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CHARACTERIZATION OF MINIATURE INHIBITORY POST-SYNAPTIC POTENTIALS IN RAT SPINAL MOTONEURONES

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SUMMARY

1. Intracellular recordings were made from motoneurones in the isolated spinal cord of neonatal rats. After action potentials had been abolished by tetrodotoxin (TTX, 10^{-6} g/ml), small (~ 0·4 mV) depolarizing potentials occurred spontaneously in motoneurones at low frequencies (~ 1·5 Hz).

2. These potentials were detectable only after the intracellular Cl^- concentration of motoneurones was raised by using KCl electrodes and most of them were blocked by strychnine, suggesting that they are inhibitory post-synaptic potentials (i.p.s.p.s). These spontaneous i.p.s.p.s under TTX are designated as 'miniature i.p.s.p.s' in order to distinguish them from i.p.s.p.s arising from spontaneous impulse activities of interneurones or afferent fibres.

3. The miniature i.p.s.p.s were still observed after Ca^{2+} in saline was substituted by Mg^{2+} or Mn^{2+}. In low Ca^{2+} and high Mg^{2+} saline, the amplitude distribution of miniature i.p.s.p.s was essentially the same as in normal saline.

4. The frequency of miniature i.p.s.p.s increased when external Ca^{2+} concentration was raised. The frequency decreased to about 60 % of the control when external Ca^{2+} was substituted by Mg^{2+} (2–4 mM), whereas it increased to more than 20-fold when substituted by Mn^{2+} (3–5 mM).

5. When the external K^+ concentration was raised, the frequency of miniature i.p.s.p.s under TTX increased non-linearly with the K^+ concentration. The maximum slope in the relation between the log frequency and log K^+ concentration was about 3·6.

6. When the osmotic pressure was increased by adding sucrose, miniature i.p.s.p.s increased in frequency. The effect of osmotic pressure was relatively mild compared with that reported for the miniature end-plate potentials (e.p.p.s) in the frog.

7. When the temperature was raised, the frequency of miniature i.p.s.p.s increased. The relation between frequency and temperature fitted approximately to a straight line in Arrhenius plot with a Q_{10} of about 2·6.

8. These characteristics of the miniature i.p.s.p.s closely resemble those of the miniature e.p.p.s. It is concluded that the miniature i.p.s.p.s recorded in motoneurones are equivalent in nature to the miniature e.p.p.s in neuromuscular junctions, thus reflecting the spontaneous release of quantal packages of the inhibitory transmitter.

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INTRODUCTION

It is generally agreed that acetylcholine (ACh) is released from the motor nerve terminal in a 'quantal' manner (del Castillo & Katz, 1954). A quantal nature of transmitter release at central synapses has also been proposed for excitatory (Kuno, 1964, 1971) as well as for inhibitory (Kuno & Weakly, 1972) synapses. However, quantal release at central synapses has recently been questioned because of the non-stepwise fluctuation of the Ia excitatory post-synaptic potentials (e.p.s.p.s) (Edwards, Redman & Walmsley, 1976*a*, *b*; Redman, 1979). The amplitude fluctuation of e.p.s.p.s has thus been attributed either to the intermittent failure of impulse invasion into the preterminal arborization (Edwards *et al.* 1976*a*, *b*; Burke & Rudomin 1977) or to non-uniform release probabilities at different boutons of the same afferent fibre (Jack, Redman & Wong, 1981). Furthermore, a lack of identification of unit synaptic potentials corresponding to the miniature end-plate potential (e.p.p.; Fatt & Katz, 1952) in mammalian central neurones has made it difficult to evaluate whether the evoked synaptic potential is indeed composed of unit potentials (Redman, 1979).

In a preliminary study (Takahashi, 1984), spontaneous miniature inhibitory post-synaptic potentials (i.p.s.p.s) have been recorded from motoneurones of the rat spinal cord after raising intracellular Cl^- concentrations. The miniature i.p.s.p.s were observed in the presence of tetrodotoxin and/or in the absence of extracellular Ca^{2+}, indicating that they are not synaptic potentials arising from impulse activities. The main purpose of the present study was to test whether the general behaviour of the miniature i.p.s.p.s is similar to that of the miniature e.p.p.s observed at the neuromuscular junction.

METHODS

Wistar rats, 5–8 days after birth, were used for the experiments. The experimental procedures were essentially the same as those previously reported (Fulton, Miledi & Takahashi, 1980; Harada & Takahashi, 1983). The lumbar cord, together with roots, was excised under ether anaesthesia and hemisected sagittaly in oxygenated saline (Otsuka & Konishi, 1974). The pial membrane on the lateral surface was removed with a pair of fine scissors and forceps to facilitate penetration with a micro-electrode. The hemisected spinal cord was immobilized, with the lateral side upward, with small pins and strips of thin paper on the Sylgard-coated bottom of a chamber (volume ~ 3 ml) and was continuously superfused at a rate of 4 ml/min with saline having the following composition (mM): NaCl, 130; KCl, 4·5; Na_2HPO_4, 1; $NaHCO_3$, 25: $CaCl_2$, 2; $MgCl_2$, 1; D-glucose, 11. The pH of the solution bubbled with 95% O_2/5% CO_2 was about 7·3. The bath temperature was normally kept at 25 ± 1 °C by a Peltier thermocouple. In high K^+ saline, NaCl was replaced with equimolar KCl. In other experiments, sucrose was used for adjusting or raising the osmolarity.

