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Putamen Mitochondrial Energy Metabolism Is Highly Correlated to Emotional and Intellectual Impairment in Schizophrenics

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In a recent study, we demonstrated that cytochrome-c oxidase (COX), an indicator of neuronal activity, is increased in several brain regions from chronic, medicated schizophrenics. In the present study, to address the functional significance of those findings, we have measured COX activity in a group of schizophrenics in whom antemortem geriatric measures of motor, intellectual, and emotional impairment had been assessed. COX activity in the putamen was strongly negatively correlated with emotional ($r = -.76$; $p < .005$) and intellectual impairment ($r = -0.76$; $p < .005$), but not with motor impairment ($r = 0.01$). No significant correlations could be found in the frontal cortex, thalamus, caudate nucleus, globus pallidus, mesencephalon, or nucleus accumbens. Dopamine D2 receptor density in the putamen, measured with [3H]raclopride, was elevated in schizophrenics as compared to controls, as were Kd values. In contrast to COX activity, D2 receptor binding was moderately, but significantly positively correlated with intellectual impairment ($r = 0.64$; $p < .05$) but not with motor impairment. Results expose a unique anomaly in the effects of neuroleptics in terms of increasing neuronal signaling in the putamen, which may underlie a reversal of cognitive deficits in schizophrenics, while at the same time, elevating D2 receptor density that seems to be detrimental.
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KEY WORDS: *Cytochrome-c oxidase; Dopamine; D2; Mitochondria; Putamen; Receptor; Schizophrenia*

Abnormalities in cognitive processes involving attention and working memory are becoming accepted as hallmarks of schizophrenia (Andreasen 1997; Saykin et al. 1991). Although the classic symptoms of the disorder, including hallucinations, delusions, and emotional blunting, have traditionally led to the assumption that multiple brain regions are involved in their expression, the possibility that these may instead result from defects in a primary brain structure has also received considerable attention. To address this issue, a wide variety of approaches have been employed with the goal of identifying brain regions that deviate from normality in schizophrenics. Primary among these have been such imaging techniques as positron emission tomography (PET) and magnetic resonance imaging (MRI). However, despite the volume of data produced and extent to which these have been applied, consensus on a physiological correlate of disease symptomatology remains to be reached. An attractive theme that has emerged as a result of post-mortem, imaging, and animal studies, is that defects in the primary structures of the brain that moderate or filter incoming information may be critical

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to the disease process. In this regard, the concept of a defective cortico-striatal-thalamic circuit in schizophrenia has become widely recognized (Carlsson and Carlsson 1990; Carlsson et al. 1997). In support of this, all of these regions, including the frontal cortex (Buchsbaum et al. 1982; Siegel et al. 1993), striatum (Siegel et al. 1993; Shihabuddin et al. 1998), and thalamus (Andreasen et al. 1996), have been implicated as potentially defective in schizophrenics.

As a complement to imaging studies, our laboratory has applied a strategy involving the post-mortem measurement of the mitochondrial respiratory chain enzyme cytochrome-c oxidase (COX) in an attempt to localize altered brain function in schizophrenia. This approach is based upon a strong body of evidence that indicates that neuronal COX is highly regulated by the energy demands of the cell and, as such, represents an endogenous marker of cellular energy metabolism over time (Wong-Riley 1989). Interest in COX as a marker of neuronal function rests upon many of the same assumptions implicit in the use of PET in the measurement of regional glucose metabolism and blood flow. Neurons are highly dependent upon oxidative phosphorylation as the primary pathway for the generation of ATP, of which 40 to 60% is utilized in the maintenance of ion gradients by ATPases. In support of this, a strong correlation has been demonstrated between the regulation of COX and Na+,K+ ATPase in brain tissue (Hevner et al., 1992). Traditionally, however, the strongest evidence that COX is coupled to neuronal energy demands comes from studies in which changes in COX activity can be induced by experimental interventions that alter neuronal activity. Perhaps the most interesting study in this regard was performed utilizing a histochemical technique to demonstrate reduced COX activity in the brains of cats in which chronic neuronal inactivity was induced in visual cortex by monocular suture (Wong-Riley 1979). In addition, studies have shown that monocular retinal impulse inhibition with tetrodotoxin results in a decrease in COX activity in specific regions of the monkey visual cortex and thalamus (Wong-Riley and Carrol 1984). In terms of localization, evidence suggests that as much as 60% of COX activity in a particular region reflects dendritic activity; whereas, glial cell activity is responsible for less than 5% (Wong-Riley, 1989).

In this article, we have primarily explored the relative contribution of energy metabolism in various brain regions to emotional, intellectual, and motor capacity in schizophrenics. Based upon extensive research alluding to an increase in dopamine receptor binding in striatal structures in schizophrenics, primarily the putamen, an attempt was also made to address the possibility that this finding may correlate with a general increase in metabolic activity in the region.

MATERIALS AND METHODS

Materials

PD10 columns were obtained from Pharmacia (Uppsala, Sweden). [^{3}H]raclopride was purchased from New England Nuclear (Boston, MA). Haloperidol (Haldol®) was obtained from Janssen (Beerse, Belgium). Cytochrome-c (horse heart) and all other compounds were purchased from Sigma Chemical Co. (St. Louis, MO).

