

## **Selective Modulation of Chemical Transmission at a Dual-Action Synapse (with Special Reference to Baclofen)**

A. I. SHAPOVALOV and B. I. SHIRIAEV

*Laboratory of Physiology of the Nerve Cell, Sechenov Institute of Physiology and Biochemistry,  
Academy of Sciences of the USSR, Thorez pr. 44, 194223 Leningrad, USSR*

**Abstract.** Monosynaptic, electrical-chemical EPSPs were produced in individual motoneurons of the isolated frog spinal cord by intracellular stimulation of single primary afferents. This preparation was used to assess the relationship between the modulation of chemical synaptic transmission and presynaptic invasion. It was found that at dual-action synapses Baclofen selectively depressed chemical transmission without affecting the electrically mediated response. The depression of chemical transmission results from a reduction in the number of transmitter quanta released by the presynaptic terminals following their adequate depolarization by the incoming action potential. Some other drugs depressing (Nembutal) and enhancing (Guanidine) chemical transmission and cooling also induce variability to release transmitter without affecting the presynaptic invasion.

**Key words:** Synaptic transmission — Single-fibre EPSPs — Unit potentials

### **Introduction**

In the central nervous system of vertebrates many synapses are formed by thin presynaptic terminals and boutons en passant, and recent studies suggest that such presynaptic arrangement can be an area of low safety factor for spike propagation. Lucher et al. (1979) suggested that the invasion of Ia terminals in the cat spinal cord is a graded process and that transmission at this synapse depends on the degree of invasion of the terminal arborisation. According to Edwards et al. (1976) the quantal fluctuations of unitary EPSP amplitudes might be due to the blockade of sensory impulses at various branching points. Davidoff and Sears (1974) suggested that Baclofen, a selective blocker of monosynaptic reflex pathways, produced its action by blocking impulse conduction in preterminal axons of the amphibian spinal cord.

These suggestions seem to fit nicely with recent morphological observations of the presynaptic structural pattern following horse redish peroxidase (HRP) labeling both in the cat (Brown and Fyffe 1978; Burke et al. 1979) and frog spinal cord (Motorina et al. 1982; Grantyn et al. 1982) where primary sensory fibres terminate in chains of varicosities connected by thin terminal axons. The electrical

loading of the large surface area of synaptic contact may make the safety factor for impulse invasion rather marginal.

The presynaptic collaterals, however, are too small to permit intracellular recording for the direct investigation of this problem. The application of different experimental approaches appears to be required to characterise the extent to which modulation of transmission at the central synapse depends on degree of presynaptic invasion. The synaptic connection between primary afferent fibres and frog motoneurons is particularly useful in studies of synaptic mechanisms because of dual mode of junctional transmission at this synapse (Shapovalov and Shiriaev 1979). We exploited this feature to assess the role of presynaptic invasion which could be monitored postsynaptically via the electrical coupling potential recorded from the motoneuron.

In addition to its usefulness in monitoring presynaptic invasion combined electrical-chemical single-fibre EPSPs are well suited for quantal analysis (Shapovalov and Shiriaev 1980). Since transmitter substance released in the monosynaptic reflex pathway in the spinal cord is still unknown, quantal analysis may provide useful means for determining whether the observed changes in chemical synaptic transmission are due to post-synaptic alterations or to variability in transmitter release.

In order to investigate this problem we used Baclofen — a derivate of  $\gamma$ -aminobutyric acid — a remarkably potent central myorelaxant, affecting monosynaptic reflex pathways (Davidoff and Sears 1974; Krnjevič 1980), as well as Nembutal, Guanidine and cooling. We present here evidence that under these conditions the modulation of chemical synaptic transmission is mainly due to a depression of transmitter release, but not to changes in presynaptic invasion.

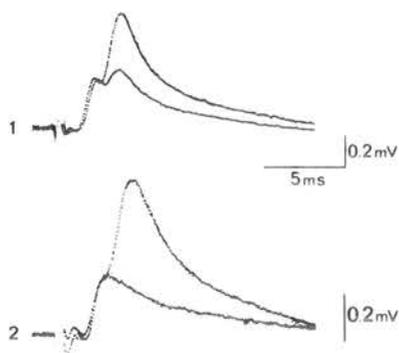
## Methods

We used techniques similar to those previously described (Shapovalov and Shiriaev 1980). Experiments were performed on adult frogs (*Rana ridibunda*) weighing 90–130 g. After laminectomy the spinal cord was removed with the attached dorsal and ventral roots and hemisected sagittally. The preparation was put in a temperature-controlled chamber and perfused with a Ringer's solution of the following composition (mmol/l): NaCl 98.0; KCl 2.0; CaCl<sub>2</sub> 1.8; MgCl<sub>2</sub> 0.5; NaH<sub>2</sub>PO<sub>4</sub> 1.2; Na<sub>2</sub>HPO<sub>4</sub> 2.0; NaHCO<sub>3</sub> 6.0; glucose 5.5; gassed with 98% O<sub>2</sub> and 2% CO<sub>2</sub> to achieve a pH of 7.4–7.6. After different periods in this normal solution, the test solution containing drugs was applied.

