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8. Speed and space clamps were improved by actively clamping the guard compartment. The signal-to-noise ratio was enhanced with the use of low-resistance microelectrodes (0.1 to 0.5 megohm) and inclusion of a high resistor (10 megohms) in the feedback loop in the current-voltage converter. The series resistance that originated from the distance between the tip of the voltage-recording microelectrode and the interior of the upper oocyte membrane was 0.5 to 1 kilohm and thus had negligible influence (<1 mV) on the clamp voltage because the membrane currents were smaller than 200 nA.
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10. The cRNAs were prepared from two plasmids bearing the *a* splice variant of the type C or cardiac α_1 subunit ($\alpha_{1C\text{-}a}$; formerly also CaCh2a) and the type *2a* cardiac β subunit (β_{2a}), here referred to simply as α_1 and β. The α_1 cDNA was digested with Hind III as described (*9*) and β cDNA with Not I (*18*). Transcription was done at 37°C in a volume of 25 μl containing 40 mM tris-HCl (pH 7.2), 6 mM $MgCl_2$, 10 mM dithiothreitol, 4 mM spermidine, adenosine triphosphate, guanosine triphosphate, cytosine triphosphate, and uridine triphosphate (0.4 mM each), 1 mM 7-methyl guanosine 5′-triphosphate, 0.5 μg of linearized DNA template, and 10 units of T7 RNA polymerase (Boehringer Mannheim). The cRNA products were extracted with phenol-chloroform, recovered by precipitation with ethanol, and suspended in double distilled water to a final concentration of 0.2 μg/μl of each species, and 50 nl was injected per oocyte. Before injection, oocytes were defolliculated by collagenase treatment (type I, 2 mg/ml for 40 min at room temperature) (Sigma). Oocytes were maintained at 19.5°C in Barth solution. Recordings were done 4 to 12 days after RNA injection.
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13. Oocytes that expressed Ca^{2+} channels showed a prominent Cl^- current, even in Cl^--free external solutions and with Ba^{2+} as the charge carrier. We eliminated this Cl^- current by injecting 37 nl of 50 mM BAPTA$(Na)_4$ [1,2-bis(*o*-aminophenoxy)-ethane-*N,N,N′,N′*-tetraacetate] before recording, as revealed by the lack of outward current in external medium containing 10 mM Ca^{2+} and 110 mM Cl^-. External solution for ionic currents had the following composition: 10 mM Ba^{2+}; 96 mM Na^+; and 10 mM Hepes titrated to pH 7.0 with methanesulphonic acid (CH_3SO_3H). Gating currents were measured in an external solution containing 2 mM $CoCl_2$, 110 mM Na^+, and 10 mM Hepes, titrated to pH 7.0 with CH_3SO_3H. The solution in contact with the oocyte interior was either 120 mM tetraethylammonium, 10 mM Hepes, 0.5 mM EGTA, and 5 mM $MgSO_4$, titrated to pH 7.0 with CH_3SO_3H; or 110 mM potassium glutamate and 10 mM Hepes, titrated to pH 7.0 with KOH. Low-access resistance to the oocyte interior was obtained by permeabilizing the oocyte with 0.1% saponin. The holding potential was −90 mV. The linear components were subtracted on-line by scaled currents elicited by small negative control pulses that were one-quarter the amplitude of the stimulating pulse.
14. In some cases, we observed asymmetric charge movement in uninjected oocytes. This movement was blocked by removal of external Na^+ or by addition of ouabain. This appears to be the charge movement associated with the activity of the Na^+, K^+ adenosine triphosphatase described by R. F. Rakowsky [*J. Gen. Physiol.* **101**, 117 (1993)]. To avoid any potential contamination in our records, we included 100 μM ouabain in all external solutions.
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16. In 10 mM Ba^{2+}, pulse depolarizations to −30 mV did not activate any measurable inward current, although in some oocytes that expressed α_1 plus β, an excess of charge was observed during the repolarization, which indicates the presence of some ionic component. Potential errors introduced by ionic current contamination were minimized by integrating only over the first 2-ms depolarizations.
17. Calcium currents arising from an endogenous α_1-like subunit whose activity can be stimulated in oocytes injected with only the β subunit could be differentiated from Ca^{2+} currents induced by the cardiac α_1 plus β subunits. Currents induced by β cRNA alone had faster kinetics and were insensitive to DHPs. Oocytes injected with cRNA encoding both cardiac α_1 and β subunits were blocked at a holding potential of −20 mV by (+) PN200-100 (2 μM), nifedipine (10 μM), and nisoldipine (1 μM).
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Abnormal Behavior Associated with a Point Mutation in the Structural Gene for Monoamine Oxidase A

