Inhibitory Miniature Potentials in the Stretch Receptor Neurons of Crayfish

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ABSTRACT Intracellular microelectrodes inserted into the soma of crayfish stretch receptor neurons record frequent fluctuations of the membrane potential. Time course, amplitude, and interval distribution indicate that they are miniature potentials. At the average resting potential the polarity of the miniature potentials depends on the anion used in the microelectrode: KCl electrodes record depolarizing, K citrate or K_2SO_4 electrodes, hyperpolarizing miniature potentials. The inhibitory postsynaptic potentials (i.p.s.p.'s) show a similar polarity change. The reversal potentials of i.p.s.p.'s and miniature potentials are equal and within 10 mv of the resting potential, more negative with K citrate (or K_2SO_4), less negative with KCl electrodes. Reversal can be accomplished by changing the membrane potential by stretching or by current passing. Injection of Cl^- into the soma or replacement of external Cl^- by propionate results in an abrupt increase of the amplitude of the miniature potentials lasting for several minutes. The miniature potentials like the i.p.s.p.'s are reversibly abolished by the application of picrotoxin and γ-aminobutyric acid. They are not affected by tetrodotoxin, nor by acetylcholine, eserine, or atropine. It is concluded that the miniature potentials represent a spontaneous quantal release of transmitter substance from inhibitory nerve terminals, and that the transmitter substance predominantly increases the Cl^- permeability of the postsynaptic membrane. The effect of the spontaneously released transmitter on the behavior of the receptor neuron is considerable. The membrane conductance is increased by up to 36% and the excitability is correspondingly depressed.

INTRODUCTION

Since the discovery of "miniature potentials" at the motor end plate of amphibian skeletal muscle (Fatt and Katz, 1952), similar subthreshold spontaneous activity at synapses has been observed in mammalian skeletal muscle (Liley, 1956), mammalian smooth muscle (Burnstock and Holman, 1962), smooth muscle of earthworm (Hidaka, Ito, and Kuriyama, 1968), and in striated muscle of birds (Ginsborg, 1960), crustaceans (Reuben and Grund-

fest, 1960; Dudel and Kuffler, 1961), and insects (Usherwood, 1963). These potentials are recognized as signs of spontaneous, quantal release of transmitter from presynaptic terminals. Miniature potentials have also been recorded from several types of nerve cells, such as the motoneurons of squid (Miledi, 1967), the frog (Katz and Miledi, 1963), and the cat (Kuno, 1964; Hubbard, Stenhous, and Eccles, 1967), and from sympathetic ganglion cells of the chick (Martin and Pilar, 1964) and the frog (Blackman, Ginsborg, and Ray, 1963). With the exception of crustacean and earthworm muscle, all the miniature potentials observed represented excitatory transmitter action.

The receptor neurons of the stretch receptor organs of crayfish receive only inhibitory synaptic input (Kuffler and Eyzaguirre, 1955; Uchizono, 1967) and show only inhibitory postsynaptic potentials (i.p.s.p.'s).

The present paper reports evidence for spontaneous quantal release of transmitter from inhibitory nerve terminals of crayfish stretch receptor neurons and presents an analysis of the ionic mechanisms involved in the action of the transmitter substance. A preliminary report of the earlier phase of this investigation was published elsewhere (Iwasaki and Florey, 1965).

METHOD

Abdominal stretch receptor organs were prepared from large specimens (4–5 inches body length) of crayfish. The species used were *Pacifastacus leniusculus*, *Orconectes virilis*, and *Procambarus clarkii*. The mode of dissection was that described in detail by Florey and Elliott in 1961. The organs were removed from the animal and mounted in a perfusion chamber. This was accomplished by clamping the muscle elements with a pair of fine forceps and stretching the stretch receptor nerve by means of a loop of thread which was hooked on to an Ag-AgCl electrode. A polyethylene sleeve was pushed over the tip of the electrode and over the proximal portion of the nerve. This arrangement made it possible to keep the entire nerve submerged in saline throughout the experiment and to use the same Ag-AgCl electrode for either stimulation or extracellular recording. The setup is shown in Fig. 1. The forceps were part of a stretching device that permitted symmetrical changes in length. With the sensory cell placed exactly in the middle between the forceps, induced changes in length produced only minimal displacement of the nerve cell.

Micromanipulated glass capillary microelectrodes were used to record membrane potentials and to pass current across the cell membrane. The electrodes were filled with 3 M KCl, 0.7 M K_2SO_4, or 2 M potassium citrate. The resistances of the electrodes chosen were between 10–20 MΩ.

