J. Physiol. (1964), **175**, *pp.* 1–16
With 7 text-figures
Printed in Great Britain

QUANTAL COMPONENTS OF THE SYNAPTIC POTENTIAL IN THE CILIARY GANGLION OF THE CHICK

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(*Received* 6 *March* 1964)

It has been established that the end-plate potential in muscle, produced by stimulation of the motor nerve, is 'quantized' (del Castillo & Katz, 1954*a*; Boyd & Martin, 1956; Liley, 1956; Dudel & Kuffler, 1961); i.e. the potential change produced by the transmitter is built up of all-or-none unit potentials which are identical in size and shape with the spontaneously occurring miniature end-plate potentials. Similar evidence has been obtained for quantization of the excitatory post-synaptic potential (e.p.s.p.) in the sympathetic ganglion of the frog (Blackman, Ginsborg & Ray, 1963*b*, *c*), and in spinal motor neurones of the frog (Katz & Miledi, 1963) and cat (Kuno, 1963). The experiments to be reported here provide evidence that the e.p.s.p. in the ciliary ganglion of the chick is built up of unit potentials.

METHODS

The experimental methods have been described in detail previously (Martin & Pilar, 1963). The ganglion was removed from the anaesthetized chick and placed in a chamber through which oxygenated saline solution flowed at a rate of about 2 ml./min. The pre- and post-synaptic nerve trunks were led into oil-filled compartments on either side and placed on platinum-wire electrodes for stimulating and recording. The usual methods were used for intracellular recording with glass micropipettes. In experiments in which the e.p.s.p. was large, initiation of action potentials was blocked by passing hyperpolarizing current through the recording pipette, using a Wheatstone bridge circuit. Unless otherwise noted, experiments were performed at room temperature, which varied from day to day in the range of 20–25° C.

One of the problems encountered when trying to record miniature e.p.s.p.'s was a low signal-to-noise ratio. With electrodes of 80 MΩ or more resistance, noise levels as high as 3 mV were encountered. Reduction of the band width of the recording system produced some improvement, but not as much as would be expected, since the electrode noise contained low-frequency components of large amplitude, particularly after penetration of the tissues. Whether some of the noise was due to intracellular penetration, as suggested by Blackman *et al.* (1963*b*), was not determined.

A second problem was encountered in determining the effects of changes in ionic composition on e.p.s.p. amplitude. Although it was occasionally possible to maintain intracellular penetrations for several hours, the duration of such penetrations was usually much less. For this reason, experiments in which high Mg and low Ca solutions were used were done without taking control records from the same cells in normal solution.

Estimates of the mean quantum content of the e.p.s.p. (m) were made both in solutions of normal ionic composition and in solutions in which the concentration of Ca was reduced and that of Mg increased. In the latter case, when m was small, the distribution of amplitudes of a series of about 200 e.p.s.p.'s was compared with the expected theoretical distribution (del Castillo & Katz, 1954*a*). In normal solutions, or in those containing *d*-tubocurarine chloride (DTC, Abbott Laboratories), estimates of m were made by a different procedure, which is outlined below.

In general, an estimate of m may be obtained in two ways. If the mean amplitude of the series of e.p.s.p.'s is $\bar{v}$, and the amplitude of the unit potential is v_1, then

$$m_1 = \bar{v}/v_1. \tag{1}$$

In addition, if it is assumed that the quantum contents of the e.p.s.p.'s in the series are distributed according to Poisson's law, the coefficient of variation of the amplitude distribution (θ) should be related to the mean quantum content by the equation

$$m_2 = [1+(cv)^2]/\theta^2 = k/\theta^2, \tag{2}$$

where (cv) is the coefficient of variation of the miniature potential amplitude distribution (cf. Edwards & Ikeda, 1962; Blackman *et al.* 1963*c*). When $\bar{v}$ is small (compared with the resting membrane potential), $m = m_1 = m_2$. For large quantum contents, when $\bar{v}$ is large, the estimate m_1 will be too small and m_2 will be too large (del Castillo & Katz, 1954*a*). This is because the unit potentials do not sum linearly (Martin, 1955). Allowance for this non-linear summation may be made by correcting the individual values of v in the series, according to the relation

$$v' = v/(1-v/V_0), \tag{3}$$

where V_0 is the difference between the resting membrane potential and the equilibrium potential for the e.p.s.p. After correction

$$m = \bar{v}'/v_1 = k/\theta'^2, \tag{4}$$

where the primes refer to the 'corrected' values of e.p.s.p. amplitude. This procedure could not in itself be applied to the experimental results with complete confidence because of the difficulty in determining V_0. Since the micropipettes usually had large tip potentials (60 mV or more) measurements of resting potential were probably inaccurate. In addition, estimates of the equilibrium potential by extrapolation of the relation between membrane potential and e.p.s.p. amplitude, with the bridge method to pass polarizing current through the micropipette, were difficult to make because of rectification in the electrodes (see Martin & Pilar, 1963, 1964*a*). In view of these difficulties, an alternative estimate of m was made, using the relation suggested by B. Katz (personal communication)

$$m = \sqrt[3]{(m_1^2 m_2)}. \tag{5}$$

Since from (1), (3) and (4)

$$m = m_1/(1-\bar{v}/V_0), \tag{6}$$

then

$$V_0 = m\bar{v}/(m-m_1). \tag{7}$$

Thus, once m was obtained from (5), an estimate of the accuracy of the value assumed for V_0 could be obtained.

