both Arg and Lys found in suppressed protein (Table 1). Nucleotide C35 in the middle of the anticodon is an identity candidate, as all known isoacceptors of $tRNA^{Arg}$ contain this residue and it appears in all four dinucleotide combinations in the expectation maximization algorithm (Fig. 1). In addition, chemical modification studies have demonstrated that C35 is important for the Arg acceptor activity of $tRNA^{Arg}$ in vitro (*18*).

From our results with suppressor $tRNA^{Arg}$, we conclude that nucleotide A20 in the tRNAs of FTOR1 and PHEA20U20aA59 contributes to the productive interaction with Arg-tRNA synthetase. Further, the minor amounts of amino acid other than Arg found in suppressed protein produced by FTOR1 and PHEA20U20aA59 may reflect the absence of C35 in these tRNAs. It is puzzling that both FTOR1 and PHEA20U20aA59 tRNAs inserted more Arg and less Lys in suppressed protein than did $tRNA^{Arg}$ (75% versus 37% Arg, respectively), but this may reflect different distributions in $tRNA^{Phe}$ and $tRNA^{Arg}$ molecules of nucleotides that repel the Lys-tRNA synthetase. Finally, neither the FTOR1 tRNA nor $tRNA^{Arg}$ has been examined to determine whether nucleotide U20a, which is adjacent to A20, contributes to acceptor identity other than serving as a structural spacer between the constant nucleotides at positions 18 and 21; likewise, positions where $tRNA^{Arg}$ and the FTOR1 tRNA share the same nucleotide (Fig. 2) have not been examined.

An inspection of all available nucleotide sequences of $tRNA^{Arg}$ (*16*) reveals that residue A20 is conserved in life forms except in yeast. This regularity may reflect a conserved feature of the acceptor identity of $tRNA^{Arg}$. The observation that nucleotide 20 contributes to the acceptor identity of *E. coli* $tRNA^{Arg}$ was anticipated from structural and theoretical considerations by Klug and co-workers (*8*). It was noted that the nucleotides at positions 16, 17, 20, 59, and 60 form a patch that arches out from the molecular surface of yeast $tRNA^{Phe}$ (Fig. 4B). The nucleotides in this patch segregate separately from a neighboring cluster of conserved nucleotides; further, there is variation in the number and type of nucleotides within the patch. Thus it was proposed that the patch constitutes a variable pocket that is part of a recognition system used to discriminate different tRNAs, possibly by the aminoacyl-tRNA synthetases. The involvement of the variable pocket in acceptor identity is not limited to $tRNA^{Arg}$, as recent reports have also demonstrated that, in addition to the anticodon (*6, 18*) and acceptor stem (*1, 3, 5, 9, 11–13, 22*), one or more nucleotides in the variable pocket contributes to the acceptor identity of *E. coli* $tRNA^{Ala}$ and $tRNA^{Phe}$ (*9, 11*) and yeast $tRNA^{Phe}$ (*10*).

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Deficit of Spinal Cord Glycine/Strychnine Receptors in Inherited Myoclonus of Poll Hereford Calves

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Inherited myoclonus of Poll Hereford calves is characterized by hyperesthesia and myoclonic jerks of the skeletal musculature, which occur spontaneously and in response to sensory stimuli. The disease shows autosomal recessive inheritance, and significant proportions of the Poll Hereford herds in many countries are thought to be carriers of the mutant gene. Studies revealed a specific and marked (90 to 95 percent) deficit in [^{3}H]strychnine binding sites in spinal cord membranes from myoclonic animals compared to controls, reflecting a loss of, or defect in, glycine/strychnine receptors. Spinal cord synaptosomes prepared from affected animals showed a significantly increased ability to accumulate [^{3}H]glycine, indicating an increased capacity of the high-affinity neuronal uptake system for glycine. In contrast, stimulus-induced glycine release and spinal cord glycine concentrations were unaltered.

