QUANTAL ANALYSIS OF TRANSMITTER
RELEASE AT AN INHIBITORY SYNAPSE IN THE CENTRAL
NERVOUS SYSTEM OF THE LEECH

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SUMMARY

The quantal nature of transmitter release has been analysed at central inhibitory synapses in the leech nervous system between an interneurone (HN) and a motoneurone (HE) that regulate the heartbeat.

1. Ganglia were bathed in leech Ringer fluid containing 20 mM-Mg and 1·8 mM-Ca and the membrane of the presynaptic HN interneurone was hyperpolarized by current injection. Under these conditions successive inhibitory potentials in the HE motoneurone, evoked by impulses in the HN interneurone, showed striking fluctuations in amplitude.

2. Assuming a Poisson distribution of the i.p.s.p.s and estimating the number of failures from the amplitude histograms of the observed responses, the mean size of the quantal unit was estimated as $0·25 \pm 0·015$ mV (s.e. of mean, $n = 26$). When m, the mean number of quanta released per trial, was varied by changing the membrane potential of the presynaptic HN cell (Nicholls & Wallace, 1978), the experimentally observed amplitude distributions could be predicted by the Poisson theory.

3. An independent estimate of the unit size was obtained by noise analysis. A long subthreshold depolarizing pulse applied to the presynaptic HN interneurone evoked a sustained hyperpolarization of the HE motoneurone, apparently caused by an increase in the rate of on-going release of quanta by the HN cell terminals. From the mean change in membrane potential and the increase in variance, the size of the unit was calculated as $0·21 \pm 0·039$ mV (s.e. of mean, $n = 11$). For ten pairs of cells an estimate of unit amplitude was made both from the Poisson analysis and the analysis of variance, again with good agreement. For these cells, the estimated unit sizes were $0·24 \pm 0·023$ mV (s.e. of mean, $n = 10$) from the failures and $0·21 \pm 0·043$ mV (s.e. of mean, $n = 10$) from the noise.

4. A similar analysis was made of the inhibitory synaptic potentials evoked in one HN interneurone by stimulation of its contralateral homologue. Transmission again appeared to be quantal; the mean unit amplitude from Poisson analysis was $0·31 \pm 0·022$ mV (s.e. of mean, $n = 19$) and from the noise $0·29 \pm 0·027$ mV (s.e. of mean, $n = 3$).

5. We conclude that transmitter is released from the terminals of the HN interneurone in quantal units that evoke miniature i.p.s.p.s of about 0·25 mV in the

post-synaptic cells. Furthermore, modulation of transmission produced by variation in the presynaptic resting potential and during presynaptic inhibition results from changes in the mean number of quanta released by each impulse.

INTRODUCTION

In the preceding paper (Nicholls & Wallace, 1978) we described the modulation of transmission at inhibitory synapses in the central nervous system of the leech. As first shown by Thompson & Stent (1976*a*, *b*, *c*), an action potential in the heart interneurone (HN cell) gives rise to a direct, chemical inhibitory synaptic potential in the heart excitor motoneurone (HE cell) and also in the contralateral HN cell. The amplitude of the inhibitory synaptic potential depends on the resting potential of the presynaptic HN cell; sustained hyperpolarization reduces the size of an inhibitory synaptic potential evoked by a superimposed spike; sustained depolarization increases the i.p.s.p. amplitude. One aim of the present investigation has been to determine if the origin of this effect is pre- or post-synaptic. Because of the complex structure of the neuropile and our ignorance of the identity of the transmitter released by the HN cell, direct determination of the sensitivity of the post-synaptic membrane is not feasible. However, quantal analysis could provide evidence for presynaptic changes. Thus, if a fixed quantum or unit could be defined which then remained constant as the synaptic potential became larger or smaller, one could infer that modulation was presynaptic.

At the neuromuscular junction the process of chemical transmission has been investigated extensively and the quantal composition of the end plate potential is well understood (Del Castillo & Katz, 1954). Attempts have also been made to extend such an analysis to synapses in the central nervous system of the cat (Kuno, 1964, 1971; Edwards, Redman & Walmsley, 1976*a*, *b*), the hatchet fish (Bennett, Model & Highstein, 1976), and *Aplysia* (Kandel, Brunelli, Byrne & Castellucci, 1976). Although in these investigations miniature potentials and quantal fluctuations were observed, two factors complicated the analysis. First, the post-synaptic cell was constantly being bombarded by spontaneously occurring synaptic potentials from many inputs, perhaps from hundreds or thousands. This background activity made the recognition of failures, essential to Poisson analysis, very difficult. Secondly, no independent measure of the size of the quantal unit was available, as there was no definitive method for distinguishing quantal units released spontaneously by the presynaptic cell of interest from those released by other unknown inputs.

In the experiments reported here, we have determined the quantal content of the inhibitory potentials evoked by stimulation of an HN interneurone in two different post-synaptic cells: the HE motoneurone and the contralateral HN interneurone. A subjective discrimination between small signals and background activity was avoided by determining the number of failures from the amplitude histogram of all responses. To obtain an independent measure of the quantal size we were able to increase the rate of spontaneous release of quanta from the presynaptic HN interneurone by depolarizing it. As a result, the post-synaptic HE cell became hyperpolarized and the 'noise' fluctuations of voltage increased. From noise analysis (Katz & Miledi, 1972) it was possible to calculate the amplitude of the unitary event

and then to test the accuracy with which this 'quantum' could be used to describe the distribution of synaptic potentials seen with repeated stimulation. The results showed that modulation of transmission, brought about by variations in presynaptic resting potential, is due to changes in the mean number of quanta released by each impulse.

