The Journal of Neuroscience, December 1991, 11(12): 3718–3726

The Involvement of $GABA_B$ Receptors and Coupled G-Proteins in Spinal GABAergic Presynaptic Inhibition

Simon Alford[a] and Sten Grillner

Nobel Institute for Neurophysiology, Karolinska Institutet, S 104 01 Stockholm, Sweden

GABA acts as a presynaptic inhibitory transmitter in the spinal cord. In the lamprey, it has recently been shown that it acts in this way at both primary sensory and motor system synapses and is important in the generation of a locomotor rhythm. Both $GABA_A$ and $GABA_B$ receptors are activated at these sites by GABA released during physiological activity. In some systems, $GABA_B$ receptor activation has been shown to lead to modulation of ion channel function indirectly through the action of a pertussis toxin (PTX)-sensitive G-protein. Here we have studied the mechanism of action of the presynaptic $GABA_B$ receptor in this system. $GABA_B$ receptor activation leads to a decrease in axonal membrane impedance and also to a reduction in the axonal action potential duration. The ionic basis for this response remains unknown, though it is not, unlike the response to $GABA_A$ receptor activation, mediated by an increase in conductance to Cl^-. The effects of $GABA_B$ receptor antagonism with phaclofen are mimicked by pretreatment of the spinal cord with PTX. Because this procedure inactivates certain classes of G-proteins, it seemed likely that the $GABA_B$ receptor–mediated effects are initiated via a presynaptic population of PTX-sensitive G-proteins. Experiments in which only presynaptic G-proteins were interfered with indicate that this is so. Stable analogs of GTP and GDP were used to activate permanently or to antagonize, respectively, the GTP binding site in the presynaptic component of these spinal synapses. We conclude that $GABA_B$ receptor–mediated synaptic suppression in the spinal cord is caused by GTP binding to presynaptic G-proteins linked to the $GABA_B$ receptor. As a result, GABA binding to the presynaptic $GABA_B$ receptor leads to a reduction in the duration of the presynaptic action potential and consequently a reduction in transmitter release.

The inhibitory amino acid neurotransmitter GABA is located throughout the vertebrate CNS, as are both of its known receptor subtypes, termed $GABA_A$ and $GABA_B$ receptors (Bowery et al., 1980, 1987; Alger and Nicoll, 1982). This feature is present in the lamprey (Alford et al., 1991). Additionally, $GABA_A$ and $GABA_B$ receptors have been demonstrated to be located on pre- and postsynaptic structures (Curtis et al., 1968, 1977). Synaptic release is reduced, after activation of $GABA_B$ receptors, from primary afferents in the mammalian spinal cord (Curtis et al., 1981) and lamprey spinal cord (Christenson and Grillner, 1991) as well as onto pyramidal neurons of the hippocampus (Bowery et al., 1980; Lanthorn and Cotman, 1981; Ault and Nadler, 1982). In the lamprey, it has also been demonstrated that the spinal motor system utilizes presynaptically directed GABAergic transmission, that both $GABA_A$ and $GABA_B$ receptor subtypes coexist on spinal axons, and that GABAergic transmission is of importance in the generation of locomotor activity (Alford et al., 1990, 1991).

L-Baclofen, or GABA, binding to $GABA_B$ receptors in mammals has been shown to mediate its effects, not directly upon an ion channel, but indirectly through the action of a guanine nucleotide–binding protein (G-protein; Andrade et al., 1986; Dolphin and Scott, 1987; Dunlap et al., 1987). Release of transmitter has been shown to be inhibited by the action of pertussis toxin (PTX)-sensitive G-proteins from dorsal root ganglion cells (Holz et al., 1989). In the CNS, the means by which $GABA_B$ receptor activation leads to presynaptic inhibition has not hitherto been directly studied at the level of the presynaptic terminal. However, regarding somatically located receptors, $GABA_B$ receptor–activated G-proteins mediate both voltage-sensitive Ca^{2+} channel inactivation in cultured neurons of the dorsal root ganglia and an inhibition of peptide release from these cells. This is also coupled to α-adrenergic receptors (Holz et al., 1989). Additionally, G-proteins coupled to $GABA_B$, adenosine, and 5-HT receptors mediate K^+ channel opening in pyramidal neurons of the hippocampus (Andrade et al., 1986; Nicoll, 1988).

Perhaps one model for the means by which L-baclofen reduces transmitter release is provided by the G-protein–mediated inactivation of voltage-gated Ca^{2+} channels following $GABA_B$ receptor activation in dorsal root ganglion neurons. However, data regarding L-baclofen depression of excitatory synaptic transmission in the hippocampus are contradictory. It has been reported that this action is mediated through a receptor insensitive to both PTX and the $GABA_B$ receptor antagonist phaclofen (Dutar and Nicoll, 1988b). Other studies indicate that high doses of the more potent $GABA_B$ antagonist 2-hydroxy-saclofen are effective at this site (Randall et al., 1990) and that intrahippocampal PTX blocks the presynaptic action of L-baclofen. Such disparity in results may at least be partly explained by the function of $GABA_B$ receptors in the hippocampus. Here they have been reported to act as autoreceptors, controlling release of GABA from the inhibitory terminal (Davies et al., 1990). Location in the synaptic cleft nearer to the site of release may lead to higher concentrations of GABA at the presynaptic receptors.

Received Mar. 21, 1991; revised June 14, 1991; accepted June 24, 1991.

This work was supported by the Swedish MRC (3026), Karolinska Institutets fonder, and the Swedish Natural Science Research Council (B-TV 3531-300); S.A. was supported by a postdoctoral fellowship from The Royal Society. The helpful comments of Dr. J. Christenson are also greatly appreciated.

Correspondence should be addressed to Prof. Sten Grillner, Nobel Institute for Neurophysiology, Box 60400, 104 01 Stockholm, Sweden.

[a] Present address: Department of Pharmacology, University of Birmingham, The Medical School, Edgbaston, Birmingham B15 2TT, United Kingdom.

Because of the inaccessibility of most vertebrate presynaptic terminals for electrophysiological studies and the difficulty of pharmacological isolation of presynaptic terminals from postsynaptic somata, it is not known by what mechanism $GABA_B$ receptor activation causes a depression of transmitter release in either the spinal cord or the hippocampus. To gain further insight into the means by which presynaptic $GABA_B$ receptor activation reduces transmitter release, we have utilized the isolated lamprey spinal cord preparation (Wallén and Grillner, 1987). This has enabled us to impale axons very close to the presynaptic terminal and to record from the target neurons of these axons (Alford et al., 1991). The effects of $GABA_B$ receptor activation have therefore been recorded in the axons, as well as on the effects of transmitter release from these same axons. It has also been possible to manipulate G-protein activation exclusively within the axonal terminals by including compounds active at G-protein binding sites within the recording microelectrodes.

This work has been presented previously in preliminary form (Alford and Grillner, 1990).