The relation given by eqn. (5) may be derived as follows:

From (3)

$$1/v' = 1/v - 1/V_0.$$

Since V_0 is constant, the variances of $1/v'$ and $1/v$ must be equal; i.e.

$$\text{var } 1/v' = \text{var } 1/v.$$

As a first approximation (Yule & Kendall, 1958; cf. Finney, 1952)

$$\text{var } 1/x = (\text{var } x)/\bar{x}^4.$$

Therefore, $$(\text{var } v')/\bar{v}'^4 = (\text{var } v)/\bar{v}^4.$$

It follows that $$\theta/\theta' = \bar{v}/\bar{v}'.$$

Since, from (3), $\bar{v}' = \bar{v}/(1-\bar{v}/V_0)$,

$$\theta/\theta' = 1-\bar{v}/V_0. \tag{8}$$

This relation is shown in Fig. 1, on which the results given by Martin (1955) are plotted. The fit of the experimental points to the theoretical relation is very good. Finally, from (2), (4) and (8)

$$\theta^2/\theta'^2 = m/m_2 = (1-\bar{v}/V_0)^2,$$

or $$m = m_2(1-\bar{v}/V_0)^2 \tag{9}$$

and, from (6), $$m^2 = m_1^2/(1-\bar{v}/V_0)^2.$$

Thus $$m^3 = m_1^2 m_2,$$

and the relation given by (5) is obtained.

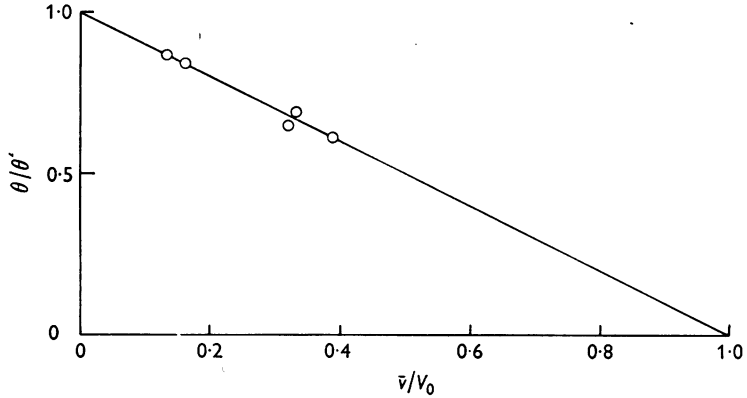

Fig. 1. Theoretical relation between coefficient of variation of e.p.s.p. amplitude distribution and mean amplitude of e.p.s.p., calculated on the basis of 'non-linear summation' of unit potentials. θ/θ' is ratio of uncorrected coefficient of variation to coefficient of variation corrected for non-linear summation. $\bar{v}/V_0$ is ratio of e.p.s.p. amplitude to driving potential (resting membrane potential minus equilibrium potential for e.p.s.p.). Experimental points are taken from Martin (1955).

RESULTS

Response to presynaptic stimulation

Stimulation of the preganglionic nerve trunk invariably produced a spike in the ganglion cell. However, when sufficient hyperpolarizing current was passed through the recording pipette, the spike was abolished, leaving an e.p.s.p. The e.p.s.p. was usually preceded by a coupling potential (Martin & Pilar, 1963). By assuming that the equilibrium potential for the e.p.s.p. was near zero membrane potential, it was possible to estimate its amplitude in the absence of the hyperpolarizing pulse. In twenty cells examined in normal solution, the mean amplitude of the e.p.s.p. at normal resting membrane potential was estimated as $18{\cdot}7 \pm 4{\cdot}1$ mV (mean $\pm$ S.D.). The mean threshold for spike initiation in the same cell was $9{\cdot}5 \pm 3{\cdot}5$ mV, so that the safety factor for spike initiation by the e.p.s.p. was about 2.

Effects of Ca and Mg ions. At other cholinergic synapses (del Castillo & Katz, 1954*a*; Boyd & Martin, 1956; Liley, 1956; Blackman *et al.* 1963*c*) the effect of increasing the concentration of Mg in the bathing solution, or decreasing that of Ca, is to reduce the quantum content of the synaptic potential. In the present experiments, no successful attempts were made to change the ionic composition of the bathing solution during the course of a single penetration (see Methods). However, increasing the Mg concentration from its normal value of 2 mM to levels approaching 20 mM appeared to have only a moderate effect on the amplitude of the e.p.s.p. (e.g. cell 4, Table 1). This lack of a striking reduction in quantum content was presumably related to the high concentration of Ca (5 mM) normally

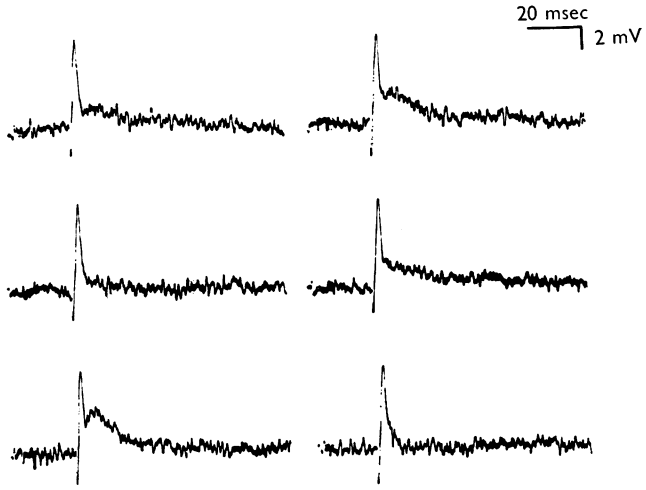

Fig. 2. E.p.s.p.'s recorded from preparation bathed in solution containing 16 mM-Mg and 2·15 mM-Ca. Initial rapid depolarization is coupling potential. E.p.s.p. arises from falling phase of coupling potential and fluctuates between 0 and 3·5 mV.

present in the bathing solution. When the increase in Mg concentration was accompanied by a decrease in Ca concentration, a marked reduction in e.p.s.p. amplitude occurred and the mean quantum content of the responses was clearly reduced.