INHERITED CONGENITAL MYOCLONUS of Poll Hereford and Poll Hereford cross calves has been reported in the United States, Australia, New Zealand, and England (*1*). This disease, previously (and erroneously) known as neuraxial edema, is characterized by hyperesthesia and myoclonic jerks of the skeletal musculature, which occur spontaneously and in response to tactile, visual, and auditory stimuli (*2*). Since tactile stimuli are sufficient to trigger convulsions, affected animals are unable to stand to feed and, consequently, do not survive. Breeding experiments show that the disease is inherited in an autosomal recessive manner (*3*). No significant pathological lesions are observed in the central nervous system of affected animals (*2*). The clinical symptoms are unaltered by antiepileptic and anticonvulsant drugs at therapeutic doses. However, the symptoms of the disorder suggest a failure of spinal interneuron inhibition and are similar to those in subconvulsive strychnine poisoning. Strychnine blocks the synaptic action of the inhibitory amino acid transmitter glycine (*4*) by interacting with the postsynaptic glycine receptor (*5*). We now report what we believe is the major

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biochemical lesion of the disorder: a specific and marked loss of, or defect in, spinal glycine/strychnine receptors (*6*). In addition, synaptosomal preparations prepared from affected animals have a specific and significantly increased ability to accumulate [^{3}H]glycine, indicating an increased capacity of the high-affinity neuronal uptake system for glycine. In contrast, the spinal cord concentration of glycine (and other amino acids) and the stimulus-induced release of glycine and other neurotransmitter amino acids were unaltered. The characteristics of this bovine disorder show several similarities to those described for the mutant mouse *spastic* (*7*).

Affected calves of both sexes (3 to 10 days after birth) were obtained from the Veterinary Laboratories breeding herd or from cattle breeders in New South Wales and Victoria and were fed regularly (three times per day) on whole milk. Aged-matched calves (unaffected Poll Hereford and male Friesian and Illawarra Shorthorn calves) were used as controls (*8*). [^{3}H]Strychnine bound specifically to a single, high-affinity site in spinal cord membranes prepared from all regions of spinal cord of control animals, including the lumbar region (Fig. 1A). The mean dissociation constant (K_d) was 4.82 ± 0.25 n*M* (n = 13) and the maximum number of binding sites (B_{max}) for the lumbar region was 355 ± 40 fmol/mg of protein (n = 8). (Error limits throughout this report are ± SE of the reported value.) The Hill coefficient was 1.00 ± 0.03 (n = 13), indicating the absence of cooperativity and the presence of only one population of sites. Glycine inhibited [^{3}H]strychnine binding with an inhibition constant (IC_{50}) of 35 μ*M*. These results are in good agreement with those reported for [^{3}H]strychnine binding to rodent spinal cord tissue (*5, 9*). However, specific [^{3}H]strychnine binding was significantly reduced in membranes from affected calves, being only 5 to 10% of control levels (at a [^{3}H]strychnine concentration approximately equivalent to the control K_d value) in samples collected as early as 1982 (Fig. 1B). This reduction in binding was unlikely to be due to a decrease in ligand affinity for the receptor because in experiments designed to measure the K_d of binding, only very low levels of specific binding to both crude and myelin-free synaptic membranes (*10*) were detected in affected calves, even at [^{3}H]strychnine concentrations as high as 35 n*M*. No change in K_d for [^{3}H]strychnine binding was reported in the mutant mouse *spastic* (*11, 12*). All unaffected Hereford calves, including a number of obligate heterozygotes, showed identical levels of [^{3}H]strychnine binding, indicating there is no partial expression of the disorder in heterozygotes. Autoradiographic studies revealed that the reduction in [^{3}H]strychnine binding occurred throughout the dorsal and ventral spinal cord of myoclonus calves (Fig. 2, A and B) as well as along the cervical-lumbar axis and in the pons-medulla (*13*). In contrast, the distribution of [^{3}H]flunitrazepam binding to benzodiazepine receptors was identical in myoclonic and control spinal cord sections (Fig. 2, C and D).