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Genetic and metabolic studies have been done on a large kindred in which several males are affected by a syndrome of borderline mental retardation and abnormal behavior. The types of behavior that occurred include impulsive aggression, arson, attempted rape, and exhibitionism. Analysis of 24-hour urine samples indicated markedly disturbed monoamine metabolism. This syndrome was associated with a complete and selective deficiency of enzymatic activity of monoamine oxidase A (MAOA). In each of five affected males, a point mutation was identified in the eighth exon of the MAOA structural gene, which changes a glutamine to a termination codon. Thus, isolated complete MAOA deficiency in this family is associated with a recognizable behavioral phenotype that includes disturbed regulation of impulsive aggression.

Studies of aggressive behavior in animals and humans have implicated altered metabolism of serotonin (*1–7*), and to a lesser extent dopamine (*4, 8, 9*), and noradrenaline (*3–5, 10–12*). These observations suggest that genetic defects in the metabolism of these neurotransmitters may affect aggressive behavior, but such mutations have not yet been reported.

We have described a large kindred in which several males are affected by a syndrome of borderline mental retardation and exhibit abnormal behavior, including disturbed regulation of impulsive aggression (*13*). Obligate female carriers in this family have normal intelligence and behavior. The genetic defect for this condition was assigned to the p11-p21 region of the X chromosome, in the vicinity of the genes for MAOA and monoamine oxidase B (MAOB). Because MAOA and MAOB are known to metabolize serotonin, dopamine, and noradrenaline, we evaluated these patients for MAO deficiency. The MAOB activity is normal in affected males from this family (*13*).

To test the hypothesis that affected males in this family have selective MAOA deficiency, we established skin fibroblast cultures from three clinically affected males, two carrier females, and one noncarrier female (*14*). Cultured human skin fibroblasts from normal individuals express both MAOA and MAOB activity in a ratio of about 80 to 90% to 10 to 20%, respectively, and the amounts of activity are stable from passage to passage during the proliferative growth phase (*15*). Treatment of fibroblast cultures with dexamethasone produces a 6- to 14-fold increase in MAOA activity and a 2- to 3-fold increase in MAOB activity (*16*). We assessed MAO activity in homogenates from skin fibroblast strains with a common substrate, tryptamine, at a concentration that favors MAOA mea-

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surement (*17*). Strains from two normal controls were used that represent very low (strain GM2037) and moderately high (strain HF24) amounts of activity, on the basis of previous analyses of more than 30 control strains with activity amounts that spanned a range of 1 to 100 pmol/min per milligram of protein (*14, 18*). These controls were grown in parallel with fibroblasts from family members to minimize activity differences due to serum components (*19*). Negligible amounts of apparent MAO activity were found in strains from three affected males in the presence or absence of dexamethasone (Table 1). The amounts of activity in two carrier females and in one noncarrier female from the same family were in the low to moderate control range and, as in control strains, were increased by treatment with dexamethasone and inhibited by more than 90% by the selective MAOA inhibitor, clorgyline.

To establish whether the lack of MAOA activity was caused by a mutation in the MAOA structural gene, we determined the coding sequence of the mRNA for MAOA by first-strand complementary DNA (cDNA) synthesis, polymerase chain reaction (PCR) amplification, and direct sequencing (*20*). Four base substitutions were detected, three of which were neutral polymorphisms (G to T at position 941, T to A at position 1077, and T to C at position 1460). However, a nonconservative C to T mutation was found at position 936. This mutation changes a glutamine (CAG) codon to a termination (TAG) codon at position 296 of the deduced amino acid sequence (*21*) (Fig. 1). Amplification and sequencing of the eighth exon (*22*), which contains nucleotides 846 to 1005, confirmed the presence of the C to T mutation at nucleotide 936 in each of five clinically affected males and in two obligatory heterozygotes. In contrast, the mutation could be excluded in 12 unaffected males in this family (Fig. 1). Two-point linkage calculations (*23*) between the clinical phenotype and the mutation in the MAOA gene reported here yield a lod score (logarithm of the likelihood ratio for linkage) of 3.55 without recombination.