The response to presynaptic stimulation of a cell bathed in a solution containing 16 mM-Mg and 2·15 mM-Ca is illustrated in Fig. 2. The response consisted of a coupling potential, followed by a small (mean amplitude 1·1 mV) e.p.s.p. which showed considerable fluctuation in amplitude. Some of the stimuli clearly failed to elicit an e.p.s.p., but because of the small signal-to-noise ratio and the fact that the response occurred near the falling phase of the coupling potential, it was somewhat difficult to distinguish 'failures' from the smallest responses. Nevertheless, a reasonable

measurement of the amplitudes of successive e.p.s.p.'s in the series could be made. This was done by averaging graphically the responses which were clearly failures, and using this average as a base line from which to measure the remaining responses.

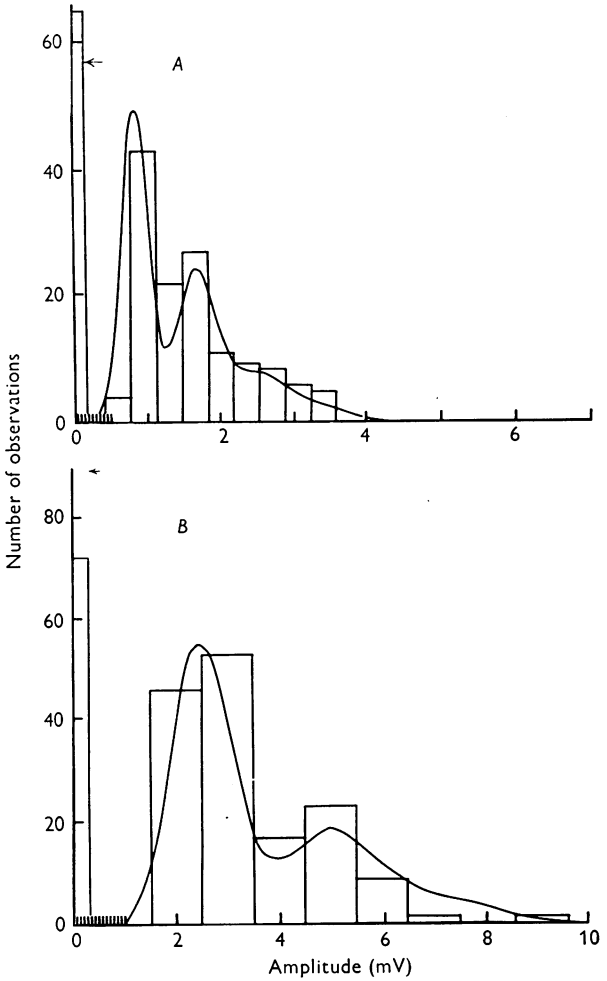

Fig. 3. Distribution of amplitudes of e.p.s.p.'s recorded from two cells (*A* and *B*) in 16 mM-Mg, 2·15 mM-Ca. The smooth curves are the theoretically expected amplitude distributions, calculated on the basis of Poisson distribution of e.p.s.p. quantum content. Broken horizontal bars indicate base-line thickness.

A histogram of the amplitude distribution of 200 of the responses illustrated in Fig. 2 is shown in Fig. 3*A*. Of the total number, 64 were classed as failures, and the remaining had amplitudes ranging from base-line noise level to approximately 3·6 mV. The distribution had clear peaks at about

1·0 and 1·6 mV, with a third possible peak at about 2·5 mV. By assuming that the peaks occurred at multiples of the mean unit potential amplitude ($\bar{v}_1$), and taking their weighted mean, $\bar{v}_1$ was estimated to be about 0·85 mV. Since the mean amplitude of the responses ($\bar{v}$) was 1·1 mV, the mean quantum content (m) was estimated from the relation $m = \bar{v}/\bar{v}_1$ as being 1·3. Knowing m, the expected number of failures (n_0) and single and multiple unit responses (n_1, n_2,...) were calculated from the Poisson relation

$$n_x = N\mathrm{e}^{-m}m^x/x!. \tag{10}$$

The smooth curve was then constructed by distributing the expected numbers of single and multiple unit responses around mean amplitudes $\bar{v}_x$ with variances σ_x^2. These parameters were calculated according to the relations

and

$$\bar{v}_x = x\bar{v}_1$$
$$\sigma_x^2 = x\sigma_1^2.$$

The variance of the single unit response (σ_1^2) was taken, arbitrarily, as 0·19 mV². The fit of the curve to the experimentally observed amplitude distribution was reasonably good (cf. del Castillo & Katz, 1954*a*; Boyd & Martin, 1956; Liley, 1956; Dudel & Kuffler, 1961; Blackman *et al.* 1963*c*), except that the number of failures observed was greater than predicted and there seemed to be a dearth of single-unit responses. This kind of deviation from theoretical prediction is to be expected when the noise level is such that some of the smaller potentials may be counted as failures. A histogram of e.p.s.p. amplitudes from another cell, together with the expected theoretical distribution, is shown in Fig. 3*B*. Again, there was reasonably good agreement between experiment and theory.

