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MEMBRANE-POTENTIAL EFFECTS ON AN INHIBITORY POST-SYNAPTIC CONDUCTANCE IN *APLYSIA* BUCCAL GANGLIA

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SUMMARY

1. Inhibitory post-synaptic currents (i.p.s.c.s) were recorded under voltage clamp using two electrodes placed in neuronal somas of the buccal ganglia of *Aplysia*, in order to study the effects of membrane potential (V_m) on decay time constant (τ).

2. From -175 to -40 mV, τ did not vary with V_m. At V_m more depolarized than -40 mV, τ decreased. Also at depolarized V_m, cell input resistance (R_{in}) decreased and many cells showed non-exponential i.p.s.c. decay, including undershoot. These results suggest that the apparently faster τ is an artifact of remote membrane poorly clamped at the low R_{in} of depolarized levels, rather than a V_m-dependent i.p.s.c. relaxation.

3. Injected current pulses produced voltage relaxations which decayed faster, including undershoot, when V_m was depolarized beyond -40 mV.

4. Step commands across the reversal potential were delivered during i.p.s.c.s. Currents reversed direction, relaxing consistent with the new V_m, thus showing that recorded current decay represents the true time course of i.p.s.c. relaxation, rather than uncontrolled slow axonal charging from a fast remote synaptic current.

5. I conclude that clamp control is poor at depolarized V_m, due to decreased R_{in}, and the faster, non-exponential decay seen includes a superimposed non-synaptic current. Tetraethylammonium injected into the presynaptic neurone produces only slight effect on the i.p.s.c. decay time constant, suggesting that the non-synaptic current is unlikely to be due to a voltage-dependent K conductance.

INTRODUCTION

It is now widely accepted, in spite of earlier beliefs to the contrary, that transmitter-activated synaptic conductance changes are partially influenced by voltage. In particular, the time course of synaptic currents in a variety of preparations has been found to be dependent upon membrane potential.

Voltage effects on synaptic current time course were first observed in recordings of frog end-plate currents (e.p.c.) (Gage & Armstrong, 1968; Kordaš, 1969, 1977; Magleby & Stevens, 1972*a*, *b*) and subsequently recorded at locust neuromuscular junction (Anderson, Cull-Candy & Miledi, 1976), in inhibitory synapses on crayfish muscle (Onódera & Takeuchi, 1976; Dudel, 1977), and at both excitatory and inhibitory synapses of *Aplysia* (Adams, Gage & Hamill, 1976; Ascher, Marty &

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Neild, 1978). In an attempt to find an hypothesis compatible with observed voltage effects on e.p.c. decay, Magleby & Stevens (1972*b*) proposed that the decay reflects the mean lifetime of the open state of a transmitter–receptor complex, and that this lifetime depends upon voltage. Measurements of fluctuations in ACh-induced current (Anderson & Stevens, 1973) and of single-channel currents themselves (Neher & Sakmann, 1976) have supported this hypothesis.

In the previous paper, Gardner & Stevens (1980) analysed the exponential decay of inhibitory post-synaptic currents (i.p.s.c.s) at hyperpolarized membrane potentials in the buccal ganglia of *Aplysia*, and suggested that the rate-limiting step for i.p.s.c. decay, as in the case of frog e.p.c., was a conformational change leading to the closing of open post-synaptic current channels. This analysis was independent of the behaviour of the post-synaptic conductance with changes in membrane potential.

Gardner & Stevens (1974) have also reported that the time constant (τ) of i.p.s.c. decay is invariant with membrane potential over the range -170 to -45 mV. In contrast, Adams *et al.* (1976), studying i.p.s.c. in homologous synapses of *Aplysia juliana* and *A. dactylomela*, reported that the decay time constant depends exponentially on voltage over the range -80 mV to 0 mV. The present study was designed in part to reconcile the two sets of findings.

In this paper, I examine the voltage dependence of the time course of post-synaptic currents. It is found that while i.p.s.c. decay is unaltered by voltage over the range -175 to -40 mV, depolarization beyond -40 mV produces a shortening of the recorded i.p.s.c. Although this effect of membrane potential appears superficially similar to synaptic voltage dependence seen in *Aplysia* (Adams *et al.* 1976) and elsewhere, I suggest that it is the result of non-synaptic, voltage-dependent conductances, seen under these experimental conditions because of imperfect clamping. The data presented are consistent with the hypothesis that the remote synaptic site of the i.p.s.c. is adequately clamped over the range -175 to -40 mV, but under imperfect voltage control at more depolarized values. What appears to be a faster decay of synaptic outward currents is in fact a superimposed non-synaptic inward current. I will consider and reject alternative explanations for the apparent voltage dependence: that it represents electrotonic coupling between neurones or an additional chemical synaptic component.

A short account of these findings has been presented to the Society for Neuroscience (Gardner, 1978).

METHODS

Animal storage, dissection, equipment, experimental protocols and analysis are in general as described in the previous paper. All experiments were performed at room temperature (20 ± 2 °C). Some aspects of the methods are of particular importance for the results described herein. Sea-water solutions were buffered either with Tris (ultra-pure grade, Schwartz-Mann) or with bicarbonate to pH $7{\cdot}8 \pm 0{\cdot}2$. Both non-voltage-dependent decays and voltage-dependent anomalies were seen with each buffer. For determination of the shape of inhibitory post-synaptic current (i.p.s.c.) decay at different voltages, fitted exponentials were used in preference to least-squares linear fits of semilogarithmic plots. Although for a trace which is known to decay exponentially with single time constant a least-squares fit has the advantage of providing a bias-free value for decay time constant, the method of fitting a computer-generated exponential

by eye provides several other important advantages (Beam, 1976). In particular, curves which deviate from exponential decay because the process being measured begins decaying at a faster, or accelerated rate, are most easily recognized when the exponential is superimposed.