The microelectrode was connected to an electrometer amplifier of high input impedance and negligible grid current. Potentials were displayed on a dual channel oscilloscope (Tektronix 502) and on an inkwriting polygraph. A bridge circuit with a series resistance of 500 MΩ was employed to permit use of the same electrode for both recording of potentials and passing current. The current was recorded across a 1 MΩ resistor in one arm of the bridge, using a balanced cathode follower of high input impedance. The applied current was derived from an electronic stimulator

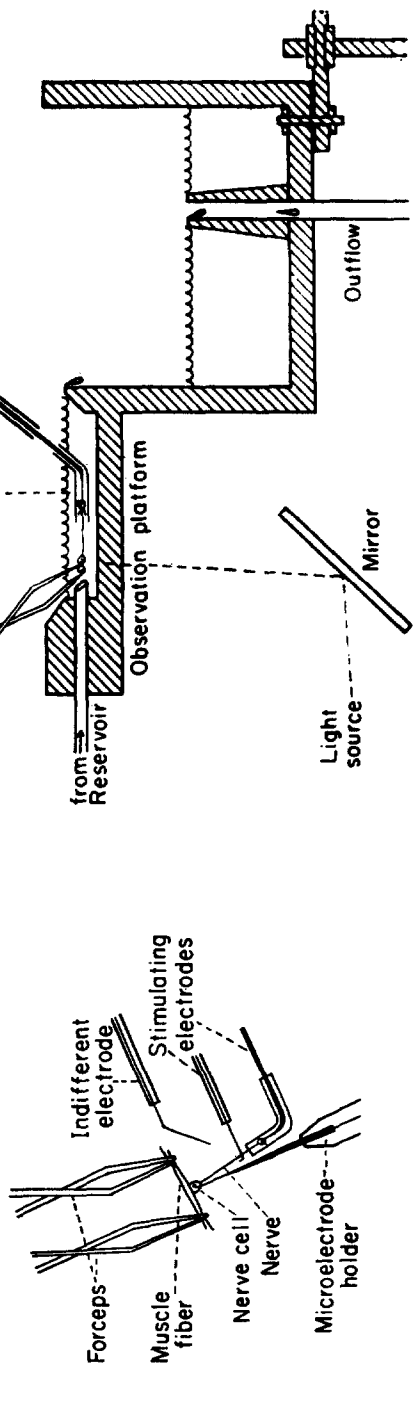

FIGURE 1 A. Electrode arrangement (seen from above) used for stimulation of the inhibitory axon and recording of potential changes from the sensory neuron. The inhibitory axon is not shown separately; it runs alongside the sensory axon. The nerve containing the sensory and inhibitory axons is hooked onto an Ag-AgCl electrode inside a polyethylene sleeve. With the exception of the microelectrode all electrodes are Ag-AgCl. 1 B. Cross-section through superfusion chamber showing how the soma of the stretch receptor neuron was placed in front of the orifice of the superfusion manifold.

(American Electronic Laboratories, Inc., Colmar, Pa., Model 102, or Nihon Koden, Model MSE-3) via an isolation unit, or from a battery-operated constant-current stimulator. The currents required were of the order of 10^{-9}–10^{-7} amp. The method of using a bridge circuit for the application of current through the recording microelectrode has intrinsic faults. Even though the bridge has been balanced before the insertion of the electrode, it becomes unpredictably unbalanced because the electrode resistance usually changes as the electrode tip penetrates the connective tissue sheath and the soma membrane. This makes it difficult to estimate the potential changes that result from the passage of current. To a certain degree it is possible to correct for this by determining the electrode resistance after withdrawal from the cell.

In some cases two electrodes were introduced into the same neuron, one for current passing and the other for recording membrane potentials. In order to increase the Cl concentration inside the cell, a negative voltage was applied to the recording KCl electrode when it was in the cell.

In several preparations it has been possible to selectively stimulate the inhibitory axon by adjusting the voltage and duration of the stimuli applied to the stretch receptor nerve.

The isolated stretch receptor organs could be maintained in good condition for several hours by the simple expedient of exposing them to a continuous stream of previously aerated saline (composition in mM/liter: NaCl 205, KCl 5.4, $CaCl_2$ 13.5, and $MgCl_2$ 2.6). For this purpose a small jet of saline was made to flow directly over the sensory cells at a rate of about 5 ml/min. The saline was delivered from a reservoir via a manifold and connecting thin polyethylene tubing. For the application of drugs a system of stopcocks was employed so as to permit instantaneous switching from a reservoir of normal saline to another which contained the particular drug dissolved in the saline. In order to avoid artifacts due to differences in flow velocity care was taken to equalize the flow rates by the use of mariotte bottles from the different reservoirs and to maintain all at the same water pressure.

The following drugs were used: acetylcholine chloride (Merck), atropine sulfate (Norwich Pharmacal Co., Norwich, N. Y.), γ-Aminobutyric acid (California Foundation for Biochemical Research, Los Angeles, Calif.), physostigmine salicylate (Burroughs Wellcome & Co., Ltd., London, England), tetrodotoxin (Sankyo Co. Ltd., Tokyo, Japan), and picrotoxin (Nutritional Biochemicals Corp., Cleveland, Ohio).