To determine whether the alteration was selective for the glycine receptor, we measured radioligand binding to other receptor sites. In the mutant mouse *spastic*, increases in the amount of other receptors, such as the γ-aminobutyric acid (GABA)–benzodiazepine receptor complex have been described (*11, 14, 15*). However, we did not detect any significant alteration in the affinity or number of [^{3}H]flunitrazepam binding sites. Similarly, in spinal cord membranes, binding parameters for $GABA_A$ receptors and muscarinic acetylcholine receptors were not significantly altered (*16*).

Because the postsynaptic receptor forms only part of the signal transduction mechanism, we also investigated kinetic processes reflecting presynaptic aspects of glycinergic neurotransmission. The postsynaptic action of amino acid transmitters is terminated by reuptake into surrounding structures (*17*). Sodium-dependent uptake of [^{3}H]glycine into purified synaptosomes (*18*) prepared from frozen bovine spinal cord was high affinity and saturable (Fig. 3A). The maximum rate (V_{max}) of [^{3}H]glycine uptake in synaptosomes from myoclonic animals was significantly higher (240% of control), but there was no significant alteration in the affinity of glycine for the uptake carrier. The specificity of this effect is shown by the fact that kinetic parameters of [^{3}H]glutamate uptake were unchanged in the same synaptosomal preparations (Fig. 3B). This increase in the capacity of the glycine trans-

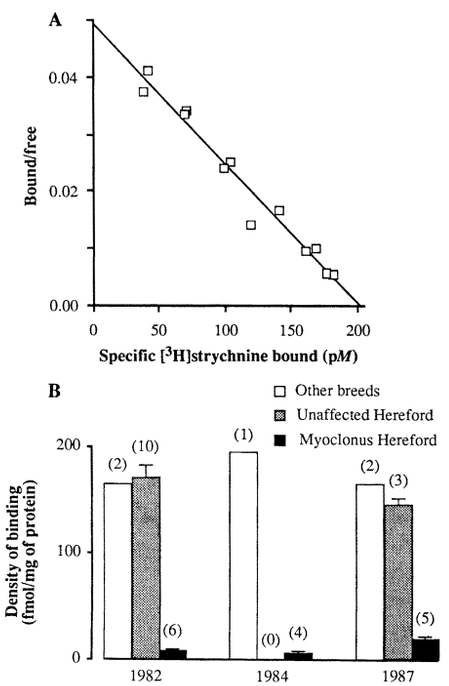

Fig. 1. [^{3}H]Strychnine binding to spinal cord from normal and myoclonic calves. (**A**) Scatchard plot of specific [^{3}H]strychnine binding (*24*) to lumbar spinal cord membranes. Strychnine concentrations ranged from 1 to 35 n*M*. Data are derived from duplicate determinations of total and nonspecific (in the presence of 10 m*M* glycine) binding, and the results from two different control membrane preparations are shown. Identical results were obtained in four similar independent experiments. (**B**) Comparison of specific [^{3}H]strychnine binding concentrations in lumbar regions of myoclonic and unaffected (normal Poll Hereford and other breeds) calves collected between 1982 and 1987. Values are the mean ± SE of the number of animals given in parentheses. The density of binding is significantly reduced in the myoclonic samples (Student's t test, $P < 0.001$).