Quantum content in normal Mg and Ca. Calculations of quantum content in eight experiments in which solutions of normal ionic composition were used are summarized in Table 1, together with similar results from four experiments in which Mg and Ca concentrations were altered. Values for m_1 and m_2 were calculated according to eqns. (1) and (2). In calculating m_1, modal values for v_1 were used, rather than mean values, if the miniature potential amplitude distribution was skewed (see below). Corrections for 'non-linear summation' were then made according to eqns. (6) and (9), the resulting values being given in the Table as m_1' and m_2'. In making the corrections, V_0 was taken simply as the total estimated membrane potential (resting potential plus hyperpolarization). Considering the probable inaccuracy of this estimate (see Methods), the corrected values of quantum content were in reasonably good agreement. Finally, a third calculation of mean quantum content, which was independent of any estimate of V_0, was made using eqn. (5). This value, given as m in the table, invariably

fell between the values m_1' and m_2', and was probably the most accurate of the three. This value was used in eqn. (7) to obtain the calculated values of V_0 shown in the last column. In the five experiments in which a complete set of measurements was obtained, these calculated values agreed well with those estimated from the resting membrane potential and the amplitude of the hyperpolarizing pulse. This would indicate either that the equilibrium potential for the e.p.s.p. was near zero membrane potential or, if it is postulated that the equilibrium potential should be approximately −15 mV (Fatt & Katz, 1951; del Castillo & Katz, 1954*b*; Nishi & Koketsu, 1960; Blackman, Ginsborg & Ray, 1963*a*), that the total membrane potential was underestimated. In view of the large electrode tip potentials, the latter interpretation seems most likely. The idea that the membrane potential might be underestimated is supported by the fact that in several other experiments in which the indicated membrane potentials were extremely low (30–40 mV), the cells were able to generate spikes with overshoots of 50 mV or more.

The mean quantum content of e.p.s.p.'s in the experiments in which solutions with normal Mg and Ca concentrations were used (cells 5–12, Table 1) was about 22. It should be noted that this estimate includes those cells which were treated with DTC. This may be justified by previous evidence that the quantum content is unaffected by DTC (e.g. Martin, 1955). This idea is supported by the limited evidence available from the present experiments; the three untreated cells in Table 1 had a mean quantum content of 20·4, while the five treated with DTC had a mean quantum content of 22·6.

Spontaneous activity

Although the evidence presented here is consistent with the idea that the e.p.s.p. is built up of a number of unit potentials, the spontaneous appearance of such unit potentials during the period of recording from a ganglion cell occurred infrequently. Prolonged observation of the base line at gains high enough to detect spontaneous activity was made in only twenty-nine of the sixty-six cells from which records were obtained. Spontaneous activity was observed in only eight cells of this group. A typical record, showing the appearance of spontaneous potentials, is presented in Fig. 4. The mean frequency of discharge was invariably low (less than 5/min at room temperature), but could be increased somewhat by raising the temperature of the bathing solution. A distribution of discharge intervals, taken from a cell at 31° C, is shown in Fig. 5. The average discharge frequency was about 12/min, and the interval distribution did not appear to deviate appreciably from the expected distribution for random events (smooth curve). A transient increase in frequency could be produced by

TABLE 1. Calculations of mean quantum content of e.p.s.p.'s from twelve cells from mean amplitude of e.p.s.p., $\bar{v}$, unit potential amplitude v_1, and estimated total membrane potential, V_0 (est). All cells hyperpolarized. Quantum contents m_1 and m_2 calculated according to eqns. (1) and (2). m_1' and m_2' are these values corrected for 'non-linear summation' (eqns. (6) and (9)). m is calculated from eqn. (5). V_0 (calc) is difference between membrane potential and equilibrium potential for e.p.s.p., calculated according to eqn. (6). Agreement between m_1', m_2' and m is consistent with Poisson distribution of e.p.s.p. quantum contents, as is agreement between the two values of V_0

Cell	Bathing solution	$\bar{v}$ (mV)	v_1 (mV)	m_1	m_2	m_1'	m_2'	m	V_0 (est) (mV)	V_0 (calc) (mV)
1	16 mM-Mg, 2·1 mM-Ca	1·1	0·84	1·3	1·4	1·3	1·4	1·3	—	—
2		2·3	2·5	0·9	1·0	0·9	1·0	0·9	—	—
3		27·2	3·8	7·2	—	10·3	—	—	90	—
4	16 mM-Mg, normal Ca	28·0	1·2	23·3	—	35·9	—	—	80	—
5		41·5	5·2	8·0	—	12·5	—	—	114	—
6	Normal	49·1	4·3	11·4	49·3	18·8	18·1	18·6	125	127
7		71·7	4·8	15·0	121·0	33·4	24·5	30·2	130	142
8	DTC (1 μg/ml.)	47·4	3·2	14·8	74·8	28·1	20·7	25·4	100	113
9		37·3	3·2	11·6	70·8	19·8	24·3	21·2	90	82
10	DTC (5 μg/ml.)	20·6	1·9	10·8	30·6	17·2	12·1	15·2	72	76
11		23·0	1·8	12·8	—	17·2	—	—	90	—
12		9·6	—	—	46·7	—	34·8	—	70	—

a brief tetanus applied to the presynaptic nerve (Martin & Pilar, 1964*b*) and this procedure was used frequently to produce a number of potentials sufficient to obtain an amplitude distribution. A second method of obtaining unit potentials for amplitude measurements took advantage of the fact that a single shock to the presynaptic nerve apparently produced a transient increase in discharge frequency. Thus, miniature potentials could be observed frequently on the falling phase of an evoked e.p.s.p., as is shown in Fig. 6. Miniature potentials were observed in this way in seventeen cells in which the spontaneous discharge was apparently absent.

