GABA_B Receptors Modulate an ω -Conotoxin-Sensitive Calcium Current That Is Required for Synaptic Transmission in the *Xenopus* Embryo Spinal Cord

M. J. Wall and N. Dale

School of Biological Sciences, Bristol University, Bristol BS8 1UG, United Kingdom

Activation of GABA_B receptors in the *Xenopus* embryo, a simple vertebrate, causes presynaptic inhibition of transmitter release from glycinergic spinal neurons and an increase in action potential threshold. To investigate the underlying mechanisms of GABA_B receptor action, we have made whole-cell voltage-clamp recordings from acutely isolated *Xenopus* embryo spinal neurons.

The GABA_B receptor agonist baclofen caused a reversible reduction in the amplitude of Ca²⁺ currents. This reduction of Ca²⁺ currents appeared to be voltage dependent as it was removed at very positive potentials. Since the specific GABA_B antagonists CGP35348, phaclofen, and 2-hydroxysaclofen all blocked the reduction in Ca²⁺ currents, we concluded that the modulation of the Ca²⁺ current was mediated by GABA_B receptors.

We have investigated the pharmacological identity of the Ca²⁺ current modulated by baclofen using the selective blocker ω -conotoxin, fraction GVIA (ω -CgTX). ω -CgTX selectively blocked voltage-gated Ca²⁺ currents without affecting the voltage-gated Na⁺ current. ω -CgTX substantially occluded the action of baclofen, suggesting that GABA_B receptors modulate an ω -CgTX-sensitive Ca²⁺ current. Since GABA_B receptors mediate presynaptic inhibition, we have studied the involvement of the ω -CgTX-sensitive Ca²⁺ current in synaptic transmission in the intact spinal cord. Inhibitory interneuron axons were stimulated to evoke monosynaptic IPSPs in motoneurons, and recorded intracellularly. Since ω -CgTX blocked inhibitory transmission, we concluded that the ω -CgTX-sensitive Ca²⁺ current plays an essential role in transmitter release. If modulation of this current were to occur in nerve terminals, it could contribute to the GABA_B receptor-mediated presynaptic inhibition of transmitter release. We next tested whether the reduction of the Ca²⁺ current could also contribute to the increase in spike threshold mediated by GABA_B receptors. Depolarizing current was injected into spinal neurons in order to evoke action potentials. In 6 of 10 neurons ω -CgTX caused a dropout of action potentials presumably by increasing the threshold.

Finally, we have investigated whether K⁺ currents could also be modulated by GABA_B receptors. In 4 of 25 neurons tested, baclofen caused a reversible enhancement of the outward currents.

Our results suggest that a major effect of GABA_B receptor activation is to reduce an ω -CgTX-sensitive Ca²⁺ current, though it can also have additional effects. This current not only is required for synaptic transmission in the spinal cord but also plays a role in determining the action potential threshold in some neurons. We therefore propose that a reduction in an ω -CgTX-sensitive Ca²⁺ current could contribute to GABA_B receptor-mediated presynaptic inhibition.

[Key words: baclofen, acutely isolated neurons, presynaptic inhibition, threshold, potassium currents, whole-cell patch clamp]

GABA is well established as an inhibitory neurotransmitter in the CNS of many organisms and is thought to act on at least two receptor subtypes, GABA_A and GABA_B (see Bormann, 1988, for review). Activation of GABA_A receptors classically produces postsynaptic inhibition (see Nicoll et al., 1990, for review) whereas the precise actions of GABA_B receptors remain unclear (for review, see Bowery, 1993). GABA_B receptors have been implicated in the presynaptic inhibition of transmitter release (Bowery et al., 1980; Davies, 1981; Gray and Green, 1987) and in the modulation of postsynaptic K⁺ currents (Gahwiler and Brown, 1985; Dutar and Nicholl, 1988; Soltesz et al., 1988). The mechanism by which GABA_B receptors mediate presynaptic inhibition of transmitter release remains controversial. GABA_B receptors reduce Ca²⁺ currents in a number of different neurons (rat dorsal root ganglia, Dolphin and Scott, 1987; myenteric plexus, Cherubini and North, 1984; cultured hippocampal neurons, Scholtz and Miller, 1991). In some neurons this reduction in Ca²⁺ currents is partially occluded by the snail toxin ω -conotoxin, fraction GVIA (ω -CgTX; Scholtz and Miller, 1991; Cox and Dunlap, 1992). Were the reduction in the Ca²⁺ current to occur in presynaptic nerve terminals, it could clearly contribute to a reduction in transmitter release. GABA_B receptors have also been implicated in the modulation of K⁺ currents, which could also reduce transmitter release by shortening the duration of the action potential (Saint et al., 1990; Gage, 1992). GABA_B receptors are selectively activated by β -*p*-chlorophenyl-GABA (baclofen; Bowery et al., 1980) and are antagonized by phaclofen (Kerr et al., 1987), 2-hydroxysaclofen (Curtis et al., 1988), and CGP35348 (Hills et al., 1991).

To investigate the mechanisms of GABA_B receptor action, we have used the vertebrate embryo *Xenopus laevis*. The *Xenopus*

Received Nov. 1, 1993; revised Apr. 15, 1994; accepted Apr. 21, 1994.

We thank Drs. I. Maitre and K. Scheiblich of Ciba Geigy for the generous gift of baclofen and CGP35348, Drs. S. R. Soffe and M. M. Usowicz for comments on earlier drafts of the manuscript, and the Medical Research Council (U.K.) and the Royal Society for generous support.

Correspondence should be addressed to N. Dale, School of Biological Sciences, Bristol University, Woodland Road, Bristol BS8 1UG, UK.

Copyright © 1994 Society for Neuroscience 0270-6474/94/146248-08\$05.00/0

embryo has a simple nervous system, with a spinal cord containing only eight morphological classes of neuron (Roberts and Clarke, 1982). It exhibits a series of well-defined locomotor behaviors and has successfully been used as a model system for studying the neuronal control of locomotion (for review, see Roberts, 1990). The *Xenopus* embryo swimming motor pattern is produced by a partially defined neural network or central pattern generator (CPG). This CPG can be considered as two antagonistic motor centers, one residing in each side of the spinal cord. Both centers receive tonic excitation (depolarization) during swimming and are coupled together by reciprocal inhibition carried by inhibitory interneurons that cross the cord ventrally (Dale, 1985; Dale et al., 1990). Thus, when neurons in one center are active, neurons in the other are inhibited and vice versa.