Materials and Methods

All experiments were performed on pieces of spinal cord isolated from adult *Ichthyomyzon unicuspis*. For details of the dissection used, see Wallén and Grillner (1987). The tissue was superfused with lamprey saline of the following composition: NaCl, 104.5 mM; KCl, 2 mM; $CaCl_2$, 2.6 mM; $MgCl_2$, 1.8 mM; glucose, 4 mM; $NaHCO_3$, 20 mM; bubbled with 95% O_2, 5% CO_2 (Wickelgren, 1979). This was continuously superfused over the preparation to enable the application of drugs and was maintained at approximately 9°C. Recordings of ventral root activity were made with saline-filled glass electrodes into which the cut ends of the roots were drawn. Intracellular recordings were made with thin-walled glass microelectrodes filled with 5 M K acetate in motoneurons while axonal recordings were made with electrodes filled with 3 M KCl, combined for certain experiments with 20 mM GTP-γ-S or 10 mM GDP-β-S. In the latter two cases, the axonal electrode was connected to a pressure ejection system to enable pressure ejection of its contents into the axon. This was performed by applying 20 msec pressure pulses and incrementing the pressure delivered to the electrode until changes in recorded axonal membrane potential were just visible during the pulse. Experiments involving pertussis toxin–pretreated spinal cords were performed after pretreating the tissue, following dissection, in saline containing 300 ng/ml PTX. The tissue was stored in this way at 5°C for 24–36 hr prior to the experiment. Control pieces of spinal cord were taken from the same animals and stored alongside the pretreated pieces in the same conditions but with no PTX added to the saline. L-Baclofen, where used, was applied via a pressure ejection pipette placed over the spinal cord close to the neuron from which recordings were made. Short (25 msec) pressure pulses released 20–50 nl of solution in each pulse, which was monitored in some cases by the addition of the dye fast green. Fictive locomotion was induced by adding 1 mM D-glutamate to the saline (Cohen and Wallén, 1980). All extracellular stimulations were made with glass microelectrodes containing 1 M NaCl and with impedances of 2–5 MΩ.

Results

Presynaptic GABA receptor activation: effect on spike width

Presynaptic $GABA_B$ receptor activation has been shown to mediate a reduction in the amplitude of postsynaptic potentials from mammalian spinal primary afferents (Curtis et al., 1977, 1981) and in the hippocampus (Ault and Nadler, 1982; Dutar and Nicoll, 1988a,b), as well as from primary afferents of the lamprey (Christenson and Grillner, 1991). We have investigated the mechanism by which this occurs. Na^+ spikes were initiated in the impaled axons by the application of brief depolarizing current steps through the recording microelectrode (Fig. 1*a*). In axons in which the application of GABA via a pressure ejection

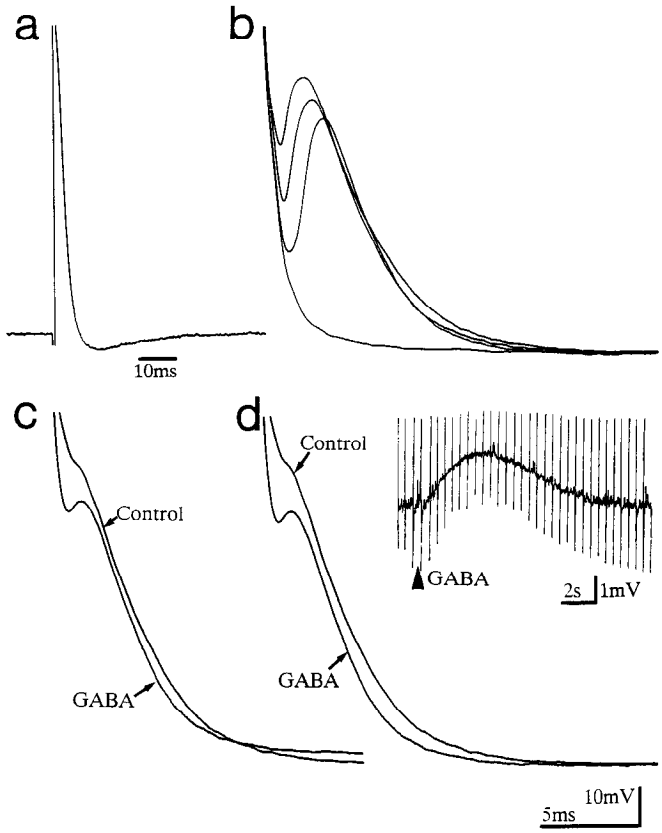

Figure 1. The effect of GABA application on axonal action potential properties. Axons were caused to spike by the passage of brief (0.2 msec) application of positive current injection through the microelectrode. *a*, Action potential at slow time base showing single phase of afterhyperpolarization. *b*, Shown at a faster time base; three consecutive action potentials activated at 400 msec intervals just before and during application of GABA. With GABA application the spikes are initiated later after the current injection and are reduced in amplitude. The baseline is corrected for the GABA-induced depolarization of the neuron. *c*, Two spikes before and during GABA showing reduced amplitude and more rapid return to membrane potential in GABA. *d*, Same two spikes as *c* but with baseline adjusted for GABA-induced depolarization to show effect on amplitude and spike width more clearly. *Inset* shows GABA-induced depolarization at a slower time base with superimposed spikes.

pipette caused a depolarizing response, this application of GABA led to an increase in the spike threshold of the axon (Fig. 1*b*; $n = 3$), a reduction in the total spike amplitude (Fig. 1*b–d*; mean reduction in spike amplitude, 9 ± 4.6 mV, ±SD) and a reduction in the spike width (Fig. 1*d*; mean reduction in spike width at half peak, 0.37 ± 0.09 msec, ±SEM). These effects were seen over a depolarization of the axon seen in a slower time base (Fig. 1, inset). In an additional 30 axons tested that showed no GABA-induced depolarization, no change in spike characteristics was observed. GABA application will, however, cause an activation of both $GABA_A$ and $GABA_B$ receptors. Consequently, these effects on the spike characteristics will result both from an increase in axonal Cl^- conductance and from conductances activated by $GABA_B$ receptors. Application of L-baclofen in the same way as above also caused a reduction in the spike width (Fig. 2; mean reduction in width at half peak, 0.26 ± 0.12 msec, ±SEM) concurrent to a depolarization of the axonal membrane potential (Fig. 2; $n = 6$). However, the effects seen on spike threshold were less marked than that seen after GABA appli-

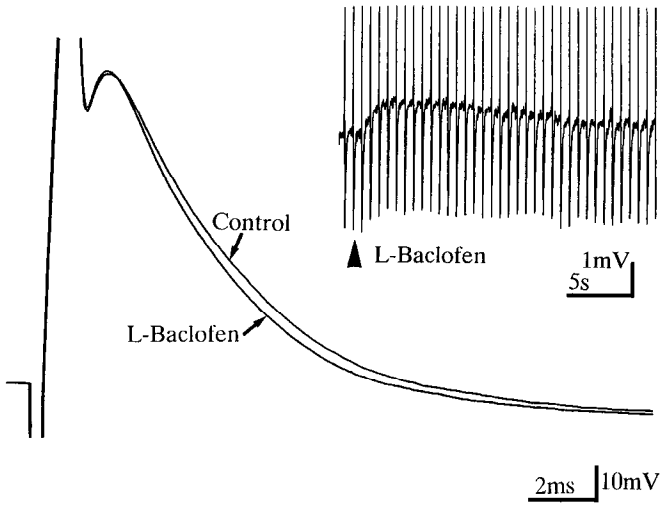

Figure 2. The effect of baclofen application on axonal action potentials. *a*, Action potentials were initiated with brief positive current passed through the microelectrode. Baclofen application causes a reduction in spike width seen as a more rapid return to membrane potential after the spike. The baseline is adjusted to remove the baclofen-induced depolarization. *Inset* shows the depolarization due to baclofen at a slower time base with superimposed spikes.

cation. In no case did the application of L-baclofen prevent spike initiation in the axons recorded here. The effect of GABA and L-baclofen was also tested on the action potentials of axons in which no depolarization was seen following agonist application. In no case (n = 14) did the application of either GABA or L-baclofen cause a change in the characteristics of such action potentials.