METHODS

Experiments were done on the medicinal leech, *Hirudo medicinalis*, at room temperature (20–25 °C). The experimental arrangement, recording techniques and Ringer fluid were as described in the preceding paper (Nicholls & Wallace, 1978). Except as otherwise indicated, all experiments were made on the third free segmental ganglion, in which the HN interneurone makes a direct synaptic connexion with the HE motoneurone (Thompson & Stent, 1976*a*, *b*).

For measuring evoked synaptic potentials the membrane potential of the post-synaptic cell was recorded via a high-gain amplifier, in most cases AC coupled ($\tau = 0.09$ sec) and through a low-pass filter ($\tau = 1.6$ msec) to reduce high frequency noise. Responses were measured by projecting photographed oscilloscope traces on to graph paper. The variance of the membrane potential was estimated by recording the high-gain trace on moving film, tracing appropriate regions of approximately 1 sec duration on graph paper and measuring the variance by hand at 2 msec intervals. The mean membrane potential was determined from a parallel DC channel recorded at relatively low gain.

I.p.s.p. amplitude was measured as the difference between the potential at two fixed times after the peak of the presynaptic spike: the point of minimum latency and the peak of the averaged i.p.s.p. (see Fig. 1). Synaptic potentials were measured to the nearest 25 μV and only runs in which the mean amplitude of the evoked responses remained constant were accepted. In plotting amplitude histograms the experimental values were three-point averaged and, for convenience, the sign reversed so that hyperpolarizing responses appear to the right along the abscissa and depolarizing responses appear to the left. Poisson distributions were drawn with the aid of a computer program (kindly provided by Dr T. D. Lamb) that included in the theoretical curves the contribution of both base line noise and scatter in the unit size (also see Edwards *et al.* 1976).

Ringer Fluid

Unless otherwise stated, the Ringer fluid used for these experiments contained (mM): NaCl, 85; MgCl$_2$, 20; KCl, 4; CaCl$_2$, 1.8; Tris maleate neutralized with NaOH, 10; glucose, 13.5. Low Na Ringer fluid had the following composition (mM): sucrose, 200; KCl, 4; CaCl$_2$, 7.5; Tris maleate neutralized with NaOH, 10; glucose, 12.

RESULTS

Fluctuations in evoked release from HN interneurone to HE motoneurone

In normal leech Ringer fluid the HN interneurone fires rhythmical bursts of impulses which give rise to large i.p.s.p.s in the HE motoneurone. If the fluid is changed to Ringer containing 20 mM-Mg, 1.8 mM-Ca, the membrane potential of the HN cell no longer undergoes spontaneous oscillations and can be held at any 'resting' potential by passing current through the recording micro-electrode. As described in the preceding paper, the size of the synaptic potential in the HE cell evoked by an action potential in the HN interneurone depends on this resting membrane potential. In the present experiments the HN cell was stimulated once every 2 sec by brief depolarizing pulses. These impulses were superimposed on a steady hyperpolarizing current that was adjusted so that many of the impulses in HN did not appear to evoke any response in HE. Under such conditions there were marked fluctuations in the amplitude of the i.p.s.p. In Fig. 1, fourteen consecutive traces have been superimposed after being aligned so that the records from HE coincide at 2 msec after

the peak of the action potential in HN. This corresponds to the minimum latency of the observed responses. The amplitude of the evoked i.p.s.p. appeared to vary in a stepwise manner. However, background activity often obscured this quantal character.

It is apparent in Fig. 1 that the resting potential of HE was not steady, but showed small random fluctuations. These fluctuations result from a continuous bombardment of excitatory and inhibitory synaptic potentials; the contribution of the electrode
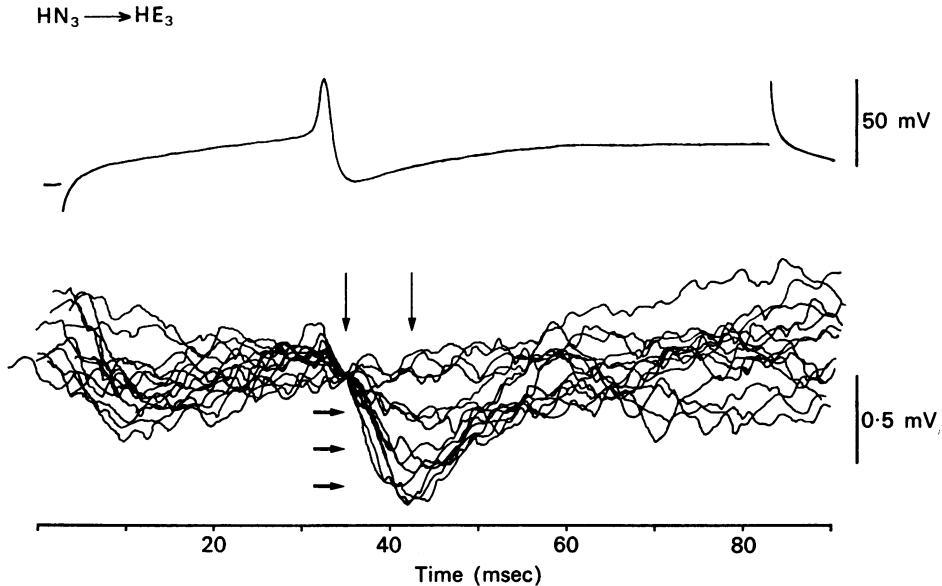
$HN_3 \longrightarrow HE_3$

Fig. 1. Fluctuations in amplitude of fourteen consecutive i.p.s.p.s recorded in the HE motoneurone evoked by stimulation of the HN interneurone. As in most other records, the Ringer fluid contained 20 mM-Mg. The traces were aligned horizontally by super-imposing the peaks of the HN action potentials and vertically by superimposing the responses in the HE cell at a point 2 msec after the peak of the action potential (the point of minimum latency). The amplitude of each synaptic potential was measured as the potential difference between the point of minimum latency and the point corre-sponding to the peak of the averaged response (thin arrows). Integral multiples of the amplitude of the quantal unit determined from the number of failures by Poisson statis-tics (see text) are indicated by the thick arrows. The amplitude distribution of all the responses from this pair of cells is shown in Fig. 2A. In this experiment, the apparent electrical coupling between HN and HE cells was an artifact, due to direct interaction between the electrodes.

resistance was much smaller. Most cells in leech ganglia are not active in 20 mM-Mg, 1·8 mM-Ringer fluid, so these potentials probably represent spontaneous release of quanta of transmitter. To avoid the arbitrary designation of some responses as failures, the amplitude in every trial was measured. An amplitude histogram of the responses was then plotted, as shown in Fig. 2. Measuring every record generates a histogram with one peak centred on 0 mV and the remainder of the responses scattered over larger amplitudes.

