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SPONTANEOUS SYNAPTIC ACTIVITY IN SYMPATHETIC GANGLION CELLS OF THE FROG

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The existence of spontaneous synaptic potentials in cells in sympathetic ganglia of the frog has been briefly reported by Nishi & Koketsu (1960). In this paper some of the characteristics of these potentials will be described.

METHODS

Sympathetic ganglion cells of the frog *Rana pipiens* were impaled with micro-electrodes in the way described in a preceding paper (Blackman, Ginsborg & Ray, 1963*a*). At high amplification there was some difficulty in detecting significant changes in membrane potential because of the very high noise level. This often exceeded 1 mV and was considerably greater than could be accounted for by the electrode resistance (usually *ca.* 30 mΩ); furthermore, the noise level when the electrode was in a cell was usually greater than when it was outside the cell, although direct measurements showed that no change in electrode resistance had taken place. Unlike the deflexions to be identified as miniature synaptic potentials, the random fluctuations of the base line were not reduced on adding tubocurarine to the bath. One procedure which did, however, reduce the noise was increasing the external K^+ concentration.

A possible explanation for the high noise level is that it reflects fluctuations in the resistance of the 'seal' around the tip of the micro-electrode, since these would be especially influential in producing fluctuations in potential in the case of impalements of cells of high resistance. The reduction in base-line noise on increasing the external K^+ concentration might then be attributed to a reduction in the resistance of the cell, which it would be expected to produce.

Whatever the explanation for the high noise level, it is possible that on some occasions random fluctuations of the base line were taken to be miniature synaptic potentials and vice versa.

RESULTS

In about a third of the cells impaled intracellular records showed small subthreshold depolarizations, occurring spontaneously, which could be distinguished from the base-line noise (Fig. 1). Some characteristics of these depolarizations are given in Table 1. Several results suggest that these spontaneous depolarizations were in fact 'miniature synaptic potentials' like those seen at the neuromuscular junction, and that they were due to quanta of acetylcholine released from the presynaptic nerve terminals.

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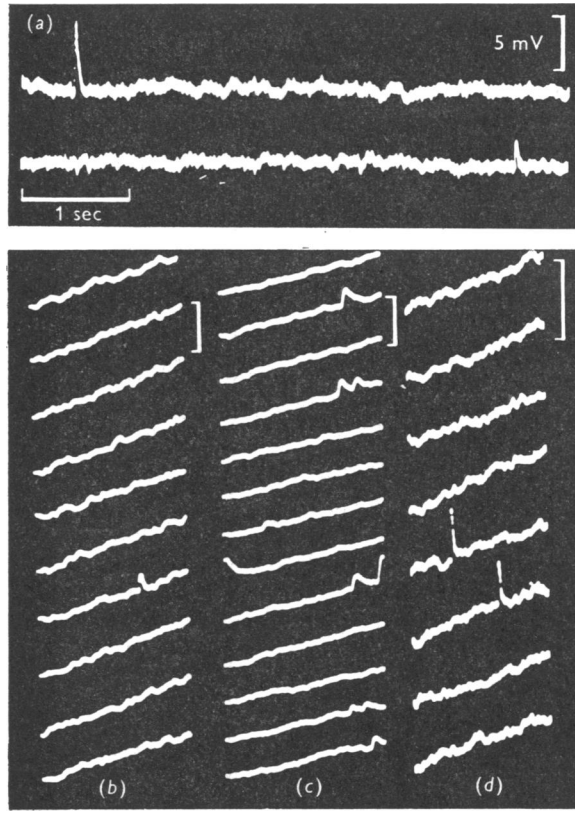

Fig. 1. Miniature synaptic potentials from four cells. (*a*) Cell 49; (*b*) cell 83, 10 mV, trace length 1250 msec; (*c*) cell 113, 10 mV, trace length 350 msec; (*d*) cell 68, 5 mV, trace length 1 sec. In (*b*)–(*d*) records read from bottom upwards and from left to right.