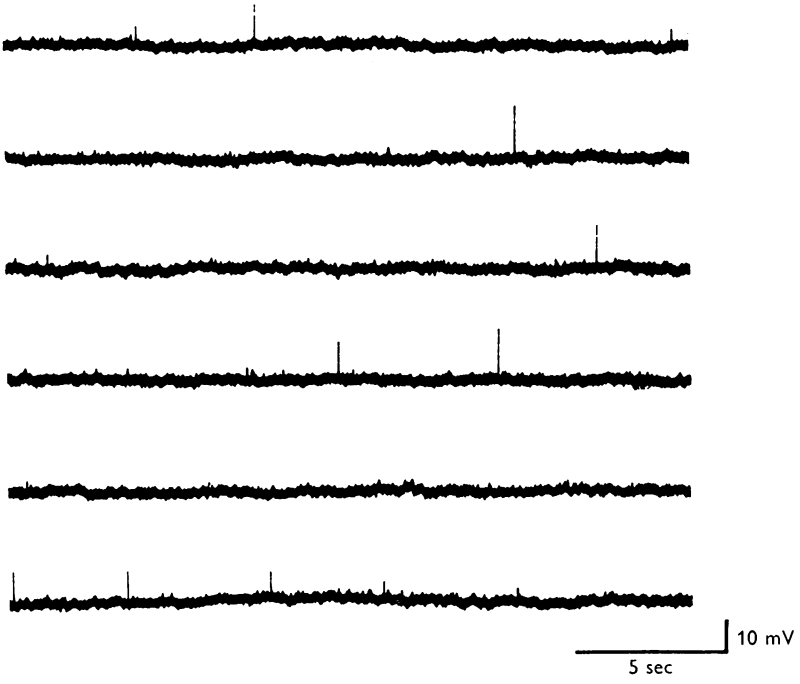

Fig. 4. Continuous record of membrane potential of ganglion cell showing spontaneous appearance of miniature e.p.s.p.'s. Temperature 30° C.

Amplitude distribution. The amplitude of the unit potentials varied widely from cell to cell over a range 0·8–5·2 mV in normal solution. This wide dispersion may have reflected some variation in the 'package size' of the transmitter. However, the major source of variation in amplitude appeared to be associated with differences in resistance of the cells from which the potentials were recorded. If it is assumed that the unit potential is produced by a shunt resistance $\Delta R = 1/\Delta G$ across the cell membrane, then the amplitude of the potential is given by

$$v_1 = V_0 R \Delta G,$$

where R is the resistance of the cell and V_0 is the difference between the resting membrane potential and the equilibrium potential for the e.p.s.p. (Katz & Thesleff, 1957). If the 'package size' of the transmitter is constant, then ΔG would be expected to be constant, and v_1 to vary directly with cell resistance. Measurement of unit potential amplitude and cell resistance, and estimated values of V_0 from thirteen cells, eight in normal

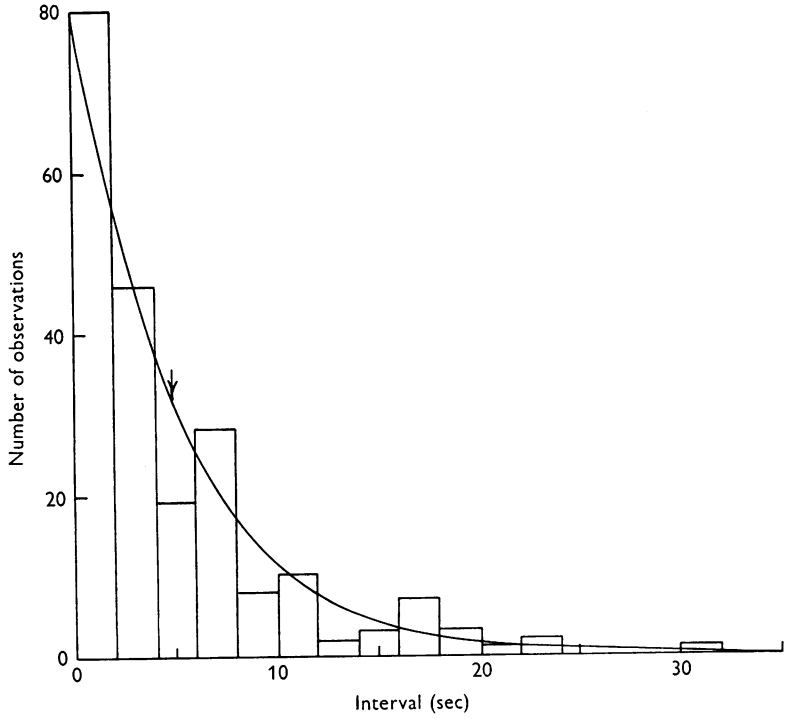

Fig. 5. Distribution of intervals between successive miniature potentials recorded as in Fig. 4. Mean interval was 5·0 msec. Temperature 31° C. The curve is the theoretically expected interval distribution for random events.

