cardium and free wall of the myocardium of hypertensive animals, although myocardial perfusion was homogeneous (Fig. 2). Dual-tracer studies with the glucose analog [^{18}F]FDG and the fatty acid analog [^{14}C]BMHDA showed a complementary distribution of the two agents in the myocardium of hypertensives (Fig. 3). These data suggest that zones of decreased fatty acid uptake are associated with increased glucose uptake.

Quantitative data were available in 17 animals (Table 1). As suggested by visual inspection of the images, the distribution of perfusion was homogeneous in the myocardium of both the normotensive and hypertensive animals. The [^{14}C]DG uptake (expressed as nanocuries per gram, after normalizing for the administered dose) was much lower than the fatty acid uptake in the myocardium of normotensive animals. The ratio of fatty acid to glucose was 9.4 in the right ventricle, 5.0 in the septum, 5.5 in the endocardial region of the left ventricle, and 10 in the epicardial region of the left ventricle. In contrast, glucose uptake was higher than fatty acid uptake in the same regions of myocardium of hypertensive animals. The ratio of fatty acid to glucose was 1.2 in the right ventricular free wall, 0.5 in the septum, 0.40 in the endocardium of the left ventricle, and 0.78 in the epicardium of the left ventricle.

Since the animals were fed the same diet, it is unlikely that a difference in circulating glucose or fatty acid concentration could account for these differences. These data suggest that substrate use is altered in prolonged severe hypertension before ischemia occurs. It is uncertain whether this is the result of a decrease in the ratio of capillaries to sarcomeres or of a defect in membrane transport, energy production, or energy utilization.

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Alterations in L-Glutamate Binding in Alzheimer's and Huntington's Diseases

Abstract. *Brain sections from patients who had died with senile dementia of the Alzheimer's type (SDAT), Huntington's disease (HD), or no neurologic disease were studied by autoradiography to measure sodium-independent L-[^{3}H]glutamate binding. In brain sections from SDAT patients, glutamate binding was normal in the caudate, putamen, and claustrum but was lower than normal in the cortex. The decreased cortical binding represented a reduction in numbers of binding sites, not a change in binding affinity, and appeared to be the result of a specific decrease in numbers of the low-affinity quisqualate binding site. No significant changes in cortical binding of other ligands were observed. In brains from Huntington's disease patients, glutamate binding was lower in the caudate and putamen than in the same regions of brains from control and SDAT patients but was normal in the cortex. It is possible that development of positron-emitting probes for glutamate receptors may permit diagnosis of SDAT in vivo by means of positron emission tomographic scanning.*

Senile dementia of the Alzheimer's type (SDAT) and Huntington's disease (HD) are among the chronic degenerative neurologic disorders that affect memory. The symptoms of SDAT, which is a relatively common disease, often resemble those seen in cortical disconnection syndromes; clinical signs of cortical dysfunction, the agnosias and apraxias, occur frequently (*1*). Pathologically, the SDAT brain is atrophic; the characteristic histological findings are numerous neuritic plaques and neurofibrillary tangles in the cortex and hippocampus (*1*). In the cerebral cortex of the SDAT brain, there are decreases in cholinergic markers (*2*), catecholamines, and somatostatin (*3*). In contrast to SDAT, HD appears to be a subcortical dementia (*4*). Pathologically, there is a loss of neurons in the caudate and putamen, and many neurotransmitter systems appear to be affected (*5*).

Glutamate is the putative neurotransmitter of both intracortical association fibers and cortical efferents to many subcortical structures, including the caudate and putamen (*6*). Because glutamate and some of its analogs are neurotoxic, it has been proposed that abnormalities in glutamate neurotransmitter function may play a causal role in neurodegenerative disorders such as HD and olivopontocerebellar atrophy (*7*). By autoradiography, we examined one aspect of the glutamatergic system—namely, the glutamate receptor (*8*)—in sections of human brains obtained post-mortem.

The brains from a series of patients who had died with SDAT, HD, or no

Table 1. Affinity constants and numbers of high- and low-affinity quisqualate binding sites (picomoles per milligram of protein) in brains from control and SDAT patients. Measurements were made by autoradiography as described (*8*); values are means ± standard error of mean; $n = 5$ for both groups.

Donor	Affinity constant		Binding sites	
	High affinity (n*M*)	Low affinity (μ*M*)	High affinity	Low affinity
Control	21 ± 11	148 ± 52	1.82 ± 0.37	3.39 ± 0.50
SDAT	75 ± 29	277 ± 144	1.38 ± 0.21	1.46 ± 0.19*

*$P < 0.01$ (independent t-test).