TABLE 1. Some characteristics of miniature synaptic potentials

Cell no.	Amplitude of evoked response (mV)		No. of miniature synaptic potentials recorded	Frequency (per sec)	Amplitude of miniature synaptic potentials (mV)		
	A	*O*			Mean	Range	S.D.
1	65	—	93	8	1·7	0·5– 6·4	0·8
113	102	—	65	1·5	2·2	1·0– 6·0	0·93
81	90	—	35	1	3·2	1·0–10	2·3
59	—	65	108	1/3	2·5	0·8–15·4	2·1
49	58	—	33	1/4	2·6	1·1– 8·0	1·1
41	—	75	44	1/5	1·9	0·9– 3·6	0·6
61	—	—	74	1/9	3·2	1·0– 8·5	1·4
68	93	—	71	1/10	2·1	0·3– 5·9	1·4
65	78	—	30	1/15	2·0	1·2– 4·2	1·1
83	57	—	23	1/15	2·6	1·4– 5·8	1·0

A, antidromic response; *O*, orthodromic response.

For example, they were seen in cells in a high Mg^{2+} concentration, in which synaptic transmission was blocked, and their time course resembled that of the evoked synaptic potentials in such cells (Blackman, Ginsborg & Ray, 1963*c*). They persisted (indeed, at an increased recurrence frequency; see below) in concentrations of K^+ which rendered the presynaptic pathway inexcitable (Fig. 2; cf. Blackman, Ginsborg & Ray, 1963*b*, Fig. 3*A*). Their amplitude was reduced by tubocurarine in concentrations which were required to depress synaptic transmission (Fig. 3).

These results would not exclude the possibility that the spontaneous electrical activity resulted from the random firing of internuncial neurones. However, there is no evidence for the existence of such neurones in sympathetic ganglia of the frog, and there is some histological evidence (Johnson, 1918) against their existence. Furthermore, the results of the following paper suggest that the miniature synaptic potentials constitute the units of the evoked response.

Frequency. The intervals between successive miniature synaptic potentials appeared to be randomly distributed (Fig. 4). Their recurrence frequency varied widely from cell to cell. In the majority of cells impaled no miniature synaptic potentials could be seen in periods of observation of 2–3 min. The duration of this 'silent period' suggests that in these cells the frequency was not greater than 1/30 sec, and probably lower (Pearson & Hartley, 1958). The highest frequency observed in normal solutions was 20/sec, but in only six of more than 100 cells was the frequency greater than 1/3 sec.

It might be argued that the failure to observe miniature synaptic potentials in the majority of cells was due to their being too small in these cells to be distinguished from random noise. In view of the effects of an enhanced external K^+ concentration, to be described below, this possibility is, in general, extremely unlikely.

Amplitude. The mean amplitudes of the miniature synaptic potentials varied as a rule between about 1 and 3 mV. In one cell (cell 100, Fig. 6), the amplitudes of about 25 % of the miniature synaptic potentials were sufficiently great to produce action potentials. In a second exceptional cell (135, Figs. 2 and 5), the mean amplitude of the miniature synaptic potentials was about 6 mV, even though the bathing fluid contained about 12 mM-K^+, and as a consequence the cell had been depolarized by 10 mV and was presumably of lower resistance than in a normal K^+ concentration. The variations in mean amplitude possibly reflect a variation in the resistance of the cells, perhaps because of differences in size (cf. Katz & Thesleff, 1957). In this connexion it is of interest that the cell of Fig. 2 supplied a post-ganglionic axon of exceptionally low conduction velocity (see Blackman *et al.* 1963*b*, Fig. 3*A*) and hence, probably, of unusually

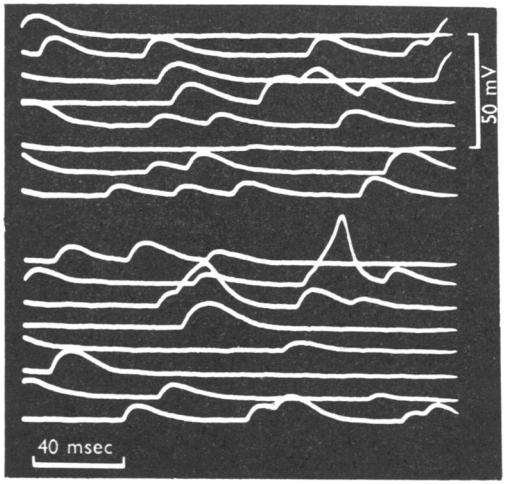

Fig. 2. Miniature synaptic potentials in a raised external concentration of potassium (11·8 mM). Note action potential generated by the almost simultaneous occurrence of several miniature synaptic potentials. Cell 135 (cf. Blackman *et al.* 1963*b*, Fig. 3).