Some additional protocols were employed for the experiments described in this paper. (1) To determine the membrane pulse response, short pulses of current were delivered by means of a pseudo-constant current active bridge, to the unclamped membrane of cells, polarized to desired membrane potential levels by means of constant-current injections. The voltage responses to these current pulses were recorded for subsequent analysis. (2) As a test for clamp quality, step commands were delivered to voltage-clamped neurones at various times during post-synaptic current flow, and current decay after the voltage jump was compared to decays of i.p.s.c. recorded at both the initial and final potential levels. To eliminate offset and contributions from non-synaptic currents, the same step commands were delivered in the absence of i.p.s.c.s and these currents subtracted from those recorded during i.p.s.c.s. (3) To try to block some non-synaptic currents in inhibitory follower neurones, tetraethylammonium chloride (TEA; J. T. Baker) was injected ionophoretically into the soma of the post-synaptic neurone by means of the voltage clamp. The current-passing electrode was filled with 10% TEA and the cell was clamped at 40–60 mV above rest for 15 or 20 min. Current for passing TEA varied from 20 to 100 nA. Note that this method differs from that used by Gardner & Stevens (1980) for TEA injection into the presynaptic neurones. For injection of TEA presynaptically, a simple test reveals whether the drug has produced action potential broadening at the presynaptic terminal of interest: if so, the i.p.s.c. peak is broadened and prolonged. For post-synaptic injection, however, no test of this type is available and we cannot state that TEA affected voltage-dependent potassium conductances at the subsynaptic membrane.

RESULTS

When a neurone from the group BL or BR 3, 6, 8, 9, 10, 11 in *Aplysia* buccal ganglia is examined under voltage clamp at membrane potentials around -130 mV, inhibitory post-synaptic currents (i.p.s.c.s) decay exponentially, with single time constant τ average 19 msec. The rate-limiting step determining i.p.s.c. decay is likely to be a conformational change leading to the closing of open synaptic current channels (Gardner & Stevens, 1980). The purpose of this study is to investigate whether i.p.s.c. decay depends upon membrane potential, and to provide an hypothesis to account for its behaviour. I have therefore analysed both i.p.s.c. and allied phenomena recorded over a wide range of membrane potentials, with emphasis upon more depolarized levels.

Behaviour of i.p.s.c. at depolarized levels of membrane potential

I.p.s.c. decay is faster with depolarization. A group of i.p.s.c.s, each recorded in the same cell at a different membrane potential, is shown superimposed in Fig. 1*A*. At all voltages, synaptic currents rise rapidly to a peak and decay more slowly. The i.p.s.c. recorded at this neurone's resting potential appears near the middle of the group, as the fifth trace from the top. At more depolarized clamp potentials, the amplitudes of the outward synaptic currents are larger, while with hyperpolarization the i.p.s.c.s invert to larger and larger inward currents. In order to investigate the dependence of the decay of synaptic currents upon membrane potential, computer-generated exponentials were superimposed on each trace and adjusted by eye for best fit (Beam, 1976; Gardner & Stevens, 1980). Values for the decay time constant (τ) determined in this way for the i.p.s.c.s of Fig. 1*A* are plotted in Fig. 1*B*. For this cell, τ was invariant with membrane potential over the

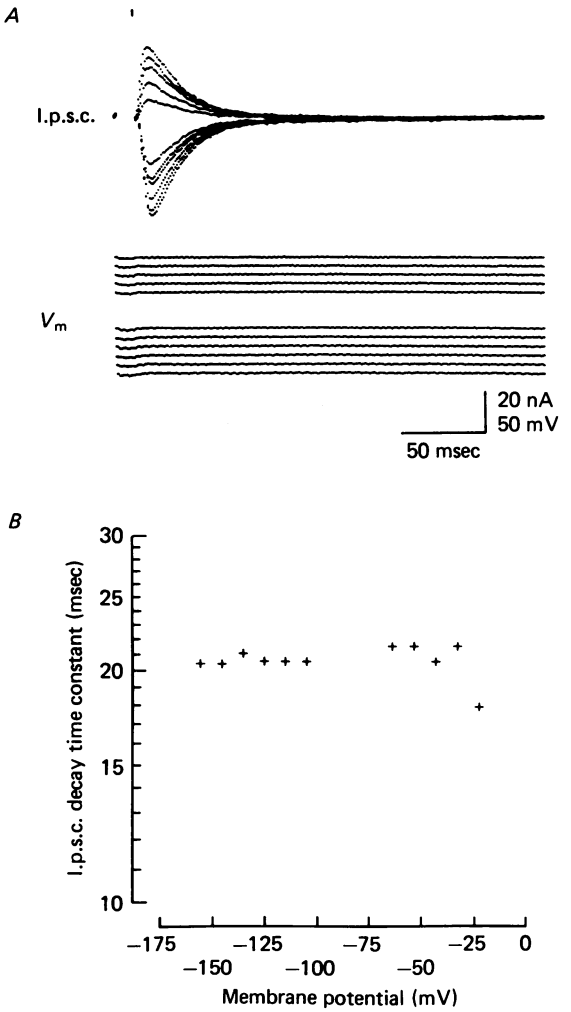

Fig. 1. Inhibitory post-synaptic current (i.p.s.c.) decay as function of membrane potential (V_m). *A*, the upper traces (i.p.s.c.) show post-synaptic currents recorded at the corresponding voltages shown in the lower traces (V_m). Eleven sweeps are superimposed, taken at V_m of (from top to bottom) −19, −29, −39, −49, −59 (resting membrane potential), −99, −109, −119, −129, −139, −149, −159 mV. Downward deflexions of i.p.s.c. indicate inward currents; all current traces have been referred to the same base line to facilitate comparison. The stimulus artifact may be seen as a gap in the i.p.s.c. traces and a slight downward deflexion of V_m. Ganglia bathed in 80 mM-Ca, 144 mM-Mg sea water at 17 °C. *B*, i.p.s.c. decay time constant for each trace from *A* is plotted (+) *vs.* V_m. Only the time constant recorded at −19 mV is measurably different from the other values.

range −29 to 159 mV, but the i.p.s.c. recorded at −19 mV was best fitted with an exponential of 15% faster τ.