solution and five in solutions containing DTC (5 μg/ml.), are given in Table 2. Because of the skewed distribution of miniature potential amplitudes discussed below, the amplitudes in the Table represent modal values, rather than means. In each case the coefficient of variation of the main peak of the distribution was about 0·3. The mean value for ΔG was $12{\cdot}5 \times 10^{-10}$ mhos, which was about 10 times smaller than the value reported by Blackman *et al.* (1963*b*), with frog sympathetic ganglion, but similar to that reported by Hunt & Nelson (personal communication) for the frog ganglion, and Kuno (personal communication) for unit potentials

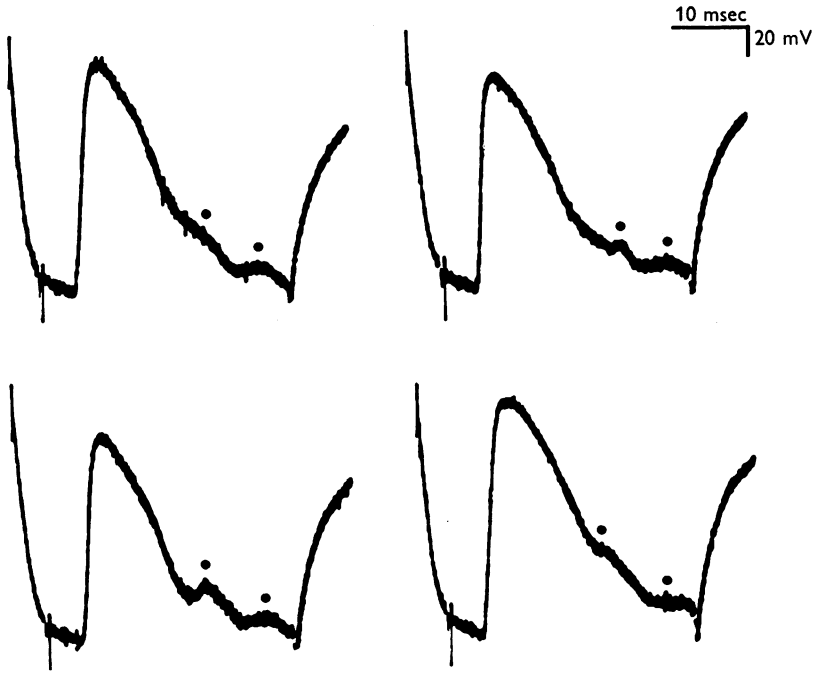

Fig. 6. E.p.s.p.'s, superimposed on electrotonic potential produced by passing hyperpolarizing current pulse through recording pipette. Dots indicate occurrence of miniature potentials on falling phase of evoked response.

TABLE 2. Amplitude of miniature potentials (v_1) in thirteen cells. Conductance change (ΔG) calculated from relation $\Delta G = v_1/V_0R$, where V_0 is membrane potential (resting potential plus hyperpolarization) and R is membrane resistance. Cells for which V_0 is given as greater than 65 mV were hyperpolarized by applied current

	v_1 (mV)	R (MΩ)	V_0 (mV)	ΔG (mhos)
A: Normal solution	1·2	24	50	$10{\cdot}0 \times 10^{-10}$
	2·0	42	60	8·0
	3·0	47	50	12·8
	3·4	45	60	12·6
	4·0	50	50	16·0
	4·0	27	120	12·3
	4·5	64	50	14·0
	5·2	32	114	14·2
Mean:	$3{\cdot}4 \pm 1{\cdot}2$	41	—	$12{\cdot}5 \pm 2{\cdot}4$
B: DTC (5 μg/ml.)	1·0	32	70	4·5
	1·8	43	90	4·7
	1·9	50	75	5·1
	3·0	80	100	3·8
	2·0	50	90	4·4
Mean:	$1{\cdot}9 \pm 0{\cdot}9$	51	—	$4{\cdot}4 \pm 0{\cdot}5$

from motoneurones in the cat spinal cord. The effect of DTC in the concentration used was to reduce ΔG to approximately 36 % of its mean control value in normal solution.

The method outlined above for determining ΔG does not take membrane capacitance into account and undoubtedly underestimates the maximum conductance change. A more rigorous approach is to determine the maximum conductance change from the relation $\Delta G_{max} = CV'_{max}/V_0$, where C is the capacitance of the cell and V'_{max} is the maximum rate of rise of the miniature potential (cf. Blackman *et al.* 1963*b*). During the present experiments, however, few records were taken with sweep speeds sufficient to measure V'_{max} because of the low discharge frequency. In addition, such measurements as were obtained were highly inaccurate because of a low signal-to-noise ratio. Another approach is to assume that the e.p.s.p. is associated with a maximum shunt conductance equivalent to about 20 MΩ (Martin & Pilar, 1963). If the mean quantum content is about 20 (Table 1), then each unit must produce a maximum shunt conductance equivalent to about 400 MΩ, or 25×10^{-10} mhos.