Intracellular recordings from lumbar motoneurones (L4 and 5) were made with electrodes filled with 3 M-KCl. The resistances of the electrodes were 25–30 MΩ after bevelling on aluminum slurry (Lederer, Spindler & Eisner, 1979). To identify a motoneurone, the ventral roots were stimulated antidromically with a suction electrode. Only those motoneurones whose resting potentials exceeded −50 mV in normal saline were used for the study. After penetrating motoneurones, tetrodotoxin (TTX, Sankyo, 10^{-6} g/ml) was applied to block the action potentials. All records were stored in an analog data recorder (TEAC, MR–10) through a filter of 3 kHz and played back on an oscilloscope for the measurements.

The perfusion fluid was changed by a magnetic valve system. After changing each solution, it took about 6 min before the frequency and amplitude of miniature i.p.s.p.s attained a new steady level.

RESULTS

Spontaneous synaptic potentials in motoneurones in the absence or presence of impulse activity

Spontaneous synaptic potentials were observed in spinal motoneurones bathed in normal saline at rest (Fig. 1*A*). These potentials recorded with KCl electrodes were all depolarizing potentials at the resting membrane potential ($-62{\cdot}2 \pm 6{\cdot}1$ mV; mean $\pm$S.D. of seventy-one motoneurones). Many of them were apparently i.p.s.p.s, since the frequency of the spontaneous potential declined markedly after administration

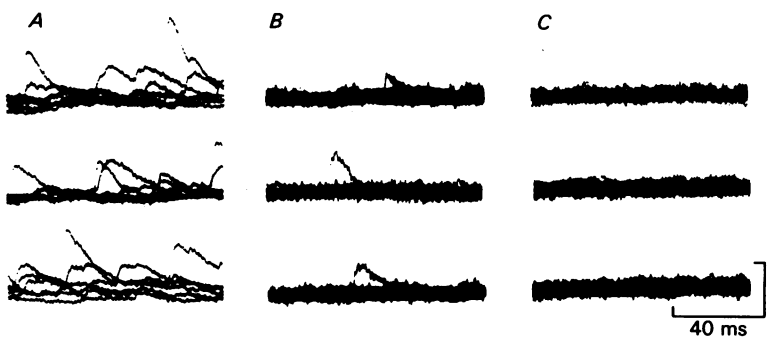

Fig. 1. Spontaneous potentials recorded in a motoneurone before (*A*) and 10 min after (*B*) the application of TTX (10^{-6} g/ml). Records in *C* were 15 min after the application of strychnine in TTX saline. 6 sweeps (*A*) and 10 sweeps (*B* and *C*) were superimposed in each record. Vertical calibration bar indicates 2·5 mV for *A* and 1 mV for *B* and *C*. In this motoneurone, the frequencies of the spontaneous potentials before and after TTX were 12·5 Hz (*A*) and 1·2 Hz (*B*), respectively. The resting membrane potentials were -62 mV (*A*) ~ -63 mV (*B* and *C*).

of strychnine (Takahashi, 1984; also, see below). Spontaneous potentials in normal saline occurred usually at high frequencies (mean, 11·1 Hz; S.D., 8·2 Hz; twenty-two motoneurones), and their amplitudes were widely dispersed (mean, 1·3 mV; S.D., 0·5 mV; twenty-two motoneurones, Figs. 1*A* and 3*A*1). After the application of TTX(10^{-6} g/ml), all action potentials evoked antidromically, orthodromically or intracellularly were abolished in a few minutes. Under such conditions, the frequency of the spontaneous potentials was markedly reduced, but definite potential blips still occurred spontaneously at low frequencies (Fig. 1*B*). The mean amplitude and frequency of the spontaneous potentials in TTX saline varied in different motoneurones. Their mean values were 0·4 mV (S.D. 0·1 mV; fifteen motoneurones) and 1·5 Hz (S.D. 0·96 Hz; thirty-two motoneurones), respectively. In a given motoneurone after TTX application, the amplitude and frequency of the spontaneous potentials remained fairly constant for over 1 h (Fig. 2*A* and *B*). These results are consistent with the idea that in normal conditions spontaneous potentials arise mostly from spontaneous impulse activities of interneurones and afferent fibres (Hubbard, Stenhouse & Eccles, 1967; Blankenship & Kuno, 1968). The impulse-independent spontaneous potentials observed in TTX saline are assumed to be similar to the

miniature e.p.p.s in origin. Thus, the spontaneous potentials observed in the presence of TTX are referred to below as the miniature synaptic potentials.