Subjects and Tissue Preparation

For the primary study relating to COX, D2 binding, and cognitive measures, putamen samples were obtained from 12 schizophrenics and 10 controls with no history of psychiatric illness. The average ages for these two groups were 82 ± 11 (SD) for schizophrenics and 80 ± 7 (SD) for controls. Post-mortem intervals were 42 ± 12 (SD) for schizophrenics and 51 ± 14 (SD) for controls. Data pertaining to patient demographics are shown in Table 1. Results in Figures 1,2, and 3 all relate specifically to these samples.

To ascertain if findings in the putamen were regionally specific, a meta-analysis was also conducted on brain samples that have previously been described biochemically but not in relation to cognitive measures (Cavelier et al. 1995; Prince et al. 1999). The number of samples employed for each brain region is listed in Table 2. For this study, brain samples were re-analyzed, and correlation coefficients were established, with emotional, intellectual, and motor impairment scales (described below).

Patients were diagnosed according to the Diagnostic and Statistical Manual (DSM) III-R. All schizophrenics had suffered from a chronic course of the disease and been treated extensively with various neuroleptics. All patients had received neuroleptic treatment ranging from 50 to 400 mg/day (chlorpromazine equivalents) within the last 2 months before death (Table 1). No evidence of Alzheimer's disease or other neuropathological features of degenerative disease could be found in either schizophrenics or controls. No evidence of substance abuse was documented in any of the patients. Brain tissue specimens from the frontal cortex, caudate nucleus, putamen, nucleus accumbens, globus pallidus, thalamus, and mesencephalon were obtained at autopsy, and dissections were performed according to anatomical landmarks: the nucleus accumbens was taken from the junction between the frontal caudate nucleus and putamen. The mesencephalon was a cross section of brainstem below the superior and inferior colliculi. This rough dissection of the mesencephalon includes portions of the A8, A9, A10, and raphe nucleus. Following dissection, brain samples were frozen in liquid nitrogen and crushed into a course powder before storage at

Table 1. Clinical Characteristics of Schizophrenics and Controls used for Putamen Studies

Patient	Sex	Age	Illness Duration	Cause of Death	Agonal Status	Neuroleptic Treatment
1	F	99	52	Heart failure	<1 week	50–400
2	M	80	60	Heart failure, bronchitis	<1 month	50–400
3	M	66	39	Myocardial infarction	Rapid (<2 days)	300
4	F	80	55	Myocardial infarction	Rapid (<2 days)	50
5	F	87	56	Pneumonia	Rapid (<2 days)	50–400
6	F	95	63	Myocardial infarction	Rapid (<2 days)	150
7	F	75	57	Pneumonia	<1 week	200
8	M	92	64	Pneumonia	Rapid (<2 days)	400
9	F	74	52	Heart failure	2 days	50–400
10	M	77	52	Pneumonia	<1 week	50
11	M	91	60	Myocardial infarction	<1 week	50–400
12	M	93	Unknown	Pneumonia	Rapid (<2 days)	50–400
13	F	89	——	Heart failure	Rapid (<2 days)	——
14	M	80	——	Heart failure	Rapid (<2 days)	——
15	F	89	——	Lung cancer, Pneumonia	<1 week	——
16	M	77	——	Lung cancer	<1 week	——
17	F	64	——	Colon cancer	<1 week	——
18	F	86	——	Myocardial infarction	Rapid (<2 days)	——
19	M	74	——	Heart failure	<1 week	——
20	M	71	——	Myocardial infarction	Rapid (<2 days)	——
21	F	90	——	Myocardial infarction	Rapid (<2 days)	——
22	F	79	——	Heart failure	Rapid (<2 days)	——

Age and duration of illness are in years. Neuroleptic dose is in mg/day in chlorpromazine equivalents. Patients 1–12 are schizophrenics, and 13–22 are controls.

−70°C. For cytochrome-c oxidase activity measurements, brain samples were homogenized in a buffer consisting of 10 mm potassium phosphate (pH 7.6), 1 mm EDTA, 0.25 m sucrose in an Ultra-Turrax set on full speed for 30 s. Homogenates were then frozen at −70°C until assayed.

Cognitive measures in schizophrenics were assessed according to the geriatric rating scale constructed by Gottfries and Gottfries (Adolfsson et al. 1981). The rating scale consists of a series of yes/no questions relating to general patient performance and contains three subscales measuring impairment of motor performance, intellectual impairment, and emotional impairment. The system was originally designed to be applied to post-mortem brain biochemical studies primarily involving neurotransmitter/metabolite levels. Values are reported posthumously during the week following death by a trained nurse in charge of individual patient care. On the basis that they were not institutionalized nor under psychiatric care before death, no values for controls were obtained. Biochemical studies were performed blind to rating scale results.