Table 2. Cortical activity of choline acetyltransferase (CAT; nanomoles per milligram of protein per hour), binding of L-[^{3}H]glutamate (picomoles per milligram of protein at 200 nM), and maximum cortical binding of isotopically labeled muscimol (MUS), flunitrazepam (FLU), and quinuclidinyl benzylate (QNB) (picomoles per milligram of protein) in brains from control patients and those with HD and SDAT. Values are means ± standard error of the means. CAT activity was determined as described (*9*). Binding site numbers were determined autoradiographically in each brain as described (*10*). Statistical analysis was by univariate one-way analysis of variance with Bonferroni inequality to compare each group with each other group. nbM, nucleus basalis of Meynert.

CAT activity	Binding in brain tissue						
	L-[^{3}H]Glutamate				[^{3}H]MUS	[^{3}H]FLU	[^{3}H]QNB
	Cortex*	Caudate	Putamen	nbM			
			Control				
7.46 ± 0.67	2.17 ± 0.07	1.27 ± 0.16	1.26 ± 0.20	0.37 ± 0.08	4.26 ± 0.80	1.53 ± 0.18	1.73 ± 0.21
			HD				
9.55 ± 1.16	2.03 ± 0.10	0.37 ± 0.05†	0.36 ± 0.07‡	0.33 ± 0.05	2.77 ± 0.24	1.79 ± 0.28	1.01 ± 0.14
			SDAT				
1.0 ± 0.17§	1.17 ± 0.18†	1.27 ± 0.16	1.10 ± 0.16	0.32 ± 0.10	2.68 ± 0.29	1.24 ± 0.14	1.55 ± 0.17

*Layers 1 and 2. †$P < 0.005$ compared to both other groups. ‡$P < 0.05$ compared to both other groups. §$P < 0.001$ compared to both other groups.

neurologic disease (control) were removed, frozen immediately, and stored at −70°C (delay from time of death was 16.0 ± 3.2 hours for control, 11.4 ± 2.9 hours for SDAT, and 20.1 ± 11.8 hours for HD). One hemisphere from each brain was preserved in Formalin and examined by routine neuropathological techniques. Five brains from normal control patients (average age, 63 ± 4 years), six from SDAT patients (average age, 71 ± 4 years), and four from patients with advanced HD (average age, 35 ± 7 years) showed either normal histology or classic signs of SDAT or HD. Patients with HD and SDAT had been hospitalized from 1 to 10 years before death. Of these ten patients, four had been receiving no medication, three had been receiving small doses of neuroleptics, and three had been receiving benzodiazepines at the time of death.

Frozen brain samples were analyzed for choline acetyltransferase (CAT) and glutamic acid decarboxylase (GAD) activities (*9*). Whole coronal brain sections (50 μm thick) were mounted by thawing them onto gelatin-coated glass lantern slides and were stored at −20°C for less than 24 hours before assay. Sections were assayed for sodium-independent glutamate binding with 200 n*M* L-[^{3}H]glutamate in 50 m*M* tris-HCl (*p*H 7.2 at 2°C) containing 2.5 m*M* $CaCl_2$ as described (*8*). Saturation studies (30 n*M* to 7.5 μ*M* [^{3}H]glutamate) and quisqualate competition studies (1 n*M* to 1 m*M*) were performed as described on 30-μm sections of superior temporal gyrus (*8*). Autoradiographs of the slides were produced and analyzed as previously described (*10*).

Specific glutamate binding varied regionally in the brains (Fig. 1). In the cerebral cortex, the amount of binding was highest in the outer two layers and lower in the deeper layers. Binding was also high in the caudate nucleus and putamen but low in the globus pallidus and nucleus basalis of Meynert (nbM). In the brains from SDAT patients, cortical binding was reduced by approximately 45 percent in the outer two layers and by about 35 percent in the inner layers (Fig. 2A). Dose-response curves for glutamate binding in the superior temporal gyri of brains from SDAT patients showed reductions in the maximum number of glutamate binding sites but no significant changes in their affinity relative to control (Fig. 2B). Computer analysis of quisqualate competition for [^{3}H]glutamate binding revealed two sites in the cerebral cortex (*11, 12*). The number of low-affinity quisqualate sites was significantly decreased (by 57 percent) in cortical tissue from SDAT patients compared to controls (Table 1). In contrast, glutamate binding in the caudate, putamen, claustrum, and nbM of brains from these patients was not significantly different from control. In brains from HD patients, binding in the claustrum, nbM, and cortical layers was normal but in the caudate and putamen was reduced (Fig. 2, A and B).