When potassium acetate electrodes were used for intracellular recording from motoneurones in TTX saline, the spontaneous potentials were hardly detectable (Takahashi, 1978). The amplitude of i.p.s.p.s in motoneurones of the cat has been

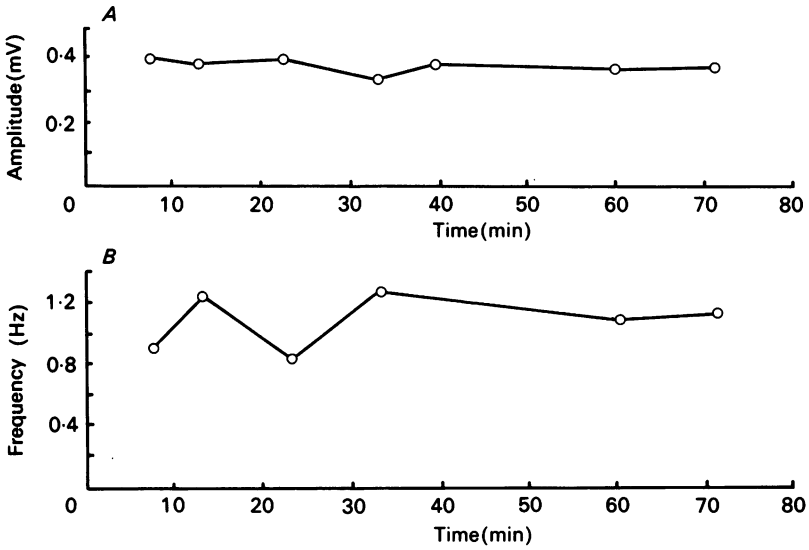

Fig. 2. Variation in mean amplitude (*A*) and frequency (*B*) of miniature i.p.s.p.s over a period of 1 h in a motoneurone. Abscissae, time after the application of TTX (10^{-6} g/ml). The mean amplitude and frequency before TTX were 2·3 mV and 12·2 Hz, respectively. Each point derived from measurements of 120–150 events. Resting potential varied from −71 to −79 mV.

shown to be affected by Cl^- injection (Araki, Ito & Oscarsson, 1961). Similarly, spontaneous potentials recorded from rat motoneurones with KCl electrode under TTX were increased in mean amplitude by Cl^- injections (Takahashi, 1984). Since the mean amplitude of the spontaneous potentials remained fairly constant in TTX saline (Fig. 2*A*), it can be presumed that the intracellular Cl^- concentration is maintained at a certain level during the recording with a KCl electrode. The application of strychnine, in concentrations (2–25 μM) at which the i.p.s.p.s in rat motoneurones are selectively blocked, abolished most of the miniature synaptic potentials (Fig. 1*C*; see also Takahashi, 1984). These results suggest that the spontaneous miniature synaptic potentials recorded under TTX are inhibitory in nature, i.e. spontaneous miniature i.p.s.p.s.

In low Ca^{2+} or high Mg^{2+} saline without TTX, both the frequency and amplitude of the spontaneous potentials were low compared with those in normal saline. However, under TTX the mean amplitude of the miniature i.p.s.p.s was virtually unaffected by changes in the Ca^{2+} or Mg^{2+} concentration. An example is shown in Fig. 3. In this experiment, the Ca^{2+} concentration was reduced from 2 to 0·5 mM, while the total divalent ion concentration was maintained at 3 mM, substituting

Mg^{2+} for Ca^{2+}. In the absence of TTX, the spontaneous potentials, particularly those of large amplitudes, were markedly reduced in the low Ca^{2+} solution (Fig. 3*A*1 and *B*1). In contrast, the amplitude distribution of miniature i.p.s.p.s (under TTX) in the low Ca^{2+} solution (Fig. 3*B*2) was approximately the same as in normal saline (Fig. 3*B*1). Thus, the amplitude of miniature i.p.s.p.s remained unchanged when the normal evoked transmitter release was suppressed by reducing the Ca^{2+} concentration.