In addition to variations in mean amplitude from cell to cell, the amplitude of individual miniature potentials recorded from a single cell showed considerable fluctuation. Histograms of the amplitude distribution of miniature e.p.s.p.'s recorded from four cells are shown in Fig. 7. In five of sixteen cells, the amplitudes were distributed more or less normally around the mean with coefficients of variation ranging from 0·2 to 0·4, as illustrated in Fig. 7*A*. In the remaining eleven cells, the distribution was positively skewed (Fig. 7*B*, *C*, *D*). One possible source of such a skewed distribution is obvious: if the signal-to-noise ratio is small, then, even though the amplitudes may, in fact, be normally distributed about the mean, the histogram will be positively skewed because the smaller potentials are lost in the base-line noise. This probably contributed to the skewness in Fig. 7*B*. However, in most cells the smaller potentials were sufficiently large to make this interpretation seem unlikely (Fig. 7*C*, *D*). In these cases, it is possible that the larger potentials were associated with the spontaneous appearance of two or more quantal units (cf. Liley, 1956). Because of the low mean frequency of the discharge, the probability of such events being due to random coincidence is virtually zero. Thus, if such an interpretation is adopted, it is necessary to postulate some kind of interaction between units.

One possible hypothesis concerning interaction between units is as follows: suppose that when a unit is released spontaneously each of the remaining units has a small probability of being 'dragged' with it. In this case, the frequency of occurrence of single, double, triple, etc., discharges should be related by the Poisson equation. The analysis of the amplitude distribution then becomes similar to that employed previously for the response of the units to nerve stimulation, except that single unit discharges now must be classed as 'failures' (no response to interaction), double unit discharges as single unit responses, and so on. Knowing the

total number of observations, N, and the number of single unit discharges, n_0, the mean quantum content of the 'responses' may be calculated from the Poisson equation (10), which gives the relation

$$m = \ln (N/n_0).$$

The number of single discharges (n_0) and the mean and variance of their amplitudes ($\bar{v}_1$ and σ_1^2) may be estimated by fitting a normal curve to the peak of the distribution. Once this is done, normal distributions may be

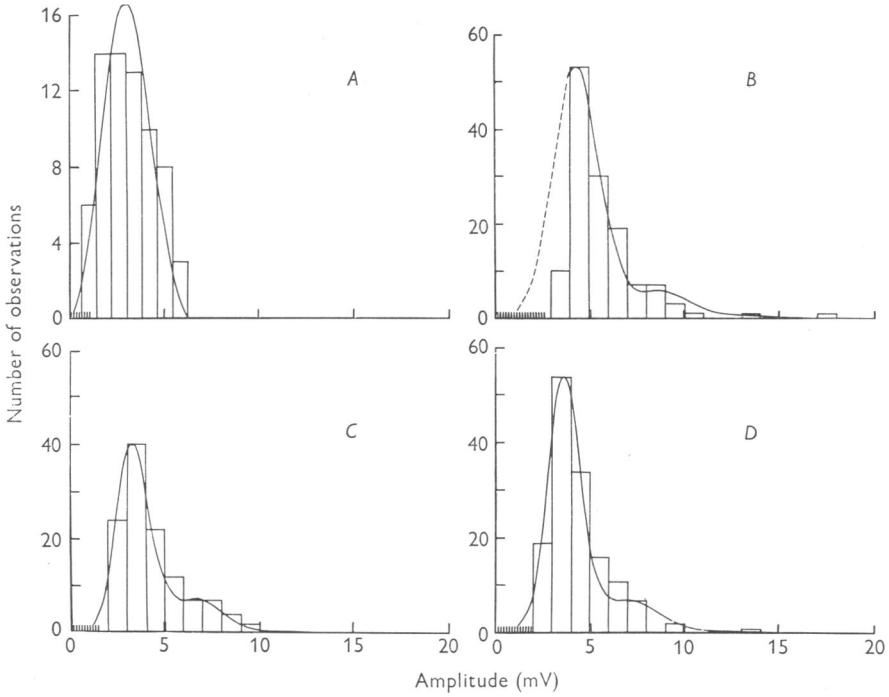

Fig. 7. Distribution of amplitudes of miniature e.p.s.p.'s recorded from four different cells. In *A*, amplitude distribution is compared with a normal distribution having a similar mean and variance. In *B*, *C* and *D*, amplitude distributions are skewed. Smooth curves are the expected theoretical distributions calculated on the basis of interaction between units (see text). Beginning of theoretical distribution in *B* (interrupted line) suggests that some of the skewness was due to loss of smaller unit potentials in base-line noise (broken horizontal bar).

drawn as before for the expected number of multiple discharges and curves constructed for the theoretically expected amplitude distribution. Thus, if the hypothesis is valid, it should be possible to predict the shape of the 'tail' of the distribution from the amplitude and width of the peak. Although the amount of data available from the present experiments was insufficient to test the hypothesis rigorously, the fit of the theoretical

distribution to the experimental results was invariably good in the five cells in which the procedure was carried out. The theoretically expected distributions (smooth curves) are compared with the experimental results obtained from three cells in Fig. 7*B*, *C* and *D*.

DISCUSSION

The results of the present experiments are consistent with the idea that the e.p.s.p. in the ciliary ganglion is built up of all-or-none units which are identical in size and shape with the spontaneously occurring unit potentials. This idea is supported by the good fit of the theoretically expected distribution of e.p.s.p. amplitudes to that observed experimentally (Fig. 3). Additional support for the hypothesis lies in the fact that various ways of calculating the mean quantum content were in good agreement, once allowance was made for non-linear summation of the unit potentials (m_1', m_2' and m, Table 1). Thus, it seems safe to conclude that the mechanism of transmission at this synapse is the same as that at other junctions.

The use of the weighted mean of the two uncorrected calculations to obtain a corrected value for the mean quantum content (eqn. 5) is of some interest in that it avoids the tedious calculations involved in correcting the amplitude of each e.p.s.p. in a series. More important, however, is the fact that it provides a good estimate of quantum content without requiring an accurate measurement of resting membrane potential or e.p.s.p. equilibrium potential.