As expected (*5*), the activity of CAT in cortices from patients with SDAT was less than that in control, but the activity in cortices from HD patients was normal (Table 2). Measurements of the CAT and GAD activities in the putamen varied and showed no significant differences

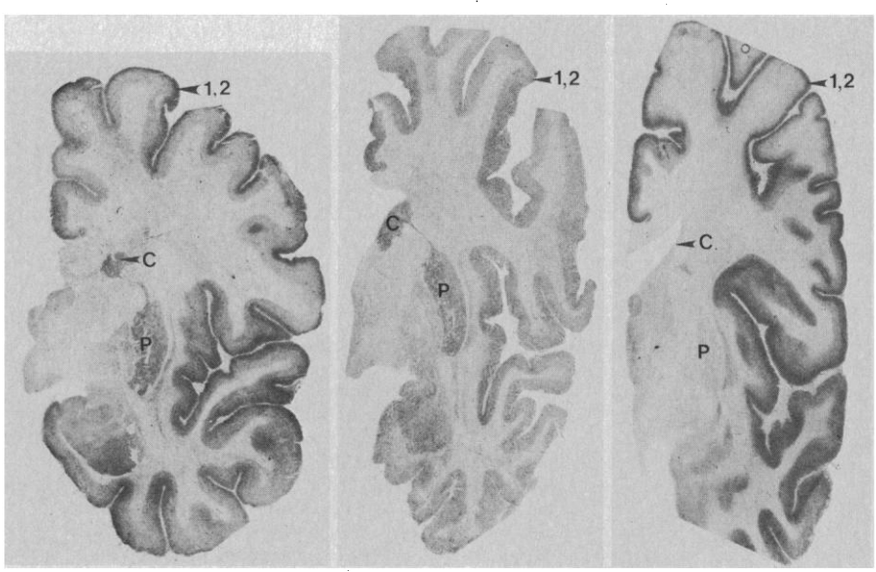

Fig. 1. Autoradiographs of coronal sections of brains from a patient with no neurologic disease (left), an SDAT patient (center), and an HD patient (right). The concentration of L-[^{3}H]glutamate was 200 n*M*. Autoradiography and measurements were performed as described (*8*). Binding in the cortex of the brain from the SDAT patient was diminished, particularly in superficial layers (labeled 1 and 2), compared to the control brain, whereas cortical binding in the brain from the HD patient was normal. Glutamate binding in the caudate (C) and putamen (P) from the HD patient was reduced compared to control, but binding in the same regions from the SDAT patient was normal.

between the groups, although the activities of these enzymes in brains from HD patients were only 57 and 17 percent of control activites, respectively (*5*). Numbers of muscarinic cholinergic, γ-aminobutyric acid, and benzodiazepine receptors were not significantly reduced in the cortices from SDAT patients compared to control (Table 2) but were reduced in the caudate and putamen of HD patients, as reported earlier (*13*).

Because glutamate is the putative neurotransmitter for both cortical association fibers and corticofugal fibers (*6*), glutamatergic pathways may be important in the transfer of information within the cortex via association fibers and between the cortex and subcortical nuclei. Furthermore, glutamate may play a role in memory formation because, in long-term potentiation (a model for memory formation), binding and release of glutamate are altered (*14*).

It has been proposed that an excitotoxic agent such as glutamate may contribute to the pathogenesis of HD (*7*). Our results apparently represent the first description of glutamate receptors in the HD brain (*12, 15*). The decrease in receptor density occurred only in the caudate and putamen, indicating that there is not a global change in this measure of glutamatergic transmission in HD. The preservation of glutamate binding in the HD cortex suggests that numbers of cortical glutamate receptors remain normal in this disease (*16*) and that chronic neurologic disease alone does not result in decreased cortical glutamate binding. The loss of receptors for glutamate and other transmitters in the HD caudate and putamen probably reflects the loss of cells that occurs in these regions in Huntington's disease.

It is not known whether the primary lesion in SDAT is cortical or subcortical; however, the decreased glutamate binding in this disease appears to be confined to cortical regions. Glutamate binding sites are enriched in dendritic zones (*8, 17*) and shrinkage of the dendritic arbor of cortical neurons has been described (*18*) in SDAT. Therefore, it is likely that the change in cortical glutamate binding in the SDAT brain represents decreased dendritic binding. Almost all the decrease in glutamate binding is due to the selective loss of a subtype of glutamate binding site (*11, 12*) that has a low affinity for quisqualate (Fig. 2B and Table 1). The relation between the loss of glutamate receptors and the etiology of SDAT is unknown. In the future, glutamate analogs may be used diagnostically to measure glutamate receptors in SDAT and HD by positron emission tomographic scanning of the brain.