This behaviour of voltage-independent τ over a range of 100 mV or more was seen in every cell studied. In some cells τ remained constant at every voltage examined, while in most cells depolarization by 20–40 mV from the resting potential resulted, reversibly, in i.p.s.c.s with faster decay.

Unlike both the standard end-plate model accounting for voltage-dependent conformational changes (Magleby & Stevens, 1972*b*) and observed behaviour of τ with V_m in several preparations (Magleby & Stevens, 1972*a*; Adams *et al.* 1976), i.p.s.c. τ does not depend exponentially on membrane potential (V_m). Instead, in most cells there appears to be a transition potential which separates voltage-independent from voltage-dependent decay (Fig. 1*B*).

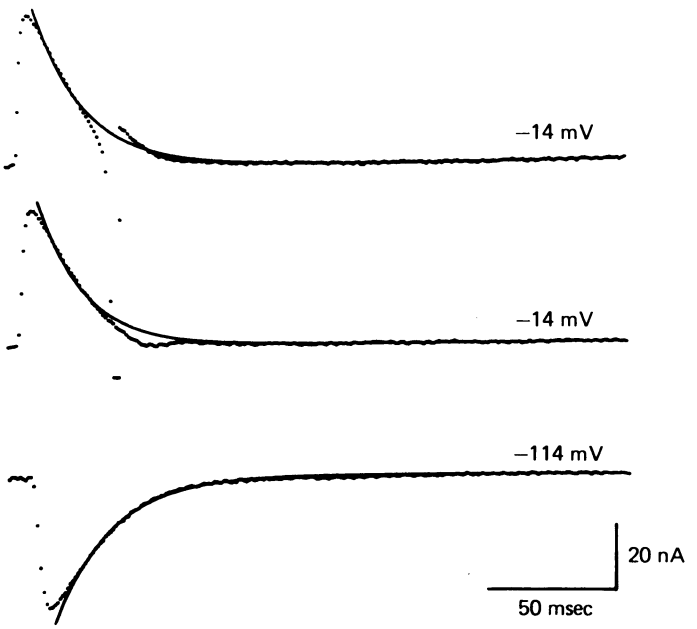

Fig. 2. Anomalous i.p.s.c. decay at depolarized V_m. Three i.p.s.c. traces are shown with superimposed exponentials. Upper and middle trace, $V_m = -14$ mV; lower trace $V_m = -114$ mV. At $V_m = -114$ mV, i.p.s.c. decay is exponential, $\tau = 22{\cdot}7$ msec. At $V_m = -14$ mV, two examples of non-exponential decay are seen. Upper trace: an uncontrolled action potential on the trailing edge of the i.p.s.c. During the uncontrolled spike, the current record briefly crosses the middle trace and saturates the recording amplifier. Middle trace: i.p.s.c. decay is faster as decay progresses and the trace undershoots the base line. Superimposed exponentials for the upper ($\tau = 19{\cdot}5$ msec) and middle ($\tau = 18{\cdot}2$ msec) traces are shown to highlight the degree of deviation of the i.p.s.c.s from exponential decay.

I.p.s.c. decay is non-exponential at depolarized voltages

Most traces recorded at levels more depolarized than the transition voltage not only decayed faster, but decayed non-exponentially: their rate of decay increased during the i.p.s.c. In some cases, one or two types of strikingly anomalous behaviour were seen. One type is characterized by currents which decay rapidly, undershoot the base line, recover, and finally decay more conventionally. In the other type of anomalous decay, the trailing edge of the i.p.s.c. gives rise to the currents of an action potential from uncontrolled remote regions. Examples from a cell in which both of these types of anomalous behaviour were recorded are shown in Fig. 2. Below the transition potential (here, -25 to -35 mV), i.p.s.c. decay is exponential and voltage-independent. For example, the lowermost trace, recorded at -114 mV,

is fitted well by a superimposed exponential. The uppermost and middle traces, each recorded at −14 mV, show an uncontrolled spike and undershoot, respectively. Here, the superimposed exponentials serve to emphasize the deviations from exponential decay. For other i.p.s.c.s which did not deviate greatly from single exponential kinetics, approximate time constants could be measured. However, for any i.p.s.c., decay was considered to be non-exponential if for any 5 msec interval during the tail the recorded current deviated from the best exponential fit, by an amount greater than the peak-to-peak noise.

