901-527 reference human leukocyte IFN (National Institutes of Health). In experiment 2, 50 μl of IFN-positive SLE serum (16 to 100 I.U./ml) was mixed with 50 μl of medium or with 50 μl of antibodies to α-, β-, or γ-IFN (*10*). After incubation at 37°C for 60 minutes, residual IFN was assayed. Dilutions of antibody to IFN capable of specifically neutralizing 100 I.U. of homologous IFN per milliliter were used (dilution of antibody to α-IFN, 1:750; antibody to β-IFN, 1:150; γ-IFN, 1:10). The neutralization factor is the IFN titer of the medium controls divided by the titer of IFN remaining after treatment with antibody to IFN. Std, standard; N.T., not tested.

Experiment	Interferon	Number of samples	Titer on human cells (I.U./ml)	Titer on human cells/titer on bovine cells	Neutralization factor		
					Antibody to α-IFN	Antibody to β-IFN	Antibody to γ-IFN
1	Stdα	2	100	1 to 2			
	Stdβ	1	100	64			
	Stdγ	1	100	>32			
	SLE	64	8 to 128	0.5 to 2			
	SLE	15	8 to 128	4 to 8			
2	Stdα	2	100		30 to 40	0	0
	Stdβ	1	100		0	10 to 20	0
	Stdγ	1	100		0 to 2	0	30 to 60
	SLE	21	16 to 128		8 to 128	0 to 2	N.T.
	SLE	6	16 to 128		8 to 16	0 to 2	0
	SLE	5	10 to 100		2 to 4	0	0

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