Intracellular recordings were made simultaneously from lumbar motoneurons and dorsal root fibres at the dorsal root entry point. Action potentials were evoked by intense depolarizing current pulse. The resulting monosynaptic unitary EPSPs were recorded from motoneurons by a separate microelectrode.

All the recordings were displayed on an oscilloscope and successive sweeps were photographed. In addition, the voltage response of the fibre and the motoneuron was frequently averaged using a DIDAC-4000 (Intertechnique).

In order to determine the statistical nature of chemical components of single-fibre EPSPs the histograms of the peak amplitude of a series of individual EPSP evoked by a presynaptic spike in a given motoneuron were plotted. The binomial probability of the evoked EPSPs has been predicted from:



**Fig. 1.** Baclofen-induced partial (1) and complete (2) blockade of the chemical component of a single-fibre EPSP in two different motoneurons. Superimposed averaged record before and following the administration of solution containing  $10^{-5}$  mol/l Baclofen (1) and  $2 \times 10^{-4}$  mol/l Baclofen (2). Note that the amplitude of electrical component remains unchanged.

$$P_{(x)} = \sum_{r=0}^n \left[ \frac{n!}{(n-r)!r!} \cdot p^r \cdot (1-p)^{n-r} \cdot \frac{1}{\sigma\sqrt{2\pi}} \cdot e^{-\frac{(x-rv)^2}{2\sigma^2}} \right]$$

The Poisson probability distribution of the evoked EPSPs has been predicted from:

$$P_{(x)} = \sum_{r=0}^n \left[ \frac{e^{-m} \cdot m^r}{r!} \cdot \frac{1}{\sigma\sqrt{2\pi}} \cdot e^{-\frac{(x-rv)^2}{2\sigma^2}} \right]$$

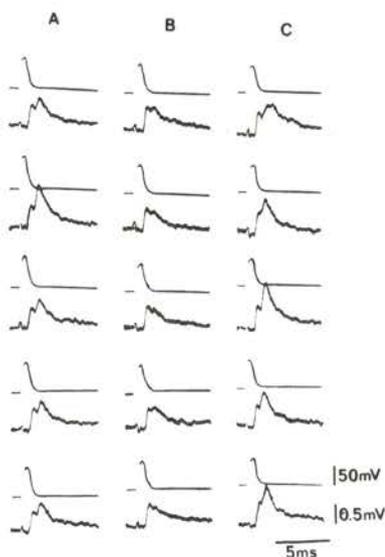
where  $P_{(x)}$  is the expected probability of the EPSPs with an amplitude of  $x$  mV,  $r$  (0, 1, 2, ...n) is the possible quantal content of each EPSP,  $\sigma$  is the standard deviation of the noise, and  $v$  is the quantal size. To determine how well the theoretical predictions fitted the observed distribution of the EPSPs, a  $\chi^2$  statistics was computed for each comparison.

## Results

### *Effects of Baclofen*

Studies on the perfused *in vitro* frog spinal cord have shown that Baclofen reduced depolarizing synaptic potentials recorded from ventral roots (Davidoff and Sears 1974). These authors suggested that Baclofen produced most of its actions presynaptically, possibly by blocking impulse conduction in preterminal axons.

The addition of Baclofen to the bathing solution invariably reduced the chemical component of the single fibre EPSP, produced by intracellular stimulation of the presynaptic axon. The averaged records of Fig. 1 illustrate the considerable depression of chemical EPSPs without any concomitant change in the coupling potential.