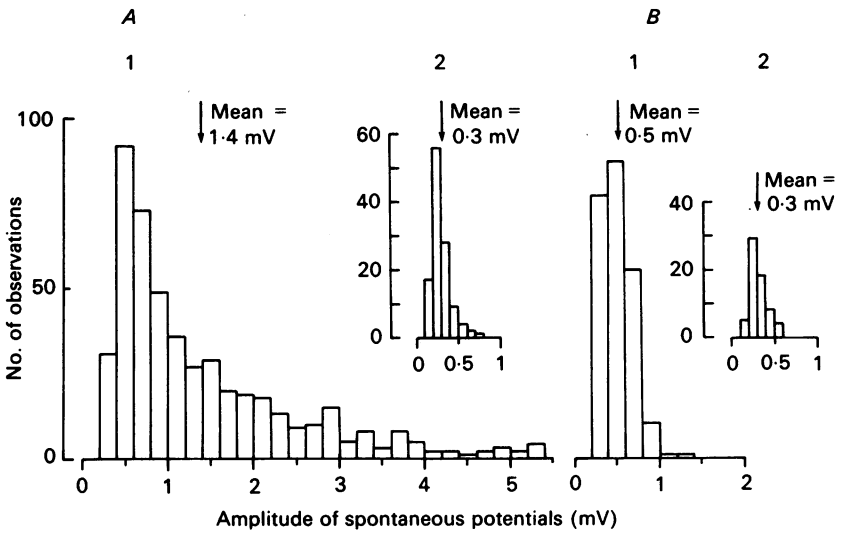

Fig. 3. Amplitude distributions of spontaneous potentials recorded from a motoneurone in the absence (*A*1 and *B*1) and presence (*A*2 and *B*2) of TTX. Abscissae and ordinates in *A*2 and *B*2 are amplitudes of miniature i.p.s.p.s and numbers of observations, respectively. Recordings were made in normal (2 mM-Ca^{2+}, 1 mM-Mg^{2+}) saline (*A*) and low Ca^{2+} (0·5 mM)–high Mg^{2+} (2·5 mM) saline (*B*). The recording sequence was *A*1, *B*1, *B*2, *A*2. Resting potential was $-71 \sim -72$ mV. Events for the histograms were sampled during 30 s (*A*1 and *B*1) or 60 s (*A*2 and *B*2). The mean frequencies were 16·2 Hz (*A*1), 2·0 Hz (*A*2), 7·1 Hz (*B*1) and 1·0 Hz (*B*2), respectively.

Random occurrence and time course of miniature i.p.s.p.s

The miniature i.p.s.p.s appear to occur randomly. This was further examined by comparing the observed interval histogram of the miniature i.p.s.p.s with the theoretical curve expected from the random occurrence. For a sample width of Δt, the theoretical number of events occurring at an interval between t and $t+\Delta t$ is given by the equation

$$n = N\Delta t/T \exp(-t/T),$$

where N is the total number of events observed and T is the mean interval (Fatt & Katz, 1952). As shown in Fig. 4, the experimental data fitted well to the theoretical curve, indicating that the miniature i.p.s.p.s occur at random without interaction between the events.

The miniature i.p.s.p.s were characterized by a fast rising phase and a slow decay. For example, in a motoneurone at 25 °C, the rise time to peak varied from 1 to 4 ms

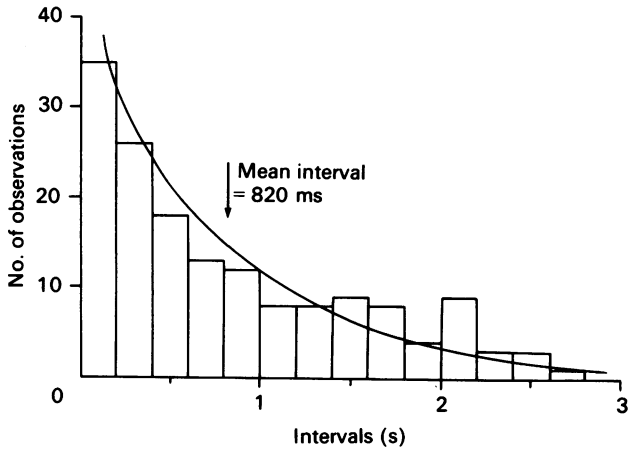

Fig. 4. Distribution of intervals between successive events in a series of 162 miniature i.p.s.p.s recorded from a motoneurone under TTX. Class interval, 0·2 s. Mean interval, 820 ms (arrow). Curve is the theoretical distribution for random events.

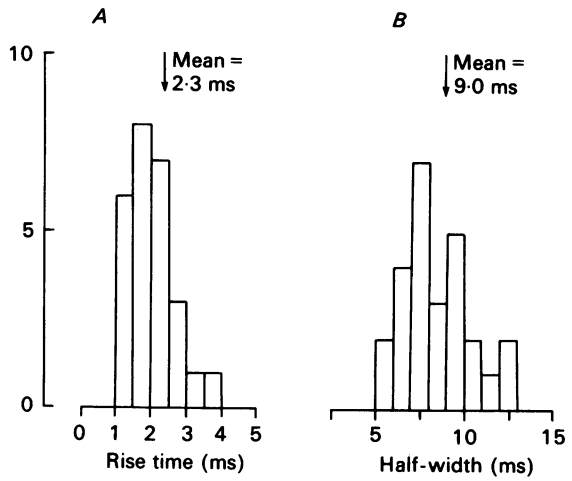

Fig. 5. Distributions of rise time (*A*) and half-width (*B*) of miniature i.p.s.p.s at 25 °C under TTX, measured from twenty-six events in a motoneurone.

with a mean of 2·3 ms (Fig. 5*A*), while the half-width ranged from 5 to 13 ms with a mean of 9·0 ms (Fig. 5*B*). Miniature i.p.s.p.s having a slow rise time usually had a long half-width, and the rise time was roughly correlated with the half-width. At high temperatures the time course of miniature i.p.s.p.s became shorter. For example, the mean half-width measured from the motoneurone shown in Fig. 5 was about 4 ms at 36 °C. Thus, the time course of the miniature i.p.s.p.s is not much different from that of the i.p.s.p.s observed in cat motoneurones *in vivo* (Eccles, 1964). The relatively constant time course of miniature i.p.s.p.s suggests that most of the observed miniature i.p.s.p.s arise from synaptic boutons terminating on restricted regions of the motoneurone.