RESULTS

General Observations

When the membrane potential of quiescent stretch receptor neurons is recorded with intracellular microelectrodes filled with 3 M KCl and at high amplification, spontaneous depolarizing fluctuations can be seen which have a steep rising and slow falling phase. In some preparations they are very frequent, in others they are noticed only sporadically. We have observed them in stretch receptors of all three species of crayfish used in our experiments. Fig. 2 shows examples of records obtained from a phasic receptor neuron. The same

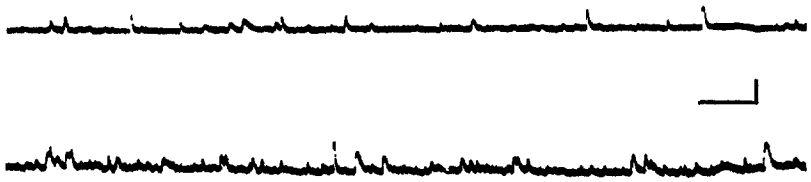

FIGURE 2. Membrane potential fluctuations (miniature potentials) recorded from two phasic receptor neurons (KCl electrode). Calibration, 1 mv and 1 sec.

kind of potential changes occurs in tonic receptors. Fig. 3 A, B, and C show histograms of amplitude, intervals, and half-decay times of 271 consecutive spontaneous membrane fluctuations recorded from a phasic receptor cell. In Fig. 3 D, the intervals between these potentials are plotted over the theoretical curve (Fatt and Katz, 1952; del Castillo and Katz, 1954) describing complete randomness of the intervals. The relatively close fit is evidence that the membrane potential fluctuations are indeed random occurrences. The wide range of time courses (5–130 msec), as shown in Fig. 3 C, is very characteristic in

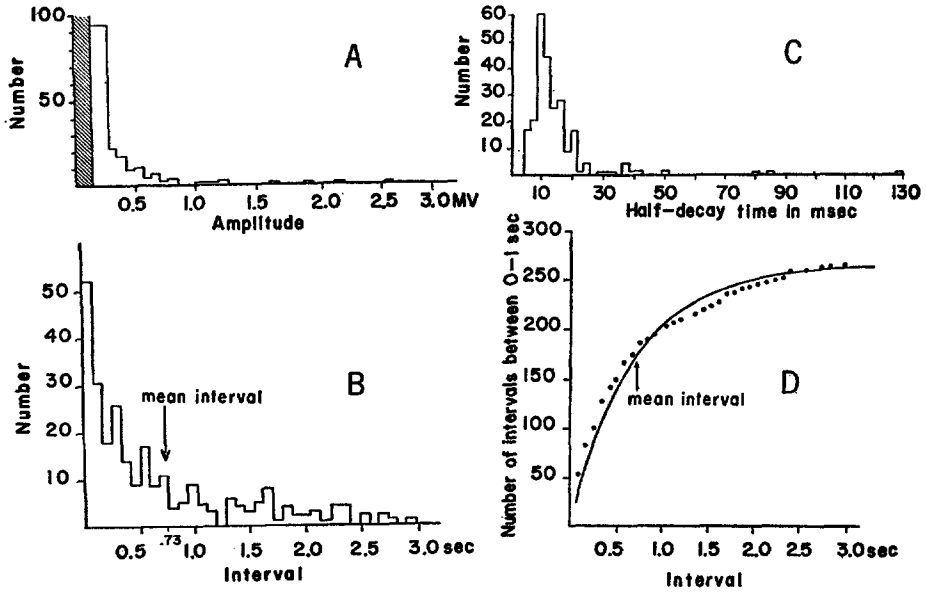

FIGURE 3. Histograms of the amplitudes (A), intervals (B), and half-decay times (C) of 271 consecutive potentials recorded from a phasic receptor neuron (KCl electrode). The shaded area in A represents the noise level. In (D) the number of intervals between zero time to time t is plotted against the intervals between successive potentials. The solid line represents the theoretical curve (Fatt and Katz, 1952) according to the equation $y = N(1 - e^{-t/T})$, where N is the total number of the potentials, t is the interval considered, and T represents the mean interval.

this type of neuron. This might be due to the wide spread of nerve terminals over soma and dendrites of the neuron; our records, however, do not indicate the expected inverse correlation between amplitude and decay time.

It should be pointed out that the sampling represented in Fig. 3 is somewhat incomplete because it must be assumed that the noise level (indicated by cross-hatching in Fig. 3 A) obscured the smaller fluctuations of membrane potential (see also later, p. 674).

In their characteristics, as is evident from the graphs in Fig. 3, these fluctuations of membrane potential fit the description of the miniature potentials

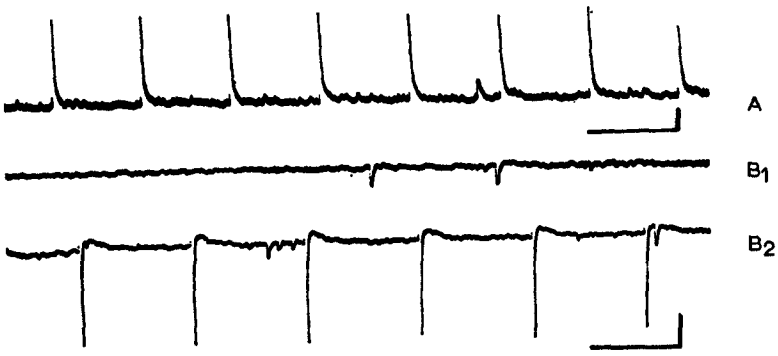

FIGURE 4. Relative amplitudes of i.p.s.p.'s and miniature potentials. A, depolarizing i.p.s.p.'s and miniature potentials recorded with KCl electrode. B, hyperpolarizing i.p.s.p.'s and miniature potentials recorded with citrate electrode (AC amplification). The amplitude ratio (i.p.s.p.: largest miniature potential) is 5.0 in the sequence represented by A, and 7.5 in the sequence represented by B. Calibration, 2 mv and 500 msec.

that occur in other types of synapses. We will, therefore, refer to them as *miniature potentials*.