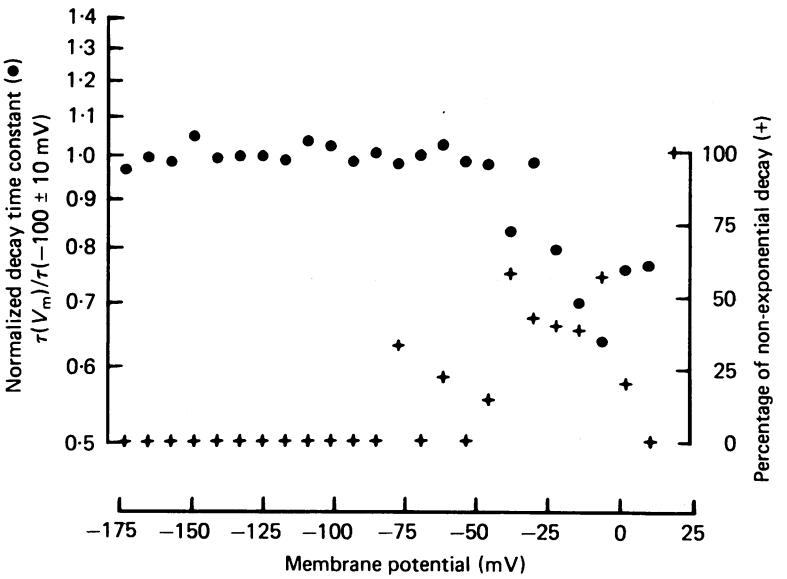

Fig. 3. Dependence of decay on membrane potential. Right-hand ordinate: the average normalized i.p.s.c. decay time constant τ is plotted (●) *vs.* V_m. Time constant is invariant with voltage over the range −180 to −40 mV and declines at more depolarized values. For each of twenty cells, decay time constants for i.p.s.c.s at each of several voltages have been normalized by dividing them by the τ for i.p.s.c.s recorded at membrane potentials between −100 and −120 mV. Each of the resulting normalized τ recorded in each of twenty-five 8 mV wide bins was then averaged and plotted *vs.* V_m. Left-hand ordinate: the percentage of i.p.s.c.s showing non-exponential decay is plotted (+) on the same V_m scale. An increase in the percentage of non-exponential i.p.s.c. decay is seen paralleling the decrease in τ above −40 mV.

In order to summarize changes in apparent decay time constant and occurrences of non-exponential decay, i.p.s.c.s were recorded in each of twenty cells at a variety of membrane potentials. Fig. 3 plots averaged normalized decay time constants *vs.* membrane potentials for i.p.s.c.s showing exponential decay. For these summary data, the transition voltage was −40 to −25 mV; τ was invariant below this potential and declined above it. On the same voltage scale the percentage of non-exponential decays at that voltage is indicated. Above the transition voltage, roughly half the i.p.s.c.s do not decay exponentially. Undershooting synaptic currents, or those giving rise to action potentials, were usually seen with considerable depolarization. However, below the transition voltage, in the range of voltage-

invariant τ, virtually all i.p.s.c.s decay exponentially. The small number of i.p.s.c.s with non-exponential decay in the range -80 to -50 mV may represent noise or additional synaptic input contaminating the decay of currents which are recorded within 15 mV of their reversal potential and consequently are of low amplitude.

Synaptic peak conductance decreases at depolarized voltages. The time course of decay is not the only measurable parameter of the inhibitory synaptic conductance which can be examined as a function of voltage. No gross changes in i.p.s.c. rise time are seen with changing clamp potential, but the sampling rate and filtering used to permit recording the whole extent of the decay tail do not allow these data to be used for accurate determination of rise time kinetics. Peak post-synaptic current, however, may be measured reliably and its dependence upon voltage determined. Gardner & Stevens (1980) found the peak i.p.s.c. to vary linearly with clamp potential over the range -175 to -45 mV. At any membrane potential, the measured peak current may be compared to the expected peak current, calculated as the product of the peak conductance determined from the slope of the I–V curve for the range -175 to -45 mV, and the voltage displacement from equilibrium. These differences were calculated for i.p.s.c.s recorded in twenty-three cells, normalized so that each cell would contribute equally to the average, regardless of the actual value of its peak conductance. The results of this procedure are plotted in Fig. 4 as a function of voltage. A horizontal line at average deviation = 0 would thus represent a voltage-independent peak conductance, while upward or downward deviations would indicate increases or decreases in peak conductance at the indicated voltages. Fig. 4 shows that, like decay τ, peak conductance is not invariant with voltage over the range studied, but is instead constant below a transition voltage, and voltage-dependent above. The transition voltage above which peak conductance decreases is -30 to -40 mV. The slope of the curve in Fig. 4 is biased by selection of the voltage range used to obtain peak conductances by least-squares fit. Attempts to obtain least-squares fits to greater ranges, including values above the transition voltage, would produce smaller calculated peak conductances. The shape of the curve in Fig. 4 would be unaltered, but would be rotated slightly anti-clockwise. Although it is possible that the I–V relation is non-linear at voltages less than -40 mV, the similarity of the transition voltages for decay and for peak conductance suggests a common mechanism.

Mechanisms accounting for i.p.s.c. behaviour at depolarized levels

The existence of the transition voltage and the possibility of non-exponential decay suggested that *observed* post-synaptic current decay at depolarized levels might not be determined by the same processes that Gardner & Stevens (1980) identified as accounting for decay at more hyperpolarized levels of membrane potential. Since decay at depolarized levels is non-exponential, it is unlikely to represent a relaxation process of independent channels, which was shown to be the process determining i.p.s.c. decay time course at hyperpolarized levels (Gardner & Stevens, 1980). Moreover, peak conductance, which also shows anomalous behaviour with depolarization, depends not upon channel closing, but upon the rate of channel opening (Dionne & Stevens, 1976). Both opening and closing processes would have to be assumed anomalously voltage-dependent, with similar transition voltages.