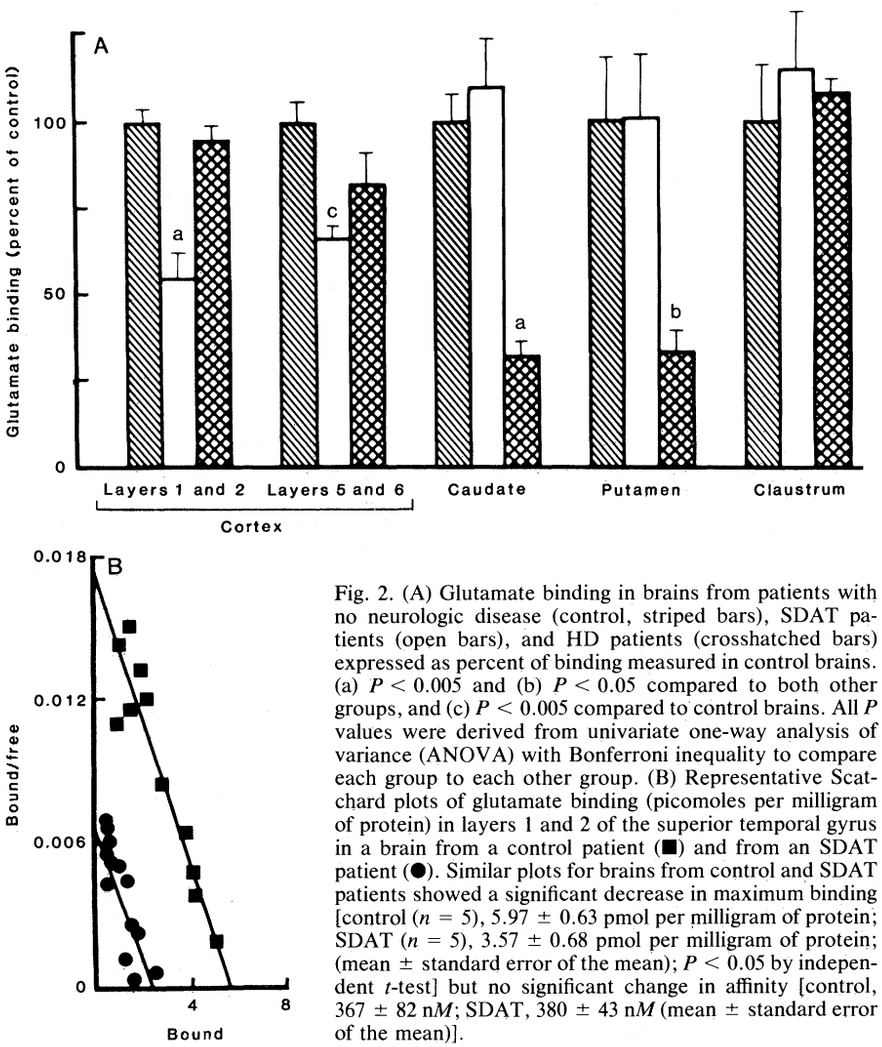

Fig. 2. (A) Glutamate binding in brains from patients with no neurologic disease (control, striped bars), SDAT patients (open bars), and HD patients (crosshatched bars) expressed as percent of binding measured in control brains. (a) $P < 0.005$ and (b) $P < 0.05$ compared to both other groups, and (c) $P < 0.005$ compared to control brains. All P values were derived from univariate one-way analysis of variance (ANOVA) with Bonferroni inequality to compare each group to each other group. (B) Representative Scatchard plots of glutamate binding (picomoles per milligram of protein) in layers 1 and 2 of the superior temporal gyrus in a brain from a control patient (■) and from an SDAT patient (●). Similar plots for brains from control and SDAT patients showed a significant decrease in maximum binding [control (n = 5), 5.97 ± 0.63 pmol per milligram of protein; SDAT (n = 5), 3.57 ± 0.68 pmol per milligram of protein; (mean ± standard error of the mean); $P < 0.05$ by independent t-test] but no significant change in affinity [control, 367 ± 82 nM; SDAT, 380 ± 43 nM (mean ± standard error of the mean)].

