from the middle of intron 47 to the 5′ splice site of intron 48 (Fig. 4). The deletion eliminates the whole of exon 48 and, consequently, 36 amino acids (residues 964 to 999) of the type II triple helical domain. We therefore conclude that, based on the identity of the affected gene product, the nature of the mutation, and its mendelian segregation with the SED phenotype, the COL2A1 deletion is the genetic lesion responsible for this particular case of dwarfism.

No biochemical data on the cartilage of this family are available; therefore, we can speculate about the metabolic consequences of the deletion only by analogy to similar mutations in the homotrimeric type III collagen (*13*). The loss of exon 48 does not alter the reading frame of mRNA or the ability of the shortened chains to participate in trimer assembly. As a result of random assortment, equal proportions (one-eighth) of the type II procollagen molecules will be homotrimers composed of either three normal length or three shortened collagen chains. Both of these molecules are expected to have normal stability and to be efficiently secreted (*13*). The remaining proportion of procollagen trimers (three-fourths) are likely to be overmodified, unstable, and excluded from secretion since they will consist of both normal and shortened chains (*13*).

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A Pertussis Toxin–Sensitive G Protein in Hippocampal Long-Term Potentiation

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High-frequency (tetanic) stimulation of presynaptic nerve tracts in the hippocampal region of the brain can lead to long-term synaptic potentiation (LTP). Pertussis toxin prevented the development of tetanus-induced LTP in the stratum radiatum–CA1 synaptic system of rat hippocampal slices, indicating that a guanosine triphosphate–binding protein (G protein) may be required for the initiation of LTP. This G protein may be located at a site distinct from the postsynaptic neuron (that is, in presynaptic terminals or glial cells) since maximal activation of CA1 neuronal G proteins by intracellular injection of guanosine-5′-O-(3-thiotriphosphate), a nonhydrolyzable analog of guanosine 5′-triphosphate, did not occlude LTP.

LONG-TERM POTENTIATION (LTP) IS a simple form of synaptic plasticity which may be an underlying event in learning and memory (*1*). Protein phosphorylation mediated by protein kinase C (PKC) has been implicated in the induction and maintenance of LTP (*2–4*), but the steps leading to PKC activation during and after the induction of LTP are unclear. Activation of PKC can be regulated by neurohumoral receptors that activate certain types of G proteins. These G proteins regulate activity of the enzyme phospholipase C (PLC), and in this way control the production of diacylglycerol. Diacylglycerol promotes the translocation of PKC from a soluble form to a more active membrane-bound form (*5*). Tetanic stimulation of presynaptic nerve tracts may lead to activation of G protein–linked receptors and PLC, giving rise to an increase in diacylglycerol production and subsequent translocation and activation of PKC.

Pertussis toxin (PT) is the causative agent in pertussis vaccine encephalopathy. This syndrome is associated with major disturbances in learning and neuronal development (*6*). Certain G proteins, including some that couple neurohumoral receptors to PLC activation (*7*), become functionally uncoupled from receptors after treatment with PT. We now report the effects of PT on LTP. We studied the stratum radiatum–CA1 synaptic system of transversely sectioned rat hippocampal slices maintained in an in vitro tissue slice chamber (*8*). The PT was stereotaxically injected at two sites directly over the right hippocampus 2 to 4 days before the preparation of brain slices (*8*). Sham controls were injected with the vehicle alone. In some experiments brain slices from the contralateral hippocampus were used as controls. As there were no significant differences between results obtained with the two groups of control slices, the control data were pooled.

Tetanus-induced LTP of the stratum radiatum–CA1 population spike was absent in slices obtained from rats injected intracerebrally with PT (3 to 4 μg) 3 to 4 days beforehand (Fig. 1 and Table 1). However, LTP could be induced in slices taken 2 days after PT injection (Table 1). This observation is consistent with the suggestion that the activity of PT is dependent on a relatively slow uptake process, which is followed by intracellular release of the active A promoter (*9*). Thus, the minimum time required for PT to exert its effects appears to be 2 days.

The PT-induced uncoupling of G proteins from their receptors abolishes hippocampal neuronal responses to baclofen (*10*), an agonist of the B subtype of the γ-aminobutyric acid ($GABA_B$) receptor. By monitoring the effect of baclofen in controls and slices obtained from PT-treated rats, we ascertained whether PT was indeed uncoupling G proteins and receptors. Baclofen (10

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μ*M*) inhibited the population spike in control slices, but this effect was significantly reduced in slices obtained at 3 and 4 days after PT injection (Fig. 1 and Table 1). This observation indicates that the protocol of intracerebral injection is adequate for uncoupling G proteins from $GABA_B$ receptors. Presumably, other PT-sensitive G proteins in the slice are similarly affected. There seems to be good agreement between antagonism of the baclofen response and blockade of LTP development by PT treatment (Fig. 1 and Table 1), suggesting that induction or expression of LTP requires activation of a PT-sensitive G protein.