$GABA_B$ receptor activation and inhibitory synaptic transmission

It is clear from the results described above that $GABA_B$ receptor activation may cause a reduction in action potential amplitude in the lamprey spinal cord. It has also been shown in a previous study that axons of the spinal motor system possess $GABA_B$ receptors (Alford et al., 1991). Here the effect of L-baclofen upon synaptic transmission in the lamprey motor system was tested. Application of L-baclofen could be used to test for the effect of presynaptic $GABA_B$ receptor activation upon synaptic transmission since in no case (n = 6 in this study) did its application change either the membrane potential or the input impedance of the impaled postsynaptic neuron as measured by passing constant-current pulses through the recording microelectrode (Fig. 3*c*; see also Christenson and Grillner, 1991). Monosynaptic compound IPSPs (Fig. 3*a*) were evoked onto motoneurons and premotoneurons impaled in the ventral horn of the spinal cord by microstimulation of the contralateral cord just rostral to the motoneuron under investigation. In order to exclude the effects of activation of excitatory axons, the spinal cord was also bathed in the excitatory amino acid antagonist kynurenate (2 mM; Perkins and Stone, 1982). This also ensured that the IPSPs were monosynaptic by eliminating all spinal fast synaptic transmission (Buchanan and Grillner, 1987; Buchanan et al., 1987). L-Baclofen, applied as above, reduced the amplitude of the IPSP recorded in all cases tested (n = 6; mean reduction, 69 ± 9%, ±SEM), and the response always showed recovery. These IPSPs have been shown to be mediated by glycine receptor activation

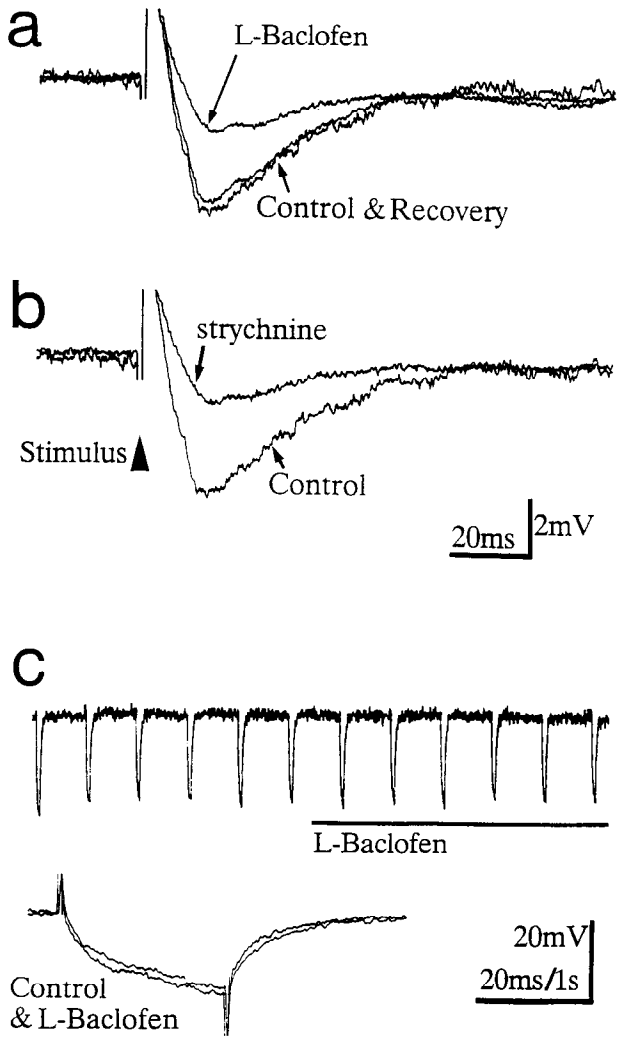

Figure 3. The application of baclofen depresses glycinergic IPSPs. *a*, Monosynaptic IPSPs were evoked onto motor or premotor neurons by stimulation of the contralateral spinal cord immediately rostral to the impaled soma. Excitatory transmission was abolished by the application of 2 mM kynurenate. Application of baclofen depressed the IPSP in a recoverable manner. *b*, These IPSPs were also depressed by the application of strychnine to the bathing medium. *c*, Membrane potential fluctuations are shown in response to application of repetitive current pulses. *Inset* shows two superimposed traces at a faster time base before and after Baclofen. Baclofen showed no effect upon membrane potential or input impedance of the soma.

(Buchanan, 1982). To confirm this finding on these IPSPs, in two cases after the washout of L-baclofen the glycine antagonist strychnine nitrate (5 μM) was applied to the bathing solution (Fig. 3*b*). The IPSPs were abolished by strychnine in both cases. Hence, a clear $GABA_B$-mediated presynaptic modulation of glycinergic inhibition has been demonstrated.

$GABA_B$ receptor activation: effects on excitatory synaptic transmission

EPSPs mediated by propriospinal axons were also depressed by the application of L-baclofen as above. Monosynaptic EPSPs that have been shown to utilize an excitatory amino acid as a transmitter (following 10 Hz stimulation without latency or am-