To estimate the number of failures, we counted the observed responses that fell to the left of 0 mV, i.e. depolarizing responses (see Fig. 2). This number was then

doubled, to correspond with hyperpolarizing background fluctuations, and added to the number of responses that fell exactly on 0 mV. This technique is based on two assumptions: (1) that the background activity is symmetrically distributed around 0 mV, and (2) that unitary responses make a negligible contribution to the responses falling to the left of 0 mV (depolarizing responses). Using the mean response amplitude and this estimate of the number of failures, the mean quantal content, size of the quantal unit, and the expected distribution of responses were
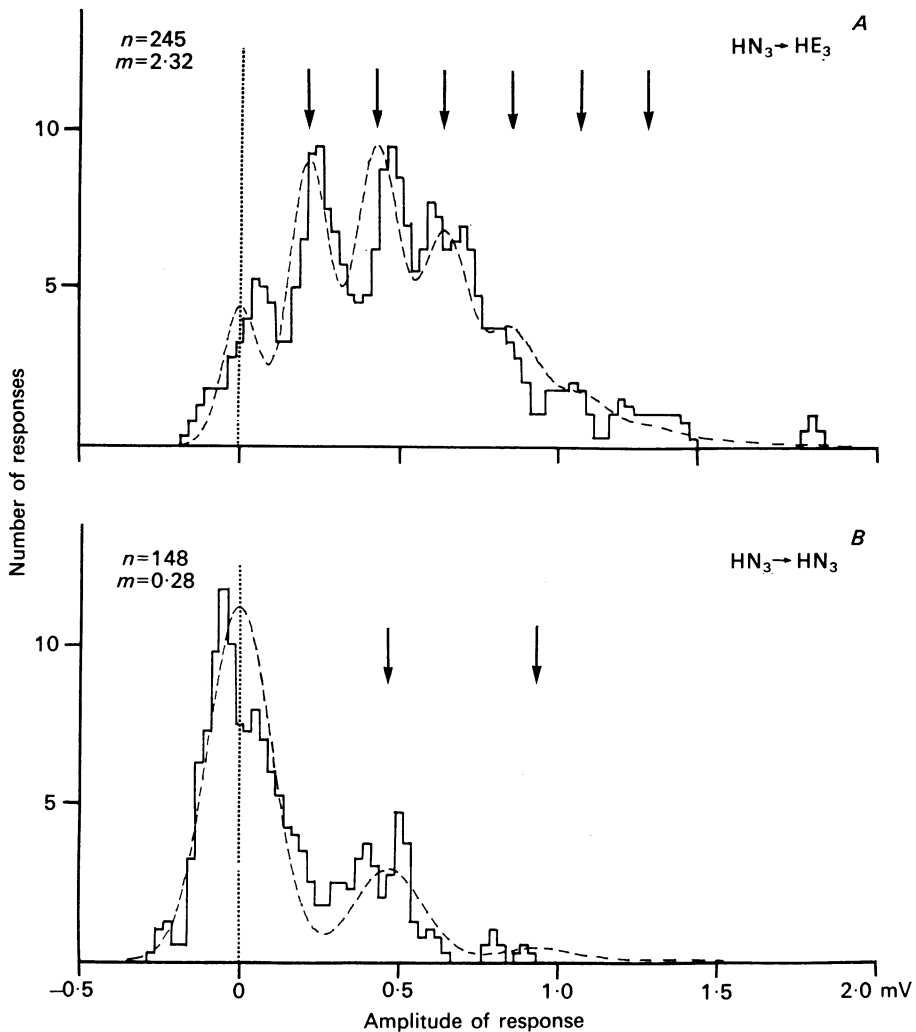

Fig. 2. Amplitude histogram of i.p.s.p.s evoked by stimulation of an HN interneurone. The solid lines are the observed responses (with the sign reversed and three-point averaged, see Methods). The dashed lines are the distributions predicted by Poisson statistics estimating the quantal content, m, from the number of failures and arbitrarily assigning a standard deviation to the unit (see Results). Arrows indicate integral multiples of the unit amplitude. A, distribution of responses in an HE moto-neurone: unit amplitude = 0·213 mV, $\sigma_{background}$ = 55 μV, σ_{unit} = 30 μV. B, distribution of responses in a contralateral HN interneurone: unit amplitude = 0·47 mV, $\sigma_{background}$ = 100 μV, σ_{unit} = 40 μV.

predicted from the Poisson theory. The dashed line in Fig. 2 is the theoretical prediction. The arrows indicate integral multiples of the unit, calculated as:

$$\frac{\text{mean i.p.s.p. amplitude}}{\ln \text{ (number of trials/number of failures)}}$$