Treatment with PT had no effect on the increase in the population spike induced by direct activation of PKC. Direct PKC activation can be achieved by exposure to tumor-promoting phorbol esters, which act as agonists at the diacylglycerol binding site on PKC (*11*). In five slices obtained at 3 or 4 days after PT injection, the population spike was increased to 203 ± 31% (mean ± SEM) of control (measured at 30 min after termination of the drug application) by a 30-min application of the phorbol ester 4β-phorbol-12,13-diacetate (PDA) at a concentration of 5 μ*M*. This potentiation occurred despite the fact that tetanus-induced LTP could not be elicited in the same slices before PDA application.

Table 1. Effect of pertussis toxin on LTP and baclofen-induced inhibition of the population spike. All values are mean ± SEM. Tetanic stimulation was at 200 Hz for 2 s.

Treatment	Stratum radiatum–CA1 population spike (% of control)		
	Baclofen at 10 μ*M*	Time after tetanic stimulation	
		10 min	30 min
3 and 4 days after vehicle injection	13 ± 6 (n = 11)	278 ± 49* (n = 11)	271 ± 39* (n = 11)
2 days after PT injection	8 ± 5 (n = 5)	238 ± 44† (n = 5)	219 ± 47† (n = 5)
3 days after PT injection	58 ± 4‡ (n = 9)	131 ± 15† (n = 9)	107 ± 11 (n = 9)
4 days after PT injection	48 ± 9‡ (n = 6)	128 ± 16 (n = 6)	115 ± 11 (n = 6)

*$P < 0.005$; significantly different from control values before tetanus (paired t test). †$P < 0.05$; significantly different from control values before tetanus (paired t test). ‡$P < 0.01$; significantly different from control values obtained at 3 and 4 days after vehicle injection (two sample t test by determinations from one-way analysis of variance).

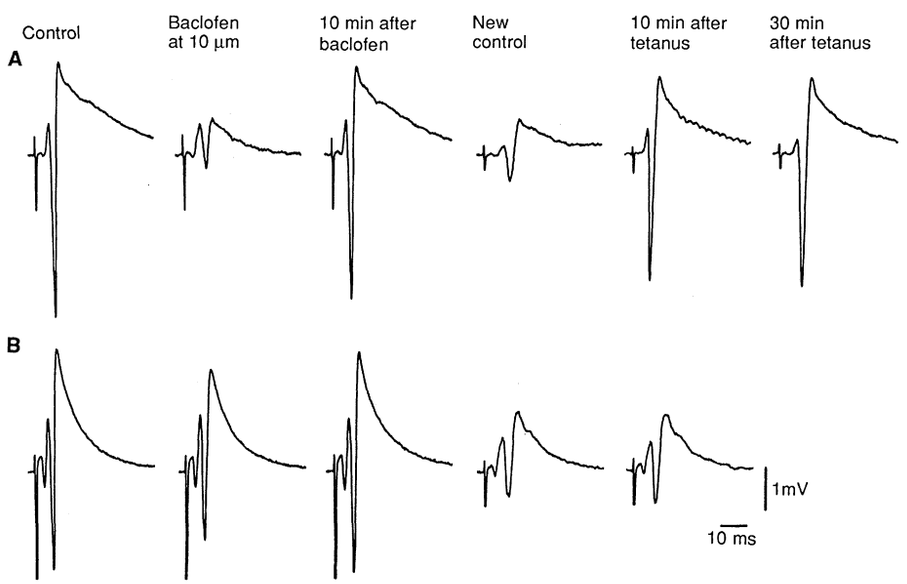

Fig. 1. Effect of pertussis toxin (PT) on baclofen-induced inhibition and long-term potentiation of the population spike. (**A**) Records of responses in a control slice obtained 3 days after stereotaxic injections of vehicle alone containing no PT. Second record was taken at 2 min during baclofen (10 μ*M*) application. Application of the drug was terminated immediately after record was obtained. After recovery from baclofen, a new control was established by decreasing stimulus intensity (fourth trace). A tetanic stimulation of 200 Hz for 2 s at twice the control stimulus intensity was delivered to the stratum radiatum input when controls were stable for at least 15 min, and the response was monitored for a further 30 min. (**B**) Records obtained in a slice from a PT-injected rat. Experimental protocol was the same as in (A). Each record is an average of five consecutive sweeps.