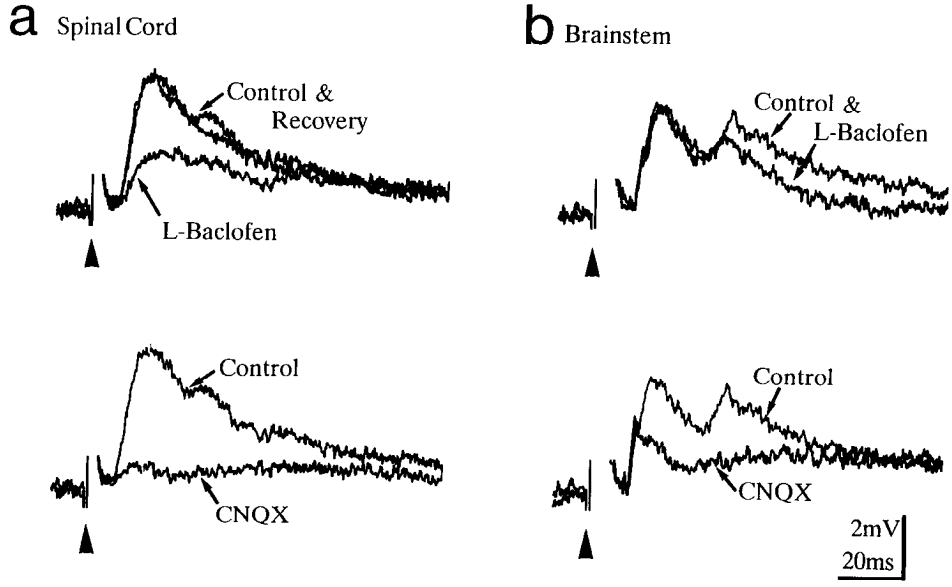

Figure 4. Baclofen application selectively depresses EPSPs evoked from within the spinal cord but not those evoked by stimulation of the brainstem. *a*, EPSPs were evoked by stimulation (*arrowhead*) of the spinal cord ipsilateral to the impaled motor or premotor neuron. The spinal cord was bathed in strychnine to abolish glycinergic IPSPs. Application of baclofen depressed this compound EPSP. The EPSP was also abolished by the application of CNQX, indicating that it was mediated by excitatory amino acid receptors. *b*, Compound EPSPs were evoked in the same neuron by stimulation of reticulospinal fibers in the brainstem. The early part of this response, known to be carried by monosynaptic reticulospinal connections, is unaffected by the same application of baclofen as in *a*. This response is, however, also sensitive to the application of CNQX.

plitude change; Buchanan, 1982; Dale and Grillner, 1986; Buchanan and Grillner, 1987) were evoked onto motoneurons by microstimulation of the ventromedial tracts of the spinal cord just caudal to the impaled motoneuron (Fig. 4*a*). The spinal cord was bathed in 5 μM strychnine in order to eliminate glycinergic transmission. Application of L-baclofen as above depressed all such compound EPSPs (n = 6; mean reduction in EPSP amplitude, 62 ± 10%, ±SEM), and the response showed recovery.

In four of these neurons, a second stimulus electrode was placed on the ipsilateral brainstem, within the fourth ventricle, in order to stimulate the large descending axons of the reticulospinal system. These reticulospinal axons have been shown to make excitatory synapses onto motor- and premotor interneurons, again utilizing an excitatory amino acid transmitter (Buchanan et al., 1987). In all cases tested, the application of L-baclofen that caused a depression of all excitatory synaptic transmission originating from propriospinal axons failed to depress transmission from reticulospinal fibers. This was also the case in an additional two motoneurons in which reticulospinal synaptic inputs were left unaffected by L-Baclofen but in which inputs from the spinal cord were not previously tested. To confirm that both propriospinal and reticulospinal axons utilized an excitatory amino acid transmitter in these cases, in two of the cells the excitatory amino acid antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 100 μM in pipette; Honoré et al., 1988) was applied to the preparation through a pressure ejection pipette close to the recorded neuron after L-baclofen washout. Chemical synaptic transmission was suppressed from both propriospinal and reticulospinal axons. That L-baclofen exerts specific effects on excitatory amino acid transmission from some but not all inputs to these neurons provides further evidence for a selective presynaptic site of action for its effect.

L-Baclofen depresses synaptic transmission between synaptically coupled pairs of neurons

In order to test for the effects of L-baclofen between synaptically coupled pairs of neurons, paired cell recordings were made between axons in the ventromedial tracts and motor- or premotor interneurons (Fig. 5*a*). In four paired cell recordings obtained in this way stimulation of the presynaptic axon through the recording microelectrode led to a postsynaptic potential (PSP) in the postsynaptic neuron (one IPSP and three EPSPs). In all four cases tested, L-baclofen caused a reduction in the amplitude of the PSP (Fig. 5*b,c*; mean reduction of PSP amplitude, 65 ± 15%, ±SEM). However, in none of these cases was the PSP completely abolished. This provides some evidence that the action of $GABA_B$ receptor activation was to reduce the quantity of transmitter released for each spike.

$GABA_B$ receptors activate PTX-sensitive G-proteins

Here we have tested the possible role of PTX-sensitive G-proteins in the action of $GABA_B$ receptors upon synaptic transmission. Pieces of isolated lamprey spinal cord were pretreated with PTX in order to ADP-ribosylate and, therefore, inactivate the GTP binding site of all PTX-sensitive G-proteins in the spinal cord (Ui, 1984). The effect of PTX pretreatment was examined on the synaptic depression mediated by L-baclofen application over motoneurons receiving compound IPSPs and EPSPs evoked from the spinal cord. Monosynaptic compound IPSPs were evoked in motor and premotor interneurons as described above. In pieces of spinal cord pretreated with PTX, L-baclofen caused a much smaller or, in two cases, insignificant depression of the compound IPSP (Fig. 6*a*). In addition to the effect of L-baclofen described without PTX pretreatment above, a further two control experiments were performed in which pieces of spinal cord from the same animals as the PTX-pretreated pieces were kept in the same conditions for the same time. These pieces of spinal cord were, however, not pretreated with PTX. A suppression of the compound IPSP was again seen in all cases in these control spinal cords.

Monosynaptic compound EPSPs (n = 2) were also evoked in pieces of spinal cord after pretreatment with PTX (Fig. 6*b*). Stimulation of propriospinal axons was performed under the conditions described above (Fig. 4). As for the compound IPSPs described after PTX pretreatment, L-baclofen again showed only a small depression of the compound EPSP. The mean reduction in amplitude of EPSPs and IPSPs in spinal cords pretreated with