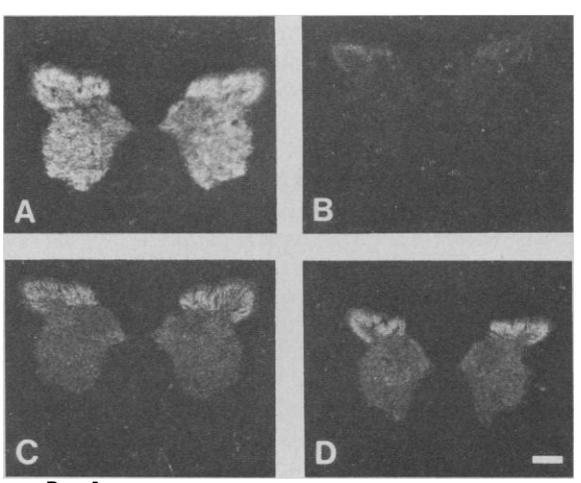

Fig. 2. Autoradiographic localization of [^{3}H]strychnine and [^{3}H]flunitrazepam binding in lumbar spinal cord from normal and myoclonic calves (*25*). (**A** and (**B**) Distribution of [^{3}H]strychnine binding in lumbar cord from normal and myoclonic calves, respectively. Nonspecific binding of [^{3}H]strychnine (in the presence of 10 m*M* glycine) produced images (data not shown) essentially equivalent to binding in sections from myoclonic calves. (**C** and **D**) Distribution of [^{3}H]flunitrazepam binding in spinal cord of normal and myoclonic calves, respectively. Similar results were obtained from sections from other regions of spinal cord and from the pons-medulla from one to three animals in each group. Bar, 1 mm.

porter may be a compensation for the lack of postsynaptic receptors.

In contrast, both potassium- and veratrine-stimulated endogenous glycine release from purified synaptosomal preparations was not significantly different in myoclonus and control cases (Fig. 3C). In both cases, stimulus-induced release was restricted to the transmitter amino acids glycine, glutamate, and GABA, whereas physiologically inactive compounds such as alanine were not released (Fig. 3C) (*19*). Furthermore, the total tissue concentrations of glycine and other transmitter amino acids were unaltered in myoclonus spinal cord (Fig. 3D). In fact, none of 20 amino acids measured were altered (Fig. 3D) (*20*). Although no detailed studies of amino acid transmitter uptake and release in spinal cord preparations from mutant mouse *spastic* have been reported, there is preliminary evidence of a 40% increase in glycine uptake (*21*).

Recent advances have suggested a molecular mechanism for the manifestation of the glycine receptor deficit in *spastic* mouse (*12, 22*). The glycine receptor is localized to the synaptic cleft, as judged by glycine receptor antibody immunohistochemistry, and the receptor is structurally similar in both control and *spastic* mice (*12, 22*). Thus it is suggested that the mutation occurs in a regulatory gene that controls the expression or stability of the glycine receptor. Receptor isolation and glycine receptor antibody studies with bovine spinal cord should help to elucidate the nature of the receptor or gene alteration in myoclonic Poll Hereford calves. The recent availability of complementary DNA probes for the strychnine receptor binding subunits (*23*) should allow quantitation of the messenger RNA coding for the receptor subunits and may assist in defining the lesion responsible for this disease. This type of molecular approach may lead to the development of a simple diagnostic test for the heterozygote and thereby facilitate prevention of this condition in Poll Hereford cattle.

Our findings suggest that the deficiency of normal inhibitory glycine receptors in spinal cord is sufficient to explain the clinical signs of this genetic myoclonic disorder. These myoclonic calves provide a useful model for investigating the plasticity of various components of other neurotransmitter systems in the functional absence of the major spinal inhibitory system.