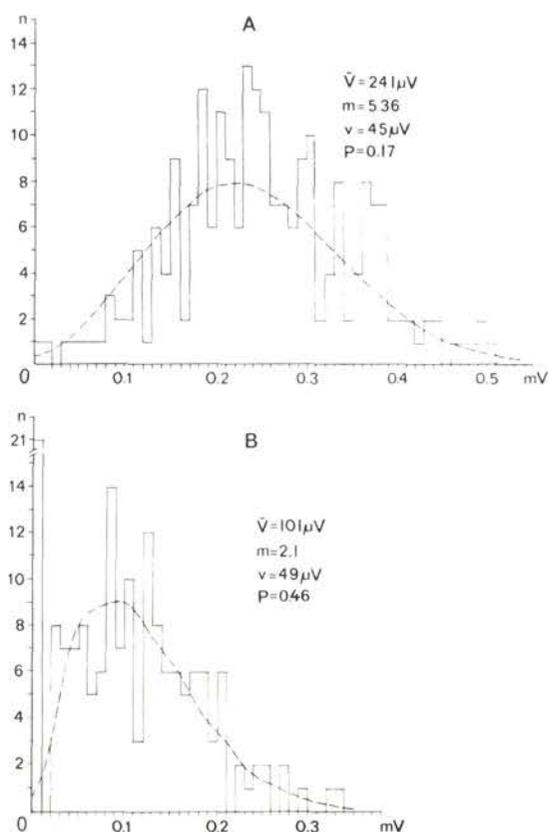


**Fig. 2.** Effect of Baclofen on individual single-fibre EPSPs. Single sweeps recorded from dorsal root fibre (*upper traces*) and a motoneuron (*lower traces*) before (**A**), after 3 min of perfusion with the solution containing  $10^{-5}$  mol/l Baclofen (**B**) and recovery 27 min after returning to of normal Ringer solution (**C**).

Increasing concentrations of Baclofen produced correspondently sharper diminutions of the chemically mediated EPSPs, but even when the block of chemical transmission at this synapse was virtually complete, the electrically mediated component of the EPSP remained unchanged (Fig. 1, 2).

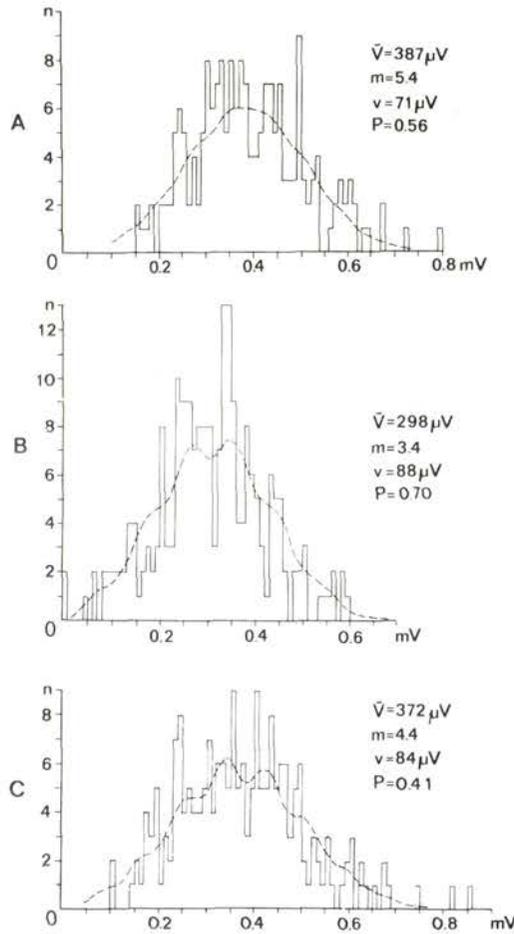
Single sweeps presented in Fig. 2 show that depression of the later, chemical component of the single-fibre EPSP by Baclofen is reversible. The records of Fig. 2 further indicate that during partial blockade produced by Baclofen the presynaptic volley either does not elicit chemical response at all or evokes the chemical EPSPs with an amplitude of 40–100  $\mu$ V. The statistical analysis of single-fibre EPSPs has previously shown (Shapovalov and Shiriaev 1980) that these values are of the same order of magnitude as a unit potential produced by a single quantum of the transmitter. Since the size of the electrical coupling potential remains unchanged, the depression of the chemical EPSP may be due either a reduction in transmitter release or a decrease in the sensitivity of the postsynaptic membrane to the naturally released transmitter. In order to resolve this question it is essential to determine the amplitude of the quantal step both before and following the application of Baclofen.

To test the quantal composition of the unitary chemical EPSP, series of



**Fig. 3.** The amplitude distribution of the chemical component of the single-fibre EPSP before (**A**) and after 5 min of perfusion with solution containing  $10^{-5}$  mol/l Baclofen (**B**). Dashed line represents the best fit using Poisson distribution.  $\bar{V}$ , average amplitude of the chemical EPSP;  $m$ , mean quantum content;  $v$ , mean quantal size;  $P$ , goodness of fit, expressed as probability of correspondence by  $\chi^2$ -test.