With the exception of certain experiments[1] on preparations obtained from *Pacifastacus leniusculus*, both miniature potentials and inhibitory postsynaptic potentials were depolarizing when KCl electrodes were used. An example showing both types of potential is given in Fig. 4 A.

When microelectrodes filled with K_2SO_4 or with K citrate were used, only small, usually hyperpolarizing, miniature potentials were observed. Under these conditions the polarity of the evoked i.p.s.p.'s also was hyperpolarizing as shown in Fig. 4 B. This suggested that the i.p.s.p.'s and miniature potentials involve a change in Cl permeability. The following evidence supports this.

[1] In a few preparations hyperpolarizing i.p.s.p.'s and depolarizing miniature potentials were recorded (KCl electrode). We assume that in these cases the conduction to the synapses near the soma was blocked by the pressure exerted when the microelectrode was forced through the tough connective tissue that surrounds the receptor organ. Cl^- diffusing from the electrode tip would mainly affect the soma from which the miniature potentials are predominantly recorded.

When the K_2SO_4 electrode was withdrawn from a cell that had shown small hyperpolarizing miniature potentials, and replaced by a KCl-filled electrode the miniature potentials reversed polarity and increased in amplitude within a few minutes. When chloride ions were injected into the neuron by passing current through the same recording KCl electrode (Eccles, 1961), the amplitude and frequency of the miniature potentials increased. In the example given in Fig. 5 the change was seen immediately after application of a negative potential (current of about 8×10^{-8} amp) for 120 sec. The observed frequency increase is probably due to the fact that small miniature potentials previously obscured by the noise level increased to an amplitude that was

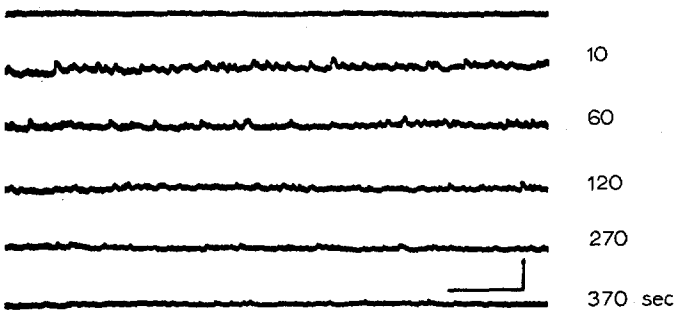

FIGURE 5. Effect of Cl^- injection on frequency and amplitude of miniature potentials. Between the first and second record a 10 v potential (calculated current 8×10^{-8} amp) was applied to the KCl microelectrode for 120 sec. The records shown were obtained at intervals after the current had been turned off. There was an initial depolarization by 10 mv which subsided gradually in parallel with the miniature potentials. Calibration, 1 mv and 500 msec.

readily discernible. The injection of Cl resulted in an immediate depolarization of about 10 mv. Depending on the magnitude and duration of the applied current recovery to the original condition of the potential and the appearance of miniature potentials took place within 3–8 min.

Replacement of 99 % of the chloride ions by propionate ions (leaving the cations unchanged) resulted in an abrupt increase in amplitude of the (depolarizing) miniature potentials as shown in Fig. 6. This usually lasted for several minutes. Partial substitution of propionate for Cl resulted in correspondingly smaller and shorter increases in amplitude and frequency of miniature potentials.

There is evidence, however, that potassium ions also contribute to the miniature potentials; depolarization of the membrane by as much as 30 mv (from the normal resting potential of around 60 mv) caused by increasing the concentration of K ions (up to 45 mM/liter) did not even initially cause reversal of the polarity of miniature potentials even though the amplitude was reduced.

Immediate reversal should have taken place if the miniature potentials were entirely due to increased Cl permeability. As will be shown below, the reversal potential normally is close to the resting potential. In the absence of potassium ions depolarizing miniature potentials were still observed indicating that a change in Cl permeability is predominantly responsible for the polarity and magnitude of miniature potentials. It is interesting that there was no noticeable increase in the frequency of miniature potentials in the high K medium in contrast to the observations of others on several kinds of excitatory synapses.

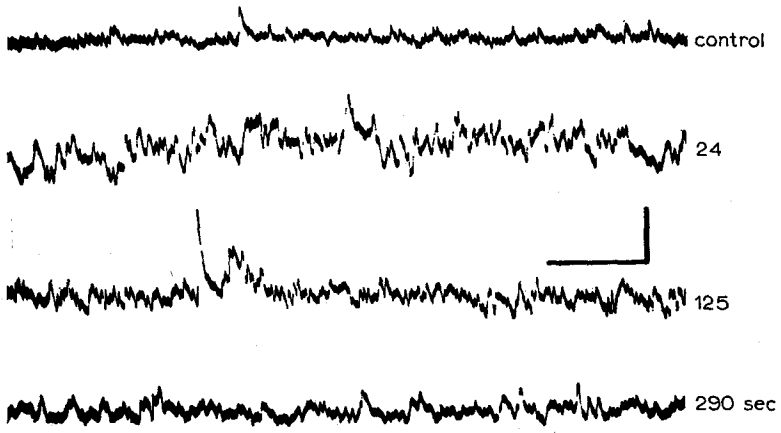

FIGURE 6. Effect of replacing 99% of the Cl by propionate. Four records obtained just before (control) and at intervals after the substitution are shown. Only a negligible depolarization (about 1 mv) occurred in this preparation. Calibration, 1 mv and 1 sec.