Immunocytochemical techniques have demonstrated the presence of five groups of GABAergic neurons within the *Xenopus* CNS including three categories in the hindbrain and two in the spinal cord (Roberts et al., 1987). *Xenopus* spinal neurons possess both GABA_A (Soffe, 1987) and GABA_B receptors (Wall and Dale, 1993); GABA_A receptors are involved in a simple stopping reflex (Boothby and Roberts, 1992). The roles of GABA_B receptors are less clear; however, their activation has a number of effects on the swimming motor pattern including an increase in action potential threshold and a reduction of transmitter release from a defined population of inhibitory glycinergic interneurons (Wall and Dale, 1993). We have used both acutely isolated *Xenopus* spinal neurons (Dale, 1991) and the intact embryo to investigate the mechanisms underlying the actions of GABA_B receptors in the *Xenopus* embryo spinal cord.

Materials and Methods

Dissociation of acutely isolated neurons. Acutely isolated spinal neurons were prepared by methods based on those described by Dale (1991). Briefly, stage 37/38 embryos (Nieuwkoop and Faber, 1956) were anesthetized in a solution of MS222 (0.5 mg/ml; Tricaine, Sigma), pinned to a rotatable Sylgard table in HEPES saline, composed of (in mM) Na⁺, 117.4; K⁺, 3; Mg²⁺, 1; Ca²⁺, 2; NO₃⁻, 2; HCO₃⁻, 2.4; Cl⁻, 124; HEPES, 10; and glucose, 10; and their spinal cords were carefully removed. The spinal cords were then transferred to a dish containing 0.1–0.3 mg/ml DNase in HEPES saline and incubated at room temperature for 3 min. The spinal cords were transferred to a dish containing 8–10 mg/ml pancreatin (Sigma; 8 × USP) in a low-chloride trituration saline, composed of (in mM) Na⁺, 117.4; MeSO₃⁻, 115; K⁺, 3; Mg²⁺, 1; Ca²⁺, 2; NO₃⁻, 2; HCO₃⁻, 2.4; Cl⁻, 9; HEPES, 10; and glucose, 10; and were incubated at room temperature for 2 min. The spinal cords were then transferred to a dish of zero-valent-cation dissociation saline, composed of (in mM) Na⁺, 115; MeSO₃⁻, 115; K⁺, 3; EDTA 2; Cl⁻, 3; glucose, 10; and PIPES, 10, pH 7.0, for 1 min; and then to a dish containing PIPES saline, composed of (in mM) Na⁺, 115; MeSO₃⁻, 115; K⁺, 3; Mg²⁺, 0.1; Ca²⁺, 0.1; Cl⁻, 3.4; glucose, 20; and PIPES, 10, pH 7.0, for 3 min. The spinal cords were then gently triturated in trituration saline containing 3 mg/ml DNase in an Eppendorf tube until the cords had dissociated. The cells were finally transferred to polylysine-coated 35 mm tissue culture dishes containing HEPES saline and allowed to settle and stick to the substrate for at least 30 min before recording.

Electrophysiological recording from dissociated neurons. Before recording from isolated spinal neurons, the HEPES saline was exchanged for a control saline composed of (in mM) Na⁺, 117.4; K⁺, 3; Mg²⁺, 1; Ca²⁺, 10; HCO₃⁻, 2.4; Cl⁻, 140; HEPES, 10; pH 7.4, at a temperature of 18–22°C. Whole-cell patch-clamp recordings were made from the isolated neurons with a List EPC7 amplifier. A CED 1401 or Data Translation 2831 digital interface and IBM compatible computer, together with the software of Dale (1991) were used to generate voltage command sequences, and display and store results on computer disk. For statistical comparisons the paired sample *t* test and the analysis of variance (Minitab) were used. All values are the mean ± SEM unless otherwise stated. Electrodes were made with thin walled glass (World Precision Instruments, TW 150F) and had a resistance of 4–8 MΩ. To

reduce the rundown of Ca²⁺ currents the higher resistance electrodes were favored. Recordings had series resistance of 6–14 MΩ; electronic compensation was used to neutralize 60–80% of this series resistance. To record K⁺ currents, the patch pipette recording solution contained (in mM) K⁺, 100; Mg²⁺, 6; MeSO₃⁻, 100; Cl⁻, 12; BAPTA, 2; ATP·Na₂, 5; GTP·Na₂, 1; and HEPES, 20. To help isolate the inward currents a Cs⁺ electrolyte composed of (in mM) Cs⁺, 100; Mg²⁺, 6; MeSO₃⁻, 100; Cl⁻, 14; EGTA 10; Ca²⁺, 1; ATP·Na₂, 5; GTP·Na₂, 1; HEPES, 20 was used to record both Ca²⁺ and Na⁺ currents. Both electrolyte solutions were adjusted to pH 7.4. Osmolarity was adjusted to 240 mOsm with water or glucose and solutions were sterile filtered.

To isolate the Ca²⁺ current, recordings were carried out in saline containing 0.1 μM TTX to block the Na⁺ current with either 5 mM TEA or 60 mM TEA substituted for 60 mM NaCl to block K⁺ currents. Neurons were clamped at a holding potential of −50 mV and exposed to two protocols: a step protocol consisting of 10 mV steps from −50 mV to +60 mV, each 70 msec long with an interstimulus interval of 2 sec, and a ramp protocol ramping from −100 mV to +100 mV in 140 msec, repeated at an interval of 3 sec. For the leak subtraction of records, 100–150 μM Cd²⁺ was applied to block inward currents at the end of experiments and the resultant equivalent traces subtracted from the records of the Ca²⁺ currents. Experiments in which the leak significantly changed were rejected. To isolate the Na⁺ currents, the external saline contained 150 μM Cd²⁺ to block the Ca²⁺ currents and 62.5 mM TEA substituted for NaCl to block K⁺ currents. Na⁺ currents were evoked by stepping from a holding potential of −50 mV to +30 mV for 30 msec. For the recording of K⁺ currents neurons were clamped at −80 mV or −50 mV and stepped in 70 msec, 10 mV steps to +40 mV with an interval of 2 sec, 0.1 μM TTX was included in the saline. Drugs were applied to isolated neurons via a nine barreled microperfusion system.

Procedure for recording from whole embryos. Whole embryos were prepared as described by Wall and Dale (1993). Briefly, stage 37/38 *Xenopus* embryos were anesthetized in MS222 (0.5 mg/ml) and their dorsal fins slit to allow access of the neuromuscular blocker α-bungarotoxin. The embryos were then transferred to 1 ml of solution of α-bungarotoxin (0.077 mg/ml) for 15–20 min until they could no longer swim in response to normal stimuli. Embryos were pinned through the notochord to a rotatable Sylgard table with etched tungsten pins and continually perfused with saline composed of (in mM) Na⁺, 117.4; K⁺, 3; Mg²⁺, 1; Ca²⁺, 5; HCO₃⁻, 2.4; Cl⁻, 130; and HEPES, 10; pH 7.4 at a temperature of 18–22°C. For intracellular recording from the spinal cord, the overlying skin and myotomes were removed with fine mounted etched tungsten pins. Intracellular recordings were made with microelectrodes of resistance 180–300 MΩ filled with 3 M K-acetate.