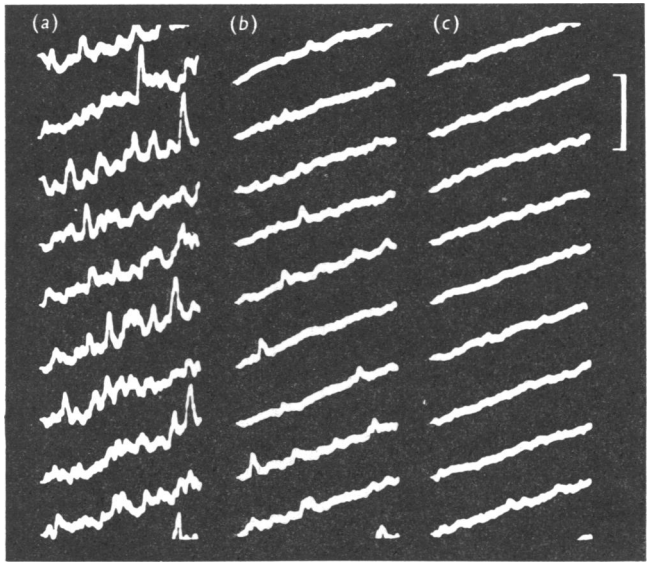

Fig. 3. Effect of tubocurarine on the miniature synaptic potentials. The external concentration of potassium was raised to 10 mM before the record (*a*) was taken, to accelerate frequency of miniature synaptic potentials. Tubocurarine (to $7{\cdot}5 \times 10^{-4}$ M) added between (*a*) and (*b*). Interval of 10 sec between (*b*) and (*c*). Voltage calibration 5 mV, trace length 550 msec.

small diameter as compared with most of the cells impaled in this investigation.

The form of the frequency distribution of the amplitudes of the miniature synaptic potentials recorded from any one cell (Fig. 5) was usually somewhat different from that of miniature end-plate potentials recorded at the neuromuscular junctions of twitch muscle fibres. There, the great majority of the amplitudes are grouped symmetrically about a mean value with a standard deviation which is rarely as large as 30 % of the mean. There are in

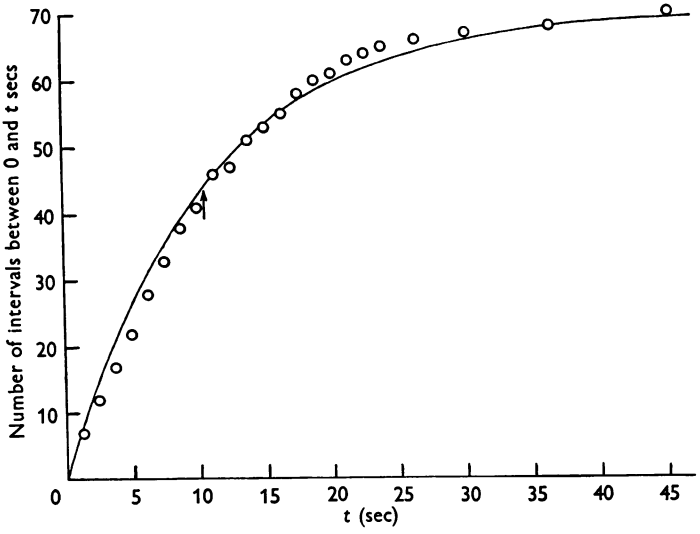

Fig. 4. Sequence of miniature synaptic potentials. Cell 68. Abscissa: t, intervals between successive miniature synaptic potentials. Ordinate: y, number of intervals less than t. Curve drawn according to the equation $y = N(1-e^{-t/T})$, where N (70) is the total number of intervals and T is the mean interval (10·4 sec).

addition in muscle fibres of the frog a few exceptionally large miniature end-plate potentials, but these may be accounted for by the random coincidence of two or more 'standard' miniature end-plate potentials (Fatt & Katz, 1952). In muscle fibres of the diaphragm of the rat, exceptionally large miniature end-plate potentials also occasionally occur, and these cannot be accounted for by a *random* coincidence of two or more discharges (Liley, 1956*a*). Liley has suggested that they might nevertheless be multiple units, due to the simultaneous spontaneous discharge of several quanta of acetylcholine.