Conditions which affect the frequency of miniature i.p.s.p.s

Various conditions are known to increase the frequency of miniature e.p.p.s (Fatt & Katz, 1952; Barton, Cohen & Van der Kloot, 1983). These include external concentrations of Ca^{2+} and K^+, osmotic pressure and temperature. We have examined whether these conditions affect the frequency of miniature i.p.s.p.s in the same manner as that of miniature e.p.p.s.

External Ca^{2+} concentration. An increase of the extracellular Ca^{2+} concentration increases the frequency of miniature e.p.p.s at the mammalian neuromuscular junctions (Boyd & Martin, 1956, Hubbard, 1961; Hubbard, Jones & Landau, 1968*a*), but little or not at all at those of the frog (Fatt & Katz, 1952). As the effect of external calcium on transmitter release is strongly voltage-dependent, such differences might arise simply from different levels of the resting potential in the presynaptic terminals. In rat motoneurones, the miniature i.p.s.p.s markedly increased in frequency when the Ca^{2+} concentration was raised 5-fold (10 mM, Fig. 6*A*2). The Ca^{2+} effect was already clear at 4 mM-Ca^{2+}. The stimulating effect of high Ca^{2+} concentration upon the frequency of miniature i.p.s.p.s was reversible after washing with normal saline (Fig. 6*A*3). Conversely, when the external Ca^{2+} was removed and substituted by Mg^{2+} (2–4 mM, Fig. 6*B*2), the frequency of miniature i.p.s.p.s decreased to about 62% (S.D. 9%; four motoneurones) of the control (Fig. 6*B*1). Reduction of the frequency of miniature e.p.p.s in low Ca^{2+} concentrations has also been reported in mammalian end-plates (Elmqvist & Feldman, 1965) as well as in those of the frog (Blioch, Glagoleva, Liberman & Nenashev, 1968; Miledi & Thies, 1971). However, when the Ca^{2+} was substituted by Mn^{2+} (3–5 mM), instead of Mg^{2+}, the frequency of miniature i.p.s.p.s markedly increased. An increase of the frequency in Mn^{2+} saline also occurs with miniature e.p.p.s (Balnave & Gage, 1973). The average frequency and amplitude of miniature i.p.s.p.s in Mn^{2+} saline was 21·4 Hz and 0·69 mV, respectively (S.D. 9·6 Hz and 0·16 mV; four motoneurones).

External K^+ concentration. An increase in extracellular K^+ concentration is well known to increase the frequency of miniature e.p.p.s (Fatt & Katz, 1952; Liley, 1956; Landau, 1969). The effect of high K^+ concentration or direct depolarization of the nerve terminal upon transmitter release has been attributed to an increase in intracellular Ca^{2+} concentration of the nerve terminal following an influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (Katz & Miledi, 1967, 1969; Miledi, 1973; Alnaes & Rahamimoff, 1975). Similarly, in rat motoneurones an increase in K^+ concentration produced a marked increase in the frequency of miniature i.p.s.p.s (Figs. 7 and 8; see also Takahashi, 1984). To eliminate a possible osmotic effect, added KCl was balanced by an equimolar reduction of NaCl. The reduction of NaCl itself did not appreciably change the frequency of miniature i.p.s.p.s within the concentration range used for the experiment. For example, when NaCl was reduced by 18 mM, while its isosmolarity was maintained by adding sucrose, the frequency of miniature i.p.s.p.s was within the normal range (control, 2·5 Hz; low NaCl saline, 2·2 Hz). An increase of the frequency of miniature i.p.s.p.s was clear at extracellular K^+ concentrations of more than 9 mM. As the K^+ concentration was raised further, a greater increase of the frequency was observed. At K^+ concentrations higher than 22·5 mM, the miniature i.p.s.p.s fused and the frequency became too high to measure.