Drugs were applied to embryos via a nine-barreled microperfusion system. The microperfusion nozzle was moved as close to the impaled cell as possible (flow was initially visualized by the inclusion of the dye fast green).

Electrical stimulation of inhibitory axons. The axons of both excitatory and inhibitory interneurons run along the outside of the spinal cord, in the marginal zone lateral tracts (Roberts and Clarke, 1982). A small suction electrode was placed on the lateral margin of the spinal cord and used to stimulate electrically the axons of interneurons. When excitatory transmission was blocked with 2 mM kynurenic acid (Sillar and Roberts, 1990) IPSPs could be recorded intracellularly in ventrally located motoneurons in response to electrical stimulation. The IPSPs appeared to be monosynaptic since they had a short and constant latency (mean 4.1 msec; SD = 0.6 msec, *n* = 126, minimum 3 msec, maximum 6 msec, pooled data from nine embryos) depending on distance between the stimulating and recording electrode (0.5–1 mm). The conduction velocities for the inhibitory axons were thus similar to the values measured for excitatory transmission (Dale and Roberts, 1985; Roberts and Sillar, 1990).

Drugs. Baclofen (1–100 μM; Ciba-Geigy), CGP35348 (200 μM; Ciba-Geigy), 1-hydroxysaclofen (200 μM; Tocris-Neuramin), phaclofen (200 μM and 1.6 mM; Tocris Neuramin), ω-conotoxin GVIA (ω-CgTX; 5 μM; Bachem), tetrodotoxin (TTX; 0.1 μM; Sigma), kynurenic acid (2 mM; Sigma), strychnine (1 μM; Sigma), cadmium chloride (Cd²⁺, 100–150 μM; Sigma), tetraethylammonium chloride (TEA; 5–60 mM; Aldrich).

Results

Baclofen reduces Ca²⁺ currents

To test whether the GABA_B agonist, baclofen acts to raise spike threshold and mediate presynaptic inhibition by reducing the

Figure 1. Baclofen reversibly reduced Ca^{2+} currents in acutely isolated spinal neurons. *A*, Ca^{2+} currents were evoked by stepping the membrane potential from -50 mV to $+10$ mV; $50 \mu\text{M}$ baclofen reversibly reduced the peak Ca^{2+} current by 50.4%. The records have been leak subtracted. *B*, A bar chart plotting as a percentage of control the inward current remaining against the concentration of baclofen. Error bars represent 1 SEM.

amplitude of Ca^{2+} currents, recordings were made from isolated spinal neurons using whole-cell voltage clamp. In the majority of neurons tested baclofen (1 – $100 \mu\text{M}$) reversibly reduced Ca^{2+} currents evoked by both ramp and step protocols in multipolar and unipolar neurons (Fig. 1*A*). In 15 neurons, $50 \mu\text{M}$ baclofen blocked $43.9 \pm 4.2\%$ of the maximum Ca^{2+} current. Since Ca^{2+} currents show washout with prolonged whole cell recording, only experiments with at least 50% recovery from the effects of baclofen during the wash were accepted. Between the concentrations of 1 – $50 \mu\text{M}$, baclofen produced a dose-dependent reduction of Ca^{2+} current (Fig. 1*B*). The receptors appear to be saturated at $50 \mu\text{M}$ as there was no significant difference between the amount of block caused by 50 and $100 \mu\text{M}$ baclofen ($p > 0.05$, ANOVA; Fig. 1*B*). Even saturating doses of baclofen blocked only about 50% of the total Ca^{2+} current. All of the inward current, presumed to be a Ca^{2+} current, could be blocked by 100 – $150 \mu\text{M}$ Cd^{2+} .

In eight of nine cells, the baclofen inhibition at $+10$ mV to $+40$ mV was accompanied by a dramatic slow-down of current activation (Fig. 2*C*). With voltage steps to $+30$ mV the mean time for the Ca^{2+} current to reach its maximum in control was $3.5 \text{ msec} \pm 0.6 \text{ msec}$ ($n = 6$) compared to a mean time to peak in $50 \mu\text{M}$ baclofen of $27.6 \text{ msec} \pm 5.5 \text{ msec}$ ($p < 0.05$, $n = 6$). The reduction in current was also less toward the end of the 70 msec pulse (Fig. 2*B*₁, *B*₂). The decrease in the rate of activation and overall depression were largely relieved at more positive voltage steps (Fig. 2*A*). At $+60$ mV the time course of baclofen modulated currents resembled those of control and the inhibition was relieved. The mean reduction of sustained current (measured at the end of the voltage pulse) by $50 \mu\text{M}$ baclofen at $+10$ mV was $39.8 \pm 5.7\%$ compared with a mean reduction of $5.5 \pm 2.4\%$ ($n = 8$, $t = 7.5$, $p < 0.001$) at $+60$ mV. These findings suggest that baclofen inhibition is voltage dependent, being relieved at very positive potentials (cf. Bean, 1989; Grassi and Lux, 1989; Pollo et al., 1992).

GABA_B antagonists block the reduction in Ca^{2+} current amplitude

We next tested whether the effects of baclofen were mediated via GABA_B receptors by studying the actions of the specific antagonists CGP35348, 2-hydroxysaclofen, and phaclofen. Both $200 \mu\text{M}$ CGP35348 and $200 \mu\text{M}$ 2-hydroxysaclofen antagonized the effects of $50 \mu\text{M}$ baclofen by $91.2 \pm 5.6\%$ ($n = 6$) and 80.4

Figure 2. The GABA_B receptor-mediated reduction in Ca^{2+} current showed voltage dependence. *A*, Ca^{2+} currents were evoked in an acutely isolated neuron by stepping from -50 mV to $+60$ mV in 10 mV steps; $50 \mu\text{M}$ baclofen caused a reversible reduction in Ca^{2+} current amplitude. At $+20$ mV baclofen slowed the activation of the current. At more positive potentials ($+40$ mV, $+50$ mV, and $+60$ mV) this slowing of activation became less pronounced and most of the block was removed. *B*₁ and *B*₂, Current-voltage plots showing the control Ca^{2+} current (squares) and the Ca^{2+} current in $50 \mu\text{M}$ baclofen (circles). The Ca^{2+} current, measured 5 msec from the beginning of the voltage step (*B*₁), is blocked to a much greater extent than the sustained current measured at 5 msec from the end of the voltage step (*B*₂). *C*, Ca^{2+} currents were evoked in a different isolated neuron by stepping from -50 mV to $+30$ mV. Application of $50 \mu\text{M}$ baclofen greatly slowed the activation of the current. All records have been leak subtracted.