In sympathetic ganglion cells, although the amplitudes of the majority of miniature synaptic potentials are grouped around a central value, the proportion of 'exceptionally' large potentials may be as great as 30 % (e.g. Fig. 5*d*). In view of the low recurrence frequency of the discharges, it is

highly improbable that any of these larger potentials were due to a *random* coincidence of several units (cf. Fatt & Katz, 1952, p. 125; Liley, 1956*b*, p. 663). It may be that an 'interaction' between discharges of the type discussed by Liley occurs in the presynaptic terminals at the sympathetic synapse, and with greater intensity than at the neuromuscular junction. There is, however, no evidence that the large miniature synaptic potentials were composed of multiple units.

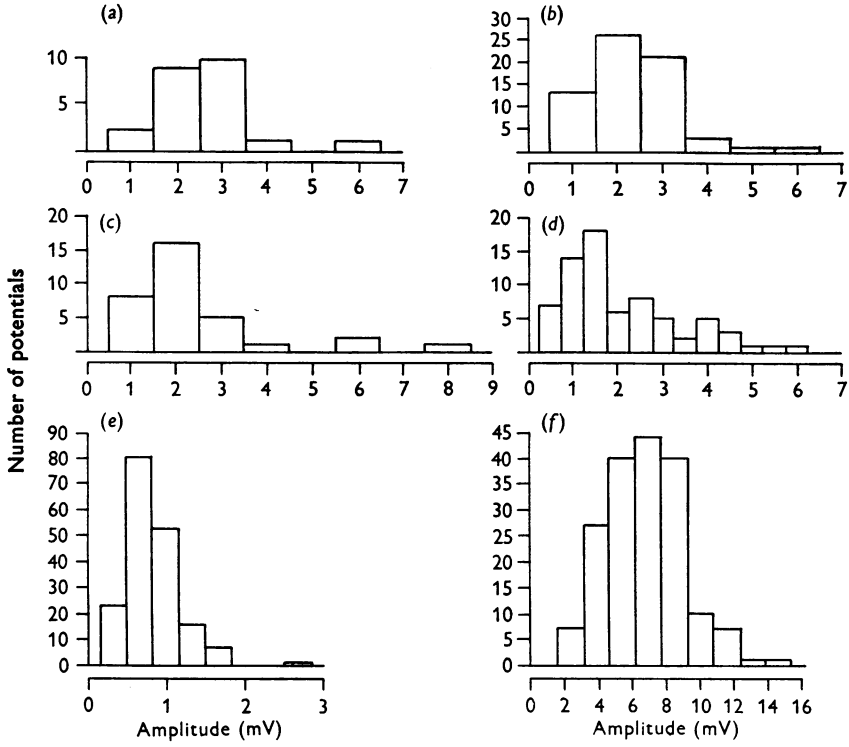

Fig. 5. Amplitude distribution of miniature synaptic potentials from six cells in normal (2 mM) and raised (*ca.* 12 mM) K. (*a*)–(*d*) 2 mM-K; (*e*)–(*f*) *ca.* 12 mM-K. (*a*) cell 83; (*b*) cell 113; (*c*) cell 49; (*d*) cell 68; (*e*) cell 118; (*f*) cell 135. (Cf. Table 1 and Figs. 1 and 3.)

Some of the larger miniature synaptic potentials generated 'local responses' which were easily detectable in records taken at a high sweep speed (e.g. Fig. 6) but which may have exaggerated some of the estimates made of the mean and maximum amplitudes of the miniature synaptic potentials recorded on a slow time base.

Time course and the conductance change underlying miniature synaptic potentials. The rise time of the miniature synaptic potentials was of the order of 10–15 msec (rarely up to 20 msec) and they decayed to half their

amplitude in about 20–30 msec. There did not appear to be any obvious relationship between the time course and the amplitude, except in those cases where 'local responses' could be detected; but in general the accuracy of measurement was limited by the slow speed of recording that was employed and by the high 'noise' level. In one cell, however, it could be clearly seen that the smaller 'miniature synaptic potentials' were not slower in time course than the larger 'miniature synaptic potentials' (Fig. 2): and in fact, often the larger ones took longer to rise to the peak (cf. del Castillo & Katz, 1956). In this cell at least the variation in amplitude observed (cf. Fig. 5) cannot have been due to varying degrees of spatial attenuation of an underlying potential change essentially less variable (cf. Burke, 1957; Ginsborg, 1960).