Cytochrome-c Oxidase Assay

Cytochrome-c oxidase was assayed according to a modification of the spectrophotometric method of Yonetani and Ray (1965). Reduced cytochrome c was prepared

Table 2. Remaining Correlations between COX Activity and Emotional, Intellectual, and Motor Impairment in Various Brain Regions in Schizophrenics

Brain Region	No. of Cases	Intellectual Impairment	Emotional Impairment	Motor Impairment
Caudate nucleus	18	0.189	0.064	0.081
Globus pallidus	11	0.415	0.559	0.437
Accumbens nucleus	12	0.033	0.121	0.163
Thalamus	10	0.086	0.181	0.450
Frontal cortex	10	0.179	0.140	0.157
Mesencephalon	14	0.334	0.294	0.540

Values represent r for linear regression lines. Significance was determined using Student's t-test. Although no significant findings were made, tendencies toward positive correlations were evident in the globus pallidus and mesencephalon with motor impairment.

by the addition of 30 mg $Na_2S_2O_4$/100 mg cytochrome c in a 10 mm potassium phosphate buffer (pH 7.6) containing 1 mm EDTA, and separated on a PD10 column (Pharmacia). Incubations were performed in 10 mm potassium phosphate buffer (pH 7.6), 1 mm EDTA, and 25 μm reduced cytochrome c at 25°C. Upon the addition of approximately 100 μg protein, the change in absorbance at 550 nm was determined on a Jasco V-550 spectrophotometer for 5 min. Initial rates were determined differentially where -d[ferrocytochrome c]/dt is derived from polynomial plots at zero time using an extinction coefficient of 19.0 $mm^{-1}cm^{-1}$ (Yonetani and Ray 1965).

[^{3}H]Raclopride Binding Assay

Experiments were carried out as described by Lepiku et al. (1996). Brain tissue was resuspended in ice-cold Tris-HCl (50 mm, pH 7.4) containing NaCl (120 mm), $MgCl_2$ (5 mm) and EDTA (1 mm). After a centrifugation at 33,000 *g* for 20 min and homogenization using a Potter-S glass-*Teflon* homogenizer (1,000 rpm, six passes), the membranes were incubated at 37° for 30 min. The pellet was then re-homogenized in the same buffer. D_2/D_3 receptor labeling was carried out in the presence of 0.5 to 10 nm [^{3}H]-raclopride (spec. act. 78.4 Ci/mmol, N.E.N.) at room temperature with approximately 0.2 mg protein per tube in a total incubation volume of 0.3 ml. Haloperidol (10 μm) was added to determine nonspecific binding. Incubation was terminated after 30 min by rapid filtration over Whatman GF/B filters using Brandel Cell Harvester (M-24S). The filters were washed with 9 ml cold incubation buffer, dried, and assayed for radioactivity by liquid scintillation spectrometry. All binding data were analyzed by nonlinear least-squares regression analysis using a commercial program *GraphPad PRISM* 2.0 (GraphPad Software, San Diego, CA)

Protein and DNA Measurements

The total amount of protein in the samples was determined according to the Markwell et al. (1978) modification of the Lowry et al. (1951) procedure using bovine albumin as a standard. Based upon extensive evidence that suggests DNA is a sensitive indicator of cell number (Downs and Wilfinger 1983; Rago et al. 1990) the total quantity of dsDNA in samples was determined fluorometrically using PicoGreen (Molecular Probes) based upon its superior selectivity for DNA over RNA (Singer et al. 1997). Samples (10 μl) were added to a 50 μl TE buffer (0.01 m Tris-HCl buffer, pH 8.0, 1 mm EDTA) containing 0.01% SDS, incubated at RT for 10 minutes and then sonicated at low power for 5 s on a Branson B15 cell disruptor. A 1 ml solution containing 0.6 μm PicoGreen was then added to the samples and incubated for 5 min at RT. Fluorescence was then determined on a Jasco FP-777 spectrofluorometer using 480 nm and 520 nm excitation and emission wavelengths. Calf thymus DNA was used as a standard, and a value of 7.23 pg DNA/cell was used to calculate cell number.

Statistics

All statistical analyses were performed using StatView v4.5.1 (Abacus Concepts) and Prism (GraphPad). Statistically significant differences in the means of biochemical data were established using Student's t-test, ANOVA, and Fisher's PLSD. Presented correlations were calculated by simple linear regression analysis, and *p*-values were obtained using paired t-tests. Significance was also assessed using Spearman rank correlation coefficients.

RESULTS

As an anticipated effect of long-term neuroleptic treatment (Prince et al. 1997a,b; Prince et al. 1998), COX activity was found to be increased in the putamen in this group of schizophrenics (Figure 1). Levels were essentially consistent with those of an earlier study (Prince et al. 1998). To allow for comparison between brain regions, material from other brain regions that had previously been examined was reanalyzed. No marked deviations from previously reported values were found. On the basis that clinical records typically demonstrate a varied dosing regimen for schizophrenic patients, it was also important to confirm that D2 receptor Kd levels were also elevated as compared to controls, offering indirect evidence of the presence of residual neuroleptics (Figure 1). Finally, D2 receptor levels, measured with 3H-raclopride, were also found to be elevated in schizophrenics, offering additional support for the handful of studies that have addressed this question (Burt et al. 1977; Mackay et al. 1982) and suggested that this results from neuroleptic treatment (Figure 1).