The data suggest that recorded i.p.s.c. decay is not determined by the same process at all voltages studied. Decay at depolarized voltages appears to reflect not only the closing rate of open post-synaptic current channels, but also an additional process, which would have the effect of speeding-up, or appearing to speed-up,

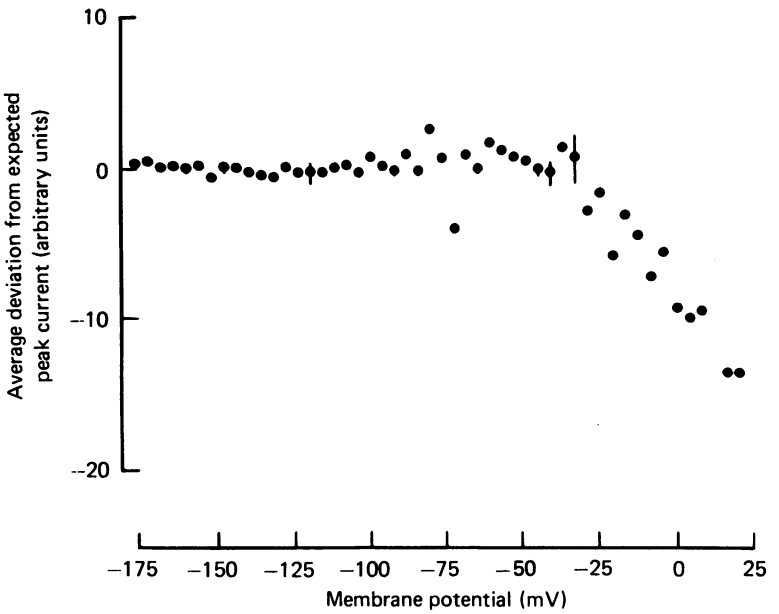

Fig. 4. Dependence of peak conductance on membrane potential. The average deviation of peak current from that expected on the basis of calculated synaptic peak conductance is plotted (●) *vs.* V_m. Peak conductance is constant over the range −30 to −180 mV and declines at more depolarized values. For each of twenty-three cells peak conductance was calculated by the method of Gardner & Stevens (1980). For each i.p.s.c. recorded in these cells the difference between observed peak current and calculated peak current assuming constant G_{peak} was normalized. Each of the resulting deviations: $(I_{peak} - G_{peak} \cdot V_m)/G_{peak}$) (1 nA/mV) recorded in each of fifty 4 mV wide bins was averaged and plotted *vs.* V_m. Each point (●) represents the average of four or five measurements; the absence of S.E. bars for a reading indicates either that the S.E. was smaller than the plotting symbol or else that a given point is the average of only two values. The arbitrary units on the ordinate would represent nA deviations in peak current for a cell with G_{peak} = 1 μmho.

decay. This additional process is likely to belong to one of three classes of phenomena: (1) artifacts of data recording, (2) additional synaptic mechanisms, such as electrotonic coupling or a multi-component synaptic potential; distributed synapses, composed of both electrotonically near and electrotonically distant elements, would also fall into this class, or (3) non-synaptic mechanisms, such as voltage- or ion-sensitive currents, activated or triggered by post-synaptic events.

Equipment artifacts are unlikely to be responsible for the apparent shortening of i.p.s.c.s. To prevent overloading amplifiers and filters, steady holding currents needed to clamp the cell membrane are backed out by means of a battery and variable resistor. At all holding potentials, therefore, the output of the current

monitor is close to the zero level, and at no stage in the current recording chain does the magnitude of any signal approach the maximum signal limit for any equipment. Apparent i.p.s.c. shortening could be due to a polarization artifact from an imperfectly reversible chlorided silver wire used for chamber ground, but this or any other explanation involving an equipment artifact does not account for the ability of the shortened i.p.s.c. to give rise to an action potential.

Similarly, a synaptic mechanism is unlikely to lead to i.p.s.c. shortening at depolarized voltages. Either electrotonic coupling or an additional chemical synaptic component with different reversal potential would be recordable at the i.p.s.c. reversal potential of -66 ± 4 mV (S.E.; $n = 19$), but no current is seen. I cannot exclude the possibility that current flow due to an electrotonic synapse might be insignificant at -66 mV but considerably larger at depolarized voltages because of lowered cell input resistance. Even though such a mechanism is plausible, it is unlikely to be responsible for i.p.s.c. shortening, as this would require inward currents. During i.p.s.c. decay, the presynaptic neurone is producing its spike after-potential, and electrotonic current flow from the presynaptic neurone would therefore be outward.

Another case to be considered is of a possible additional synaptic component which would produce a late conductance *decrease* to Cl. At depolarized levels, this conductance might produce net inward currents which could account for both an apparent decrease in synaptic peak conductance and an apparently faster decay, including overshoot. However, unless this postulated conductance decrease were itself voltage-dependent, it should produce, at hyperpolarized voltages, a net outward current which would appear to shorten i.p.s.c. duration and reduce peak conductance. This effect is not seen. It is therefore unlikely that synaptic mechanisms are responsible for the anomalous behaviour of i.p.s.c.s at depolarized voltages.

Finally, the possibility remains that some non-synaptic property of the neurone affects the apparent decay of i.p.s.c.s. Experiments which investigate non-synaptic membrane properties are described below.

Behaviour of non-synaptic membrane at depolarized levels of membrane potential

Under voltage clamp, quasi steady-state I–V curves were obtained in resting neurones, without stimulating presynaptic cells. The currents measured thus include those flowing through passive channels and voltage-dependent conductances, but do not include any post-synaptic currents. In all cases, cell input resistance was high and more or less constant over a voltage range of -175 to -45 mV. At voltages more depolarized than -28 ± 4 mV ($n = 8$), cell input resistance fell, due to activation of conventional voltage-dependent conductances. These conductances are turned on at voltages which agree roughly with the transition voltage above which i.p.s.c. decay changes, suggesting the possibility that axon cable properties above this voltage might be altered sufficiently to decrease the effectiveness of voltage control of a distant synapse.