$\pm 7.7\%$ ($n = 6$), respectively (Fig. 3*A*, *C*). However, phaclofen was a much weaker antagonist having no effects on the actions of baclofen at $200 \mu\text{M}$ ($n = 4$) and only producing a $60 \pm 9.9\%$ ($n = 4$) antagonism at 1.6 mM (Fig. 3*B*).

The actions of ω -conotoxin on voltage-gated inward currents

The snail toxin, ω -conotoxin, fraction GVIA (ω -CgTX) is claimed to be a selective Ca^{2+} channel blocker (Feldman et al., 1987) and has been used to pharmacologically separate Ca^{2+} currents in a number of neurons (Barish, 1991; Cox and Dunlap, 1992; Mintz and Bean, 1993). We have investigated the effects of ω -CgTX on the inward currents of acutely isolated *Xenopus* spinal neurons. Application of $5 \mu\text{M}$ ω -CgTX reduced the maximum Ca^{2+} current evoked in response to both voltage steps and ramps by a mean of $56 \pm 4.4\%$ ($n = 19$; Fig. 4*A*₁). The ω -CgTX-mediated block did not appear voltage dependent as even with large depolarizations there was still a significant block (Fig. 4*A*₂). The effects of ω -CgTX were partially reversible; in six neurons $38.3 \pm 6.5\%$ of the $5 \mu\text{M}$ ω -CgTX-mediated block was removed upon wash.

Feldman et al. (1987) showed that application of ω -CgTX could produce small, irreversible reductions of the Na^{+} current in chick dorsal root ganglia cells. To test whether ω -CgTX might also block voltage-gated Na^{+} currents in *Xenopus* neurons, $5 \mu\text{M}$ ω -CgTX was applied to neurons in which the Na^{+} currents had been pharmacologically isolated. In six neurons the application of $5 \mu\text{M}$ ω -CgTX did not significantly reduce the peak Na^{+} current (mean change $-1 \pm 0.4\%$, $t = 0.56$, $p > 0.05$; Fig.

Figure 3. GABA_B receptor antagonists blocked the baclofen-mediated reduction in Ca²⁺ current amplitude. Voltage ramps from -100 to $+100$ mV were used to evoke Ca²⁺ currents in acutely isolated neurons. *A*, Ca²⁺ currents in the presence of $50 \mu\text{M}$ baclofen (1), $50 \mu\text{M}$ baclofen and $200 \mu\text{M}$ CGP35348 (2), and the wash (3). *B*, Ca²⁺ currents in $50 \mu\text{M}$ baclofen (1), $50 \mu\text{M}$ baclofen and 1.6 mM phaclofen (2) and the wash (3). *C*, Ca²⁺ currents in $50 \mu\text{M}$ baclofen (1), $50 \mu\text{M}$ baclofen and $200 \mu\text{M}$ 2-hydroxysaclofen (2) and the wash (3). All records are an average of five consecutive traces and have not been leak subtracted. The irregularities in the traces of (C) are due to spontaneous synaptic currents.

4B). Thus, in *Xenopus* spinal neurons ω -CgTX is a selective blocker of voltage-gated Ca²⁺ channels.

ω -CgTX substantially occludes the GABA_B receptor-mediated reduction in Ca²⁺ current

We next investigated the pharmacological identity of the Ca²⁺ current inhibited by GABA_B receptors by studying the actions of ω -CgTX. We first applied $50 \mu\text{M}$ baclofen alone and then once again after the application of $5 \mu\text{M}$ ω -CgTX, to determine whether the blocking action of the two drugs was additive or occlusive. Because the $5 \mu\text{M}$ ω -CgTX-mediated block was partially reversible, ω -CgTX was continually applied during the application of baclofen. When applied alone, $50 \mu\text{M}$ baclofen reduced the maximal Ca²⁺ current by a mean of $48 \pm 5.4\%$ ($n = 11$; Fig. 5A). After the baclofen had been washed off, $5 \mu\text{M}$ ω -CgTX reduced Ca²⁺ currents by a mean of $58.3 \pm 4.1\%$ ($n = 11$; Fig. 5B). Fifty micromolar baclofen then applied in the presence of $5 \mu\text{M}$ ω -CgTX reduced the currents by $65.6 \pm 2.3\%$ of control ($n = 11$; Fig. 5B), an increased block of only 7.3% . In all 11 neurons tested, the amount of current blocked by baclofen was greatly reduced in the presence of $5 \mu\text{M}$ ω -CgTX but not totally abolished ($p < 0.001$, $t = 6.7$; Fig. 5). Since the blocking actions of ω -CgTX and baclofen were not additive and ω -CgTX greatly

Figure 4. ω -CgTX blocked voltage-gated Ca²⁺ channels but did not block the Na⁺ current. *A*₁, Ca²⁺ currents were evoked in an acutely isolated neuron by stepping from -50 mV to $+45$ mV in 5 mV steps; $5 \mu\text{M}$ ω -CgTX caused a reduction in Ca²⁺ current amplitude. *A*₂, Plot of maximal Ca²⁺ current against voltage for control (squares) and $5 \mu\text{M}$ ω -CgTX (circles). Curves are the product of the best-fitting Boltzmann equation to describe channel activation, and the best fitting Goldman-Hodgkin-Katz equation to describe permeation of the channel by Ca²⁺. *B*, Graph plotting maximum Na⁺ current amplitude against time. Na⁺ currents were evoked by stepping from -50 mV to $+30$ mV for 30 msec every 10 sec. Insets show the evoked Na⁺ currents represented by the arrowed points. Application of $5 \mu\text{M}$ ω -CgTX (bar) had no effect on the amplitude or kinetics of the Na⁺ current.

occluded the effect of baclofen, we therefore conclude that GABA_B receptors block an ω -CgTX-sensitive Ca²⁺ current. We cannot exclude the possibility that GABA_B receptors may also weakly block an ω -CgTX-insensitive current. In 3 of the 11 neurons tested, the application of $50 \mu\text{M}$ baclofen in the presence of ω -CgTX produced a small increase in the net evoked inward current (mean = 12.5 ± 5.5 pA), suggesting baclofen may have additional, as yet uncharacterized, actions.

ω -CgTX and baclofen block inhibitory synaptic transmission

The reduction of the ω -CgTX-sensitive Ca²⁺ current by GABA_B receptors could, in principle, contribute to presynaptic inhibition if this current was present in nerve terminals. We therefore tested the role of the ω -CgTX-sensitive Ca²⁺ current in synaptic transmission. IPSPs were evoked in the intact spinal cord by electrical stimulation of contralateral rostral or ipsilateral caudal inhibitory axons (see Materials and Methods). These IPSPs were blocked by $1 \mu\text{M}$ strychnine (Fig. 6C) and hence were glycinergic. Neurons were current clamped at either a constant depolarized membrane potential (-50 mV to -30 mV) or a constant hyperpolarized membrane potential (-100 mV) to move the membrane potential from the chloride reversal potential and thus increase the size of evoked IPSPs.