The primary finding in the present study was the significant correlation between COX activity and intellectual and emotional impairment, but not motor impairment, in the putamen of chronically medicated schizophrenics (Figure 2). Results using Spearman rank correlation coefficients were $p = .0067$ and $p = .0121$ for intellectual and emotional impairment, respectively. This effect was specific for the putamen, because none of the other regions investigated were found to correlate significantly with these parameters (Table 2). In this regard, no significant correlations could be found between these three measures and COX in the frontal cortex, caudate nucleus, nucleus accumbens, globus pallidus, thalamus, or mesencephalon. However, a tendency toward a positive correlation was evident in the globus

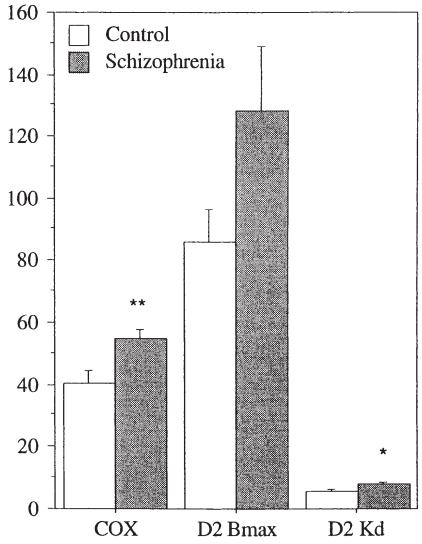

Figure 1. COX activity (nmol/min/mg), D2 binding (fmol/mg), and D2 Kd coefficients (nm) in the putamen of schizophrenics. Significance was determined using Student's t-test. * $p < .05$; ** $p < .005$. Significance for Bmax was $p = .054$.

pallidus and mesencephalon with regard to motor impairment, but significance did not fall below the $p < .1$ level (Table 2). In contrast with the correlation between COX and cognitive measures in the putamen, a moderate but significant positive correlation was observed between D2 receptor binding and emotional impairment in the region (Figure 2). This was not evident for motor impairment, but a tendency was apparent for intellectual impairment (Figure 2). A unique finding was also made in terms of a correlation between COX activity and D2 binding in the putamen (Figure 3). In this regard, a significant positive correlation could be demonstrated in controls, but this was apparently absent in schizophrenics. Neither of these two latter relationships, D2 binding and emotional impairment nor COX and D2 binding, were significant when Spearman rank correlation coefficients were used. Nonetheless, these results are still presented, and discussion is raised around them based on the significance evidenced by simple linear regression analysis.

Although no assessment of dendritic density was made in this study, a measure that should be reflected by both COX and D2 binding, attempts were made to correlate cell density with COX, D2 binding, and cognitive measures. The underlying assumption in this analysis was that protein measures alone would not suffice if a generalized change in synaptic density had occurred. On this basis, the relationship between protein and DNA quantity was anticipated to reveal a possible deviation. In this regard, no significant findings were made (data not shown). A high correlation was found between protein and DNA quantity (data not shown), suggesting that both measures are closely related.

A final correlation study was performed between COX activity in various brain regions and D2 binding in the putamen. Significant positive correlations were observed between the mesencephalon and thalamus and D2 binding ($p < .005$ in both cases; data not shown). In addition, neither COX nor D2 binding were correlated with post-mortem interval or age (data not shown).

DISCUSSION

In the present study, we provide evidence that implicates the specific involvement of the putamen in the regulation of cognitive functions that may be of relevance for schizophrenia. The concept that neuroleptics impart their effects via an enhancement of energy metabolism in specific brain structures, particularly the striatum is supported by a firm base of data (Prince et al. 1997a,b; Prince et al. 1998; DeLisi et al. 1985; Holcomb et al. 1996). On the basis that patients in this study were highly medicated before death, results strengthen the hypothesis that an elevation in energy metabolism in the putamen, possibly reflecting enhanced excitatory transmission, may underlie some of the beneficial effects of neuroleptics. However, this study is limited by several factors. The primary measure of energy metabolism (COX activity) is still subject to scrutiny based upon limited knowledge about stability of the protein in the brain over time. This is particularly relevant considering the extended post-mortem durations of patients. In addition, results reflect findings in a geriatric group and may not be indicative of what occurs in a younger population.

The search for associations between schizophrenic behavior and brain physiology has seen a wide variety of approaches over the years. The field has primarily benefited from such brain imaging techniques as PET and MRI and, from these, several cohesive themes have emerged. In this regard, the concept of "hypofrontality" has played a central role in our understanding of the disease (Siegel et al. 1993; Buchsbaum et al. 1992). However, assumptions about complex patterns of altered brain function are giving way to theories that attempt to define schizophrenia based upon alterations in a fundamental cognitive process (Sabri et al. 1997; Andreasen et al. 1997; Andreasen 1997). A convincing argument for this being the case stems from findings that a cognitive deficit in schizophrenia remains stable, even in the presence of fluctuating symptoms (Weinberger and Gallhofer 1997).