In another series of experiments, unclamped neurones were perturbed with a brief (2–5 msec) current pulse and the resulting changes in membrane potential were recorded. Fig. 5 shows both current pulses and resulting voltage traces for

a cell polarized to each of two voltages by steady injected current. Near the resting potential, and at hyperpolarized levels, V_m shows smooth decay to base line. However, when a current pulse was injected into a cell depolarized more than 20 mV from rest the response was more complex. Decay was significantly faster (Fig. 5*B*), and in five of eight cells included substantial undershoot. In four out of eight cells,

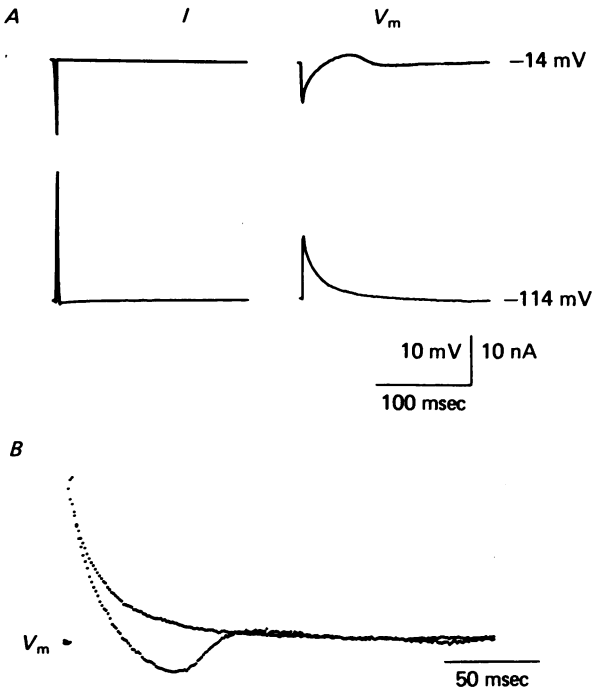

Fig. 5. Membrane pulse response at two values of V_m. *A*, the right-hand traces show the unclamped membrane voltage response (V_m) to the short-duration current pulse (I) shown in the left-hand traces. Top traces recorded with $V_m = -14$ mV, bottom traces at -114 mV. The membrane response at -114 mV shows smooth decay while that at -14 mV displays more complex behaviour, including undershoot. *B*, the two current traces from *A* have been superimposed to allow comparison of decay phases. The -14 mV trace has been inverted and its amplitude adjusted to allow superimposition of peaks.

the trailing edge of the hyperpolarizing response produced action potentials. Both the i.p.s.c. and the voltage response of the membrane to an outward current step thus share several properties. For a wide range of membrane potentials more hyperpolarized than a transition potential, both decay smoothly without undershoot. At depolarized levels, both decay faster than at hyperpolarized levels, both show undershoot and both can give rise to action potentials on their trailing edges. The transition voltage separating these two types of behaviour is close to the inflexion point on the neuronal steady-state $I-V$ curve where voltage-dependent conductances increase.

I.p.s.c. decay at hyperpolarized voltages is not an artifact of axonal charging. The similarity between synaptic and non-synaptic properties could be explained by

poor clamping of the synapse not only at hyperpolarized voltages, but also at any voltage. Gardner & Stevens (1980) showed theoretically that the subsynaptic membrane is likely to be a small fraction of a length constant distant from the soma even for transient voltage changes of the speed of i.p.s.c. decay. If this theoretical analysis were in error, and the synapse sufficiently remote, then fast transient events

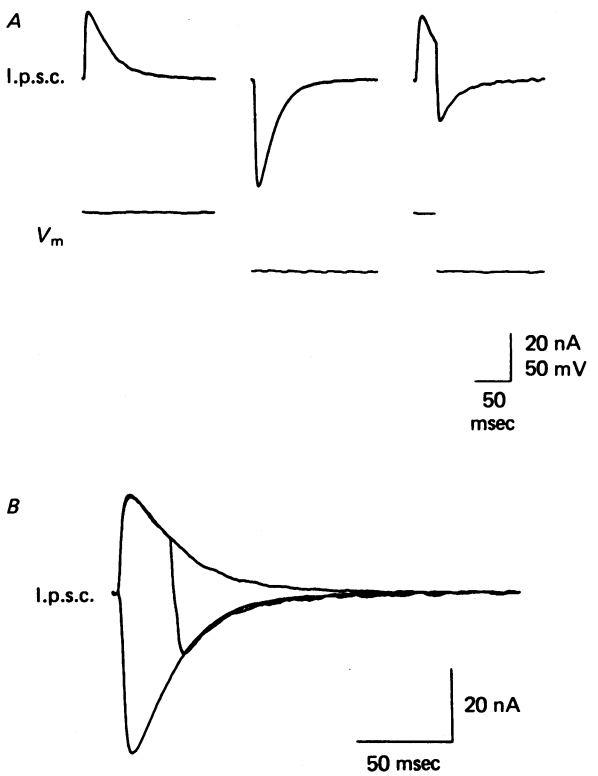

Fig. 6. Voltage jumps during i.p.s.c. *A*, three i.p.s.c.s are shown, each recorded with a different V_m protocol. Left-hand traces, i.p.s.c. recorded at $V_m = -46$ mV. Middle traces, i.p.s.c. recorded at $V_m = -106$ mV, beyond reversal potential. Right-hand traces, during the i.p.s.c., V_m was jumped from -46 to -106 mV. After the jump, the i.p.s.c. decays in a direction consistent with the new value of V_m. Currents recorded during an identical jump in the absence of any i.p.s.c. were subtracted from the i.p.s.c. recorded during the jump in order to eliminate base-line offsets and non-synaptic charging currents. Each current trace is the average of four i.p.s.c.s. *B*, the three i.p.s.c.s from *A* are superimposed. After the jump, i.p.s.c. decay is identical to that of the i.p.s.c. recorded entirely at the new membrane potential.