The dispersion of the base line noise and unit amplitude were chosen arbitrarily to give the best fit by eye; usually the standard deviation of the unit size equalled approximately one tenth the amplitude of the unit.
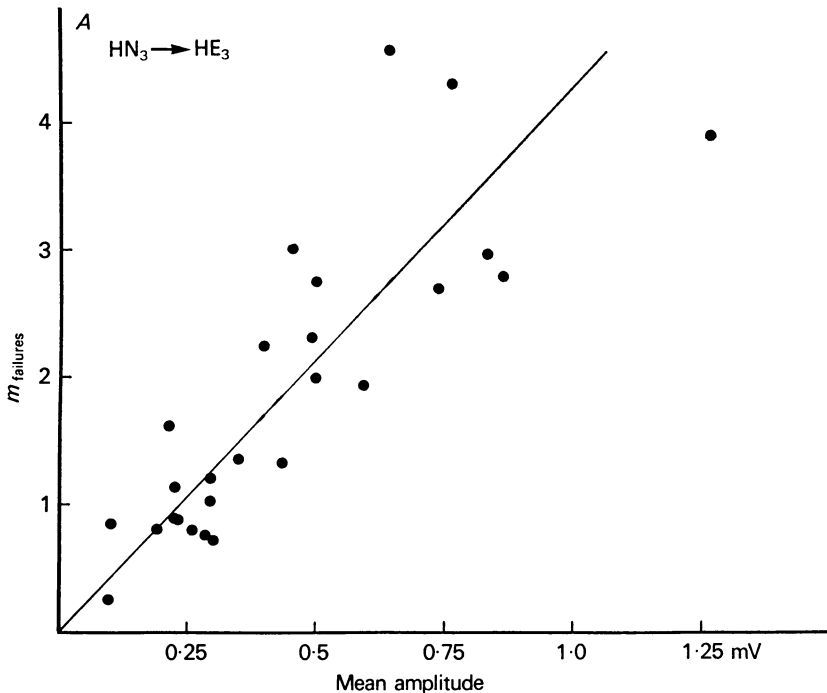

Fig. 3. Summary of results of quantal analyses. A and B, mean amplitude of the i.p.s.p.s plotted against quantal content estimated from the failures. The continuous lines are a least-squares fit to the data (required to pass through the origin). A, data from i.p.s.p.s recorded in HE motoneurones, slope corresponds to a unit amplitude of 0·23 mV. B, data from i.p.s.p.s recorded in contralateral HN interneurones in the third (filled circles) and fourth (open circles) free segmental ganglia. Slope corresponds to a unit amplitude of 0·26 mV. C, log mean quantal content, m, plotted against the log coefficient of variation of i.p.s.p. amplitude. Solid line corresponds to a slope of − 0·5, as predicted for a Poisson distribution. The estimates of unit amplitude obtained by least-squares fit of the pooled data are slightly lower than the mean of the individual estimates.

Results of all experiments analysed in this way are summarized in Fig. 3. As expected, the mean quantal content estimated from the failures varied linearly with the mean amplitude (Fig. 3A). The mean unit size was 0·25 ± 0·015 mV (s.e. of mean, $n = 26$) for experiments on twenty-one pairs of cells. The slope of the least-squares fit to the pooled data gave a slightly smaller estimate of the unit size (see Fig. 3A). All measurements were made in leech Ringer fluid with 20 mM-Mg, 1·8 mM-Ca. The

range of values of mean quantal content was obtained by varying the resting membrane potential of the presynaptic HN interneurone by current injection. Thus, the modulation of transmission brought about by variation in the presynaptic resting potential resulted from changes in the mean number of quanta released by each
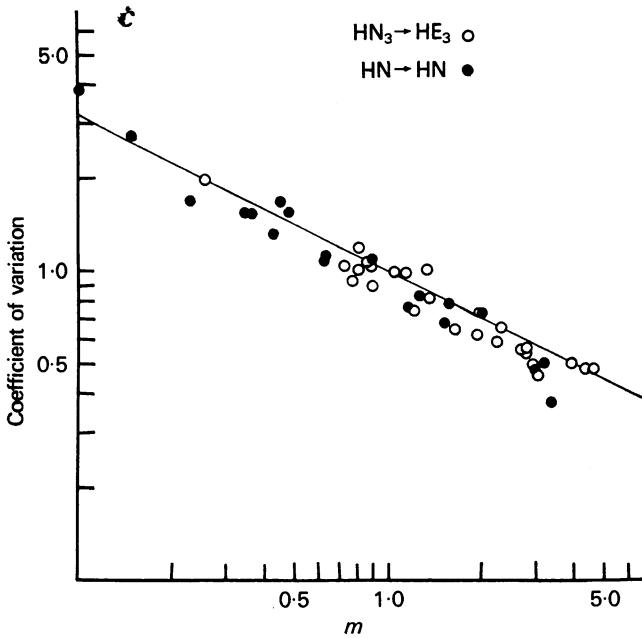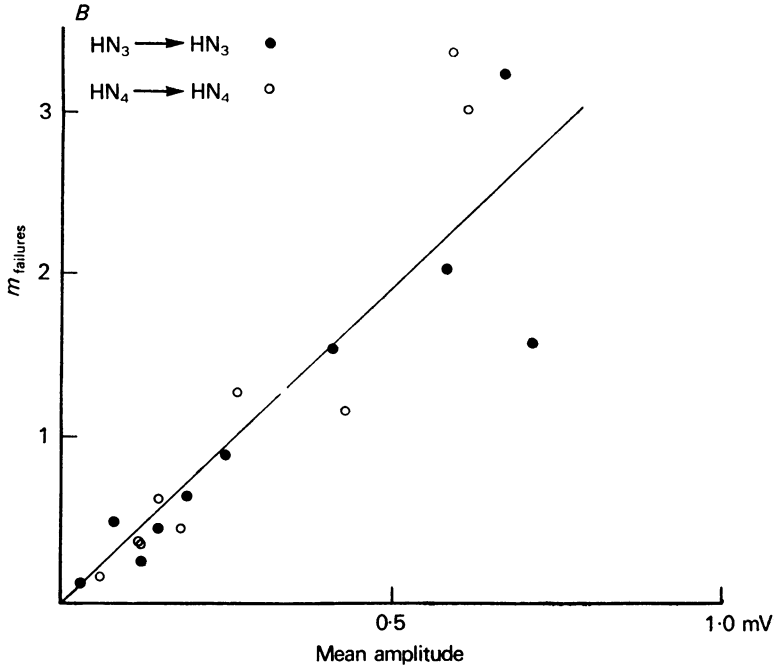

Fig. 3 *B* and *C*. For legend see facing page.

impulse. For a Poisson distribution the coefficient of variation (s.d./mean) varies logarithmically with m; the observed values, plotted in Fig. 3C (open circles), are in good agreement with the theoretical prediction.