To investigate the locus of the G protein required for LTP, we injected guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS), a nonhydrolyzable guanosine 5′-triphosphate (GTP) analog, directly into CA1 neurons. This should result in an irreversible activation of postsynaptic G proteins. Again, a response mediated by $GABA_B$ receptors was used as an index of G protein activity. For this set of experiments, however, LTP and baclofen responses were monitored in a single cell by intracellular recording methods (*8*).

Baclofen (50 μ*M*) application to the slice produced a hyperpolarization of the CA1 neuronal membrane and a decrease in input resistance that was reversible over several minutes (Fig. 2A). This observation is consistent with the results of Andrade *et al.* (*10*). When GTPγS (10 m*M*) was included in the filling solution of the recording micropipette, the same dose of baclofen gradually lost its ability to produce the above effects (Fig. 2A). Occlusion of the baclofen-induced hyperpolarization, which suggests a complete activation of G proteins normally coupled to $GABA_B$ receptors, occurred after 20 to 60 min and was not dependent on the frequency of agonist application. Development of occlusion of the $GABA_B$ response was accompanied by a gradual hyperpolarization of the membrane and a decrease in input resistance. There was good agreement in each cell between the time after impalement required for stabilization of the membrane potential and input resistance of the cell and complete occlusion of the baclofen response. This delay presumably reflects the time required for GTPγS to diffuse out of the tip of the recording electrode, equilibrate with the cell contents, and activate G proteins. Newberry and Nicoll (*12*) have demonstrated that $GABA_B$ receptors are located mainly in the dendritic zone of CA1 pyramidal neurons. Therefore, occlusion of the baclofen-induced response by GTPγS suggests that the nucleotide is reaching the dendritic postsynaptic membrane involved in generating excitatory postsynaptic potentials (EPSPs) evoked by stratum radiatum stimulation.

Despite the occlusion by GTPγS of the postsynaptic effects of baclofen, the EPSP was still suppressed by application of the $GABA_B$ agonist (Fig. 2B). Baclofen (50 μ*M*) reduced the EPSP amplitude to 16 ± 3% (mean ± SEM, n = 9) in control cells and to 20 ± 3% (n = 14) in cells loaded with GTPγS. The postsynaptic hyperpolarization produced by baclofen in these two groups of cells was −6.0 ± 0.5 mV (mean ± SEM, n = 13) and −0.5 ± 0.2 mV (n = 15), respectively. Therefore, when the post-

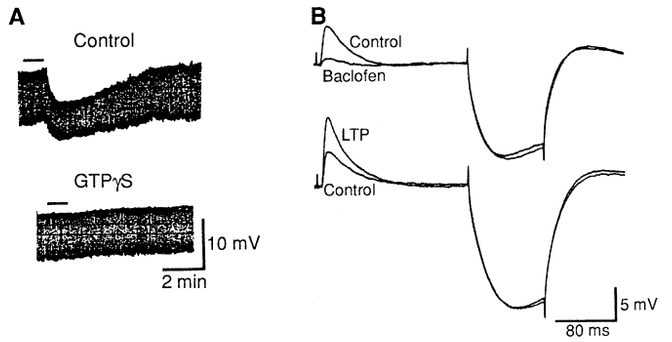

Fig. 2. Effect of intracellular loading of GTPγS on baclofen-induced responses and LTP in CA1 neurons. (**A**) The CA1 neuronal response to a 1-min application of baclofen (50 μ*M*, bar) in a control cell with a 3*M* KCl recording electrode (top) and a cell impaled with a 3*M* KCl electrode containing GTPγS (10 m*M*) (bottom). Complete occlusion of the baclofen response in the latter cell took 30 min. (**B**) After sufficient GTPγS loading to completely occlude the postsynaptic hyperpolarization and decrease in input resistance produced by baclofen, the response of the EPSP to baclofen (top) and tetanus-induced LTP (bottom) was monitored. Both top and bottom records were from the same cell. The control EPSP in the bottom record was obtained at 15 min after baclofen application. A tetanic stimulation of 200 Hz for 2 s at twice the control stimulus strength was delivered to the stratum radiatum input and the response monitored for an additional 60 min. A 100-ms, −0.3-nA current pulse was delivered through the recording electrode in both (A) and (B) to monitor input resistance. Each EPSP record in (B) is an average of five consecutive traces.

synaptic response was fully occluded, suppression of EPSP amplitude through activation of presynaptic $GABA_B$ receptors did not differ to a statistically significant extent from normal. Thus, the postsynaptic injection of GTPγS had little, if any, effect on presynaptic G proteins.