Reversal of Miniature Potentials

Addition of tetrodotoxin (10^{-7} g/ml) to the perfusion fluid made it possible without the interference of spike potentials to shift the steady membrane potential by altering the length of the receptor muscles.

When KCl-filled electrodes were used, it was found that a relatively small depolarization was sufficient to cause the miniature potentials to diminish and to reverse. Fig. 7 shows an example in which the miniature potentials appeared inverted during stretch when the neuron was depolarized by 9 mv.

Similar results were obtained when the membrane potential was altered by passing a current (through a bridge circuit or a second electrode). In the case shown in Fig. 8 A, the resting potential was −68 mv. Hyperpolarization to −73 and −78 mv greatly augmented the miniature potentials while depolarization to −66 mv abolished, and depolarization to −59 mv reversed them. The i.p.s.p.'s had a reversal potential similar to that of the miniature potentials, as is shown in Fig. 8 B.

When K citrate electrodes were used, the miniature potentials reversed when the membrane was hyperpolarized by 1–10 mv, depending on the preparation.

Because of the small size of most miniature potentials relative to the noise level, particularly near the reversal potential, it was not possible to establish with certainty their mean amplitude. Therefore, in attempts to determine the reversal potential the largest amplitudes measured at each potential level were selected and plotted against the respective membrane potential. The data obtained with KCl and with K citrate electrodes are shown in Fig. 9. Referred to the resting potential (relaxed preparation, no current applied) reversal always occurred at less than 10 mv depolarization and hyperpolarization, respectively.

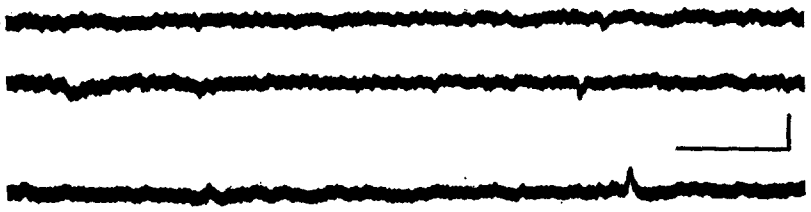

FIGURE 7. Reversal of miniature potentials by stretch depolarization. The lowest record was obtained at the resting potential of a moderately stretched preparation. After stretching a steady depolarization of 9 mv persisted and the miniature potentials appeared inverted, as shown in the upper two records (KCl electrode). Calibration, 1 mv and 1 sec.

Pharmacological Observations

Picrotoxin has been known to block the action of inhibitory synapses of crustaceans and to abolish the ability of the postsynaptic membrane to react to the transmitter substance of the inhibitory neurons (Elliott and Florey, 1956; Grundfest, Reuben, and Rickles, 1959; Robbins and Van der Kloot, 1958).

All the miniature potentials were reversibly abolished by the application of picrotoxin in a concentration of 10^{-6} g/ml. Fig. 10 shows a typical record obtained before, during, and after application of picrotoxin. The drug also blocked the i.p.s.p.'s.

When picrotoxin was applied a small change in membrane potential was often observed. One neuron, for example, which had shown depolarizing miniature potentials (KCl electrode) was hyperpolarized by 1–2.5 mv after application of picrotoxin (10^{-6} g/ml). In another slightly stretched preparation in which only minute miniature potentials could be observed with a citrate electrode, the neuron began to fire spontaneously after application of picrotoxin, indicating some depolarization. Close examination of many records reveals a conspicuous reduction of the apparent noise level during applica-

tion of picrotoxin, indicating that in the absence of the blocking agent numerous almost imperceptible miniature potentials contribute to the normal "noisy" appearance of the trace (Fig. 11). These facts can be explained by assuming a continuous liberation of transmitter from inhibitory nerve ter-