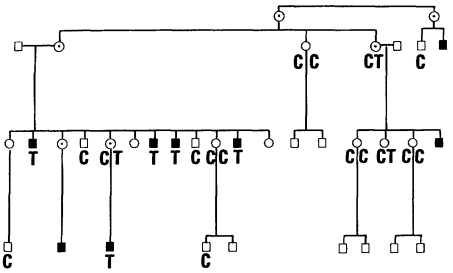

Fig. 1. Segregation of a mutation in the MAOA structural gene in a family with X-linked borderline mental retardation and prominent behavioral disturbance. All affected males and obligate carriers have a C to T mutation at nucleotide position 936. In 12 normal males, only the normal C is present.

These results document complete and selective deficiency of MAOA in affected males. Interestingly, MAOA activity in two carrier females was not different from that of a noncarrier female and two unrelated controls. Therefore, carrier females are not detectable by enzymatic activity in cultured fibroblasts. Whether this is due to high activity of the normal allele, incomplete X-inactivation, or other factors is unknown. Selective MAOA deficiency in this family results in a marked disturbance of monoamine metabolism. Increased urinary excretion of normetanephrine and tyramine and decreased concentrations of 5-hydroxyindole-3–acetic acid (5-HIAA), homovanillic acid (HVA), and vanillylmandelic acid (VMA) have been documented by analysis of 24-hour urine samples (*13*). Although measurements of cerebrospinal fluid metabolites are not available for this family, the urinary findings presumably reflect altered central neurotransmitter metabolism. Selective inhibition of MAOA in male rats has been shown to increase concentrations of noradrenaline, dopamine, and serotonin in the brain (*24*).

Five patients with X chromosomal deletions including MAOA and MAOB as well as the Norrie disease gene have been described that had severe mental retardation (*25*). The relatively mild symptoms in males with selective MAOA deficiency, and the absence of psychiatric symptoms or mental retardation in two brothers with a complex deletion involving the Norrie disease gene and part of the MAOB structural gene that leaves MAOA intact (*26*), may reflect the overlapping substrate specificities and tissue distribution of the MAOA and MAOB isozymes. The behavioral phenotype in this family is characterized by borderline mental retardation and a tendency toward aggressive outbursts, often in response to anger, fear, or frustration. These behavioral responses have been noted in each of eight affected males for whom clinical data are available and have occurred in affected subjects living in different parts of the country at different times (*13*). It should be stressed that the aggressive behavior varied markedly in severity and over time, even within this single pedigree. Other types of impulsive behavior that occurred in individual cases included arson, attempted rape, and exhibitionism.

Table 1. MAO activity in cultured skin fibroblasts. For the detection of MAOA activity, cells were harvested at confluency (−DEX) or after an additonal 7 to 9 days of exposure to 50 nM dexamethasone (+ DEX) as described (*15*). Activity amounts for the affected males were all below detection limits (<30% above a blank that had no homogenate). All values are given as the average ± SD with the number of assays in parentheses.

Subjects	Treatment	
	−DEX	+DEX
	(picomoles per minute per milligram of protein)	
	Affected males	
BB	<1	<1
AW	<1	<1
AX	<1	<1
	Carrier females	
AY	10 ± 3 (4)	114 ± 35 (7)
AZ	32 ± 9 (4)	122 ± 43 (5)
	Noncarrier female	
BA	27 ± 16 (4)	189 ± 32 (5)
	Normal controls	
GM2037	3 ± 3 (4)	24 ± 11 (7)
HF24	36 ± 10 (4)	317 ± 42 (5)

It has been postulated that aggression in animals can be subdivided into several subtypes (*1*). In humans, impulsive aggression rather than premeditated aggression and violence has been linked to low concentrations of 5-HIAA in cerebrospinal fluid (*2*). This observation is usually taken to indicate a reduction in central serotonergic function in impulsive aggression. Our data suggest that reduced 5-HIAA concentrations may also be caused by absent MAOA activity in these subjects.