Both $50 \mu\text{M}$ baclofen (Fig. 6A) and $5 \mu\text{M}$ ω -CgTX (Fig. 6B) reversibly reduced the amplitude of evoked IPSPs in their normal or reversed form. The mean reduction in IPSP size in $50 \mu\text{M}$ baclofen was $61.1 \pm 6.5\%$ ($n = 12$) and in $5 \mu\text{M}$ ω -CgTX was $70.4 \pm 6.2\%$ ($n = 7$). Unlike $5 \mu\text{M}$ ω -CgTX, baclofen $50 \mu\text{M}$ did not reduce the evoked IPSP amplitude in all neurons tested: 6 of 18 neurons tested were resistant to block. The effects of baclofen were fully reversible, whereas only $45.6 \pm 5.4\%$ ($n = 3$) of the ω -CgTX block was removed (see Fig. 6B, trace 3) after washing for a long time (15 – 20 min).

To test whether the reduction in IPSP amplitude by baclofen was a specific effect of GABA_B receptor activation, we studied

Figure 5. ω -CgTX substantially occluded the action of baclofen. Ca^{2+} currents were evoked by voltage ramps from -100 mV to $+100$ mV. **A**, Control Ca^{2+} currents in an acutely isolated spinal neuron (1); $50 \mu\text{M}$ baclofen reduced the peak Ca^{2+} current by 65.3% (2). Ca^{2+} currents returned to nearly control amplitude after wash (3). **B**, In the same neuron, Ca^{2+} currents were reduced by 64.5% in $5 \mu\text{M}$ ω -CgTX (1); $50 \mu\text{M}$ baclofen applied in the presence of $5 \mu\text{M}$ ω -CgTX gave a further block of only 16.3% to reduce the current by 80.8% of control (2). Cadmium ($100 \mu\text{M}$) blocked all inward current (3). All records are an average of five consecutive traces and are not leak subtracted. **C**, Bar chart plotting the mean percentage of control current blocked by $50 \mu\text{M}$ baclofen alone, $5 \mu\text{M}$ ω -CgTX alone, and $50 \mu\text{M}$ baclofen applied in the presence of $5 \mu\text{M}$ ω -CgTX for 11 embryos. Error bars represent 1 SEM.

the actions of CGP35348 and phaclofen. Two hundred micromolar CGP35348 reversed the $50 \mu\text{M}$ baclofen-mediated reduction in IPSP amplitude (mean reduction in block $89.8 \pm 5.1\%$, $n = 7$). As with the GABA_B receptor-mediated inhibition of Ca^{2+} currents, $200 \mu\text{M}$ phaclofen produced little or no antagonism (mean reduction in block $11.2 \pm 11.2\%$, $n = 6$). We thus conclude that the ω -CgTX-sensitive Ca^{2+} current is involved in inhibitory synaptic transmission and that modulation of this current by GABA_B receptors could play a substantial role in presynaptic inhibition.

The ω -CgTX-sensitive Ca^{2+} current is involved in action potential threshold

We have previously demonstrated that GABA_B receptors increase the spike threshold of spinal neurons. Since the net balance of inward and outward currents determines the spike threshold, modulation of the ω -CgTX-sensitive current could be effective in altering the action potential threshold. To investigate the possible role of the ω -CgTX-sensitive current in setting the threshold, constant depolarizing current pulses just sufficient to evoke an action potential were injected into spinal neurons. In 6 of 10 neurons tested the application of $5 \mu\text{M}$ ω -CgTX reversibly caused a loss of action potential firing that was restored either by injection of more depolarizing current or by washing off the ω -CgTX (Fig. 7). We therefore conclude that the ω -CgTX-sensitive current is active around the spike threshold and may help to determine the probability of spike generation.

ω -CgTX disrupts and then abolishes the swimming motor pattern

The above findings show that ω -CgTX can mimic some of the actions of baclofen by causing both presynaptic inhibition and

Figure 6. Both ω -CgTX and baclofen blocked inhibitory synaptic transmission in the intact spinal cord. Monosynaptic IPSPs were evoked by electrically stimulating the surface of the spinal cord and recorded in probable motoneurons with a microelectrode. Control IPSP (traces 1 in A–C), amplitude was greatly reduced in $50 \mu\text{M}$ baclofen (trace A2), $5 \mu\text{M}$ ω -CgTX (trace B2), and $1 \mu\text{M}$ strychnine (trace C2). After wash IPSP amplitude recovered (traces A3 and B3). Records are an average of 10 consecutive sweeps.

an increase in action potential threshold. We therefore asked to what extent ω -CgTX could also mimic the actions of GABA_B receptors on the swimming motor pattern.

In control swimming episodes ventral rhythmic spinal neurons received tonic excitation, glycinergic midcycle inhibition and fired one action potential per cycle (Fig. 8A₁, B₁). The application of baclofen caused a selective loss of the midcycle inhibition (Fig. 8A₂) and then reduced the excitatory drive (Fig. 8A₃; see also Wall and Dale, 1993). Longer applications of baclofen caused an abolition of swimming in response to sensory stimuli (see Wall and Dale, 1993). The application of $5 \mu\text{M}$ ω -CgTX also caused a progressive reduction in both the excitatory and inhibitory synaptic drive during swimming. At intermediate stages of drug action, around 1 min, $5 \mu\text{M}$ ω -CgTX had reduced both the amplitude of tonic depolarization by a mean of $34.4 \pm 12.4\%$ ($n = 5$) compared to control and the midcycle inhibition by a mean of $32.4 \pm 16.5\%$ ($n = 5$; Fig. 8B₂). As more time elapsed and the ω -CgTX gained better access to the spinal cord, the synaptic drive became progressively smaller (Fig. 8B₃) until after 2–3 min all motor activity in response to electrical stimuli or dimming the lights was completely abolished (Fig. 8B₄). Unlike baclofen, ω -CgTX did not selectively block inhibitory transmission during swimming but affected both excitatory and inhibitory drive equally. This may reflect a non-selective action of ω -CgTX on all types of neurons and synaptic transmission. These reductions in synaptic drive and the loss of swimming were not readily reversible and presumably resulted from the almost complete blockade of synaptic transmission. We thus conclude that the ω -CgTX-sensitive Ca^{2+} current is essential for the functioning of the swimming motor circuitry.