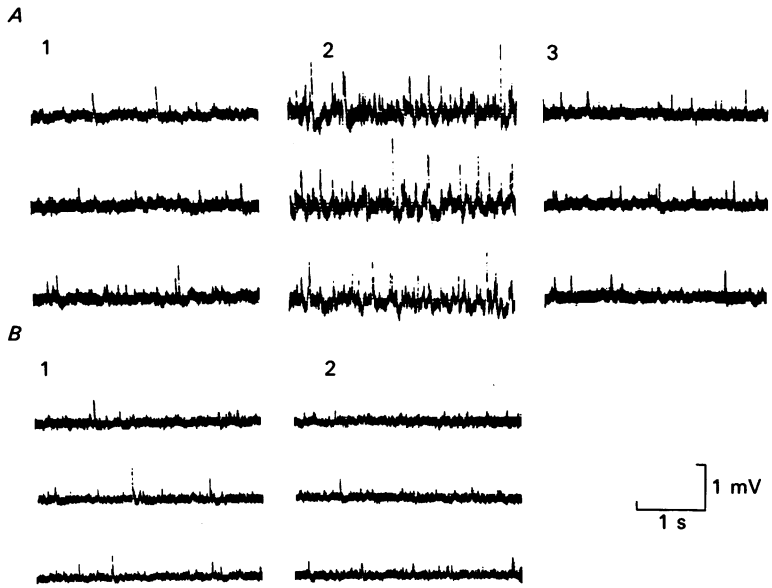

Fig. 6. Effects of external Ca^{2+} concentration on the miniature i.p.s.p.s under TTX. Records in *A* and *B* were obtained from different motoneurones. Traces in *A*2 were recorded 15 min after changing from normal (*A*1) to high Ca^{2+} (10 mM) saline. Traces in *A*3 were recorded 25 min after washing with normal saline. Traces in *B*2 were recorded 20 min after changing solution from normal (*B*1) to nominally Ca^{2+}-free saline containing Mg^{2+} (5 mM). Isomolarity was maintained by adding sucrose. The mean frequencies were 1·2 Hz (*A*1 and 3), 9·2 Hz, (*A*2), 1·4 Hz (*B*1) and 0·65 Hz (*B*2), respectively. Resting potentials were −59 mV (*A*1), −54 mV (*A*2), −56 mV (*A*3) −60 mV (*B*1) and −58 mV (*B*2), respectively.

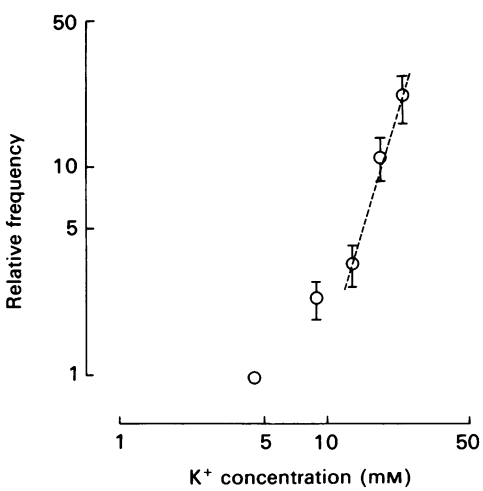

Fig. 7. Relation between the external K^+ concentration and the ratio of the mean frequency of miniature i.p.s.p. under TTX relative to the control (4·5 mM) in double logarithmic plot. The mean frequency in control saline solution was 1·4 Hz (S.D. 0·7 Hz; eleven motoneurones). Each point derived from three–five motoneurones. Vertical bars indicate S.D. In high K^+ saline isosmolarity was maintained by reducing NaCl concentration. The dashed line was drawn by the least-squares method. Average resting potentials of motoneurones were −67, −55, −52, −49 and −38 mV for the K^+ concentrations of 4·5, 9, 13·5, 18 and 22·5 mM, respectively.

The relation between K^+ concentration and frequency was non-linear, the maximum slope being about 3·6 in double logarithmic plot (Fig. 7, dashed line). This Figure is comparable to that reported for the miniature e.p.p.s in the frog (4.0, Liley, 1956, after Katz, 1962) but smaller than that in human end-plates (6·0, Cull-Candy, Miledi, Trautman & Uchitel, 1980).

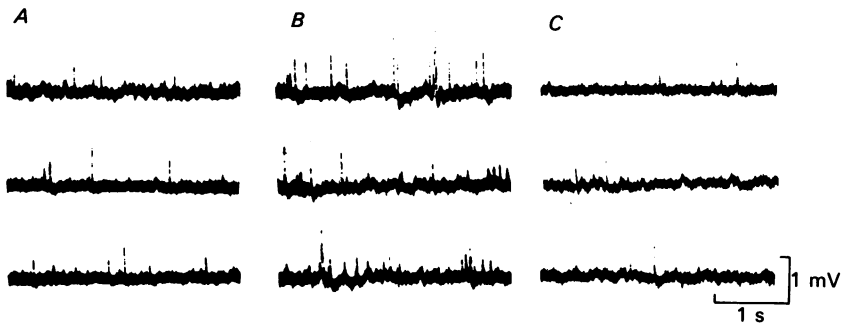

Fig. 8. Effects of external K^+ concentration on the frequency of miniature i.p.s.p.s under TTX in the presence (*B*) and absence (*C*) of external Ca^{2+}. Extracellular K^+ concentrations were 4·5 mM in *A* and 13·5 mM in *B* and *C*. Ca^{2+} concentrations were 2 mM in *A* and *B* and nominally free (Mg^{2+}, 5 mM) in *C*. The mean frequencies and resting potentials were 3·6 Hz, −55 mV (*A*), 13·5 Hz, −30 mV (*B*) and 1·6 Hz, −35 mV (*C*), respectively.