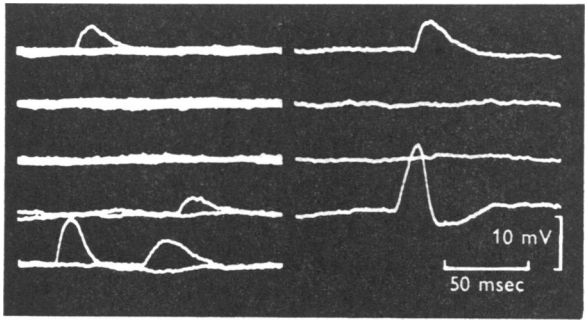

Fig. 6. Miniature synaptic potentials with 'local responses'. Cell 100, Ringer's fluid with 23 mM-Mg^{2+}. The very large miniature synaptic potentials have a faster rate of fall, and sometimes a 'positive phase' due to a 'local response'.

The maximum rates of rise of a number of miniature synaptic potentials of about the mean amplitude, recorded from six different cells, varied from cell to cell from 0·56 to 2·0 V/sec. If the capacity across the cell membrane is taken as 0·5 nF (Nishi & Koketsu, 1960), and the 'equilibrium potential' for acetylcholine is taken as the absolute value of the resting potential minus 10 mV, the additional conductance across the cell membrane underlying the mean miniature synaptic potentials in these cells (see Fig. 10, Blackman *et al.* 1963*a*) varied from 0·5 to $1{\cdot}8 \times 10^{-8}$ mho. These values are smaller than the corresponding value for the miniature end-plate potentials at the neuromuscular junction of the frog (about $1{\cdot}4 \times 10^{-7}$ mho; Takeuchi & Takeuchi, 1960).

If it is tentatively assumed that the step of the response of the ganglion cell to orthodromic stimulation consists of summed miniature synaptic potentials (i.e. that the quantal hypothesis applies to the release of transmitter at the synapse), it is of interest to compare the maximum rates of rise of the miniature and the evoked synaptic potentials. The ratio between

the two values should give an estimate of the mean number of miniature synaptic potentials which go to make up the response (i.e. the mean quantal content). In the six cells referred to above this number was found to vary from cell to cell between 6 and 34.

Factors modifying the frequency and amplitude. Several procedures which are known to increase the frequency of occurrence of the miniature end-plate potentials at the vertebrate neuromuscular junction (e.g. Fatt & Katz, 1952; Liley, 1956*a*, *b*) acted similarly on the miniature synaptic potentials in the sympathetic ganglion cells of the frog. For example, an increase in the tonicity of the Ringer's fluid caused an increase in the recurrence frequency. It was also observed in several cells that trains of rapid preganglionic stimulation produced a transient burst of the miniature synaptic potentials. The most interesting phenomenon, however, was the striking increase in the recurrence frequency which occurred when the external concentration of potassium was raised.

Effects of potassium. When the external concentration of potassium was raised from its normal value of 2 mM to about 10–12 mM, miniature synaptic potentials were invariably seen whether or not they had been present previously. The concentration required to produce an obvious increase in frequency was always in excess of that required to block presynaptic transmission. This block is most easily explained by supposing that depolarization of the presynaptic nerves has taken place (see Blackman *et al.* 1963*b*) and it therefore seems reasonable to assume that the increased recurrence frequency caused by the increased concentration of K^+ is also due to the depolarization of the presynaptic terminals. In nine cells, in 10–14 mM-K^+, the recurrence frequency of the miniature synaptic potentials varied from a minimum of 1/sec (in this cell, two miniature synaptic potentials were seen in a period of 87 sec before the addition of potassium) to more than 50/sec. Since the recurrence frequency of the miniature synaptic potentials was very low before the addition of K^+, it is difficult to make a reliable comparison of the amplitudes before and after the addition of K^+. Some indication of a reduction in amplitude is, however, given by the results from one cell, in which seven miniature synaptic potentials with a mean amplitude of 2·4 mV were seen before the application of potassium; after the application of K^+ to 10·4 mM the mean amplitude of a hundred discharges was 1·5 mV. A small reduction in amplitude might be expected on two counts, namely (1) the reduction in the resting potential (of the order of 10 mV) of the post-synaptic cell, and (2) a reduction in the cell resistance.