Baclofen modulates K^+ currents in a minority of neurons

Although our results show that GABA_B receptors clearly modulate Ca^{2+} currents, we have also tested whether baclofen could modulate voltage-gated K^+ currents. We have used two approaches to investigate the possible modulation of K^+ currents: direct study of the K^+ currents in isolated neurons and an examination of the action potential after hyperpolarization (AHP) of neurons in the intact spinal cord. Baclofen (50 – $100 \mu\text{M}$) enhanced the outward currents in only 4 of 25 neurons tested. In the intact spinal cord, $50 \mu\text{M}$ baclofen reversibly increased the AHP amplitude in only one of 15 neurons. Together these observations suggest that K^+ currents are modulated in only a small minority of neurons.

Discussion

Activation of GABA_B receptors has two main actions on *Xenopus* embryos: presynaptic inhibition of transmitter release and

Figure 7. ω -CgTX increased action potential threshold. Constant depolarizing current pulses (marked by solid bars) were injected into a neuron in the intact spinal cord through the recording microelectrode. In control the neuron fired an action potential in response to current injection (A). The application of $5 \mu\text{M}$ ω -CgTX first increased the latency of the action potential (B), and then caused action potential failure (C). Either wash (D), or an increase in depolarizing current allowed the neuron to fire action potentials again. Capacitative switching artifacts are present at the beginning and end of the current pulses and have been partly erased.

an increase in action potential threshold (Wall and Dale, 1993). To understand how GABA_B receptors mediate these effects, we have used acutely isolated neurons to study the ionic currents modulated by GABA_B receptors. GABA_B receptor activation in many neurons clearly results in a reversible block of Ca²⁺ currents. In contrast, the voltage-gated K⁺ currents of most cells appear to be unaffected by baclofen.

The pharmacological identity of the Ca²⁺ current modulated by GABA_B receptors

Five micromolar ω -CgTX blocked about 60% of the peak Ca²⁺ current of acutely isolated spinal neurons. This is very similar to the block seen in cultured *Xenopus* neurons (Barish, 1991). As the GABA_B receptor-mediated block of Ca²⁺ currents is occluded by ω -CgTX, this may suggest that GABA_B receptor activation blocks an N-type current (Tsien et al., 1988; Aosaki and Kasai, 1989; Williams et al., 1992a). However, ω -CgTX has been shown to block the N-type Ca²⁺ current irreversibly in a number of different neurons (Aosaki and Kasai, 1989; Sher and Clementi, 1991; Williams et al., 1992a). In our neurons approximately 40% of the ω -CgTX-mediated block was removed upon wash. It is therefore possible that ω -CgTX is blocking more than one type of Ca²⁺ current: one reversibly and the other irreversibly. While the irreversibly blocked component may correspond to the N type Ca²⁺ current, the identity of the reversibly blocked current is not clear. In some neurons there is evidence that ω -CgTX will transiently block L-type currents (Tsien et al., 1988; Aosaki and Kasai, 1989; Williams et al., 1992b). However, it seems unlikely that ω -CgTX is blocking an L-type current in our experiments because, like cultured *Xenopus* neurons (Barish, 1991), acutely isolated *Xenopus* neurons either do not possess or only have a very small dihydropyridine-sensitive Ca²⁺ current (N. Dale, unpublished observation). It is possible that ω -CgTX is reversibly blocking a subtype of N-like channels; for example, Ellinor et al. (1993) have recently described a rapidly inactivating Ca²⁺ current that is reversibly blocked by ω -CgTX and Plummer et al. (1989) have described a component of N-type Ca²⁺ current that is reversibly blocked by ω -CgTX in peripheral neurons. Resolution of this problem awaits the availability of agents that can discriminate between the components of the ω -CgTX-sensitive current.

Our results show that GABA_B receptors modulate ω -CgTX-sensitive Ca²⁺ currents. Similar observations have been made

Figure 8. Both baclofen and ω -CgTX disrupted the swimming motor pattern. Fictive swimming was recorded in an intact embryo using a microelectrode and started by dimming the lights. During control fictive swimming episodes (A₁, B₁) neurons fired an action potential once per cycle, received midcycle inhibition, and were tonically depolarized. Application of $50 \mu\text{M}$ baclofen first caused a loss of midcycle inhibition (A₂) and then a loss of excitation and a dropout of action potentials (A₃). The effects of baclofen were reversed upon wash (A₄). After applying $5 \mu\text{M}$ ω -CgTX for 63 sec there was a reduction in both excitation and inhibition during swimming (B₂). After 200 sec, ω -CgTX had greatly reduced the swimming synaptic drive (B₃). Longer applications of $5 \mu\text{M}$ ω -CgTX caused a complete loss of swimming in response to dimming the lights (lights dimmed at arrow, B₄).

for cultured hippocampal neurons (Scholtz and Miller, 1991), chick dorsal root ganglia neurons (Cox and Dunlap, 1992), and rat spinal cord neurons (Mintz and Bean, 1993). In our experiments, a small component of the current could still be modulated by the activation of GABA_B receptors in the presence of ω -CgTX. This modulation of the remaining current may have been due either to an incomplete block of the ω -CgTX-sensitive current by the dose of ω -CgTX that we used, or to an action on an ω -CgTX-insensitive Ca²⁺ current. In other preparations, for example, cultured hippocampal neurons, baclofen also weakly modulates an ω -CgTX-insensitive current (Scholtz and Miller, 1991). In acutely isolated postnatal rat spinal cord neurons, baclofen not only inhibits both an N-type and a P-type Ca²⁺ current (Usovich, 1992), but also has actions on a further high-threshold Ca²⁺ current that is resistant to blockers of N-, P-, and L-type Ca²⁺ channels (Mintz and Bean, 1993).

The inhibition of Ca²⁺ current is voltage dependent

Although we have not investigated the kinetics in detail, the reduction of the Ca²⁺ current by GABA_B receptors appears to be voltage dependent and involve a slowing of the rate of activation. Modulation of Ca²⁺ currents by several transmitters including GABA and noradrenaline can exhibit voltage dependency (Bean, 1989; Grassi and Lux, 1989; Elmslie et al., 1990; Pollo et al., 1992). To explain this voltage dependency, Bean (1989) has proposed a model in which transmitters such as GABA inhibit Ca²⁺ currents by shifting Ca²⁺ channels from a "willing mode" into a "reluctant mode" of opening. When channels are in this reluctant mode they are not easily opened by small depolarizations but can still be opened by large depolarizations. The physiological significance of this voltage depen-

dence remains unclear; however, a train of action potentials fired at a high enough frequency could conceivably reduce the GABA_B-mediated inhibition of the Ca^{2+} current. During fictive swimming in the *Xenopus* embryo the frequency of action potential firing (10–20 Hz) appears to be insufficient to remove the block, as inhibitory transmission remains blocked during the application of baclofen (Wall and Dale, 1993). However, during struggling behavior much higher frequency bursts of action potentials are fired (Soffe, 1993). Under these conditions the inhibition of Ca^{2+} currents could possibly be weakened.