The frequency of miniature i.p.s.p.s increased in high K^+ saline only when extracellular Ca^{2+} was present (Fig. 8). Removal of Ca^{2+} by substitution by Mg^{2+} (3–5 mM) virtually abolished the effect of K^+ (Fig. 8*C*). The frequency of miniature i.p.s.p.s in Ca^{2+}-free (Mg^{2+}) high K^+ (13·5 mM) saline was comparable to that in Ca^{2+}-free (Mg^{2+}) normal K^+ saline (Fig. 6*B*2). Furthermore, in Ca^{2+}-free Mn^{2+} saline, an increase of K^+ concentration did not increase the frequency of miniature i.p.s.p.s at all.

Osmotic pressure. An increase in the osmotic pressure has been reported to produce a pronounced increase in the frequency of miniature e.p.p.s (Fatt & Katz, 1952; Furshpan, 1956; Liley, 1956; Blioch *et al.* 1968; Hubbard, Jones & Landau, 1968*b*). In rat motoneurones, the effect of osmotic pressure was tested on miniature i.p.s.p.s. When the osmotic pressure was raised by more than 10%, the frequency of miniature i.p.s.p.s increased significantly (Fig. 9). Several minutes after changing solution, the frequency reached a plateau and then stayed nearly constant for at least 15 min. Increased frequency with high osmotic pressure was not accompanied by any appreciable change in the membrane potential, and the effect was reversible. The frequency of miniature i.p.s.p.s increased in proportion to the rise in osmotic pressure, but tended to reach a plateau at high levels (Fig. 9*B*). The relation differs from the much steeper increase in frequency of miniature e.p.p.s observed at frog neuromuscular junctions (Fatt & Katz, 1952; Furshpan, 1956), but is comparable to the behaviour of miniature e.p.p.s in the rat diaphragm, in which the frequency–osmolarity relation also reaches a plateau at high osmolarity levels (Hubbard *et al.* 1968*b*).

Temperature. As the temperature of the superfusing saline was raised, the

miniature i.p.s.p.s became not only faster in time course but also occurred more frequently. The relation between the frequency of miniature i.p.s.p.s and temperature apparently fitted to a straight line between 21 and 36 °C (Fig. 10, dashed line) in Arrhenius plot. Below 21 °C the behaviour was more complicated, showing an occasional increase of frequency in response to lowering temperature. This differs

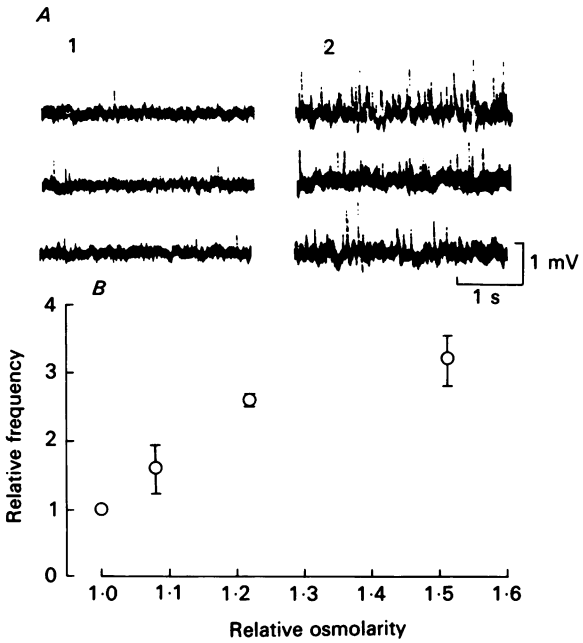

Fig. 9. Effect of high osmotic pressure on the frequency of miniature i.p.s.p.s under TTX. Sample records derive from a motoneurone before (*A* 1) and after (*A* 2) adding 120 mM-sucrose (osmolarity relative to control, 1·5). Mean frequencies and resting potentials were 1·3 Hz, −61 mV (*A*1) and 6·3 Hz, −58 mV (*A* 2), respectively. Ordinate in *B* indicates the frequency of miniature i.p.s.p.s relative to that in normal saline. The mean frequency in control solution was 1·3 Hz (S.D. 0·79 Hz; eight motoneurones). Osmolarities in each solution were examined by an osmometer and were plotted on the abscissa.

from miniature e.p.p.s in the frog (Fatt & Katz, 1952) but is similar to what has been reported for miniature e.p.p.s at the mammalian neuromuscular junction (Liley, 1956; Ward, Crowley & Johns, 1972). Since the membrane potential of motoneurones tended to decline at temperatures lower than 20 °C, a similar depolarization may have occurred in the presynaptic terminals, thereby increasing the frequency of miniature i.p.s.p.s. Another possibility would be that the Ca^{2+} uptake system within the nerve terminals may have been suppressed at low temperatures, resulting in Ca^{2+} accumulation in the cytoplasm (Alnaes & Rahamimoff, 1975). The average Q_{10} estimated from the straight lines between 21 and 36 °C in three motoneurones was 2·6 (S.D., 0·17). This value is comparable to that reported for miniature e.p.p.s (3·0, Fatt & Katz, 1952; 3–4, Liley, 1956; 1·5–3·1, Boyd & Martin, 1956; but 13·1, Hofmann, Parsons & Feigen, 1966).