Quantal analysis of transmission between HN interneurones

The HN cells in the third and fourth segmental ganglia make reciprocal inhibitory connexions with their contralateral homologues (Thompson & Stent, 1976c). As at the synapses between the HN interneurone and the HE motoneurone, changes in the membrane potential of the presynaptic HN cell modulated the size of the i.p.s.p. in its homologue, the contralateral HN cell. Transmission at this synapse also appeared to be quantal. Fig. 2B shows the observed responses (filled histogram) and
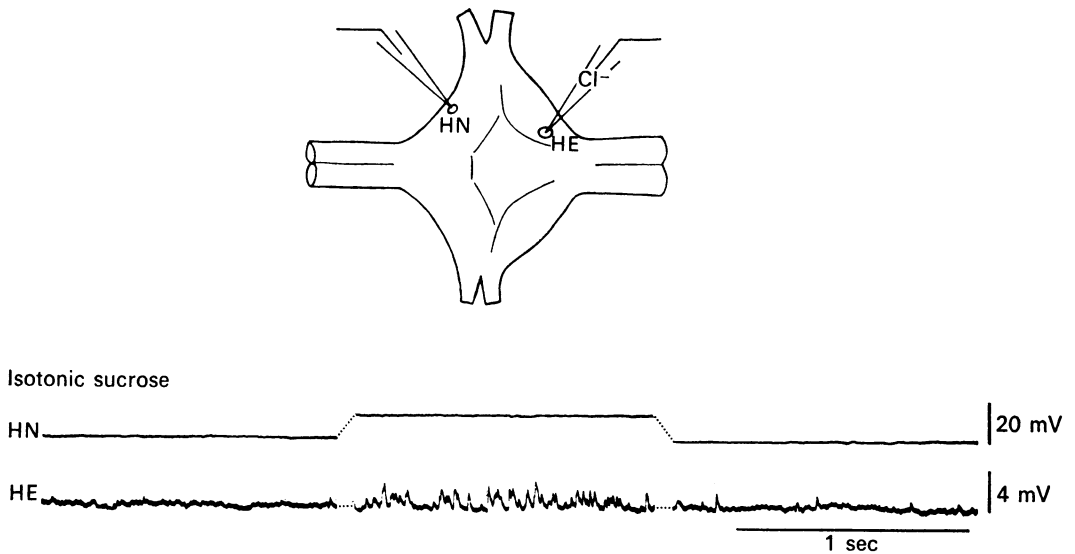

Fig. 4. Demonstration of tonic transmitter release in the absence of nerve impulses. The third segmental ganglion was bathed in a solution of 200 mM-sucrose, 4 mM-KCl, 10 mM-Na Tris maleate, 7·5 mM-CaCl₂. Cl was injected in to the HE motoneurone to reverse the sign and increase the amplitude of the i.p.s.p. Depolarization of the presynaptic HN interneurone with a sustained current pulse evoked a barrage of depolarizing potentials. Dotted lines represent short breaks in the records.

the amplitude distribution predicted by Poisson analysis using the number of failures to estimate the mean quantal content and the unit size. The mean amplitude of the unit in HN cells estimated from the failures was $0·31 \pm 0·022$ mV (s.e., $n = 19$) for experiments on fourteen pairs of cells (see Fig. 3B and C). This value is slightly larger than that recorded in the HE cell.

Independent estimate of unit size

The results presented so far are consistent with the hypothesis that transmitter release from the HN interneurone is quantal and can be described by Poisson statistics. Nevertheless, the absence of clearly distinguishable quantal events prompted experiments designed to obtain an independent estimate of the amplitude unit. The high background activity in the HE cell made it impossible to recognize

individual quanta released spontaneously from HN. However, if the frequency of release of quanta from HN were to be increased without influencing release from other sources, then the contribution of HN to the background activity could be analysed.

The experiment of Fig. 4 demonstrates one method for producing release of transmitter from the terminals of the HN neurones. Here the conditions were designed to minimize spontaneous firing of neurones within the ganglion by replacing Na in the Ringer fluid by sucrose. In addition, the amplitudes of the reversed i.p.s.p.s were increased by injection of chloride into the post-synaptic HE cell (Nicholls & Wallace, 1978). Fig. 4 shows that depolarization of the presynaptic HN cell led to a maintained burst of reversed i.p.s.p.s resembling miniature potentials, in the HE
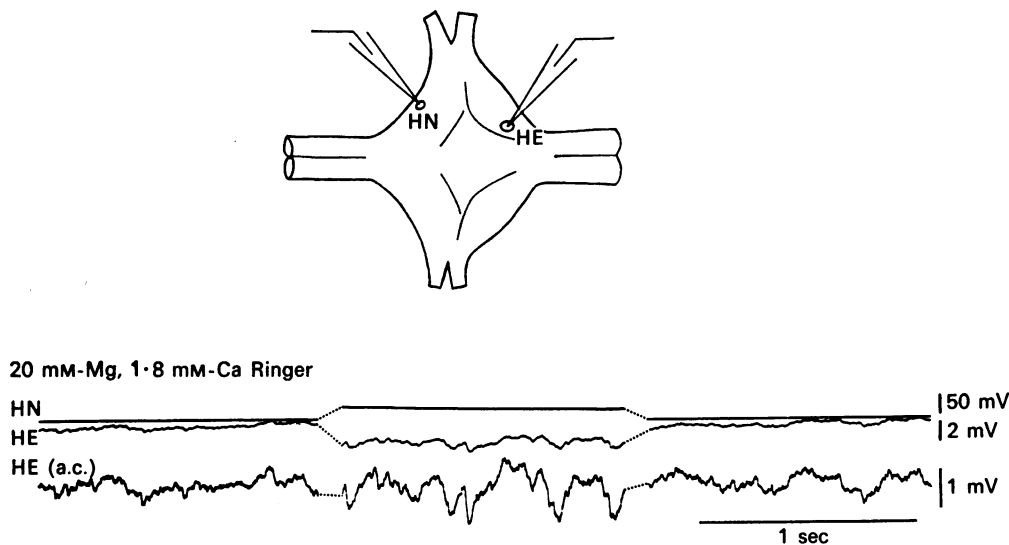