Although studies in which brain imaging has been employed to describe correlations between symptoms and brain alterations in schizophrenics are relatively

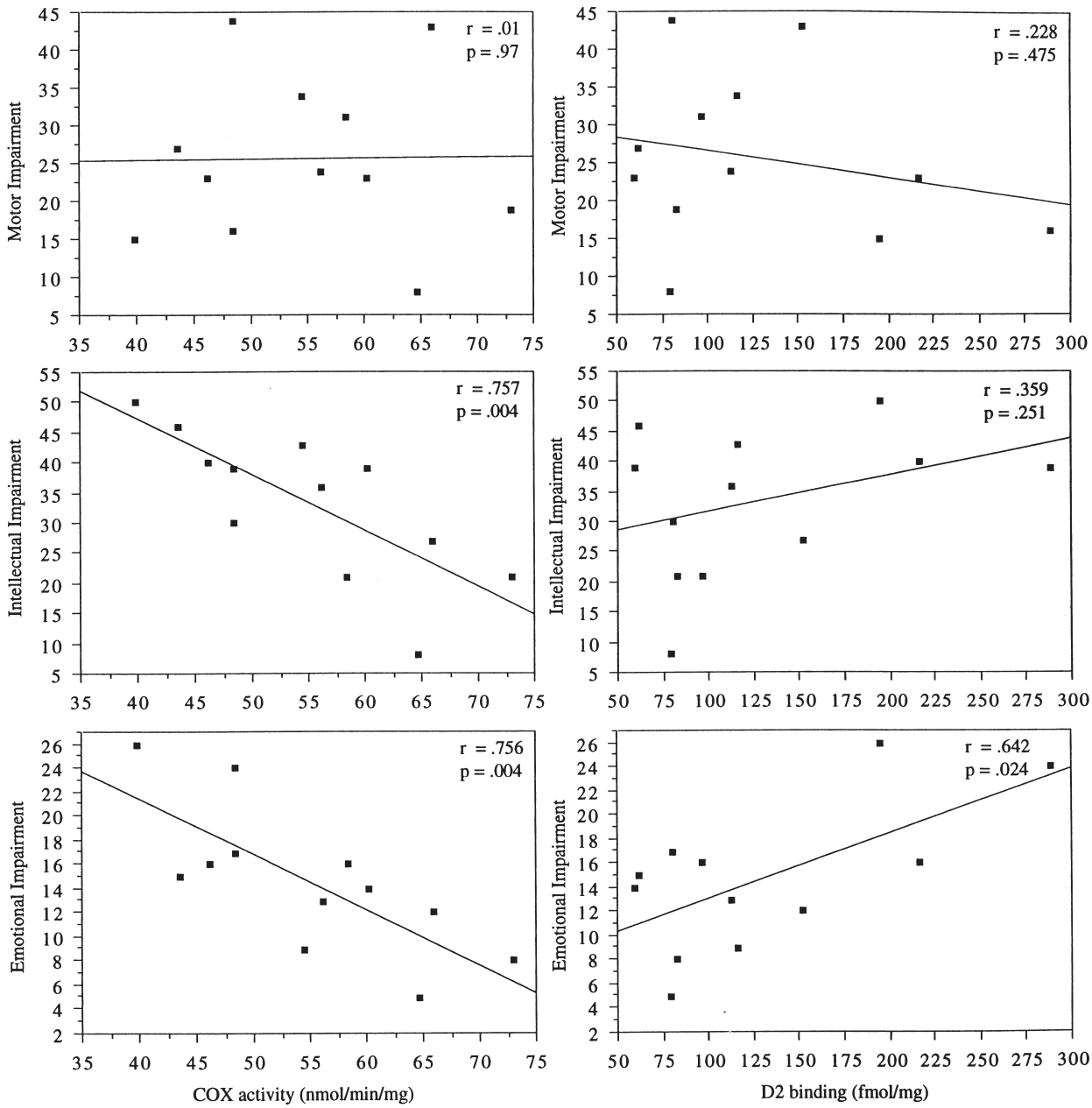

Figure 2. Correlations between COX (left column) and D2 binding (right column) and motor, intellectual, and emotional impairment in the putamen of schizophrenics. Graphs represent linear regression lines from simple regression analysis. Significance was determined by Student's t-test.

common (Sabri et al. 1997; Okubo et al. 1997), findings that correlate basic cognitive measures with brain changes are rare. In this regard, correlations have been found between cognitive neuropsychological indexes and striatal size (Stratta et al. 1997) and glucose metabolic rate (Nordahl et al. 1996). Although cortical regions may also play an important role in schizophrenic pathology (Nordahl et al. 1996; Shenton et al. 1992; Humphries et al. 1996), evidence implicating the striatum is perhaps more substantial (Siegel et al. 1993). This is important considering the fact that our present results suggest a pronounced correlation with emotional and intellectual impairment and COX in the putamen, but not in the frontal cortex. The critical involvement of the putamen in information processing in the brain is well documented (Brown et al. 1997). In this regard, the concept of the putamen as a regulator of information to the thalamus is particularly pertinent to schizophrenic pathology (Carlsson et al. 1997; Smith and Bolam 1990).