An ω -CgTX-sensitive Ca^{2+} current could be involved in neuron recruitment

We have demonstrated that ω -CgTX selectively blocks voltage-gated Ca^{2+} currents without reducing other inward currents such as the Na^{+} current. Application of ω -CgTX led to the loss of action potential firing, an effect that was reversible with the injection of more depolarizing current. Since the threshold at which a neuron fires an action potential will depend on the relative contributions of the inward and outward currents our results suggest that the reduction of an ω -CgTX-sensitive Ca^{2+} current increases action potential threshold. Of course, a variety of other currents will also influence spike threshold, including the Na^{+} current, ω -CgTX-insensitive Ca^{2+} currents and K^{+} currents. Transmitters such as GABA that reduce an ω -CgTX-sensitive Ca^{2+} current would increase action potential threshold leading to the possible loss of action potentials and the dropout of neurons during swimming. The increase in K^{+} current amplitude seen in a small proportion of neurons may also contribute to the increase in spike threshold. If an increase in the amplitude of the ω -CgTX-sensitive Ca^{2+} current was mediated by modulatory transmitters, previously quiescent neurons could thus be recruited into the motor pattern during swimming. In the *Xenopus* embryo, the switch from swimming to struggling appears to involve recruitment of neurons to the motor network (Soffe, 1993). Such a switch in motor patterns could conceivably result from modulations of a variety of currents that are active around spike threshold including the ω -CgTX-sensitive Ca^{2+} current.

Mechanisms of presynaptic inhibition

Since application of ω -CgTX to the intact spinal cord greatly attenuated both the inhibitory synaptic transmission and the synaptic drive during swimming, we have concluded that ω -CgTX-sensitive Ca^{2+} currents play a key role in synaptic transmission. However, the effects of ω -CgTX on synaptic transmission were partially reversible, suggesting that both the irreversibly and reversibly blocked Ca^{2+} currents could be involved in synaptic transmission. Furthermore, a small remnant of synaptic transmission remained in the presence of ω -CgTX, suggesting that an ω -CgTX-insensitive Ca^{2+} current might also be involved in transmitter release. Thus, it is plausible that as many as three different Ca^{2+} currents could play a role in transmitter release.

We know that GABA_B receptors are present on the terminals of inhibitory interneurons where they are able to modulate spontaneous release (Wall and Dale, 1993) and that the ω -CgTX-sensitive Ca^{2+} current is essential for transmitter release and so must also occur at the nerve terminals. Although we have not shown it directly, it seems plausible that the ω -CgTX-sensitive Ca^{2+} current could be modulated by the GABA_B receptors present on the nerve terminals and thus contribute to the presynaptic

inhibition of transmitter release (cf. Edmonds et al., 1990). It is possible that GABA_B receptors could also modulate other Ca^{2+} currents, which would also reduce transmitter release. Since baclofen also enhanced voltage-gated K^{+} currents, this too, in analogy with molluscan systems (Hochner et al., 1986a,b), could contribute to a reduction in transmitter release by reducing action potential duration. This may, however, be a relatively unimportant mechanism as K^{+} currents are only modulated in a small proportion of neurons.

We have previously shown that baclofen reduced the probability of spontaneous release from inhibitory interneurons (Wall and Dale, 1993). As these previous experiments were done at the resting potential and in the presence of 1 μM TTX and sufficient Cd^{2+} to block the voltage-gated Ca^{2+} currents, the reduction in the probability of spontaneous release must presumably occur by a mechanism independent of Ca^{2+} current modulation (cf. Scholtz and Miller, 1992). We therefore suggest that although baclofen reduces a Ca^{2+} current that could plausibly contribute to a modulation of transmitter release, additional mechanisms such as direct modulation of the synaptic machinery, for example, by reducing the probability of vesicle docking, are also very likely to contribute to presynaptic inhibition (cf. Man-Son-Hing et al., 1989; Dale and Kandel, 1990).

References

- Aosaki N, Kasai H (1989) Characterization of two kinds of high-voltage-activated Ca^{2+} channel currents in chick sensory neurons: differential sensitivity to dihydropyridines and ω -conotoxin GVIA. *Pflügers Arch* 414:150–156.
- Barish ME (1991) Voltage gated calcium currents in cultured embryonic *Xenopus* spinal neurons. *J Physiol (Lond)* 444:523–543.
- Bean BP (1989) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature* 340:153–156.
- Boothby KM, Roberts A (1992) The stopping response of *Xenopus laevis* embryos: pharmacology and intracellular physiology of rhythmic spinal neurons and hindbrain neurons. *J Exp Biol* 169:65–86.
- Bormann J (1988) Electrophysiology of GABA-A and GABA-B receptor subtypes. *Trends Neurosci* 11:112–116.
- Bowery NG (1993) GABA_B receptor pharmacology. *Annu Rev Pharmacol Toxicol* 33:109–147.
- Bowery NG, Hill DR, Hudson A, Doble A, Middlemass DN, Shaw J, Turnbull M (1980) (–)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* 283:92–94.
- Cherubini E, North RA (1984) Inhibition of calcium spikes and transmitter release by gamma aminobutyric acid in the guinea pig myenteric plexus. *Br J Pharmacol* 82:101–105.
- Cox D, Dunlap K (1992) Pharmacological discrimination of N-type from L-type calcium current and its selective modulation by transmitters. *J Neurosci* 12:906–914.
- Curtis DR, Gynther BD, Beattie J, Kerr DIB, Prager RH (1988) Baclofen antagonism by 2-hydroxy-saclofen in the cat spinal cord. *Neurosci Lett* 92:97–100.
- Dale N (1985) Reciprocal inhibitory interneurons in the *Xenopus* spinal cord. *J Physiol (Lond)* 363:61–70.
- Dale N (1991) The isolation and identification of spinal neurons that control movement in the *Xenopus* embryo. *Eur J Neurosci* 3:1025–1035.
- Dale N, Kandel ER (1990) Facilitatory and inhibitory transmitters modulate spontaneous transmitter release at cultured *Aplysia* sensorimotor synapses. *J Physiol (Lond)* 421:203–222.
- Dale N, Otterson OP, Roberts A, Storm-Mathisen J (1986) Inhibitory neurons of a motor pattern generator in *Xenopus* revealed by antibodies to glycine. *Nature* 324:255–257.
- Dale N, Roberts A, Soffe SR (1990) The anatomy, development and role of glycinergic neurons in the *Xenopus* embryo spinal cord. In: Glycine neurotransmission (Otterson OP, Storm-Mathisen J, eds), pp 329–353. London: Wiley.
- Davies J (1981) Selective depression of synaptic excitation in cat spinal