20 mM-Mg, 1·8 mM-Ca Ringer

HN ————————————————————————————— |50 mV
HE ————————————————————————————— |2 mV
HE (a.c.) ————————————————————————————— |1 mV

1 sec

Fig. 5. Demonstration of tonic transmitter release from presynaptic HN cell in normal Ringer solution containing 20 mM-Mg, 1·8 mM-Ca. Prolonged subthreshold depolarization of the presynaptic HN cell gives rise to sustained hyperpolarization of the HE cell (low gain, DC trace) and an increase in the random voltage fluctuations of the membrane potential (lower trace: high gain, AC coupled recording). Dotted lines represent short breaks in the records.

cell. The results suggested that the rate at which quanta were liberated from the HN terminals could be increased by depolarizing the cell body. In low sodium solution, however, it is impossible to evoke synaptic potentials by stimulating the presynaptic cell and thus to test the agreement between the amplitude of the miniature i.p.s.p. and the unit predicted from Poisson distributions of the type shown in Fig. 2.

Fig. 5 shows the results of a similar experiment, but in the usual high Mg Ringer solution and without chloride injection. Current was passed through the electrode in the presynaptic HN cell to produce a long subthreshold depolarization. The membrane potential of the post-synaptic HE motoneurone was recorded at both low gain and high gain (AC coupled). When the HN cell was depolarized, the HE motoneurone became hyperpolarized and the random fluctuations in membrane potential became more pronounced. Apparently, depolarization of HN increased the

'spontaneous' rate of release of quantal units and the HE cell was hyperpolarized by this barrage of miniature i.p.s.p.s.

Assuming that the mean hyperpolarization, V, is made up of linearly additive elementary quanta occurring at random intervals, each with an instantaneous rise to amplitude, a, and an exponential decay, then the size of the unit can be related to the change in variance ($\bar{E}^2$) according to Campbell's Theorem (see Katz & Miledi, 1972),

$$a = 2\bar{E}^2/V.$$

The mean unit amplitude, determined for eleven pairs of cells was 0.21 ± 0.039 mV (s.e. of mean, $n = 11$). Fig. 6 shows the results of all twenty-two experiments with
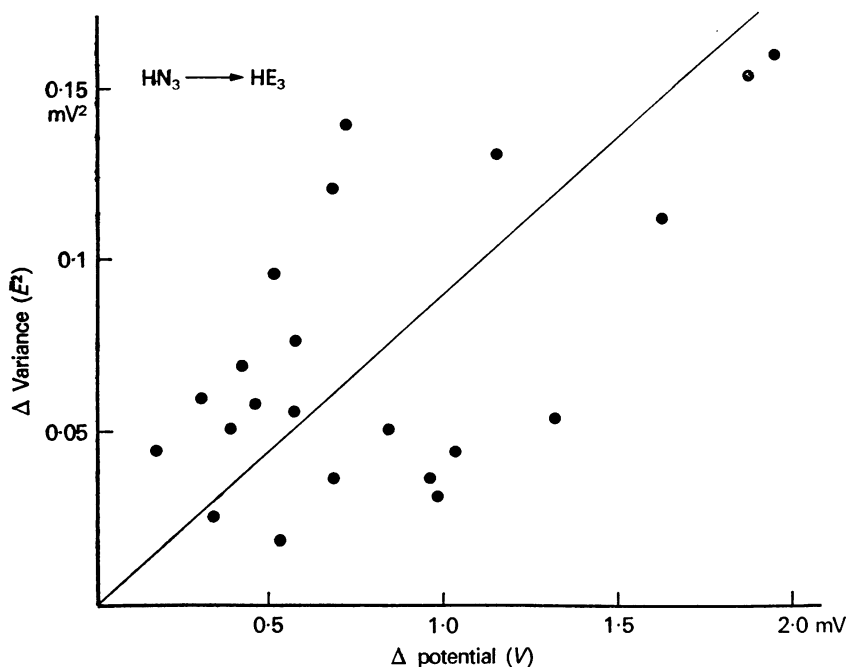

Fig. 6. Summary of the results of experiments on noise analysis. The mean hyperpolarization (V) evoked in HE motoneurones by sustained depolarization of the ipsilateral HN interneurone is plotted against the increase in variance of the membrane potential ($\bar{E}^2$) in the HE motoneurone. The slope of the continuous line (least-squares fit required to pass through the origin) corresponds to a unit amplitude of 0.18 mV. The estimate of unit amplitude by least-squares fit of the pooled data is slightly lower than the mean of the individual estimates.

eleven pairs of cells, plotting the change in the variance against the mean hyperpolarization of the HE cell membrane potential during sustained depolarization of HN. Although there is considerable scatter, the results are consistent with the hypothesis that the hyperpolarization of HE is due to a barrage of quanta with unit amplitude of approximately 0.2 mV.