Although the case for the putamen is formidable, several anomalies in the present study confound the evidence. The foremost of these is the lack of correlation

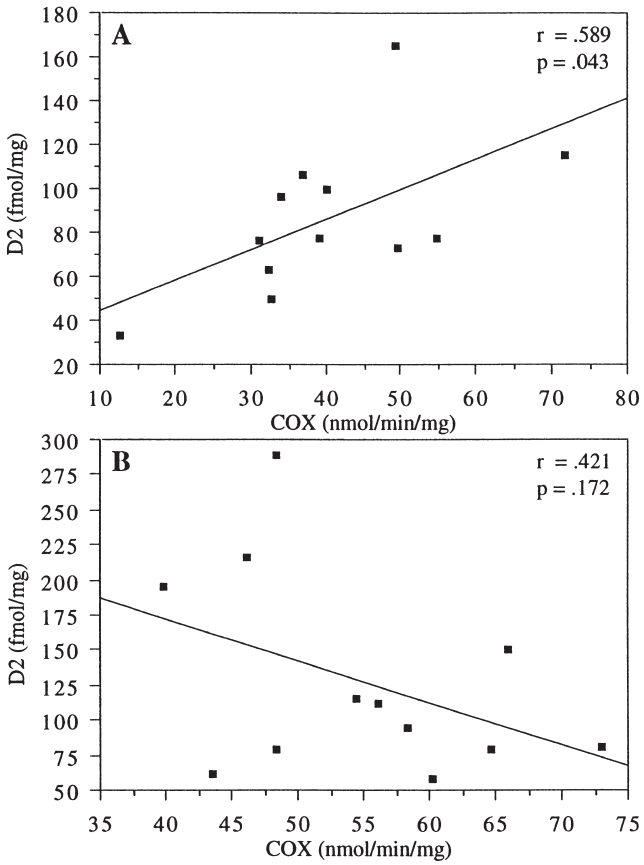

Figure 3. Correlations between COX and D2 binding in the putamen of **(A)** controls and **(B)** schizophrenics. Graphs represent linear regression lines from simple regression analysis. Significance was determined using Student's t-test.

between psychiatric parameters and COX activity in the caudate nucleus or nucleus accumbens, both of which contain high levels of dopamine receptors. However, we have previously reported that COX activity is decreased in the caudate, but elevated in the putamen in schizophrenics, a fact that may aid in explaining this dilemma (Prince et al. 1999). In this regard, it was suggested that the effects of neuroleptics in the caudate may be subordinate to those upon the putamen in terms of elevating functional activity (Prince et al. 1998a). This is of importance based upon recent evidence that the caudate is involved in working memory (Levy et al. 1997). Thus, an alternative explanation in this matter is that a lack of correlation in the caudate in schizophrenics may itself be "pathological" and suggestive of damaged circuitry. Indeed, the lack of ability of neuroleptics to reverse a potential deficit in the caudate may underlie some of their therapeutic shortcomings in terms of inconsistent effects on cognitive function (King 1990; Hindmarch 1994).

The second anomalous finding in this study was the positive correlation between emotional impairment and D2 receptor binding in the putamen. To our knowledge, this is the first time that elevated D2 binding has been shown to be detrimentally associated with a behavioral parameter. Although the elevation of dopamine receptors by neuroleptics is well established (Mackay et al. 1982), the behavioral consequences have been more difficult to define (Burt et al. 1977). In this regard, the main focus has been on such extrapyramidal disturbances as tardive dyskinesia, which are assumed to result from chronic neuroleptic treatment. On the basis that no correlation was found between D2 levels and motor impairment, present results suggest that extrapyramidal disturbances may not be directly coupled to D2 receptor elevation in the putamen. Instead, it seems that an elevation in D2 receptor binding may be more relevant for emotional blunting, which is also a common side effect of neuroleptic treatment (Casey 1995).

The final finding in this study that deserves attention is the correlation between energy metabolism and D2 binding in the putamen in controls and the absence of a correlation in schizophrenics. Although the biochemical basis for this effect is difficult to ascertain, it is likely to result from neuroleptic treatment. This may also have relevance for the finding of a positive association between D2 levels and emotional impairment. That this effect is dependent upon neuroleptics is supported by the finding that D2 levels in normal individuals are negatively correlated with "detachment," a measure of social withdrawal according to the Karolinska Scales of Personality (KSP) (Farde et al. 1997). Thus, it seems that under normal circumstances, D2 binding and COX are correlated, reflecting functioning circuitry that may be disturbed by neuroleptics. The chain of events can perhaps be envisioned as follows: as D2 receptors are blocked, the initial effect is to enhance the effect of the glutamatergic system in the putamen, resulting in an increase in metabolic rate in the region and, thus, an increase in the need for metabolic machinery; that is, COX. An increase in D2 levels may then occur disproportionately with this effect. In terms of significance, the primary location of mitochondria in the brain is within dendrites, implying that increases in energy metabolism are also predominantly dendritic (Wong-Riley 1989). The concept that increased synaptic density in the putamen may result from neuroleptic treatment receives support from findings of increased synaptophysin levels after haloperidol treatment (Eastwood et al. 1994).