- neurones by baclofen: an iontophoretic study. *Br J Pharmacol* 72: 373–384.
- Dolphin AC, Scott RH (1987) Calcium channel currents and their inhibition by (–) baclofen in rat sensory neurons: modulation by guanine nucleotides. *J Physiol (Lond)* 386:1–17.
- Dutar P, Nicoll RA (1988) A physiological role for GABA-B receptors in the central nervous system. *Nature* 332:156–158.
- Edmonds B, Klein M, Dale N, Kandel ER (1990) Contributions of two types of calcium channels to synaptic transmission and plasticity. *Science* 250:1142–1147.
- Ellinor PT, Zhang JF, Randall AD, Zhou M, Schwarz TL, Tsien RW, Horne WA (1993) Functional expression of a rapidly inactivating neuronal calcium channel. *Nature* 363:455–458.
- Elmslie KS, Zhou W, Jones SW (1990) LHRH and GTP- γ S modify calcium current activation in bullfrog sympathetic neurons. *Neuron* 5:75–80.
- Feldman DH, Olivera BM, Yoshikami D (1987) Omega *Conus geographus* toxin: a peptide that blocks calcium channels. *FEBS Lett* 2:295–300.
- Gage PW (1992) Activation and modulation of neuronal K⁺ channels by GABA. *Trends Neurosci* 15:46–51.
- Gawwiler BH, Brown DA (1985) GABA_B-receptor-activated K⁺ current in voltage-clamped CA₁ pyramidal cells in hippocampal cultures. *Proc Natl Acad Sci USA* 82:1558–1562.
- Grassi F, Lux HD (1989) Voltage-dependent GABA-induced modulation of calcium currents in chick sensory neurons. *Neurosci Lett* 105:113–119.
- Gray J, Green AR (1987) GABA_B receptor mediated inhibition of potassium-evoked release of endogenous 5-hydroxytryptamine from mouse frontal cortex. *Br J Pharmacol* 91:517–522.
- Hills J, Larkin M, Howson W (1991) A comparison of the relative activities of a number of GABA-B antagonists in the isolated vas deferens of the rat. *Br J Pharmacol* 102:631–634.
- Hochner B, Klein M, Schacher S, Kandel ER (1986a) Action-potential duration and the modulation of transmitter release from the sensory neurons of *Aplysia* in presynaptic facilitation and behavioral sensitization. *Proc Natl Acad Sci USA* 83:8410–8414.
- Hochner B, Klein M, Schacher S, Kandel ER (1986b) Additional component in the cellular mechanism of presynaptic facilitation contributes to behavioral dishabituation in *Aplysia*. *Proc Natl Acad Sci USA* 83:8794–8798.
- Kerr DIB, Ong J, Prager RH, Gynther BD, Curtis DR (1987) Phaclofen: a peripheral and central baclofen antagonist. *Brain Res* 405:150–154.
- Man-Son-Hing H, Zoran MJ, Lukowiak K, Haydon PG (1989) A neuromodulator of synaptic transmission acts on the secretory apparatus as well as ion channels. *Nature* 341:237–239.
- Mintz IM, Bean PB (1993) GABA_B receptor inhibition of P-type Ca²⁺ channels in central neurons. *Neuron* 10:889–898.
- Nicoll RA, Malenka RC, Kauer JA (1990) Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiol Rev* 70:513–565.
- Nieuwkoop PD, Faber J (1956) Normal tables of *Xenopus laevis* (Daudin). Amsterdam: North-Holland.
- Plummer MR, Logothetis DE, Hess P (1989) Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. *Neuron* 2:1453–1463.
- Pollo A, Lovallo M, Sher E, Carbone E (1992) Voltage-dependent noradrenergic modulation of ω -conotoxin-sensitive Ca²⁺ channels in human neuroblastoma IMR32 cells. *Pfluegers Arch* 422:75–83.
- Roberts A (1990) How does a nervous system produce behaviour? A case study in neurobiology. *Sci Proc (Oxford)* 74:31–51.
- Roberts A, Clarke JDW (1982) The neuroanatomy of an amphibian embryo spinal cord. *Proc R Soc Lond [Biol]* 296:195–212.
- Roberts A, Sillar KT (1990) Characterization and function of spinal excitatory interneurons with commissural projections in *Xenopus laevis* embryos. *Eur J Neurosci* 2:1051–1062.
- Roberts A, Dale N, Ottersen OP, Storm-Mathisen J (1987) The early development of neurons with GABA immunoreactivity in the CNS of *Xenopus laevis* embryos. *J Comp Neurol* 261:435–449.
- Saint DA, Thomas T, Gage PW (1990) GABA_B receptors modulate a transient potassium current in cultured mammalian hippocampal neurons. *Neurosci Lett* 118:9–13.
- Scholtz KP, Miller RJ (1991) GABA_B receptor-mediated inhibition of Ca²⁺ currents and synaptic transmission in cultured rat hippocampal neurons. *J Physiol (Lond)* 444:669–686.
- Scholtz KP, Miller RJ (1992) Inhibition of quantal transmitter release in the absence of calcium influx by a G protein-linked adenosine receptor at hippocampal synapses. *Neuron* 8:1139–1150.
- Soffe SR (1987) Ionic and pharmacological properties of reciprocal inhibition in *Xenopus* embryo motoneurons. *J Physiol (Lond)* 382: 463–473.
- Soffe SR (1993) Two distinct rhythmic motor patterns are driven by common premotor and motor neurons in a simple vertebrate spinal cord. *J Neurosci* 13:4456–4469.
- Soltesz I, Haby M, Leresche N, Crunell V (1988) The GABA-B antagonist phaclofen inhibits the late K⁺ dependent IPSP in cat and rat thalamic and hippocampal neurons. *Brain Res* 448:351–354.
- Tsien RW, Lipscombe D, Madison DV, Bley KR, Fox AP (1988) Multiple types of neuronal calcium channels and their selective modulation. *Trends Neurosci* 11:431–438.
- Usovich MM, Sugimori M, Cherskey B, Llinas R (1992) P-type calcium channels in the somata and dendrites of adult cerebellar Purkinje cells. *Neuron* 9:1185–1199.
- Wall MJ, Dale N (1993) GABA_B receptors modulate glycinergic inhibition and spike threshold in *Xenopus* embryo spinal neurons. *J Physiol (Lond)* 469:275–290.
- Williams ME, Brust PF, Feldman DH, Patthi S, Simerson S, Maroufi A, McCue AF, Velicelebi G, Ellis SB, Harpold MM (1992a) Structure and functional expression of an ω -conotoxin-sensitive human N-type calcium channel. *Science* 257:389–395.
- Williams ME, Feldman DH, McCue AF, Brenner R, Velicelebi G, Ellis SB, Harpold MM (1992b) Structure and functional expression of α_1 , α_2 , and β subunits of a novel human neuronal calcium channel subtype. *Neuron* 8:71–84.