Comparison of unit size from Poisson distribution and noise analysis

The assumption that transmitter is released by the HN interneurone in quantal units is supported by the general agreement between the mean amplitude of the unit as determined from the Poisson analysis (0·25 mV) and that determined from the change in variance during prolonged presynaptic depolarization (0·21 mV). Since
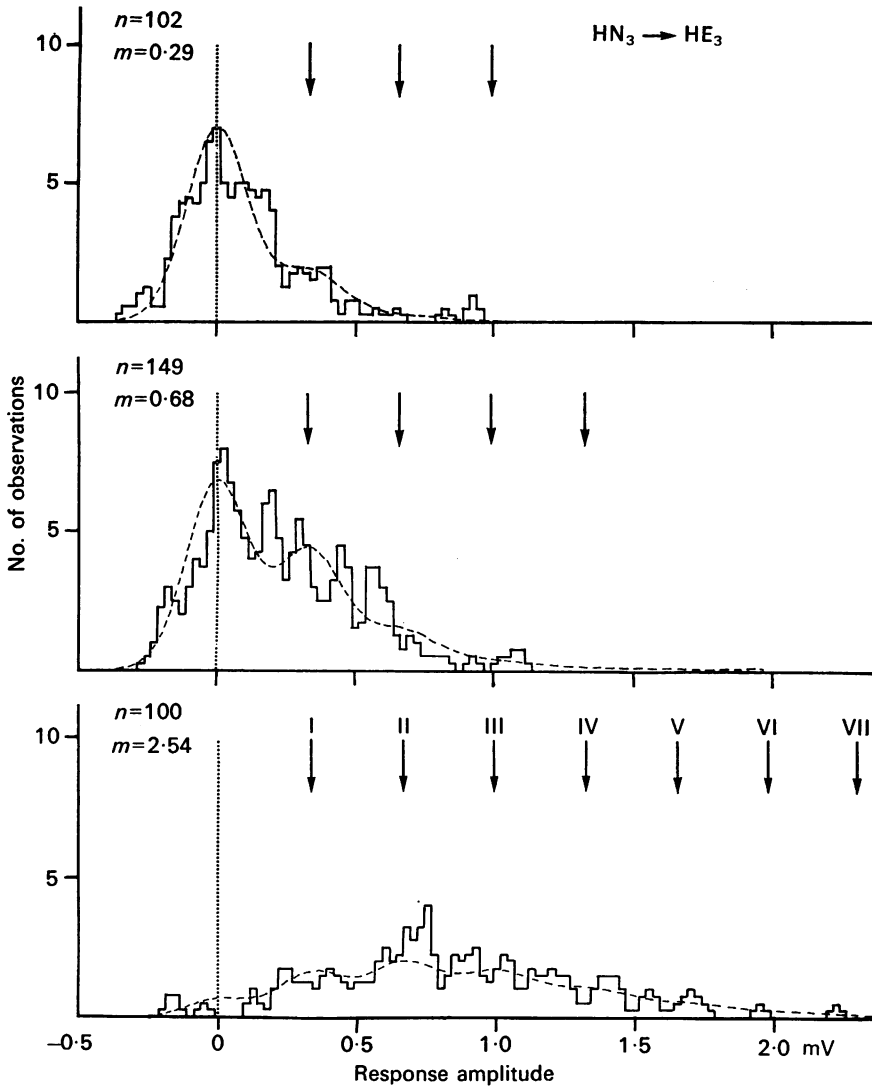

Fig. 7. Results from one pair of cells for which a quantal unit amplitude was estimated from both Poisson distribution and noise analysis. Amplitude distributions are plotted for three runs (continuous line histograms). The mean quantal content was varied between runs by adjusting the membrane potential of the presynaptic HN cell. The arrows indicate integral multiples of the unit as determined by noise analysis. The dashed lines are the Poisson distributions predicted by the unit amplitude determined from the noise and the mean amplitude of the response (unit amplitude = 0·33 mV, $\sigma_{background}$ = 0·11 mV, σ_{unit} = 0·05 mV).

some of the scatter in the estimates of quantal size may result from real differences in the unit size from preparation to preparation, thirteen experiments were made in which the quantal unit was determined by both methods for each pair of cells.

Results from one pair of HN and HE cells are shown in Fig. 7. For these two cells three Poisson analyses were made; the mean quantal content was varied between runs by adjusting the membrane potential of the presynaptic HN cell. In addition, between runs three estimates of the unit size were made by noise analysis. The estimates of the amplitude of the quantal unit were 0.32 ± 0.035 mV (s.e. of mean,
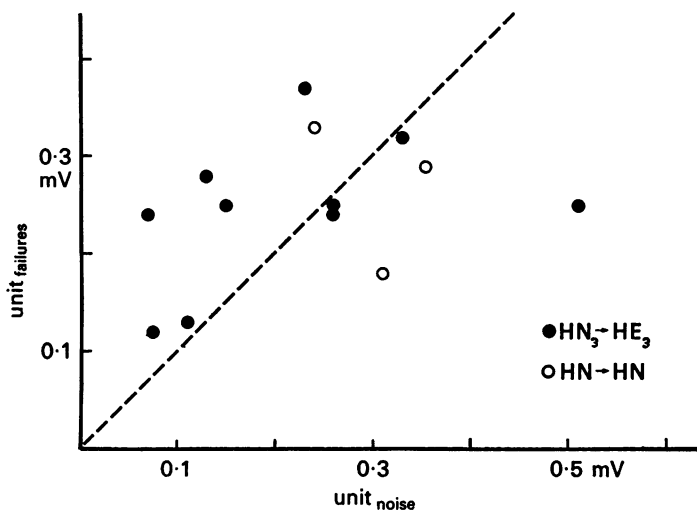

Fig. 8. Summary of results from thirteen pairs of cells for which a determination of unit amplitude was made by both Poisson analysis and noise measurements. Filled circles, $HN_3 \rightarrow HE_3$; open circles, $HN \rightarrow HN$. The dashed line corresponds to agreement of the two estimates.