It was unfortunately not possible to make a detailed study of the relation between the external K^+ concentration, and hence the depolarization, and the recurrence frequency. However, it seems probable that the rela-

tion between the depolarization and the recurrence frequency is, as at the neuromuscular junction, extremely steep. Figures 7 and 8 show the time course of development of the effect of raising the external concentration of K^+ in one cell. The recurrence frequency increased gradually over a period of 45 sec, starting about 2 min after the addition of K^+ to the bath. From the results described in the preceding paper (see Blackman *et al.* 1963*b*, Fig. 2) it is evident that the major change in concentration around the cell occurs within 10–20 sec. During the period illustrated in Fig. 7, therefore, the concentration will have almost attained its final level and will have been increasing over only a very narrow range. Nevertheless, during this period the recurrence frequency of the miniature synaptic potentials increased by a factor of at least 10.

An alternative explanation of these results might be that there is a long delay between the onset of depolarization and the increase in recurrence frequency. This explanation may, however, be discounted by the results of experiments in which the concentration was raised to higher levels. In one cell an obvious increase occurred within 25 sec of raising the K^+ concentration to 14 mM. Most of this period would presumably be required for the major part of the depolarization of the presynaptic terminals to occur by virtue of the 'diffusion delay'.

Effects of magnesium. Several attempts were made to estimate the effects of an enhanced Mg^{2+} concentration on the frequency and amplitude of miniature synaptic potentials, with very variable results. In two cells in which the original recurrence frequency was 1/2 sec and 1/9 sec, and in which more than 50 miniature synaptic potentials were recorded both before and after increasing the concentration of Mg^{2+} from 0 to 20 mM, no effect was observed either on the frequency or amplitude. In one of these cells the response to orthodromic stimulation continued to be an action potential, after the increase in Mg^{2+} concentration, although the synaptic step rose at a slower rate. This is in contrast to the results usually obtained (see Blackman *et al.* 1963*c*). In the other cell no response to orthodromic stimulation was obtained before the addition of Mg^{2+}. The results from a third cell indicated that in some cases Mg^{2+} does have some effects. The initial observations were made in a Mg^{2+} concentration of about 12 mM, and the cell was stimulated orthodromically at a rate of 1/sec. The responses were action potentials, and in addition 75 miniature synaptic potentials were observed in a period of about 5 min. Their mean amplitude was 1·9 mV with a range of 1·0–5·0 mV. The concentration of Mg^{2+} was then raised to 26 mM. The responses to orthodromic stimuli became synaptic potentials only (see Blackman *et al.* 1963*c*) and the recurrence frequency of the miniature synaptic potentials and their mean amplitude were reduced: only 9 miniature synaptic potentials were observed in about 5 min, with a

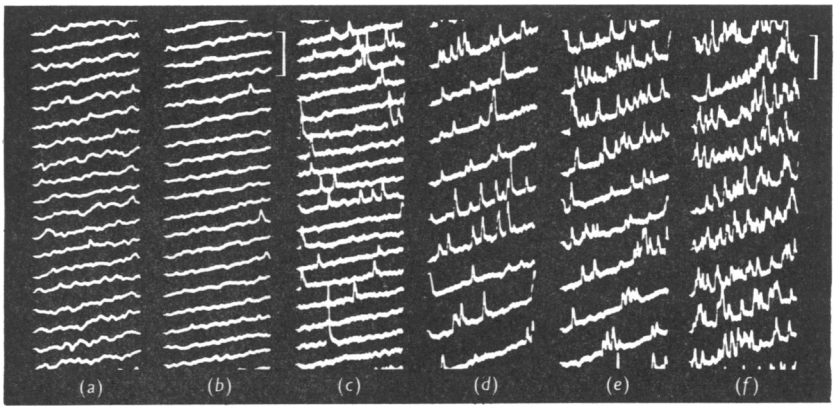

Fig. 7. Time course of effect of increasing the external K^+ concentration on the recurrence frequency of miniature synaptic potentials. Cell 87. The external K^+ concentration was raised to 10·4 mM between (*a*) and (*b*). Voltage calibration 15 mV for (*a*) and (*b*), 5 mV for the remaining groups of traces. Trace length 570 msec. Records read from bottom upwards. Times at which they were recorded are shown in Fig. 8 (*c*)–(*f*).