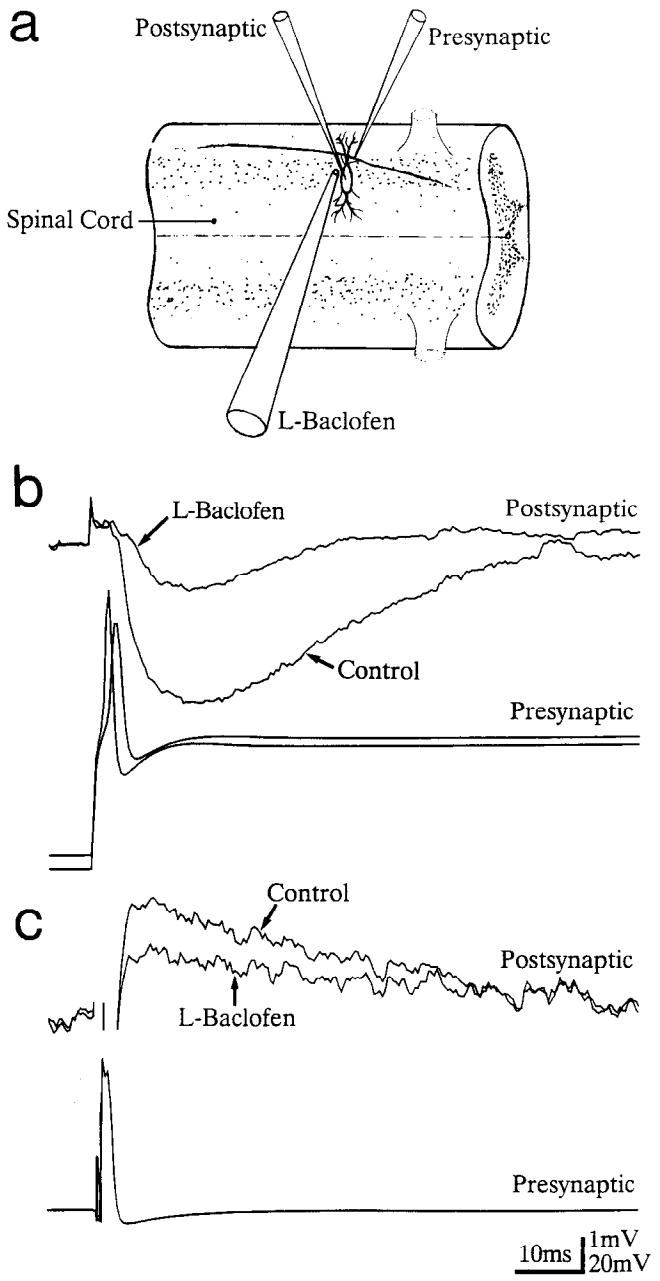

Figure 5. Baclofen depresses paired IPSPs and EPSPs recorded between spinal axons and nearby axons. *a*, Representation of the preparation used. Postsynaptic motor or premotor neurons were impaled with microelectrodes. A presynaptic axon was then impaled that made monosynaptic contact with the postsynaptic neuron and, for all the paired recordings described, was within the dendritic field of the postsynaptic cell. Baclofen was applied through a pressure pipette positioned immediately over the impaled soma. Paired IPSPs (*b*) and EPSPs (*c*) obtained in this way were depressed by the application of baclofen. In both *b* and *c*, the *upper record* shows the recording from the postsynaptic neuron following a spike (*lower record*) in the presynaptic axons. Records are averages of 50–80 sweeps.

PTX was significantly less than in untreated tissue (0.001 level, Student's *t* test; mean reduction in PSP amplitude following L-baclofen application in PTX-pretreated spinal cord, 8.4 ± 1.5%, ±SEM). It therefore seems likely that $GABA_B$ receptor activation in the presynaptic terminal of these axons leads to GTP binding to PTX-sensitive G-proteins and a subsequent reduction in transmitter release through an undefined path.

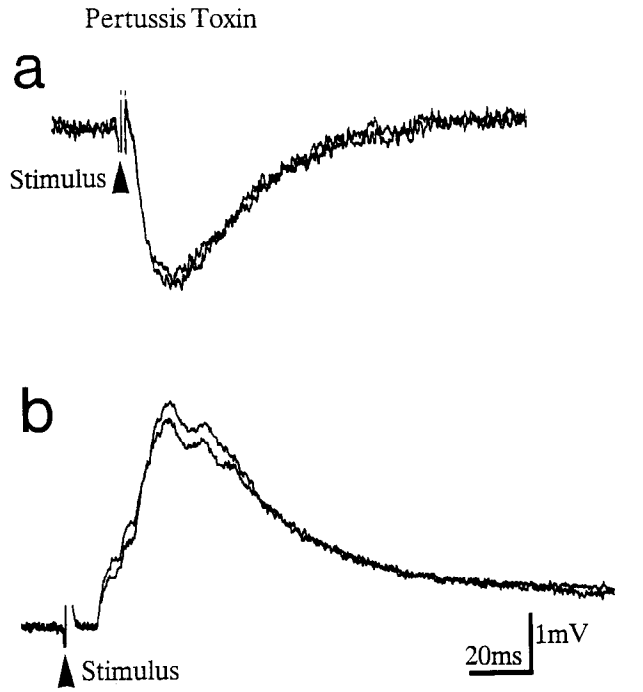

Figure 6. The baclofen-induced depression of monosynaptic compound IPSPs is sensitive to treatment with PTX. Isolated spinal cord pieces were pretreated with PTX for 24–36 hr prior to the experiment. After this treatment, compound IPSPs (*a*) and EPSPs (*b*) evoked from within the spinal cord as in Figures 3 and 4 showed little or no effect caused by the application of baclofen.

Inactivation of presynaptic G-proteins partially reverses the effect of L-baclofen

A more detailed test of this hypothesis required the manipulation of the presynaptic terminal of a coupled synaptic pair of neurons. In order to achieve this, paired cell recordings were made between axons in the ventromedial tracts and motoneurons or premotoneurons such that the postsynaptic neuron was immediately adjacent to the impaled axon (see Fig. 5*a*). The axon was always impaled within the typical extent of the dendritic tree of spinal motoneurons (see Russell and Wallén, 1983; Wallén et al., 1985; within approximately 100 μm from the postsynaptic electrode) in order to maximize the possibility that compounds ejected from the presynaptic recording microelectrode would diffuse into the presynaptic terminal. The presynaptic microelectrode contained 3 M KCl and 10 mM GDP-β-S. GDP-β-S is a stable analog of GDP that has been shown to prevent GTP binding to G-proteins (Eckstein et al., 1979). In this way, G-protein activation in the presynaptic terminal may be inhibited by ejection of the electrode contents into the presynaptic axon.

In order to test whether ejection of GDP-β-S into the presynaptic axon showed any effect on the characteristics of the PSP evoked in the postsynaptic neuron, experiments were performed ($n = 4$) in which PSPs were evoked from the presynaptic axon (Fig. 7). GDP-β-S was then ejected from the presynaptic recording microelectrode, in the absence of baclofen and exogenous GABA by the application of pressure pulses through the electrode. No effect was seen on the evoked PSP in any of these paired recordings (Fig. 7*c*).