at the subsynaptic site might not be under adequate control. At any voltage, then, the recorded clamp current would represent current needed to charge axonal membrane between synapse and soma because its potential had been changed by the poorly controlled i.p.s.p. Since at hyperpolarized voltages the i.p.s.c. was shown to decay exponentially with a single time constant (Gardner & Stevens, 1980), one process is likely to be responsible for decay. If axonal charging of the intervening membrane between soma and synapse determines apparent i.p.s.c. time course, the

actual duration of the synaptic conductance must be much shorter than the time to charge membrane, and the single exponential decay would represent slow axonal charging in response to a synaptic current transient. If however the synapse is well clamped over the range -175 to -45 mV, axonal charging time will be much faster than the duration of the synaptic current, and current flow through the synapse will determine the time course of the current measured at the soma. The two hypotheses are thus differentiated by the relative speeds of synaptic current flow and axonal charging.

In order to distinguish between these two hypotheses, the membrane potential of the post-synaptic cell was stepped across the reversal potential during i.p.s.c.s. Both initial and final values of V_m were more hyperpolarized than the transition voltage. The remote synapse hypothesis would require actual synaptic current flow across the subsynaptic membrane to have terminated early in the recorded decay, so that subsequent voltage jumps to the other side of the reversal potential would not reverse current flow or alter subsequent axonal charging. Fig. 6 shows the result of such an experiment. The left-hand and middle traces of Fig. 6*A* show i.p.s.c.s recorded above and below the reversal potential. When the potential is stepped during the i.p.s.c., as the right-hand traces show, the current reverses and subsequent decay is consonant with the new value of potential, indicating that post-synaptic current is still flowing at that point in the decay.

Fig. 6*B* superimposes the three current traces from Fig. 6*A* in order to show the adequacy of the clamp: within 5–10 msec of the voltage step the synaptic current has settled and is indistinguishable from the current tail of the i.p.s.c. that was clamped at this voltage for its entire duration. The same rapid adjustment, producing currents consistent with post-step voltages, was seen in each of five neurones studied, not only for jumps across the reversal potential but also for jumps in which the reversal potential was the initial or final value. These results demonstrate that the i.p.s.c. is adequately clamped at voltages more hyperpolarized than the transition voltage, and that the recorded i.p.s.c. at these voltages accurately reveals the time course of the synaptic conductance.

Recorded i.p.s.c. decay at depolarized voltages is modified by a non-synaptic conductance

The hypothesis remaining to account for i.p.s.c. behaviour at depolarized voltages may be stated in four parts: (1) In cells depolarized past a transition voltage, lowered axonal membrane resistance increases the electrotonic distance between synapse and soma. At this increased distance the i.p.s.p. is incompletely clamped. (2) The incompletely clamped i.p.s.p. slightly hyperpolarizes the post-synaptic cell at the synaptic site. (3) This hyperpolarization alters voltage-dependent non-synaptic conductances. (4) Current flow recorded in the soma is the sum of current due to these non-synaptic conductances and the synaptic current. Contributions of non-synaptic currents of the right magnitude and direction can produce either apparent i.p.s.c. shortening or, if large enough, apparent undershoot.

In order to account for the results I have presented, the non-synaptic current must be either an inward current activated, or else an outward current turned off, by hyperpolarization in a neurone which has been depolarized above a certain

voltage. Two likely candidates include the voltage-dependent potassium current, I_K, which would be turned off by hyperpolarization, or else an inward Na or Ca current, which hyperpolarization would increase by removal of inactivation. In either case the transition voltage would be related to the neuronal threshold for firing. In order to distinguish between these two possibilities, TEA was injected into the post-synaptic cell in an attempt to block I_K at the synaptic site. Although soma K currents were reduced, results at the synapse were mixed. Of ten cells injected, three showed slightly longer decay time constants after TEA; one was shorter. An effect of TEA abolishing i.p.s.c. shortening, or shifting the transition voltage to a more depolarized level, would argue in favour of I_K as the voltage-dependent current responsible. However, in seven of the ten cells, no effect on transition voltage was seen. In two cells the transition voltage was shifted in the depolarizing direction, while in one other the shift was hyperpolarizing. This lack of a clear modification by TEA coupled with the action potential generation seen, and presumably induced by the same current, argues that an increased inward current, produced by the removal of inactivation, is responsible for the apparent shortening of synaptic current duration at depolarized voltage.

DISCUSSION

The synaptic conductance change is likely to be voltage-insensitive

These data, from inhibitory synapses in *Aplysia* buccal ganglia, present a counter-example to recent findings of voltage sensitivity of a number of synaptic conductances. The results show no voltage dependence of either the peak value or the time constant of decay of the i.p.s.c. over a membrane potential range of -175 to -45 mV. At more depolarized voltages, i.p.s.c. time course appears to shorten, but the non-exponential decay and overshoot seen in the decay phase permit ascribing the shortening to a non-synaptic mechanism. The i.p.s.c. is assumed to be under poor control at depolarized voltages due to a fall in the cell's input resistance. The resulting synaptic hyperpolarization is assumed to influence a voltage-dependent non-synaptic conductance which adds transient inward current to the synaptic outward current recorded.