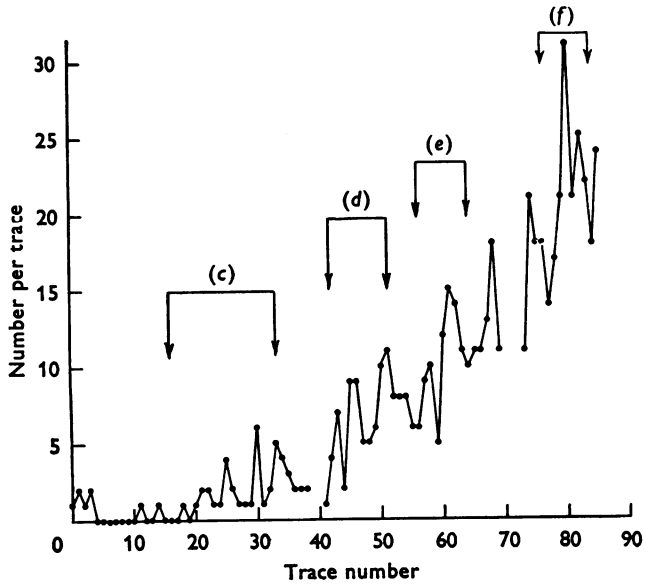

Fig. 8. The time course of development of the effect of raised external K^+ concentration on the recurrence frequency of miniature synaptic potentials in cell 87 (cf. Fig. 7, *c*–*f*). The number of miniature synaptic potentials per trace has been shown against each successive trace. Each trace represents 570 msec. Record starts 2 min after addition of K^+ (see text).

mean amplitude of 1·0 mV and a range of 0·6–1·8 mV. The effects on the normal recurrence frequency of Mg^{2+} cannot be inferred from the results of this experiment, since it is probable that the initial frequency observed was related to the occurrence of orthodromic stimuli. The reduction in mean amplitude, however, probably reflects the fact that the post-synaptic sensitivity to acetylcholine is depressed in high concentrations of Mg^{2+} (cf. del Castillo & Katz, 1954). A more striking effect of Mg^{2+} was that on the recurrence frequency of miniature synaptic potentials, when this had been previously increased by raising the K^+ concentration. The recurrence frequency on the addition of Mg^{2+} was invariably reduced. In one experiment, for example, six miniature synaptic potentials were observed in an initial period of 52 sec in normal concentrations of K^+ and (zero) Mg^{2+}. When the K^+ concentration was raised to 14 mM, the recurrence frequency was increased, 85 discharges occurring in a period of 15 sec. When Mg^{2+} was then added to the bath to raise its concentration to 18 mM, the recurrence frequency was reduced, only three discharges occurring in a period of 2 min.

DISCUSSION

There is evidently a very close correspondence between the spontaneous release of acetylcholine at the sympathetic synapse in the frog and that at the neuromuscular junction. The acceleration of the recurrence frequency of the miniature synaptic potentials by a high external K^+ concentration, presumably acting by depolarizing the presynaptic nerve terminals, is strong presumptive evidence in favour of the idea (see Liley, 1956*b*; Katz, 1962) that the nerve impulse releases acetylcholine in the form of quanta which, when released spontaneously, produce miniature synaptic potentials. Further evidence in favour of this idea is described in the following paper (Blackman *et al.* 1963*c*).

It has frequently been suggested that the origin of the quantum of transmitter released from the nerve terminals at the neuromuscular junction (see e.g. Katz, 1962) is the characteristic 'synaptic vesicle'. The present results provide no evidence against this idea, since synaptic vesicles can be seen in the presynaptic terminals in sympathetic ganglia of the frog (De Robertis & Bennet, 1955; Taxi, 1961).

Although no miniature synaptic potentials have been observed in records from mammalian sympathetic ganglion cells (see Eccles, 1955), it is tempting to suppose that they may be 'induced' by presynaptic depolarization, and that this depolarization accounts for the spontaneous output of acetylcholine from mammalian ganglia when they are perfused with K^+-enriched solutions (Brown & Feldberg, 1936).

SUMMARY

1. The existence of miniature synaptic potentials in sympathetic ganglion cells of the frog has been confirmed. Their mean amplitude varied as a rule from 1 to 3 mV, with a standard deviation of 30–50 % of the mean. Their recurrence frequency was usually less than 1/3 sec.

2. The miniature synaptic potentials were reduced in amplitude by curarization, and were presumably due to the action of 'packets' of acetylcholine.

3. The release of these packets was spontaneous, since miniature synaptic potentials persisted in concentrations of K^+ which blocked presynaptic conduction. An increase in recurrence frequency, usually to more than 50 times the value in a normal K^+ concentration, occurred when K^+ was raised to 10–14 mM. This effect was reversed by high Mg^{2+} concentrations.

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