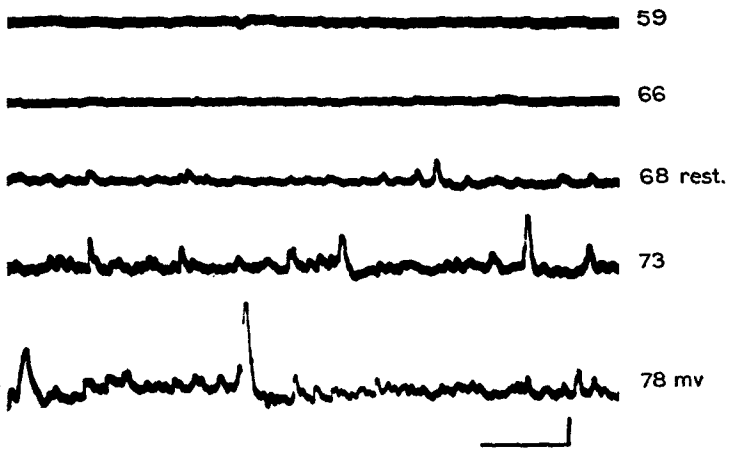

FIGURE 8 A

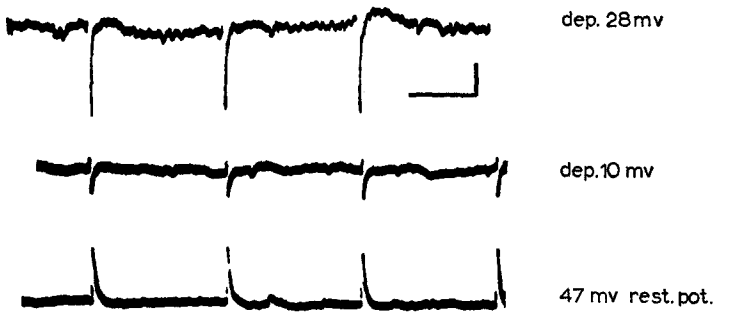

FIGURE 8 B

FIGURE 8. Amplitude changes and reversal of miniature potentials produced by current passing through the recording electrode (A) or through a second intracellular microelectrode (B). Reversal of miniature potentials and i.p.s.p.'s occurred at about the same membrane potential, as shown in (B). Calibrations, (A) 2 mv, 500 msec; (B) 1 mv, 500 msec. The resting potential in (B) is somewhat low, presumably due to the insertion of the second electrode.

minals which would keep the membrane potential close to the equilibrium potential of the ion or ions responsible for the transmitter action. The blocking of this transmitter action by picrotoxin would shift the membrane potential toward its "normal," or uninhibited value. The common experience that the discharge frequency of stretch receptors *in situ* increases slightly when picrotoxin is applied (extracellular recording from the axon) is in line with this

interpretation. Direct evidence for this interpretation was obtained in experiments in which the membrane resistance was measured. The addition of picrotoxin to the perfusion medium (10^{-5} g/ml, pH 7.2) caused a reversible increase of 15–36 % in membrane resistance. A resistance change of 79–89 % (decrease) resulted when GABA (3×10^{-5} g/ml) was applied. The data are summarized in Table I.

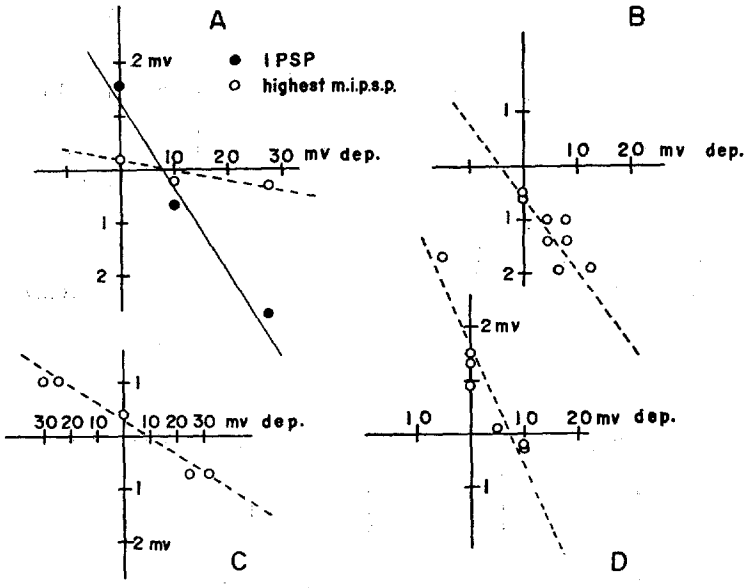

FIGURE 9. Graphic estimation of reversal potential of miniature potentials recorded from different preparations with KCl (A, C, and D) and citrate (B) electrodes. In each series the largest miniature potentials were plotted against the respective membrane potential. In A the amplitude of the i.p.s.p.'s (solid circles) was plotted also. The membrane potential was altered by current passing. The preparation represented by A is the same as that of Fig. 8 B.

GABA has been reported to imitate the action of the natural inhibitory transmitter on stretch receptor neurons and to shift the membrane potential towards the reversal potential of the i.p.s.p.'s (Kuffler and Edwards, 1958; Hagiwara, Kusano, and Saito, 1960). We found that GABA reversibly abolished all miniature potentials and i.p.s.p.'s and that it shifted the membrane potential in the direction of the polarity of the miniature potentials; cells with hyperpolarizing miniature potentials (KCl electrode) were depolarized, cells with hyperpolarizing miniature potentials (K_2SO_4 or K citrate electrodes) were hyperpolarized.