Several findings speak against a voltage effect on synaptic channel lifetime as the mechanism responsible for shortening i.p.s.c. duration. These include (1) voltage independence over a wide range, with shortening seen only above a transition voltage, (2) non-exponential decay of i.p.s.c.s, with the decay phase undershooting and even giving rise to action potentials, (3) an inconsistency in the responses to depolarization in different cells and (4) a non-synaptic membrane response to injected current which closely parallels i.p.s.c. behaviour. Hypotheses ascribing apparent decay shortening to an electrotonic or additional chemical synaptic component, or to an artifact of the membrane time constant or the clamp equipment, were also considered to be less likely than the non-synaptic current activation explanation.

These findings, then, do not invoke the direct membrane potential effect on synaptic current lifetime which has been a finding characteristic of several recent studies (Kordaš, 1969; Magleby & Stevens, 1972*a*, *b*; Anderson *et al.* 1976; Onodera

& Takeuchi, 1976; Adams *et al.* 1976; Dudel, 1977; Ascher *et al.* 1978). Since Gardner & Stevens (1980) identified the rate-limiting step for synaptic current decay as a conformational change leading to the closing of open synaptic channels, the lack of voltage dependence suggests that the conformational change is not influenced by membrane potential. If the conformational change is assumed to involve movement of a protein or other charged molecule, the change would be uninfluenced by membrane potential only if the dot-product of the electric field vector and the dipole did not change during movement (Jackson, 1962). Examples of permissible changes would be motion of a molecule such that its dipole moment vector either shifted without rotation in the membrane, or rotated without changing the angle it makes with a line drawn normal to the membrane. Movement of this sort, and its consequent lack of sensitivity to membrane potential, is entirely plausible behaviour for a membrane component. I cannot, however, exclude the possibility that i.p.s.c. decay time constant is in fact voltage-sensitive. A shift in dipole moment of about 10 debye could produce a 25% decrease in decay time constant over a 150 mV span; such a decrease would be too small to detect because it would be masked by the greater apparent shortening produced by the mechanism described above. Measurements of ACh current noise at depolarized voltages could reveal if i.p.s.c. average lifetime shortened, but noise was not recorded at these voltages, as the amplitude of fluctuations of non-synaptic currents is larger than the ACh fluctuations. At present, therefore, there is no evidence that the rate-limiting step for *Aplysia* i.p.s.c. decay is voltage-sensitive.

From the data, it is not possible to tell whether the decrease in synaptic peak conductance with depolarization is due to inward non-synaptic currents obscuring the true peak, or whether there exists a true voltage-dependent synaptic mechanism affecting peak conductance. Such a mechanism could be a voltage-dependent channel opening rate (Dionne & Stevens, 1975), rectification in open channels, or a decreased ACh concentration due to electrically induced ACh movement at depolarized voltages. All three possibilities are compatible with a channel-closing rate-limiting step.

Comparisons to other Aplysia synaptic conductances

Two recent investigations of synaptic currents in *Aplysia* ganglia have provided evidence for synaptic conductance changes which are voltage dependent, unlike the one I have reported (Adams *et al.* 1976; Ascher *et al.* 1978). Comparison of my results with those of Adams *et al.* (1976) is especially significant, because both studies were carried out at inhibitory synapses of *Aplysia* buccal ganglia. They reported an average 23% decrease in i.p.s.c. decay time constant when membrane potential was shifted from -40 mV to 0 mV, and calculated from this value a dipole moment change of 39 debye. Contrasting their results with Gardner & Stevens' (1974) early brief report of no voltage dependence over a 120 mV range, a finding that this paper confirms, they suggested the differences might be ascribable to different cells. They recorded from neurones of *A. dactylomela* and *A. juliana*, rather than *A. californica*, so differences in cells may indeed account for the contrasting results.

However, other differences in technique, besides species selection and voltage range, may be responsible. Adams *et al.* (1976) did not evoke i.p.s.c.s by intracellular

stimulation of identified presynaptic neurones, but relied on spontaneously appearing i.p.s.c.s or those evoked by shocks to peripheral nerve trunks (D. J. Adams, personal communication). However, large i.p.s.p.s are seen in these buccal neurones, which are not produced by activity in identified interneurones (Gardner, 1971). These synaptic events may have different properties from those reported here, including voltage dependence. Evoking i.p.s.c.s by nerve stimulation in these ganglia is also likely to activate both ispsilateral interneurones, in addition to other cells, producing compound synaptic events in follower cells. A final difference in technique involves bathing solution. Adams *et al.* (1976) bathed their preparation in sea water containing 11 mM-Ca, while I used 60 mM-Ca in order to increase synaptic conductance and quiet spontaneous activity, and the increased Ca concentration may affect the mechanism for voltage dependence.

In abdominal and buccal neurones of *Aplysia*, Pellmar & Wilson (1977) have reported an excitatory synaptic response to serotonin ionophoresis which is affected by voltage in an unconventional manner parallel to the one I have described. They find an excitatory post-synaptic conductance increase whose amplitude and time course appear roughly constant from -90 to -40 mV. At more depolarized voltages, they see an increased amplitude and a prolongation and change of shape of the synaptic response. At the same time, they record an apparent conductance decrease which they say could be accounted for by synaptic activation of a voltage-sensitive Na conductance. Over the same range of membrane potential the inhibitory cholinergic synaptic response I have described shows parallel but reversed behaviour: shortened rather than prolonged decay time and smaller rather than larger peak conductance. Since the response I have described is also inhibitory rather than excitatory, it is possible that a common mechanism is being altered in opposite ways by the two types of synapses.

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