Further studies are required to determine whether complete isolated MAOA deficiency is associated with similar behavioral patterns in other families, or even in animal models. Also, it is presently unclear whether all of the biochemical alterations caused by the MAOA deficiency state are required to cause the apparent increase in liability to impulsive aggressive behavior. The inhibition of MAO has not been reported to cause aggressive behavior in adult humans (*27*) but deficiencies throughout life might have different consequences. Only limited data are available on MAO activity and aggression regulation in animals. MAO inhibition increased shock-induced aggression in male rats in one study (*28*). Other studies of aggressive behavior have stressed the importance of reduced serotonergic transmission (*1–7*), increased dopaminergic transmission (*4, 8, 9*), or increased noradrenergic transmission (*3–5, 11, 12*) in animals as well as in humans.

Another factor that could be involved in causing increased impulsive aggression is rapid eye movement (REM) sleep deprivation. MAOA inhibitors have been shown to suppress REM sleep in human subjects (*29*), whereas REM sleep deprivation increases shock-induced fighting in rats, especially in combination with dopaminergic stimulation (*9*).

Taken together, data obtained in this family suggest a relation between isolated

complete deficiency of MAOA activity and abnormal aggressive behavior in affected males. This observation raises a number of important questions. First, the frequency of MAOA deficiency in the population has to be determined. Second, given the wide range of variation of MAOA activity in the normal population (*18*), one could ask whether aggressive behavior is confined to complete MAOA deficiency. Third, animal models could help to determine the various neurochemical alterations that are induced by selective MAOA deficiency and their secondary effects on the organism. Such studies might also suggest possibilities for treatment of the metabolic disturbance caused by the MAOA deficiency state. Finally, the possibility of hypertensive crises in selective MAOA deficiency through increased sensitivity to dietary and pharmacologic amines has not yet been investigated.

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14. After informed consent was obtained, skin fibroblasts were established from punch biopsies of family members. Normal control cell lines were chosen on the basis of very low activity (GM2037) and moderately high activity (HF24) (*18*). All strains were in the proliferative stage of growth and were grown in parallel in Dulbecco's modified essential medium with 10% fetal calf serum, penicillin, and streptomycin (Gibco, Paisley, Scotland). For measurement of MAO activity, cells were harvested at confluency or after an additional 7 to 9 days of exposure to 50 nM dexamethasone, as described (*15*). Cell homogenates from two or more harvests were sonicated, and the protein was determined by the method of Bradford (*30*).
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17. We measured MAO activity by the toluene extraction procedure in fibroblast homogenates with 100 μg of protein per assay (*16*). All assays were done in triplicate within the range of linearity for time. A buffer blank was used routinely, but in some cases additional blanks of 10^{-6} M clorgyline (Sigma, St. Louis, MO) or 10^{-6} M deprenyl were included. For a common substrate we used 30 μM [ethyl-^{3}H]tryptamine (35 Ci/mmol, New England Nuclear, Boston, MA) for both MAOA and MAOB activity, although at this concentration MAOA is favored by its higher substrate affinity. Values obtained in these experiments for control cell strains were about sevenfold those of previous studies (*14, 31*). This is probably because of the different lots of serum used and the effects of hormones contained in serum on MAO activity (*19*).
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Mutations in the Glucose-6-Phosphatase Gene That Cause Glycogen Storage Disease Type 1a

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Glycogen storage disease (GSD) type 1a is caused by the deficiency of D-glucose-6-phosphatase (G6Pase), the key enzyme in glucose homeostasis. Despite both a high incidence and morbidity, the molecular mechanisms underlying this deficiency have eluded characterization. In the present study, the molecular and biochemical characterization of the human G6Pase complementary DNA, its gene, and the expressed protein, which is indistinguishable from human microsomal G6Pase, are reported. Several mutations in the G6Pase gene of affected individuals that completely inactivate the enzyme have been identified. These results establish the molecular basis of this disease and open the way for future gene therapy.

Glucose-6-phosphatase (E.C. 3.1.3.9), the key enzyme in the homeostatic regulation of blood glucose concentrations, catalyzes the terminal step in gluconeogenesis and glycogenolysis (*1–3*). Deficiency of G6Pase causes GSD type 1a (von Gierke disease), an autosomal recessive disorder with an incidence of one in 100,000 to 300,000 (*1, 2*). This metabolic disease typically manifests during the first year of life with severe hypoglycemia and hepatomegaly caused by the accumulation of glycogen. Individuals with GSD type 1a exhibit

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