The idea that the appearance of multiquantal spontaneous discharges may be due to some kind of 'drag' effect seems to be consistent with the present experimental results. Moreover, the hypothesis seems to agree well with miniature potential amplitude distributions observed in the frog sympathetic ganglion, even when 3- and 4-unit discharges occur (e.g. Blackman *et al.* 1963*b*, Fig. 5*d*). There are, however, other explanations possible for this phenomenon, and further experimental work is necessary to determine which is the most appropriate. It should be noted that this kind of interaction should not alter the apparent random distribution of discharge intervals (Fig. 5) provided the duration of the interaction is brief.

One alternative hypothesis is that the spontaneous potentials appear as the result of activations of a 'release mechanism'. In this case, the total number of such activations, N, and the number of failures, n_0, would be unknown. The observed miniature potentials would then have quantum contents of 1, 2, etc., their relative numbers again being given by the Poisson equation. However, the ratio of the number of double unit discharges to single unit discharges is no longer predictable, since the number of 'failures' is unknown. This hypothesis would, of course, fit the present

results, since its conditions are less demanding; i.e. the selection of n_1 and n_2 can be made in a completely arbitrary fashion. However, its acceptance would require that the agreement between the experimental distribution and the distribution predicted by the 'drag' hypothesis was entirely due to chance.

SUMMARY

1. Intracellular recording techniques were used to record excitatory post-synaptic potentials (e.p.s.p.'s) and spontaneously occurring miniature synaptic potentials from cells in the ciliary ganglion of the chick.

2. When the amplitude of the evoked e.p.s.p.'s was reduced by increasing the concentration of Mg in the bathing solution and reducing that of Ca, the e.p.s.p. amplitude fluctuated in a manner predicted by the Poisson equation. In addition, the Poisson relation appeared to apply to amplitude fluctuations in solutions of normal ionic composition.

3. The spontaneous occurrence of miniature synaptic potentials was infrequent. However, they occurred frequently on the falling phase of evoked e.p.s.p.'s and their frequency of spontaneous appearance could be increased by warming the preparation or by applying a brief tetanic stimulus to the presynaptic nerve.

4. The amplitude distribution of the miniature potentials was usually positively skewed. This skewness appeared to be due largely to the occurrence of multiple-unit discharges and could be explained by assuming that there was interaction between the units.

5. The results are consistent with the hypothesis that the e.p.s.p. is built up of all-or-none units, or 'quanta'. The mean quantum content of the e.p.s.p. in normal solution was about 20.

This work was supported by U.S.P.H.S. grants No. NB-01554 and NB-04470.

REFERENCES

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*a*). Synaptic transmission in the sympathetic ganglion of the frog. *J. Physiol.* **167**, 355–373.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*b*). Spontaneous synaptic activity in sympathetic ganglion cells of the frog. *J. Physiol.* **167**, 389–401.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963*c*). On the quantal release of the transmitter at a sympathetic synapse. *J. Physiol.* **167**, 402–415.

BOYD, I. A. & MARTIN, A. R. (1956). The end-plate potential in mammalian muscle. *J. Physiol.* **132**, 74–91.

DEL CASTILLO, J. & KATZ, B. (1954*a*). Quantal components of the end-plate potential. *J. Physiol.* **124**, 560–573.

DEL CASTILLO, J. & KATZ, B. (1954*b*). The membrane change produced by the neuromuscular transmitter. *J. Physiol.* **125**, 546–565.

DUDEL, J. & KUFFLER, S. W. (1961). The quantal nature of transmission and spontaneous miniature potentials at the crayfish neuromuscular junction. *J. Physiol.* **155**, 514–529.

EDWARDS, C. & IKEDA, K. (1962). Effects of 2-PAM and succinylcholine on neuromuscular transmission in the frog. *J. Pharmacol.* **138**, 322–328.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intracellular electrode. *J. Physiol.* **115**, 320–370.

FINNEY, D. J. (1952). *Statistical Method in Biological Assay*, pp. 24–32. New York: Hafner.

KATZ, B. & MILEDI, R. (1963). A study of spontaneous miniature potentials in spinal motoneurones. *J. Physiol.* **168**, 389–422.

KATZ, B. & THESLEFF, S. (1957). On the factors which determine the amplitude of the 'miniature end-plate potential'. *J. Physiol.* **137**, 267–278.

KUNO, M. (1963). The quantal nature of monosynaptic transmission in spinal motoneurons of the cat. *Physiologist*, **6**, 219.

LILEY, A. W. (1956). The quantal components of the mammalian end-plate potential. *J. Physiol.* **133**, 571–587.

MARTIN, A. R. (1955). A further study of the statistical composition of the end-plate potential. *J. Physiol.* **130**, 114–122.

MARTIN, A. R. & PILAR, G. (1963). Dual mode of synaptic transmission in the avian ciliary ganglion. *J. Physiol.* **168**, 443–463.

MARTIN, A. R. & PILAR, G. (1964*a*). An analysis of electrical coupling at synapses in the avian ciliary ganglion. *J. Physiol.* **171**, 454–475.

MARTIN, A. R. & PILAR, G. (1964*b*). Presynaptic and post-synaptic events during post-tetanic potentiation and facilitation in the avian ciliary ganglion. *J. Physiol.* **175**, 17–30.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.* **55**, 15–30.

YULE, G. U. & KENDALL, M. G. (1958). *Introduction to the Theory of Statistics*, 14th ed., p. 329. London: Griffin.