Wiersma, Furshpan, and Florey (1953) reported that acetylcholine excited the stretch receptor neurons of crayfish, that eserine potentiated, and atropine blocked these effects. In experiments on *Pacifastacus* preparations we found

that acetylcholine had very little excitatory action; usually it caused only a transitory increase in the impulse frequency accompanied by a slight transient depolarization (up to 4 mv) when applied in a concentration of 5×10^{-4} g/ml. No detectable change in membrane resistance occurred. Neither acetyl-

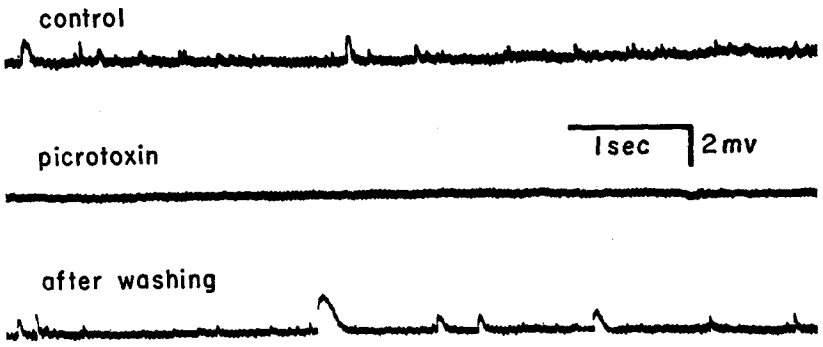

FIGURE 10. Picrotoxin (10^{-5} g/ml) reversibly abolishes the miniature potentials KCl electrode).

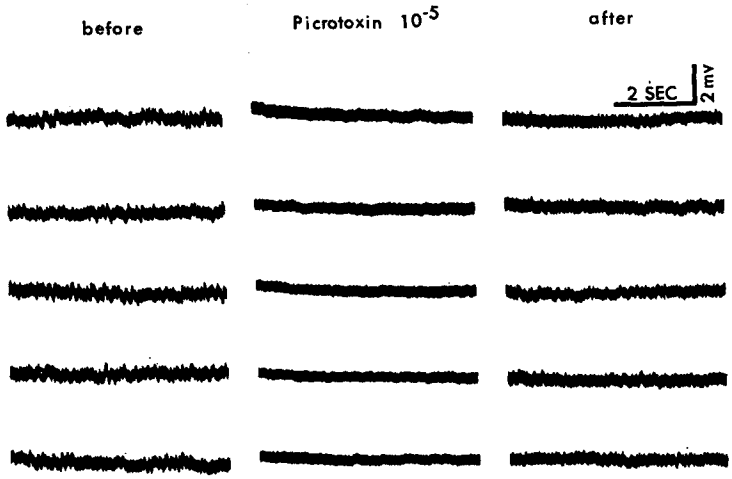

FIGURE 11. Five records showing subsequent segments of oscilloscope traces obtained when the preparation was superfused with control saline, with picrotoxin 10^{-5} g/ml, and again with control saline. Note the great "noise" reduction caused by picrotoxin. The positioning of the traces does not indicate the level of membrane potential. In all cases picrotoxin caused a reversible polarization of between 1 and 2 mv (KCl electrode).

choline (up to 10^{-4} g/ml) nor eserine or atropine (up to 10^{-5} g/ml) had any effect on the frequency, amplitude and interval of miniature potentials. For instance, the mean amplitude and interval of 100 consecutive miniature potentials were 0.57 mv and 0.67 sec before, and 0.54 mv and 0.66 sec several minutes after onset of superfusion with eserine (10^{-5} g/ml).

Tetrodotoxin, an agent extracted from the puffer fish, is known to specifically abolish the sodium conductance change responsible for spike initiation without affecting resting membrane conductance (Nakajima, Iwasaki, and Obata, 1962; Narahashi, Moore, and Scott, 1964). It also does not affect the postsynaptic membrane which reacts with the transmitter substance released

TABLE I

EFFECT OF PICROTOXIN AND OF GABA ON MEMBRANE RESISTANCE AND MEMBRANE POTENTIAL

The resistance was determined by applying hyperpolarizing current pulses of 9 namp (preparation I) and of 3.3 namp (preparation II) with a duration of 1 sec and by measuring the extent of unbalance of the bridge in relation to the value of unbalance appearing upon withdrawal of the electrode (KCl).

Perfusion medium *	Effective membrane resistance	Resistance change	Membrane potential
	MΩ	%‡	mv
Preparation I			
Control	3.3		59.4
Picrotoxin	4.0	+15	59.9
Control	3.7		59.0
GABA	0.4	−89	48.5
Control	3.7		55.0 declining to 52.0
Picrotoxin	4.8	+30	53.0
Control	3.9		51.0
GABA	0.8	−79	44.6
Control	3.6		50.0
Preparation II			
Control	6.9		
Picrotoxin	9.6	+36	
Control	7.2		
Picrotoxin	10.3	+35	
Control	8.1		69.0

* Control = normal saline; picrotoxin: 10^{-5} g/ml; GABA: 3×10^{-5} g/ml. In all cases the pH was 7.2.

‡ Difference from the average value of preceding and following control value.

from nerve terminals (Furukawa, Sasaoka, and Hosoya, 1959; Katz and Miledi, 1966) and does not affect the initiation of the receptor potential of crustacean stretch receptor cells and of Pacinian corpuscles of the cat (Loewenstein, Terzuolo, and Washizu, 1963). Tetrodotoxin applied in concentrations as low as 10^{-7} g/ml abolished spike generation in stretched preparations within a few seconds. Even in higher concentrations (10^{-6} g/ml), however, it did not change the amplitude and frequency of miniature potentials.