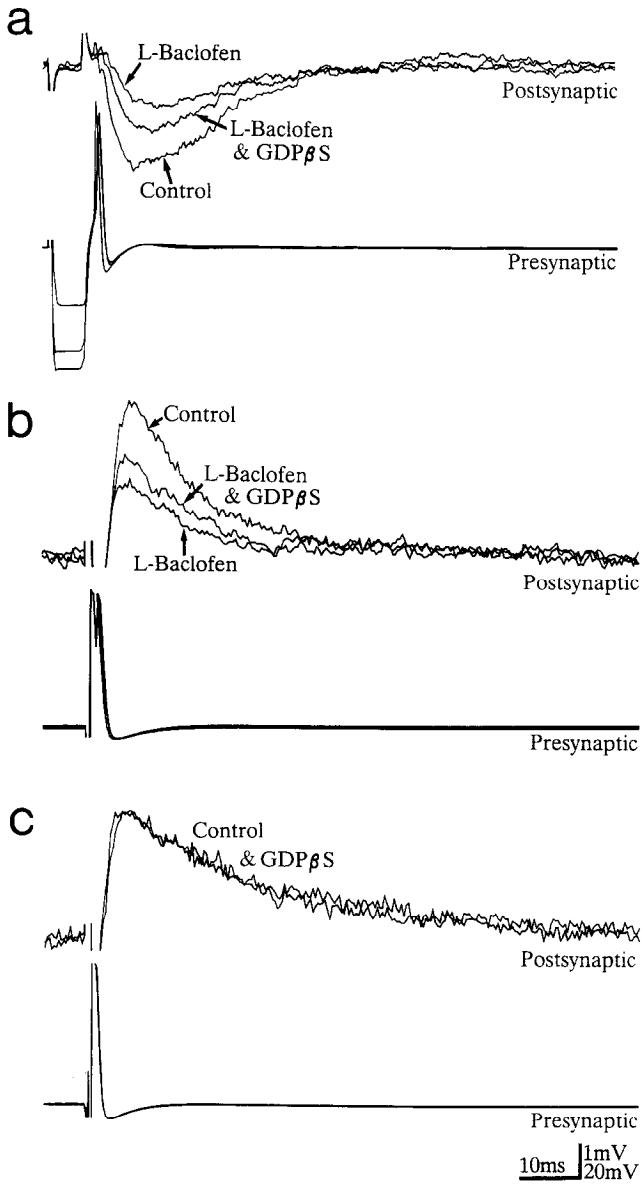

Figure 7. Ejection of GDP-β-S into the presynaptic axon partially reverses the depression of paired PSPs induced by baclofen. *a* and *b* show paired IPSPs and EPSPs, respectively, between spinal axons and spinal soma (*upper records*) and presynaptic evoked spikes (*lower records*). Pairs were obtained as described in Figure 6; however, the electrode used to impale the presynaptic axon contained 20 mM GDP-β-S. Paired PSPs were depressed by the application of Baclofen. Under continued application of baclofen, loading the axon with GDP-β-S by pressure application through the microelectrode led to a partial recovery of the PSP. *c* shows that the application of GDP-β-S through the recording pipette had no effect upon the amplitude of the PSP in the absence of baclofen. All records are averages of 50–80 sweeps.

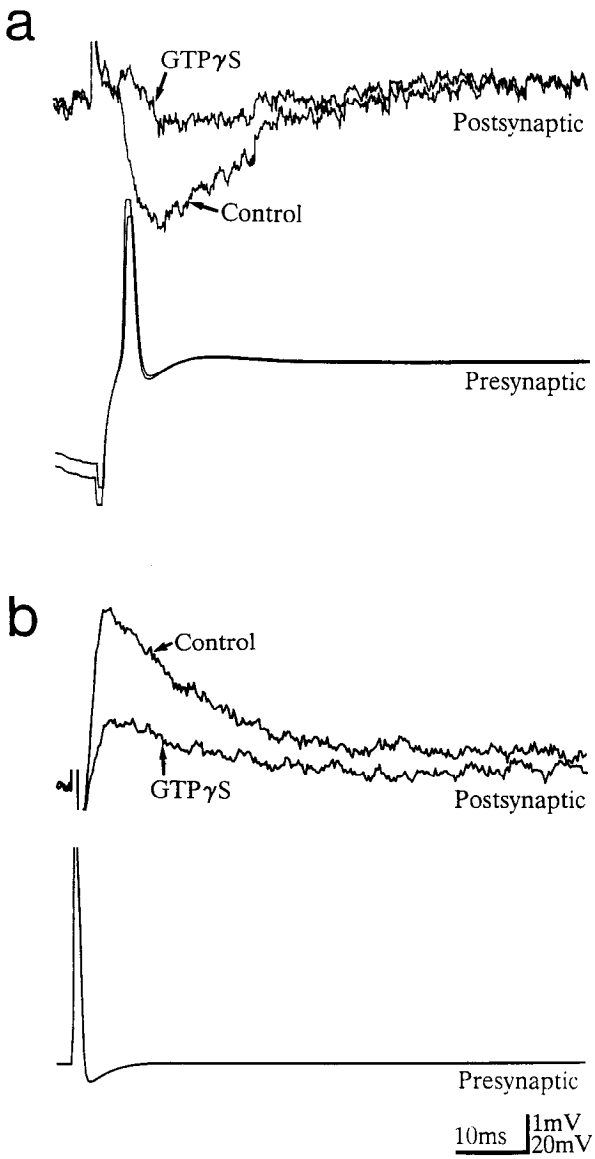

Figure 8. Activation of axonal G-proteins is sufficient to depress synaptic transmission. Paired recordings were obtained between axons and spinal soma as for Figures 6 and 7 in which IPSPs (*a*) and EPSPs (*b*) were obtained (*upper records*) after evoked presynaptic spikes (*lower records*). The presynaptic electrode now contained 50 mM GTP-γ-S. After obtaining control PSPs, GTP-γ-S was ejected into the axon by pressure application through the pipette. GTP-γ-S application caused a depression of the PSPs. Records are averages of 50–80 sweeps.

Furthermore, similar, paired cell recordings were made during which L-baclofen was applied. PSPs evoked as described above were recorded before and after L-baclofen was applied by pressure ejection over the recording site (six EPSPs, two IPSPs). In four cases, the application of L-baclofen caused no suppression of the PSP recorded in the adjacent motoneuron. This should be contrasted with the results considered above in which L-baclofen application always caused a suppression of the paired PSP when the presynaptic recording electrode was located farther from the synapse and contained only 3 M KCl (Fig. 4). In the remaining four pairs (three excitatory and one inhibitory; amplitudes were reduced to 47%, 32%, 60%, and 60% of control), the pressure ejection of L-baclofen caused a reduction in the amplitude of the recorded PSP (Fig. 7*b*). The presynaptic axon was then loaded with GDP-β-S by passing pressure pulses through the axonal recording electrode. The amplitude of the evoked PSP then showed partial recovery in amplitude in three cases tested (amplitudes returned to 67%, 62%, and 87% of control, in the first three of the depressed cells above) with no effect in the fourth.

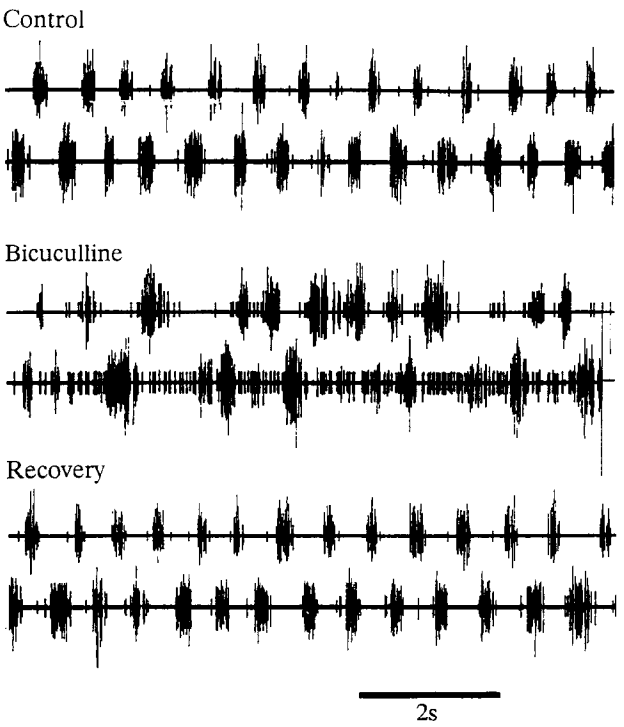

Figure 9. Pretreatment of the spinal cord with PTX mimicked the application of the $GABA_B$ antagonist baclofen upon fictive locomotion. Fictive swimming was evoked by the application of glutamate (1 mM) in pieces of spinal cord after pretreatment with PTX (*control*). Application of the $GABA_A$ receptor antagonist bicuculline, which otherwise does not affect fictive swimming, caused a marked disruption of this activity. Washout of bicuculline restored regular burst activity characteristic of the spinal cord.