$n = 3$) from the failures and 0.33 ± 0.033 (s.e. of mean, $n = 3$) from the noise. In Fig. 7 the observed distribution of responses has been plotted in solid lines as an amplitude histogram. The dashed lines are Poisson distributions drawn using the unit calculated from the noise analysis. The agreement between the theoretical curves and observed results is consistent with the suggestion that changes in the presynaptic resting potential vary the mean quantal content of the i.p.s.p.

Results from all thirteen pairs of cells are summarized in Fig. 8, which compares the amplitudes of the unit determined from the noise to that determined from the number of failures by Poisson analysis. There is considerable scatter in the data, primarily in the estimates obtained from noise analysis. For the ten HN_3 to HE_3 pairs (filled circles), the mean unit amplitude was 0.24 ± 0.023 mV (s.e. of mean, $n = 10$) from the failures and 0.21 ± 0.043 mV (s.e. of mean, $n = 10$) from the noise analysis. For the three pairs of HN cells, the mean unit amplitude was 0.27 ± 0.045 mV (s.e. of mean, $n = 3$) from the failures and 0.29 ± 0.027 mV (s.e. of mean, $n = 3$) from the noise analysis.

DISCUSSION

When the quantal components of synaptic interactions between nerve cells are analysed in the central nervous system, complications arise from the anatomy and from the constant bombardment of inputs from a variety of sources. These complications have been reduced in the present experiments by applying objective methods for measuring the amplitude of individual responses and for estimating the number of failures. To estimate the failures from the distribution of responses around 0 mV we assumed that the background activity was symmetrically distributed around 0 mV. This assumption was tested by measuring the potential difference between two points on the base line at a particular time before the presynaptic spike and separated by the same time interval as that used to measure the responses. These measurements were done routinely and gave an indication of the variance in the base line. Measured in this way the base line noise had a mean of 0 mV and a standard deviation of approximately 0·1 mV. This is approximately the value that gave the best fit by eye when plotting the Poisson distributions and is also comparable to the value obtained from measurements made in the course of noise analysis ($\sigma_{base line} = 0\cdot1-0\cdot2$ mV).

An independent estimate of the quantal size was obtained by the technique of membrane noise analysis introduced by Katz & Miledi (1972). The mean amplitude of the quantal unit determined by noise analysis did not differ significantly from the estimate obtained from Poisson analysis (two sided Student's t test, $t = 1\cdot17$, $P = 0\cdot2-0\cdot3$).

Using these methods we have analysed the interaction of the HN interneurone with two target neurones, the HE motoneurone and the contralateral HN cell. At each of these monosynaptic inhibitory connexions, transmission appears to occur by release of quanta that produce miniature inhibitory synaptic potentials of approximately 0·25 mV in the post-synaptic cell. Under normal conditions the amplitude of the i.p.s.p. recorded in the HE cell is 5–10 mV, which would result from the simultaneous release of more than forty quanta.

Throughout this analysis the results were not corrected for non-linear summation. The reversal potential for the i.p.s.p.s was approximately −75 mV and the resting potential about −40 mV. For the Poisson analyses the largest i.p.s.p.s were of the order of −2 mV, which represents less than a 10% change in driving force. The effects of non-linear summation are negligible in this range and so were ignored.

The effect of non-linear summation on the estimate of the quantal amplitude from noise measurements is more severe. Katz & Miledi suggest that the estimate of the unit amplitude should be multiplied by the third power of the conventional correction factor (i.e. by $[V_0/(V_0 - V)]^3$, see Katz & Miledi, 1972). However, in our experiments, the membrane potential of the post-synaptic cell was routinely adjusted by passing steady currents through the recording electrode in order to prevent spontaneous firing. This made accurate determination of the membrane potential and driving force difficult. Failure to correct for non-linear summation may account for our finding that the mean amplitudes of the units estimated from the noise were less than those determined from the Poisson statistical treatment. When the data from the noise measurements were re-analysed making a reasonable estimate of the correction

factor, slightly larger values for the amplitudes of the quantal units were obtained: 0.23 ± 0.040 mV (S.E. of mean, $n = 11$) as the size of the quantal unit recorded in the HE motoneurone, 0.36 ± 0.063 mV (S.E. of mean, $n = 3$) in the HN interneurone.

The synaptic interaction between the HN and HE cells displays several remarkable characteristics. As described by Thompson & Stent (1976b) and Nicholls & Wallace (1978), synaptic potentials show little facilitation or depression during trains, although their size is very sensitive to the presynaptic resting membrane potential. In this paper we have shown that the variation in i.p.s.p. amplitude is a presynaptic phenomenon; more quanta are released per impulse as the presynaptic terminal is depolarized. Thus during presynaptic inhibition, when one HN cell hyperpolarizes its contralateral homologue, fewer quanta are liberated. Furthermore, quanta are continuously being released and the rate of this 'spontaneous' release also depends on the presynaptic potential.

The quantal fluctuations we have observed in transmission between nerve cells in the central nervous system suggests that there is a striking uniformity among the synaptic specializations between the pre- and post-synaptic cells, such that the conductance change produced by a quantum from each release site causes a similar potential change when recorded in the soma of the post-synaptic cell. One possibility would be that synaptic connexions are made at only one location on the dendritic arborization of the HE cell, or at comparable sites on several branches. Experiments are in progress to determine the location and fine structure of the synaptic terminals of the HN cell on the HE and HN cells to see what anatomical specializations are correlated with these remarkable physiological properties.

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