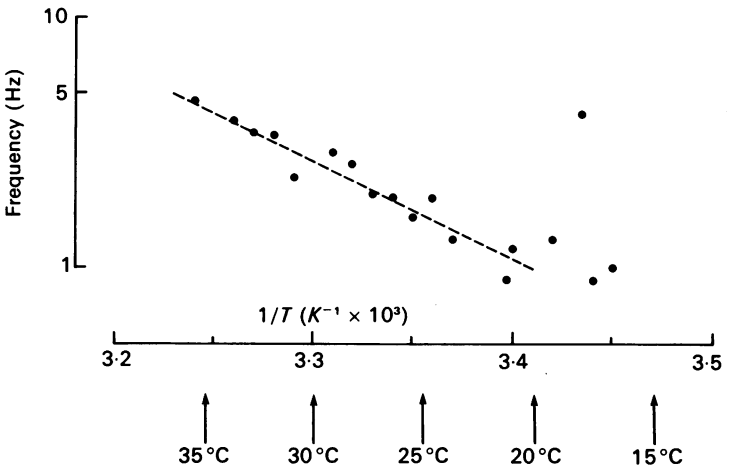

Fig. 10. Arrhenius plot of the mean frequency of the miniature i.p.s.p.s recorded in a motoneurone under TTX. Temperature in the bath was changed by changing currents in a Peltier element. The dashed line was drawn by the least-squares method.

DISCUSSION

Synaptic 'noise' in mammalian central neurones (Brock, Coombs & Eccles, 1952) has been attributed to the synaptic potentials produced by spontaneous impulse bombardment arising from interneurones or afferent fibres (Hubbard *et al.* 1967). It has been suggested that the possible quantum action of the transmitter is too small to be detected (Brock *et al.* 1952; Redman, 1979). In the previous (Takahashi, 1984) and present studies, spontaneous miniature i.p.s.p.s were clearly recorded, using KCl electrodes, after impulse activities were abolished by TTX. The miniature i.p.s.p.s cannot be attributed to the residual impulse activity of interneurones nor to spontaneous depolarization of the nerve terminals, since they still exist after synaptic transmission was blocked by removal of external Ca^{2+}. Therefore, it is likely that the miniature i.p.s.p.s observed in motoneurones under TTX are produced by spontaneous release of individual packages of the inhibitory transmitter from the synaptic boutons.

Spontaneous potentials resistant to TTX or synaptic blockade have also been reported in the motoneurones of the isolated frog spinal cord (Katz & Miledi, 1963; Colomo & Erulkar, 1968), cat spinal cord *in situ* (Blankenship & Kuno, 1968), as well as in spinal motoneurones (Shapovalov, Shiriaev & Tamarova, 1979) or hippocampal neurones (Brown, Wong & Prince, 1979) in slice preparations. However, little has been reported about their characteristics, particularly in comparison to those of the miniature e.p.p.s. The present results demonstrate that the general characteristics of the miniature i.p.s.p.s in rat motoneurones are similar to those of miniature e.p.p.s in many respects. First, the amplitudes of both types of miniature potentials were not affected by lowering extracellular Ca^{2+} and raising Mg^{2+} concentrations (Fatt & Katz, 1952). Secondly, the frequencies of both miniature potentials increased in high external Ca^{2+} concentrations (Boyd & Martin, 1956;

Hubbard, 1961; Hubbard *et al.* 1968*a*; but see Fatt & Katz, 1952). Thirdly, the frequencies of both miniature potentials decreased when external Ca^{2+} was substituted by Mg^{2+} (Elmqvist & Feldman, 1965; Blioch *et al.* 1968; Miledi & Thies, 1971), but increased when substituted by Mn^{2+} (Balnave & Gage, 1973). Fourthly, an increase in K^+ concentration markedly increased the frequency of both the miniature potentials only when extracellular Ca^{2+} was present (del Castillo & Katz, 1954). Fifthly, the frequency of both miniature potentials increased under high osmotic pressures (Fatt & Katz, 1952; Furshpan, 1956; Blioch *et al.* 1968; Hubbard *et al.* 1968*b*). Sixthly, both miniature potentials behaved similarly when the temperature was changed. These similarities in characteristics between miniature i.p.s.p.s and e.p.p.s further support the idea that the miniature i.p.s.p.s in motoneurones occur as a result of the release of transmitter quanta as do the miniature e.p.p.s.

Since miniature i.p.s.p.s are now clearly recorded from motoneurones, the analysis of the unitary potentials of monosynaptically evoked i.p.s.p.s (e.g. Kuno & Weakly, 1972) would be helpful for testing further the quantum hypothesis of transmitter release at central synapses (Kuno, 1971).

While most of the spontaneous miniature potentials were abolished by strychnine, occasionally small potential blips were observed in the presence of strychnine and TTX (see also Takahashi, 1984). Whether these potentials are excitatory or residual inhibitory ones still awaits to be elucidated. The dominant occurrence of the miniature i.p.s.p.s in the motoneurones could possibly be attributed to the dendritic localization of the excitatory inputs. However, it is also possible that the quanta of excitatory transmitter are too small or their release too infrequent to be detected (Redman, 1979).

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