Activation of G proteins in the postsynaptic CA1 neuron by GTPγS did not occlude the expression of LTP induced by tetanic stimulation of the stratum radiatum input (Figs. 2B and 3). It seems unlikely, therefore, that G proteins involved in LTP are present in the postsynaptic neuron.

A difference in early potentiation (<10 min after tetanus) was seen between the GTPγS-loaded and control cells, in that control cells exhibited early potentiation whereas GTPγS-treated cells did not (Fig. 3). The potentiation of synaptic responses seen after tetanic stimulation of an input is thought to consist of at least two different NMDA (*N*-methyl-D-aspartate) receptor–dependent components (*13*). Induction of the early transient component is not sensitive to PKC inhibitors (*13*). Induction of the long-lasting sustained enhancement is blocked by these compounds. An occlusion or an inhibition of the early potentiation may occur as a result of the GTPγS loading, suggesting that a postsynaptic G protein–dependent pathway can influence the initial PKC-independent potentiation of EPSPs. This G protein may, however, be PT-insensitive, since brain slices that did not exhibit LTP because of PT pretreatment still showed a transient potentiation lasting a few minutes after the tetanic stimulation. Loading the postsynaptic neuron with GTPγS was also associated with a reduction of EPSP amplitude of 31 ± 4% (mean ± SEM, $n = 8$). It is likely, therefore, that the postsynaptic pathway that is activated by GTPγS is inhibiting the early potentiation rather than occluding it.

Our results provide evidence that activation of a G protein, which is not located in the postsynaptic neuron, is required for the development of LTP; this G protein will be referred to as G_{LTP} for convenience. Given the evidence that PKC activation may be responsible for LTP and that PT-sensitive G proteins can regulate PKC activity, we suggest that G_{LTP} mediates activation of PKC. In this scheme, our results suggest that activation of PKC occurs at a site distinct from the postsynaptic neuron. Current evidence is not inconsistent with this possibility.

Phorbol esters, which are activators of PKC (*11*), produce a potentiation of the synaptic response still evident after their removal. However, this potentiation is not maintained for as long as tetanus-induced LTP (*14*). It has been proposed (*4*) that activation of PKC alone is not sufficient to maintain LTP, but that phorbol ester–induced potentiation mimics the expression of LTP. It is possible that LTP is associated with an uncoupling between regulatory and catalytic domains of PKC, leading to permanent enzyme activity (*4*). Activation of PKC either presynaptically or postsynaptically by phorbol esters appears to produce changes resembling LTP (*15, 16*); however, since phorbol esters are lipophilic, a localized application into postsynaptic cells (*15*) does not preclude the possibility that the compound could cross cell membranes and traverse the synaptic cleft to gain access to the presynaptic terminal. This may also be true for phorbol ester application onto presynaptic axons (*16*). Hu *et al.* (*17*) show that injections of PKC into CA1 neurons produce changes consistent with LTP. The alterations induced by the postsynaptic injections of PKC, however, may not be mediated by the same mechanisms as LTP, for the following reasons. (i) High concentrations of PKC are found in the brain, especially in the hippocampus (*18*), so further addition of the enzyme might not be expected to produce any significant effects. Furthermore, it has been demonstrated that it is the activated form of PKC that elicits a potentiation of synaptic transmission (*3, 15, 16*). In the study of Hu *et al.* (*17*), no activators were administered. (ii) There was a gradual hyperpolarization of the neuronal membrane as PKC entered the cell. In contrast, membrane potential does not change during LTP. (iii) Injection of the enzyme eventually induced the generation of multiple spikes in the neuron in response to synaptic activation. This is also uncharacteristic of LTP, but could be a manifestation of epileptic discharges. Therefore, studies to date have not determined conclusively the site where activation of PKC can lead to an LTP-like potentiation.