In summary, the present study provides evidence that an increase in energy metabolism in the putamen may underlie an augmentation of intellectual and emotional capacity in schizophrenics. Evidence that this occurs as a result of chronic neuroleptic treatment is substantial (Prince et al. 1997a,b; Prince et al. 1998; Delisi et al. 1985; Holcomb et al. 1996). In contrast, an elevation in D2 receptor binding seems to have a detrimental effect

upon emotion. Together, these findings illuminate the possibility of identifying pharmacological approaches that strive to increase energy metabolism in the putamen, while at the same time, minimizing an upregulation of D2 receptors.

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REFERENCES

Adolfsson R, Gottfries CG, Nystrom L, Winblad B (1981): Prevalence of dementia disorders in institutionalized Swedish old people. The work load imposed by caring for these patients. Acta Psychiat Scand 63:225–244

Andreasen NC, O'Leary DS, Cizadlo T, Arndt S, Rezai K, Ponto LL, Watkins GL, Hichwa RD (1996): Schizophrenia and cognitive dysmetria: A positron-emission tomography study of dysfunctional prefrontal-thalamic-cerebellar circuitry. Proc Nat Acad Sci USA 93:9985–9990

Andreasen NC, O'Leary DS, Flaum M, Nopoulos P, Watkins GL, Boles Ponto LL, Hichwa RD (1997): Hypofrontality in schizophrenia: Distributed dysfunctional circuits in neuroleptic-naive patients. Lancet 349:1730–1734

Andreasen NC (1997): Linking mind and brain in the study of mental illnesses: A project for a scientific psychopathology. Science 275:1586–1593

Brown LL, Schneider JS, Lidsky TI (1997): Sensory and cognitive functions of the basal ganglia. Curr Opin Neurobiol 7:157–163

Buchsbaum MS, Haier RJ, Potkin SG, Nuechterlein K, Bracha HS, Katz M, Lohr J, Wu J, Lottenberg S, Jerabek PA, Trenary M, Tafalla R, Reynolds C, Bunney WE (1992): Fontostriatal disorder of cerbral metabolism in never-medicated schizophrenics. Arch Gen Psychiat 49:935–942

Buchsbaum MS, Ingvar DH, Kessler R, Waters RN, Cappelletti J, van Kammen DP, King AC, Johnson JL, Manning RG, Flynn RW, Mann LS, Bunney WE Jr, Sokoloff L (1982): Cerebral glucography with positron tomography. Use in normal subjects and in patients with schizophrenia. Arch Gen Psychiat 39:251–259

Burt DR, Creese I, Snyder SH (1977): Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. Science 196:326–328

Carlsson M, Carlsson A (1990): Interactions between glutamatergic and monoaminergic systems within the basal ganglia—Implications for schizophrenia and Parkinson's disease. Trends Neurosci 13:272–276

Carlsson A, Hansson LO, Waters N, Carlsson ML (1997): Neurotransmitter aberrations in schizophrenia: New perspectives and therapeutic implications. Life Sci 61:75–94

Casey DE (1995): Motor and mental aspects of extrapyramidal syndromes. Int Clin Psychopharmacol 10:105–114

Cavelier L, Jazin EE, Eriksson I, Prince J, Bave U, Oreland L, Gyllensten U (1995): Decreased cytochrome-c oxidase activity and lack of age-related accumulation of mitochondrial DNA deletions in the brains of schizophrenics. Genomics 29:217–224

DeLisi LE, Holcomb HH, Cohen RM, Pickar D, Carpenter W, Morihisa JM, King AC, Kessler R, Buchsbaum MS (1985): Positron emission tomography in schizophrenic patients with and without neuroleptic medication. J Cereb Blood Flow Metab 5:201–206

Downs TR, Wilfinger WW (1983): Fluorometric quantification of DNA in cells and tissue. Anal Biochem 131:538–547

Eastwood SL, Burnet PW, Harrison PJ (1994): Striatal synaptophysin expression and haloperidol-induced synaptic plasticity. NeuroReport 5:677–680

Farde L, Gustavsson JP, Jonsson E (1997): D2 dopamine receptors and personality traits. Nature 385:590

Hevner RF, Duff RS, Wong-Riley MTT (1992): Coordination of ATP production and consumption in brain: Parallel regulation of cytochrome oxidase and Na+,K+-ATPase. Neurosci Lett 138:188–192

Hindmarch I (1994): Instrumental assessment of psychomotor functions and the effects of psychotropic drugs. Acta Psychiat Scand Suppl 380:49–52

Holcomb HH, Cascella NG, Thaker GK, Medoff DR, Dannals RF, Tamminga CA (1996): Functional sites of neuroleptic drug action in the human brain: PET/FDG studies with and without haloperidol. Am J Psychiat 153:41–49

Humphries C, Mortimer A, Hirsch S, de Belleroche J (1996): NMDA receptor mRNA correlation with antemortem cognitive impairment in schizophrenia. NeuroReport 7:2051–2055

King DJ (1990): The effect of neuroleptics on cognitive and psychomotor function. Br J Psychiat 157:799–811