DISCUSSION

All evidence indicates that the miniature potentials observed in crayfish stretch receptor neurons are due to the spontaneous release of transmitter substance from *inhibitory* nerve terminals; the ionic mechanism and the pharmacological behavior of miniature potentials and of i.p.s.p.'s appear to be identical and the reversal potential of both i.p.s.p.'s and miniature potentials is close to the resting membrane potential. The insensitivity of the miniature potentials to tetrodotoxin shows that they are not caused by spontaneous firing of branches of the inhibitory neuron. In this respect our findings are similar to those of Katz and Miledi (1966) who have already shown that tetrodotoxin does not affect the miniature potentials arising in amphibian motor end plates.

The experiments with Cl injection and the replacement of external Cl show that the most important ion responsible for the miniature potentials is Cl. The participation of K is likely, however, since depolarization by increased external K concentration does not effect reversal of the miniature potentials as would be expected if Cl were the only contributing ion. Hagiwara et al. (1960) have already found that in addition to the increased permeability to K described by Edwards and Hagiwara (1959) the inhibitory transmitter action involves a change in the permeability to Cl.

Estimates of the number of quanta normally released by a nerve impulse at various types of synapses have been derived by statistical analyses based on the relative size of postsynaptic potentials and miniature potentials. For the mammalian and amphibian motor end plate they range from 100 to 300 quanta (del Castillo and Katz, 1954; Martin, 1955; Boyd and Martin, 1956; Liley, 1956). Burke (1957) estimated that 215–460 quanta are released from the tonic motor terminals on a 1 cm length of slow amphibian muscle fiber. From the data of Dudel and Kuffler a value of about 80 can be derived for the number of quanta released per impulse from the motor terminals on a single muscle fiber of the adductor of crayfish chela.

A lower value of 20 was postulated for calyciform synapses of the chick ciliary ganglion (Martin and Pilar, 1964). In the case of the crayfish stretch receptor neurons in which the transmitter action is *inhibitory* the analysis is difficult because the reversal potential is close to the resting membrane potential so that summation can be expected to be nonlinear, and because the smaller miniature potentials are masked by the noise level so that the amplitude distribution curve does not give a standard distribution (Fig. 3 A). However, the peak of the amplitude distribution occurs at a value not greater than one tenth the amplitude of the largest miniature potential (Fig. 3 A); this corresponds to a ratio of i.p.s.p. to unit miniature potential of about 80 considering that the amplitude ratio of i.p.s.p. to the largest miniature potential

recorded in the same series of records ranged from 3 to 13, with an average of 8 (± 3 SE). Thus *at least* 80 quanta can be expected to be released per nerve impulse from the inhibitory terminals; the actual number must be considerably higher for the reasons already given. Number and distribution of inhibitory nerve terminals over soma and dendrites of the receptor neuron are still unknown so that it is not possible to estimate the number of quanta released from each synaptic site.

Measurement of the quantum content of i.p.s.p.'s by means of external microelectrodes is not possible because the dense connective tissue sheath that surrounds soma and dendrites prevents the electrodes from reaching the immediate vicinity of the synapses.

That the change in membrane conductance produced by picrotoxin is so large was unexpected. Analysis of all the oscilloscope traces obtained before, during, and after picrotoxin application (see Fig. 11) shows, however, that much of what appears to be noise is not electronic noise but noise that can be abolished by treatment of the preparation with picrotoxin. In the light of all the results obtained in our investigation we conclude that this nonelectronic noise is in fact due to the spontaneous release of transmitter from inhibitory nerve terminals and represents, therefore, synaptic noise which is composed of very frequent, nearly imperceptible miniature potentials. Some idea of their normal frequency can be gained by inspection of the bottom trace (Fig. 8 A) obtained from an artificially polarized receptor neuron; the smaller potentials cannot be individually recognized because of the frequent occurrence of summation and superposition. The frequency of the miniature potentials readily explains the magnitude of the conductance change produced by picrotoxin. Even though this spontaneous transmitter release may decrease the normal membrane resistance by as much as 36 % (see p. 675), this still represents only a fraction of the maximal conductance change caused by the application of GABA. In the intact animal the spontaneous release of transmitter may be considerably less. In the course of the dissection the stretch receptor organs are subjected to considerable mechanical stress. This may well be responsible for the intensity of spontaneous transmitter release. Enhancement of miniature potential frequency due to mechanical stress has already been reported by Fatt and Katz (1952) for the amphibian motor end plate.

That the stretch receptor neurons are normally depressed by spontaneously released inhibitory transmitter is perhaps surprising but not unique. Grundfest et al. (1959, Fig. 6, p. 1311) have already shown that picrotoxin causes a noticeable decrease in the membrane conductance of lobster muscle which is also innervated by inhibitory neurons that release a transmitter that is blocked by picrotoxin. Observations on the crayfish heart also led to the speculation that the slight excitation caused by picrotoxin might be due to blocking of spontaneously released transmitter from inhibitory terminals (Florey, 1966).

The possibility that picrotoxin also affects the neuronal membrane directly, closing certain ion channels (or preventing their opening) cannot be excluded.

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