Activation of presynaptic G-proteins by GTP-γ-S mimics L-baclofen application

To investigate the effect of the activation of G-proteins located in the presynaptic terminal, further paired recordings, as above, were made in which the presynaptic microelectrode contained GTP-γ-S. Again, the postsynaptic neuron was impaled with an electrode containing 5 M K acetate, while the presynaptic axon was recorded with an electrode containing 3 M KCl and 20 mM GTP-γ-S. GTP-γ-S is a stable analog of GTP that permanently binds to and, consequently, inactivates G-proteins (Jakobs et al., 1983). Immediately after impaling the pair of cells, the axon was stimulated through its recording microelectrode in order to evoke an action potential and a PSP onto the postsynaptic neuron (Fig. 8*b*). The presynaptic axon was again loaded with the contents of the recording microelectrode by passing pressure pulses through its barrel. Stimulation of the presynaptic axon now to led to the generation of a PSP of markedly smaller amplitude in the motoneuron than was observed prior to ejection of the GTP-γ-S (Fig. 8*b*; six EPSPs, three IPSPs; amplitudes were reduced by 81 ± 4.6%, ±SEM, of control).

A physiological role of PTX-sensitive G-protein activation

$GABA_B$ receptor activation has been shown to play a role in the coordination of locomotor activity in the lamprey spinal cord (Alford et al., 1991). Antagonism of $GABA_B$ receptors in combination with blockade of $GABA_A$-mediated activity leads to a disruption of fictive locomotion. This is in contrast to the blockade of $GABA_A$ receptors alone, which has little or no effect upon locomotor activity (Grillner and Wallén, 1980). In order to test whether this mechanism relies upon the activation of PTX-sensitive G-proteins, experiments were performed on the effect of G-protein inactivation upon fictive locomotion. This activity, the neuronal correlate of locomotion, comprises alternation of burst activity in opposite pairs of ventral roots. Fictive locomotion may be activated by the bath application of excitatory amino acids to the isolated spinal cord. The pieces of spinal cord, pretreated with PTX (300 ng/ml, 36 hr), were superfused with saline containing 1 mM D-glutamate in order to initiate fictive locomotion (Fig. 9; Cohen and Wallén, 1980). In untreated pieces of spinal cord, blockade of only $GABA_A$ receptors does not noticeably disrupt such fictive locomotion (Grillner and Wallén, 1980; Alford et al., 1991), though simultaneous blockade of both $GABA_A$ and $GABA_B$ receptors does disrupt this activity. In this study, the application of the $GABA_A$ receptor antagonist bicuculline methiodide (20 μM) to the PTX-pretreated pieces of spinal cord led to a marked disruption in the regularity of the fictive locomotor rhythm (Fig. 9), as after perfusion of the spinal cord simultaneously with bicuculline methiodide and phaclofen (mean variance of the mean interburst interval before and after the application of bicuculline, 0.007 and 0.097, respectively; significantly different for each piece of spinal cord at the 0.05 level, F distribution). Washout of the bicuculline revealed recovery of the activity (Fig. 9). Pieces of spinal cord kept in identical conditions for the same time and from the same animals as those discussed above did not show disruption of the fictive locomotion. This finding would thus suggest that the physiological $GABA_B$ receptor activation normally occurring during locomotion has been blocked and that both $GABA_A$ and $GABA_B$ receptors are important for regularity of the fictive locomotor rhythm.

Discussion

It has recently been demonstrated that GABA, applied exogenously and most likely released endogenously, causes depolarizing responses in axons of the lamprey spinal cord (Alford et al., 1991). These responses are evoked by the application of agonists to both $GABA_A$ and $GABA_B$ receptors, although the responses to the different agonists are mediated by different mechanisms. In this article, we have addressed the mechanism and effect of such presynaptic $GABA_B$ receptor activation on presynaptic spike characteristics and upon subsequent synaptic transmission onto postsynaptic neurons.

Application of GABA to the spinal cord reduced spike width and height and increased the spike threshold to intracellular positive current injection. L-Baclofen application is most effective in reducing spike width. All of these characteristics imply a presynaptic inhibitory role of GABA receptor activation, including $GABA_B$ receptor activation, because a reduction in spike size will result in reduced presynaptic Ca^{2+} entry and consequent transmitter release. Subsequent analysis of synaptic transmission confirmed that the application of L-baclofen to the spinal cord suppresses both excitatory and inhibitory synaptic transmission in the motor system. These effects are in agreement with results obtained showing a role for presynaptic $GABA_B$ receptors in primary afferent transmission in the lamprey (Christenson and Grillner, 1991) and other vertebrates (Curtis et al., 1977, 1981).

It is clear from this study that the $GABA_B$-mediated suppression of synaptic transmission observed in the lamprey spinal cord (Christenson and Grillner, 1991; Alford et al., 1991) is, in

turn, mediated by the action of PTX-sensitive G-proteins. These G-proteins are located on the presynaptic terminal, and so their activation represents a mechanism of presynaptic inhibition. The data presented in this article indicate that the site of action of the G-proteins must be within the axonal membrane because in the experiments involving intracellular GTP-γ-S and GDP-β-S the compounds were only exposed to the presynaptic components of the synapses studied.

The means of action by which G-protein activation leads to a reduction of transmitter release in the spinal cord is less clear. The net result of presynaptic $GABA_B$ receptor activation, however, is to reduce the duration of the presynaptic action potential. This may result either from a reduction in voltage-gated Ca^{2+} channel opening or from an increase in axonal membrane conductance. The result of either of these changes would be to reduce the amount of Ca^{2+} entering the presynaptic terminal, which would cause a reduced quantity of transmitter release from the terminal.

These data do not exclude the possibility, however, that other transmitters may control synaptic release by acting at a presynaptic site also utilizing G-proteins. Indeed, it is possible that the involvement of G-proteins in the control of synaptic release may be widespread within the CNS. 5-HT, which may activate G-proteins, is seen to act as an inhibitor of release from reticulospinal axons of the lamprey (Buchanan and Grillner, 1991) and to be coupled to the same group of G-proteins as $GABA_B$ receptors in mammalian hippocampus (Andrade et al., 1986). Additionally, in the cat spinal cord noradrenaline may act as a mediator of synaptic transmission, acting presynaptically (Bras et al., 1989) and its receptors have been shown to be coupled to G-proteins in cultured dorsal root ganglion cells (Dolphin and Scott, 1987; Scott and Dolphin, 1987).

In this study, we have provided evidence for a presynaptic locus for $GABA_B$ receptor–mediated presynaptic inhibition in the vertebrate spinal cord. It is also shown that this reduction is caused after activation of PTX-sensitive G-proteins, and evidence is provided for a presynaptic locus of action of L-Baclofen in the spinal cord.