Lepiku M, Rinken A, Järv J, Fuxe K (1996): Kinetic evidence for isomerization of the dopamine receptor—raclopride complex. Neurochem Int 28:591–595

Levy R, Friedman HR, Davachi L, Goldman-Rakic PS (1997): Differential activation of the caudate nucleus in primates performing spatial and nonspatial working memory tasks. J Neurosci 17:3870–3882

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with the folin phenol reagent. J Biol Chem 193:265–75

Mackay AV, Iversen LL, Rossor M, Spokes E, Bird E, Arregui A, Creese I, Synder SH (1982): Increased brain dopamine and dopamine receptors in schizophrenia. Arch Gen Psychiat 39:991–997

Markwell MAC, Maas SM, Biebar LL, Tolbert NE (1978): A modification of the Lowry procedure to simplify protein determination in membrane and in protein samples. Anal Biochem 87:206–211

Nordahl TE, Kusubov N, Carter C, Salamat S, Cummings AM, O'Shora-Celaya L, Eberling J, Robertson L, Huesman RH, Jagust W, Budinger TF (1996): Temporal lobe metabolic differences in medication-free outpatients with schizophrenia via the PET-600. Neuropsychopharmacology 15:541–554

Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, Someya Y, Sassa T, Sudo Y, Matsushima E, Iyo M, Tateno Y, Toru M (1997): Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385(6617):634–636.

Prince JA, Blennow K, Gottfries CG, Karlsson I, Oreland L (1999): Mitochondrial function is differentially altered in the basal ganglia of chronic schizophrenics. Neuropsychopharmacology 21:372–379

Prince JA, Yassin MS, Oreland L (1997a): Neuroleptic-induced mitochondrial enzyme alterations in the rat brain. J Pharm Exp Ther 280:261–267

Prince JA, Yassin MS, Oreland L (1998): A histochemical demonstration of altered cytochrome oxidase acitivty in the rat brain by neuroleptics. Eur Neuropsychopharmacol 8:1–6

Prince JA, Yassin M, Oreland L (1997b): Normalization of cytochrome-c oxidase activity in the rat brain by neuroleptics after chronic treatment with PCP or methamphetamine. Neuropharmacology 36:1665–1678

Rago R, Mitchen J, Wilding G (1990): DNA fluorometric assay in 96-well tissue culture plates using hoechst 33258 after cell lysis by freezing in distilled water. Anal Biochem 191:31–34

Sabri O, Erkwoh R, Schreckenberger M, Owega A, Sass H, Buell U (1997): Correlation of positive symptoms exclusively to hyperperfusion or hypoperfusion of cerebral cortex in never-treated schizophrenics. Lancet 349:1735–1739

Saykin AJ, Gur RC, Gur RE, Mozley PD, Mozley LH, Resnick SM, Kester DB, Stafiniak P (1991): Neuropsychological function in schizophrenia. Selective impairment in memory and learning. Arch Gen Psychiat 48:618–624

Shenton ME, Kikinis R, Jolesz FA, Pollak SD, LeMay M, Wible CG, Hokama H, Martin J, Metcalf D, Coleman M, McCarley RW (1992): Abnormalities of the left temporal lobe and thought disorder in schizophrenia. A quantitative magnetic resonance imaging study. N Engl J Med 327:604–612

Shihabuddin L, Buchsbaum MS, Hazlett EA, Haznedar MM, Harvey PD, Newman A, Schnur DB, Spiegel-Cohen J, Wei T, Machac J, Knesaurek K, Vallabhajosula S, Biren MA, Ciaravolo TM, Luu-Hsia C (1998): Dorsal striatal size, shape, and metabolic rate in never-medicated and previously medicated schizophrenics performing a verbal learning task. Arch Gen Psychiat 55:235–243

Siegel BV, Buchsbaum MS, Bunney WE Jr, Gottschalk LA, Haier RJ, Lohr JB, Lottenberg S, Najafi A, Nuechterlein KH, Potkin SG (1993): Cortical-striatal-thalamic circuits and brain glucose metabolic activity in 70 unmedicated male schizophrenic patients. Am J Psychiat 150:1325–1336

Singer VL, Jones LJ, Yue ST, Haugland RP (1997): Characterization of PicoGreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation. Anal Biochem 249:228–238

Smith AD, Bolam JP (1990): The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 13:259–265

Stratta P, Mancini F, Mattei P, Daneluzzo E, Casacchia M, Rossi A (1997): Association between striatal reduction and poor Wisconsin Card Sorting Test performance in patients with schizophrenia. Biol Psychiat 42:816–820

Weinberger DR, Gallhofer B (1997): Cognitive function in schizophrenia. Int Clin Psychopharmacol 12:S29–S36

Wong-Riley M (1979): Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res 171:11–28

Wong-Riley M (1989): Cytochrome oxidase: An endogenous metabolic marker for neuronal activity. TINS 12:94–101

Wong-Riley M, Carrol EW (1984): Effect of impulse blockage on cytochrome oxidase activity in monkey visual system. Nature 307:262–264

Yonetani T, Ray G (1965): Studies on cytochrome oxidase. J Biol Chem 240: 3392–3399