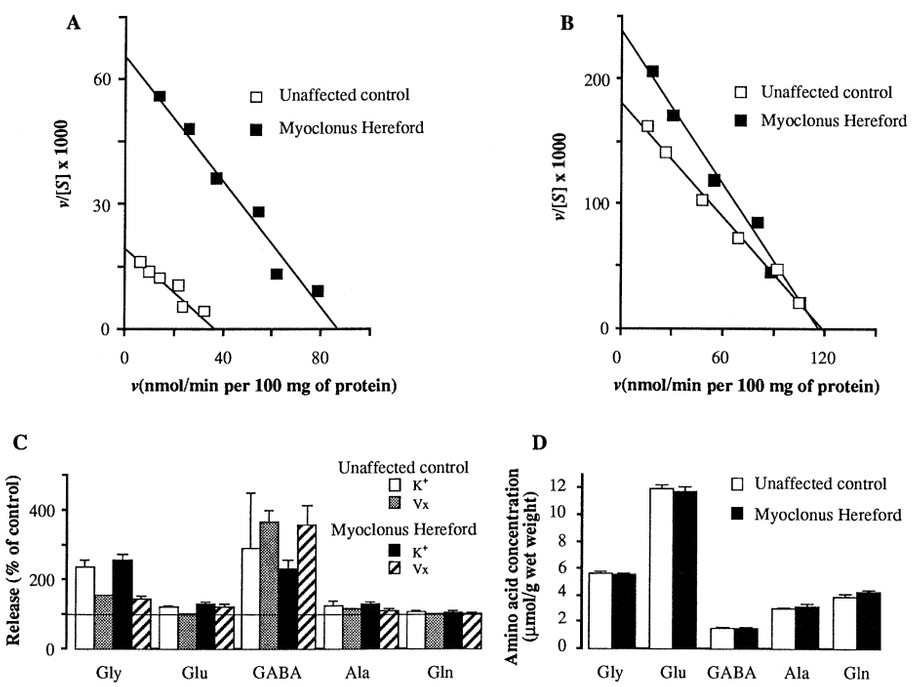

Fig. 3. Amino acid uptake and release and tissue concentrations in spinal cord of normal and myoclonic calves. (**A** and **B**) Eadie-Hofstee plots of [^{3}H]glycine and [^{3}H]glutamate uptake, respectively, measured in spinal cord synaptosomes from normal and myoclonic Poll Hereford calves (*26*). For [^{3}H]glycine uptake, K_m = 38 ± 10 and 40 ± 10 μ*M* (NS), and V_{max} = 37 ± 7 and 88 ± 16 nmol/min per 100 mg of protein ($P < 0.05$), for control (n = 3) and myoclonus (n = 4), respectively. For [^{3}H]glutamate uptake, K_m = 8.4 ± 0.9 and 7.8 ± 0.9 μ*M* (NS), and V_{max} = 119 ± 9 and 116 ± 10 nmol/min per 100 mg of protein for control and myoclonus, respectively. Initial velocity of uptake, v; and substrate concentration, [*S*] (μ*M*). (**C**) Veratrine (Vx)- and potassium (K^+)-stimulated release of glycine and other amino acids from spinal cord synaptosomes (*27*). Glycine and GABA release were significantly ($P < 0.05$, t test) enhanced by depolarizing stimulation, although differentially affected by the two stimuli. However, there was no statistically significant difference between control and myoclonus in either the extent or pattern of release. Results represent mean ± SE (*19*). (**D**) Amino acid concentrations in spinal cord of myoclonic and normal calves. Total tissue amino acid concentrations were determined in perchloric acid extracts (*28*) of thoracic spinal cord (n = 5) from normal and myoclonic calves (*20*). Data are presented for glycine (Gly), glutamate (Glu), γ-aminobutyric acid (GABA), alanine (Ala), and glutamine (Gln).

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20. Concentrations of many other amino acids including taurine, threonine, serine, valine, methionine, leucine, isoleucine, tyrosine, phenylalanine, histidine, lysine, and arginine were also measured and found to be identical in unaffected and myoclonus spinal tissue (NS by *t* test).
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26. Synaptosomes were purified from 10% homogenates of rapidly thawed tissue in 0.32*M* sucrose as described (*10*) and resuspended in ice-cold 0.32*M* glucose. In each preparation, the sodium-dependent, high-affinity uptake of both [^{3}H]glycine (19.0 Ci/mmol, Amersham) and [^{3}H]glutamate (53.0 Ci/mmol, Amersham) were measured by incubating synaptosome suspensions in Krebs-Hepes buffer for 2 min at 37°C; control incubations were carried out in medium in which all sodium ions were replaced by tris (*18*).
27. For release studies, synaptosomes (*10*) were incubated at 37°C for 20 min in Krebs-bicarbonate buffer containing 2.5 m*M* Ca^{2+}. Portions of KCl solution (to give a final concentration of 47.5 m*M*) or veratrine solution (to give a final concentration of 75 μ*M*) were then added, and the incubation continued for an additional 10 min. Addition of NaCl solution or normal medium served as the respective controls. Amino acid levels were determined in acidified samples of medium, after brief centrifugation at room temperature, on a Varian high-performance liquid chromatograph by means of cation exchange in lithium buffers and post-column derivatization with *ortho*-phthalaldehyde.
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Synaptic Transmission Between Dissociated Adult Mammalian Neurons and Attached Synaptic Boutons