Concurrent postsynaptic depolarization and presynaptic activation are required for successful induction of LTP in the CA1 region (*19*). An elevation of Ca^{2+} in CA1 neurons is sufficient to produce an LTP-like state (*20*). It is possible that the "associative" nature of LTP is mediated by a Ca^{2+}-dependent, G protein–independent release of a substance from the postsynaptic neuron, which retrogradely diffuses to the presynaptic terminal (*21*) or other structures

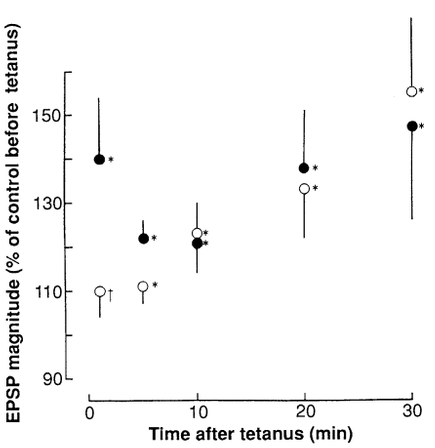

Fig. 3. Effect of GTPγS loading into CA1 neurons on the development of LTP. A tetanic stimulation (200 Hz for 2 s at twice the control stimulus intensity) was delivered to the stratum radiatum input following complete occlusion of the CA1 neuronal response to baclofen. The EPSP was allowed to recover for at least 15 min after drug application before the tetanus was given. Each point shown represents mean ± SEM. *$P < 0.05$; significantly different from control values before tetanus (paired *t* test). †$P < 0.05$; significantly different from control group (no GTPγS) (unpaired *t* test). Open circles, GTPγS; closed circles, control.

(*22*) to produce the G_{LTP}-dependent changes responsible for LTP.

Intracerebroventricular injections of PT block LTP in mossy fiber–CA3 but not stratum radiatum—CA1 synapses (*23*). We were also unable to counteract LTP in the CA1 region by ventricular injection of the toxin (*24*). The CA3 region of the hippocampus in rats is strategically located close to the lateral ventricle, whereas the CA1 area is not. Therefore, PT in the ventricle would have better access to the CA3 region. However, intracerebral injections of the toxin directly above the hippocampus, as were used in this study, localize it in the vicinity of CA1 neurons. Taken together, our study and that of Ito *et al.* (*23*) suggest a G protein is involved in LTP in two separate synaptic systems in the hippocampus. Several differences have been found between LTP in the CA1 and CA3 areas. (i) LTP induction is associative in the CA1 area but not in mossy fiber–CA3 synapses (*25*). (ii) NMDA receptor blockade inhibits LTP induction in the CA1 region but not in the mossy fiber–CA3 system (*25, 26*). (iii) Intracellular injections of Ca^{2+} chelators block LTP in the CA1 region but not in the mossy fiber–CA3 system (*20, 27*). A role for G proteins in these two apparently different pathways provides a common link in the LTP process. Furthermore, PKC activation by phorbol ester application can produce a potentiation of responses in both the stratum radiatum–CA1 and mossy fiber–CA3 synaptic systems. There is a good correlation between the distributions of phorbol ester binding sites in the brain and G_o (*28*), a G protein that is inactivated by PT and can regulate PLC activity (*7*). Perhaps G_o and G_{LTP} are similar or identical proteins.

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Autonomic Regulation of a Chloride Current in Heart

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In isolated heart cells, β-adrenergic receptor stimulation induced a background current that was suppressed by simultaneous muscarinic receptor stimulation. Direct activation of adenylate cyclase with forskolin also elicited this current, suggesting regulation by adenosine 3′,5′-monophosphate (cAMP). This current could be recorded when sodium, calcium, and potassium currents were eliminated by channel antagonists or by ion substitution. Alteration of the chloride equilibrium potential produced changes in the reversal potential expected for a chloride current. Activation of this chloride current modulated action potential duration and altered the resting membrane potential in a chloride gradient–dependent manner.

IN HEART, STIMULATION OF β-ADRENergic receptors activates adenylate cyclase, resulting in the production of cAMP and subsequent protein kinase activation. This pathway is involved in the regulation of several ionic currents. Furthermore, the response to β-adrenergic stimulation of at least two of these, the Ca^{2+} current (I_{Ca}) (*1*) and the delayed rectifier K^+ current (I_K) (*2–4*), can be antagonized by acetylcholine (ACh) acting through muscarinic receptors (*4–5*). The transient outward current (I_{to}) is also enhanced by β-adrenergic stimulation (*6*). The I_{to}, which rapidly activates during membrane depolarization and helps repolarize the membrane potential (*7*), was initially suggested to be a Cl^- conductance (*8*) but it is now believed to be carried predominantly by K^+ ions (*9*). Nevertheless, single Cl^- channels with a unitary conductance of 55 pS can be observed in planer lipid bilayers

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