200–250 responses were recorded before, during the addition of Baclofen and after returning to normal Ringer solution. Chemical EPSP amplitude histograms were plotted as shown in Fig. 3 and 4 and fitted by either Poisson or binomial equations in the way outlined in the methods. It can be seen that the decrease in the mean amplitude of the response was accompanied by an increase in the number of failures and a decrease in the relative number of larger responses in the distribution. The mean quantum content  $m$  was also diminished. However, the amplitude of a single quantal response  $v$  calculated by dividing the mean amplitude of the distribution  $\bar{V}$  by the mean quantal content  $m$  was not diminished after addition of Baclofen. In Fig. 3 the values  $v$  thus obtained were  $45 \mu\text{V}$  before and

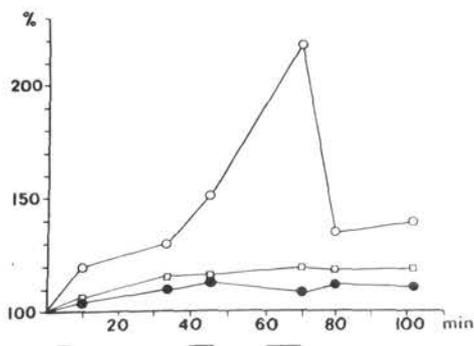


**Fig. 4.** Histograms of amplitude distributions of the chemical component of single-fibre EPSP. The responses to single fibre stimulation were obtained in the same motoneuron in normal Ringer solution (A), 6 min after the addition of  $10^{-5}$  mol/l Baclofen (B) and following 10 min wash in the normal Ringer solution (C). The binomial prediction (dashed line) fits the distribution.

49  $\mu\text{V}$  after addition of Baclofen, indicating that the sensitivity of the post-junctional membrane to the neurotransmitter had not been altered by the addition of the drug and, thus, that its effect was entirely presynaptic.

The EPSP fluctuation pattern presented in Fig. 3 fitted the Poisson law. Similar results were observed when amplitude distribution of EPSP could be fitted to the binomial law.

Another example of distribution of the peak responses measured within

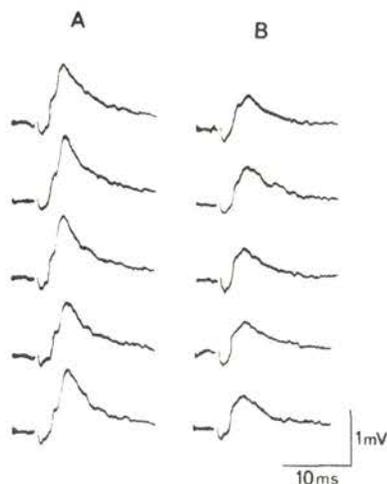


**Fig. 5.** Effect of Baclofen ( $10^{-5}$  mol/l) on the resting membrane potential ( $\bullet$ ), the amplitude of electrical component of the single-fibre EPSP ( $\square$ ) and on amplitude of quantal unit ( $\circ$ ), expressed as a percentage of control values obtained in normal Ringer solution. The durations of Baclofen application are represented by black bars.

defined latency bounds following the presynaptic action potential is shown in Fig. 4. This distribution fits predictions based on binomial statistics ( $P=0.56$ ). After the administration of Baclofen the mean amplitude of EPSPs was reduced from 387 to 298  $\mu$ V and a series containing failures of occurrence appeared (Fig. 4B). However, the amplitude of unit potentials was not decreased. In fact it was increased relative to the control distribution. There is a tendency to return to control values when Baclofen was washed out (Fig. 4C). These results indicate that postsynaptic depression does not play any role in the blocking action of Baclofen. Moreover, unit potentials were frequently observed to increase following the exposure to Baclofen. Fig. 5 which graphically illustrates data from one of these experiments shown considerable augmentation of the size of quantum potential during the partial blockade produced by Baclofen. This increase exceeds the rise in the membrane resting potential and in the amplitude of the electrical EPSP. The mechanism of this augmentation is not clear, but it is consistent with the notion that there is no decrease in the sensitivity of postsynaptic membrane to the neurotransmitter.

#### *Effects of Nembutal and Guanidine*

The results obtained with Baclofen suggest that changes in transmitter release may be produced without any influence on the presynaptic invasion. It would be reasonable to investigate some other drugs affecting synaptic transmission. In the cat spinal cord Nembutal was shown to depress single-fibre EPSPs reducing their mean quantum content (Weakly 1969). In the frog spinal cord large doses of



**Fig. 6.** Effect of Nembutal on the single-fibre EPSP. Single sweeps recorded in normal Ringer solution (A) and after 43 min of perfusion with solution containing 1.2 mmol/l Nembutal (B).