References

Alford S, Grillner S (1990) $GABA_B$ receptor–mediated presynaptic modulation is due to a G protein coupled mechanism in isolate spinal cord of the lamprey *I. unicuspis*. J Physiol (Lond) 426:53P–54P.

Alford S, Sigvardt KA, Williams TL (1990) GABAergic control of rhythmic activity in the presence of strychnine in the lamprey spinal cord. Brain Res 506:303–306.

Alford S, Christenson J, Grillner S (1991) Presynaptic $GABA_A$ and $_B$ receptor mediated phasic modulation in axons of spinal motor interneurones. Eur J Neurosci 3:107–117.

Alger BE, Nicoll RA (1982) Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied *in vitro*. J Physiol (Lond) 399:247–266.

Andrade R, Malenka RC, Nicoll RA (1986) A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. Science 234:1261–1265.

Ault B, Nadler JV (1982) Baclofen selectively inhibits transmission at synapses made by axons of CA3 pyramidal cells in hippocampal slices. J Pharmacol Exp Ther 223:171–178.

Bowery NG, Hill DR, Hudson AL, Doble A (1980) (−)Baclofen decreases neurotransmitter release in mammalian CNS by an action at a novel GABA receptor. Nature 283:92–94.

Bowery NG, Hudson AL, Price GW (1987) $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. Neuroscience 20:365–383.

Bras H, Cuvallari P, Jankowski E, McCrea D (1989) Comparison of effects of monoamines on transmission in spinal pathways from group I and II muscle afferents in the cat. Exp Brain Res 76:27–37.

Brodin L (1990) Three types of GABA-immunoreactive cells in the lamprey spinal cord. Brain Res 508:172–175.

Buchanan JT (1982) Identification of interneurones with contralateral caudal axons in the lamprey spinal cord: synaptic interactions and morphology. J Neurophysiol 47:961–975.

Buchanan JT, Grillner S (1987) Newly identified 'glutamate interneurones' and their role in locomotion in the lamprey spinal cord. Science 236:312–314.

Buchanan JT, Grillner S (1991) 5-HT depresses reticulospinal excitatory postsynaptic potentials in motoneurons of the lamprey. Neurosci Lett 112:71–74.

Buchanan JT, Brodin L, Dale N, Grillner S (1987) Reticulospinal neurones active excitatory amino acid receptors. Brain Res 408:321–325.

Christenson J, Grillner S (1991) Primary afferents evoke excitatory amino acid receptor mediated EPSPs, modulated by presynaptic $GABA_B$ receptors in lamprey. J Neurophysiol, in press.

Christenson J, Bongianni F, Grillner S, Hökfelt T (1991) Putative GABAergic input to axons of spinal interneurones and primary sensory neurones in the lamprey spinal cord as shown by intracellular Lucifer yellow and GABA immunohistochemistry. Brain Res 538:313–318.

Cohen AH, Wallén P (1980) The neuronal correlate of locomotion in fish. "Fictive swimming" induced in an *in vitro* preparation of the lamprey spinal cord. Exp Brain Res 41:11–18.

Curtis DR, Hosli L, Johnston GAR, Johnston IH (1968) The hyperpolarisation of spinal motoneurones by glycine and related amino-acids. Exp Brain Res 5:235–258.

Curtis DR, Lodge D, Brand SJ (1977) GABA and spinal afferent terminal excitability in the cat. Brain Res 130:360–363.

Curtis DR, Lodge D, Bornstein JC, Peet MJ (1981) Selective effects of (−)-baclofen on spinal synaptic transmission in the cat. Exp Brain Res 42:158–170.

Dale N, Grillner S (1986) Dual component synaptic potentials in the lamprey mediated by excitatory amino acid receptors. J Neurosci 6: 2653–2661.

Davies CH, Davies SN, Collingridge GL (1990) Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J Physiol (Lond) 424:513–531.

Dolphin AC, Scott RH (1987) Calcium channels currents in their modulation by (−)baclofen in rat sensory neurons: modulation by guanine nucleotides. J Physiol (Lond) 386:1–17.

Dunlap K, Holz GG, Rane SG (1987) G proteins as regulators of ion channel function. Trends Neurosci 10:241–244.

Dutar P, Nicoll RA (1988a) A physiological role for $GABA_B$ receptors in the central nervous system. Nature 332:156–158.

Dutar P, Nicoll RA (1988b) Pre- and post synaptic $GABA_B$ receptors in the hippocampus have different pharmacological properties. Neuron 1:585–591.

Eckstein F, Cassel D, Levkovitz H, Lowe M, Selingen Z (1979) Guanosine 5-0-(2 thiodiphosphate). An inhibition of adenylate cyclase stimulation by guanine nucleotides and fluoride ions. J Biol Chem 254:9829–9834.

Grillner S, Wallén P (1980) Does the central pattern generator for locomotion in the lamprey depend on glycine inhibition? Acta Physiol Scand 110:103–105.

Holz GG, Kream RM, Spiegel A, Dunlap K (1989) G proteins couple α-adrenergic and $GABA_B$ receptors to inhibition of peptide secretion from peripheral sensory neurons. J Neurosci 9:657–666.

Honoré SN, Davies TS, Drejer J, Fletcher EJ, Jacobsen P, Lodge D, Nielsen FE (1988) Quinoxalinediones: potent competitive non-*N*-methyl-D-aspartate glutamate receptor antagonists. Science 241:701–703.

Jakobs K, Shultz G, Gangler B, Pfeffger T (1983) Inhibition of NN protein stimulated human platelet adenylate cyclase by epinephrine and stable GTP analogs. Eur J Biochem 134:351–354.

Lanthorn TH, Cotman CW (1981) Baclofen selectively inhibits excitatory synaptic transmission in the hippocampus. Brain Res 225: 171–178.

Nicoll RA (1988) The coupling of neurotransmitter receptors to ion channels in the brain. Science 241:545–551.

Perkins NM, Stone TW (1982) An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. Brain Res 247:184–187.

Randall AD, Schofield JG, Davies CH, Collingridge GL (1990) The baclofen depression of excitatory synaptic transmission in rat hippocampus can be reversed by 2-hydroxy-saclofen. J Physiol (Lond) 426:51P.

Russell DF, Wallén P (1983) On the control of myotomal motoneurones during fictive swimming in the lamprey spinal cord *in vitro*. Acta Physiol Scand 117:161–170.

Scott RH, Dolphin AC (1987) Activation of a G protein promotes agonist responses to calcium channel ligands. Nature 330:760–762.

Ui M (1984) Islet-activating protein, pertussis toxin: a probe for functions of the inhibitory guanine nucleotide regulatory component of adenylate cyclase. Trends Pharmacol Sci 5:277–279.

Wallén P, Grillner S (1987) *N*-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. J Neurosci 7:2745–2755.

Wallén P, Grillner S, Feldman JL, Bergelt S (1985) Dorsal and ventral myotome motoneurons and their input during fictive locomotion in lamprey. J Neurosci 5:654–661.

Wickelgren WO (1979) Physiological and anatomical characteristics of reticulospinal neurones in lamprey. J Physiol (Lond) 270:89–114.