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Control of Cytochrome P_1-450 Gene Expression by Dioxin

Abstract. *The environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) may produce its effects by altering gene expression in susceptible cells. In mouse hepatoma cells, TCDD induces the transcription of the cytochrome P_1-450 gene, whose product, aryl hydrocarbon hydroxylase, contributes both to the detoxification and to the metabolic activation of carcinogenic polycyclic aromatic hydrocarbons. A DNA fragment containing sequences flanking the 5′ end of the cytochrome P_1-450 gene was isolated and analyzed. This DNA fragment contains a* cis*-acting control element with at least three functional domains: a putative promoter, an inhibitory domain upstream from the promoter that blocks its function, and a TCDD-responsive domain still farther (1265 to 1535 base pairs) upstream of the promoter. These findings, together with results from earlier studies, imply that transcription of the cytochrome P_1-450 gene is under both positive and negative control by at least two* trans*-acting regulatory factors.*

Halogenated dibenzodioxins have generated interest because of their potential toxicity and their presence as environmental contaminants. Effects of the prototypical dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), such as epithelial metaplasia, tumor promotion, teratogenesis, and enzyme induction, suggest that the compound alters gene expression in responsive cells (*1*). The binding of TCDD to an intracellular protein receptor and the accumulation of TCDD-receptor complexes in the nucleus are required for TCDD action (*1, 2*). We have studied the mechanism by which TCDD increases the expression of the cytochrome P_1-450 gene in mouse hepatoma cells. In wild-type (Hepa 1c1c7) cells, TCDD induces about a 20-fold increase in the rate of transcription of the cytochrome P_1-450 gene, which is followed by corresponding increases in the content of cytochrome P_1-450 messenger RNA (mRNA) and in the activity of aryl hydrocarbon hydroxylase (E.C. 1.14.14.1) (*3, 4*).

We have isolated two classes of variant cells that show decreased responsiveness to TCDD (*3, 5*). Class I variants form few TCDD-receptor complexes; those that do form accumulate within the nucleus, as is the case with wild-type cells. These variant cells show decreased accumulation of cytochrome P_1-450 mRNA and lower aryl hydrocarbon hydroxylase activity in response to TCDD. Class II variants form a normal number of TCDD-receptor complexes; however, the complexes do not accumulate in the nucleus. These variant cells do not transcribe the cytochrome P_1-450 gene in response to TCDD. Thus, induction of cytochrome P_1-450 gene transcription by TCDD requires the localization of TCDD-receptor complexes in the nucleus.

The results of nuclear transcription assays indicate that concurrent exposure to TCDD and cycloheximide superinduces the rate of transcription of the cytochrome P_1-450 gene to a level about ten times greater than the maximum level induced by TCDD alone. Superinduction requires functional TCDD-receptor complexes because it does not occur in class II variant cells (*6*). These findings indicate the existence of a second control mechanism for cytochrome P_1-450 gene transcription. Taken together, our results imply that at least two *trans*-acting factors regulate cytochrome P_1-450 gene expression: (i) the TCDD-receptor complex and (ii) a labile repressor protein that negatively modulates the action of the TCDD-receptor complex.

We have isolated a third class of variant cells that overtranscribe the cytochrome P_1-450 gene in response to TCDD. These cells are designated high activity variant (HAV) cells. Our analyses suggest that these variants contain an altered *cis*-acting genomic element that increases their responsiveness to TCDD (*7*). We have isolated from HAV cells a genomic element that regulates the response of the cytochrome P_1-450 gene to TCDD. This control element has novel properties compared to other elements known to regulate the expression of eukaryotic genes.

A genomic library was constructed by partial digestion of HAV cellular DNA with Mbo I, insertion of 10- to 16-kb (kilobase) fragments into the Bam HI site in bacteriophage λ L47.1, and packaging in vitro (*8*). After growth on *Escherichia coli* C600, plaques containing cytochrome P_1-450 DNA were isolated by repetitive screening with nick-translated cytochrome P_1-450 complementary DNA (*9*). Figure 1A shows the restriction map of one clone, designated λ CPM 17. Hybridization of nick-translated cytochrome P_1-450 cDNA to restriction digests of λ CPM 17 and hybridization of nick-translated λ CPM 17 restriction fragments to RNA from uninduced and TCDD-induced cells determined the orientation of the fragments within the clone and identified a 2.58-kb Hind III fragment that appeared to contain the 5′ end of cytochrome P_1-450 gene.

To determine whether the 2.58-kb Hind III fragment contains regulatory information, we inserted it into the plasmid pSV0cat at the Hind III site immediately upstream of the chloramphenicol acetyltransferase (CAT) gene (*10*). Recombinants were isolated as ampicillin-resistant colonies in *E. coli* HB101, and the