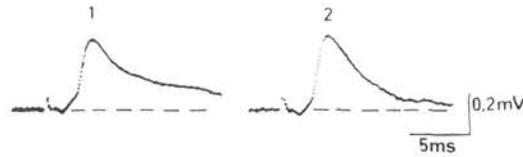
Nembutal, while strongly affecting polysynaptic transmission, preserved monosynaptic EPSPs derived from the stimulation of dorsal roots (Fadiga and Brookhart 1960).

In the present experiments the addition of Nembutal to the perfusing medium markedly depressed the chemical component of the single-fibre EPSP but did not affect its early, electrical component. The successive sweeps of Fig. 6 illustrate a representative example of selective depression of chemical transmission at a dual-action synapse by Nembutal. It may be concluded, therefore, that like Baclofen this barbiturate does not affect presynaptic invasion but depresses the release of transmitter by presynaptic endings.

Guanidine which is known to enhance synaptic transmission (Kusano 1970; Banks 1978) increased the amplitude of chemical component of single-fibre EPSP but did not change its electrical component. The averaged records of Fig. 7 illustrate the single-fibre EPSP before and following administration of Guanidine suggesting that facilitation of chemical transmission may be achieved without any change in presynaptic invasion.

#### *Effects of temperature*

A decrease in temperature was shown to depress synaptic transmission at chemical synapses of the frog spinal cord, but not electrical transmission (Shapovalov et al. 1978). We used single-fibre EPSP to investigate the effect of temperature on electrical and chemical transmission at the same synapse.



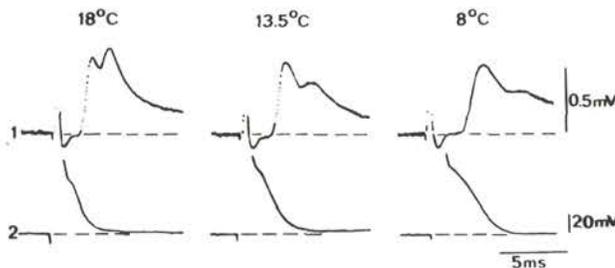
**Fig. 7.** Effect of Guanidine on the single-fibre EPSP. Averaged records before (1) and after 5 min of perfusion with solution containing 0.2 mmol/l Guanidine.

The averaged records of Fig. 8 demonstrate that as the temperature of the bath is lowered, there is a progressive decrease in the amplitude of the chemical component. On the contrary the electrical component of the EPSP and the amplitude of presynaptic spike are not decreased. There is, however, a considerable prolongation of both electrical coupling potential and the presynaptic spike.

The synaptic delay measured as an interval between the onset of the electrical component and beginning of the chemical component is greatly increased (from 1.6 ms at 18° to 3.9 ms at 8°C,  $Q_{10}$  being 2.43).

## Discussion

The data described in the present communication indicate that Baclofen decreases quantal transmitter release in the monosynaptic pathway in the frog spinal cord. The decrease in transmitter release was dissociated from changes in the presynaptic action potential monitored by the electrical component of the single-fibre EPSP. Similar effects were produced by Nembutal and by cooling whereas exposure of the spinal cord to Guanidine resulted in an enhancement of chemical synaptic transmission without concomitant changes in the electrical coupling potential.



**Fig. 8.** Effect of reduction of temperature on the single-fibre EPSP. Action potentials recorded from dorsal root fibre (*lower traces*) and corresponding EPSPs recorded from a motoneuron (*upper traces*). Note the prolongation of the presynaptic spike and increase in the latency of the chemical component of the EPSP.

There is now ample evidence that the quantal content of the EPSP is sensitive to agents inhibiting calcium influx into presynaptic endings (Katz 1969; Marshall et al. 1980; Shapovalov and Shiriaev 1980; Shapovalov et al. 1982). It may be supposed, therefore, that the agents used in the present study interfere in some way with the role of calcium ions in the triggering of transmitter release. It was found recently that barbiturates significantly depressed depolarization-induced calcium influx into synaptosomes (Leslie et al. 1980). It is worth noting that both barbiturates and Baclofen can affect receptors for  $\gamma$ -amino-butyric acid, suggesting that they may have a similar mechanism of action on the presynaptic terminals.

The fact that depression and enhancement of chemical synaptic transmission does not require any change in the presynaptic action potential is consistent with the previously reported data on post-synaptic potentiation at a dual-action synapse (Shapovalov and Shiriaev 1980).

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