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In most studies of synaptic currents in mammalian central neurons, preparations have been used in which synaptic currents are recorded at some distance from the synapse itself. This procedure introduces problems in interpretation of the kinetics and voltage-dependent properties of the synaptic current. These problems have now been overcome by the development of a preparation in which presynaptic vesicle-containing boutons have been coisolated with the soma of individual neurons, thus providing the opportunity to study synaptic currents under conditions of both adequate voltage control and internal ionic perfusion. Spontaneous synaptic currents mediated by γ-aminobutyric acid and excitatory amino acids were recorded from neurons isolated from a mammalian medial solitary tract nucleus. Calcium- and depolarization-dependent spontaneous currents of several to hundreds of picoamperes occurred with rapid rise times of 0.8 to 3 milliseconds and decays at least ten times as long.

THE USE OF DISPERSED ADULT MAMmalian neurons to characterize the properties of central neurons is becoming widespread (*1*). Normally, the synaptic inputs can only be determined indirectly through the response to application of exogenous transmitter substances. However, we report here a neuronal isolation procedure that leads to retention of synaptic function.

Neurons isolated from the medial, dorsomedial, and commissural subnuclei of the medial nucleus tractus solitarius (MNTS) had cell bodies 10 to 13 μm in diameter and bipolar processes of 10 to 100 μm (Fig. 1A). A similar morphology is predominant in stained sections from the MNTS (*2*). Structures were apparent that were the synaptic endings of the in vivo afferent inputs to these neurons (Fig. 1B). These boutons have retained vesicles believed to be associated with neurotransmitter release. Retention of vesicles was dependent on isolation and incubation of the tissue in a low Ca^{2+} (0 to 200 μ*M*) and high Mg^{2+} (5 m*M*) solution, rather than solutions containing 1 to 2 m*M* Ca^{2+} as used by others (*1*).

Neurons with neurites shorter than 20 μm were selected for our patch clamp studies. These neurons had capacitive charging currents with single time constants and an average capacitance of 6 to 8 pF. These capacitances are consistent with values expected from an ellipsoid geometry and an assumed specific capacitance of 1 μF/cm^2 (*3*). These conditions ensure adequate temporal and spatial control of postsynaptic membrane potentials for the currents we investigated.

Both spontaneous inward and outward current deflections were observed in these neurons at a holding potential of −40 mV, which in their rapid onset and slow decay resembled synaptic currents (*4, 5*). Previous electrophysiological and histochemical experiments were consistent with the possibility that glutamate, or an analog of glutamate (*6*), and γ-aminobutyric acid (GABA) (*7*) were neurotransmitters in the MNTS.

The inhibitory amino acid, GABA (*8*), and the excitatory amino acids (EAA), glutamate or its structural analogs (*9*), are proposed to be the major transmitters at fast synapses in the mammalian brain. GABA activates a Cl^- conductance at the $GABA_A$ receptor (*8, 10*). Glutamate is an agonist for multiple receptors that activate nonspecific cation conductances (*9, 11*). In our conditions, the Cl^- reversal potential was ~ −90 mV and, if equal permeabilities for Na^+, K^+, and Cs^+ (*12*) are assumed, the cation reversal potential was ~0 mV. At a mem-

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