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Alteration of the fast excitatory postsynaptic current by barium in voltage-clamped amphibian sympathetic ganglion cells

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1 Barium-induced alterations in fast excitatory postsynaptic currents (e.p.s.cs) have been studied in voltage-clamped bullfrog sympathetic ganglion B cells.

2 In the presence of 2–8 mM barium, e.p.s.c. decay was prolonged and in many cells the e.p.s.c. decay phase deviated from a single exponential function. The decay phase in these cases was more accurately described as the sum of two exponential functions. The frequency of occurrence of a complex decay increased both with increasing barium concentration and with hyperpolarization.

3 Miniature e.p.s.c. decay also was prolonged in barium-treated cells.

4 E.p.s.c. amplitude was not markedly affected by barium (2–8 mM) in cells voltage-clamped to −50 mV whereas at −90 mV there was a progressive increase in peak size with increasing barium concentration.

5 In control cells the e.p.s.c.-voltage relationship was linear between −20 and −100 mV; however, this relationship became progressively non-linear with membrane hyperpolarization in barium-treated cells. The e.p.s.c. reversal potential was shifted to a more negative value in the presence of barium.

6 There was a voltage-dependent increase in charge movement during the e.p.s.c. in barium-treated cells which was not present in control cells.

7 We conclude that the voltage-dependent alteration in e.p.s.c. decay time course, peak amplitude and charge movement in barium-treated cells is due to a direct postsynaptic action of barium on the kinetics of receptor-channel gating in postganglionic sympathetic neurones.

Introduction

Barium is a potent pharmacological blocker of voltage-dependent potassium channels in a variety of tissues (Sperelakis *et al.*, 1967; Krnjevic *et al.*, 1971; Standen & Stanfield, 1978; Armstrong & Taylor, 1980; Adams *et al.*, 1982). Further, with moderate concentrations and brief exposures, barium can be used to block selectively some currents such as the 'M' current whereas longer durations of exposure are required to block other membrane potassium conductances, such as the delayed rectifier (Adams *et al.*, 1982).

Barium also is commonly used in place of external calcium to study the kinetic properties of voltage-gated calcium channels (Hagiwara & Byerly, 1981; Reuter *et al.*, 1982; Hagiwara & Ohmori, 1982). Barium has permeability properties similar to calcium, but unlike calcium, it produces much less calcium channel inactivation or activation of outward potassium currents (Tillotson, 1979; Gorman & Hermann, 1979).

In a preliminary examination of the influence of different divalent cations on ganglionic transmission, we observed that barium altered the amplitude and decay time course of excitatory postsynaptic currents (e.p.s.cs) in voltage-clamped postganglionic neurones. These alterations could not be attributed to a barium-induced block of non-synaptic membrane potassium channels (Sperelakis *et al.*, 1967;

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Krnjevic *et al.*, 1971; Standen & Stanfield, 1978; Armstrong & Taylor, 1980; Adams *et al.*, 1982). Consequently, in addition to its other pharmacological actions, it appeared that barium also directly influenced the kinetic properties of the nicotinic e.p.s.c. in ganglion cells. As barium is used so commonly as a pharmacological tool, we felt it of interest to investigate the postsynaptic action of barium on ganglionic cells in more detail. We have found that in barium-treated sympathetic postganglionic cells there is a voltage- and concentration-dependent alteration in e.p.s.c. size, decay time course and amount of charge moved. We suggest that all of these effects can be attributed to a direct action of barium on nicotinic receptor-channel gating in postganglionic sympathetic neurones.

Methods

All experiments were done *in vitro* on B cells of the VIII, IX and X ganglia of the sympathetic chain of the bullfrog, *Rana catesbeiana* in a HEPES-buffered solution. No attempt was made to identify the cells as either fast B type or slow B type cells (Dodd & Horn, 1983). The composition of the control solution was (mM): NaCl 120, KCl 2.5, $CaCl_2$ 1.8, HEPES 1.0, pH = 7.3, 21–23°C. $BaCl_2$ (2–8 mM) was added directly to normal Ringer solution containing 1.8 mM calcium. In each experiment, e.p.s.cs were initially obtained from at least three untreated cells and then e.p.s.cs were recorded from other cells in the same preparation after an approximately 15 min equilibration period. The influence of barium was then tested in a number of different cells over a subsequent exposure period which ranged up to 110 min in any given concentration. The results obtained from different preparations were pooled.

Individual B cells (30–60 μm) were identified with a compound microscope using brightfield illumination at a magnification of 150–200 × and impaled with two glass microelectrodes filled with 3 M KCl (resistances of 6–15 MΩ). The cells were voltage-clamped using a two microelectrode voltage clamp as described previously (MacDermott *et al.*, 1980; Connor *et al.*, 1983). The following criteria were used to select currents for analysis; (1) the voltage deviation was less than 0.5% of the driving force (membrane potential-reversal potential), (2) the shift in the reference voltage after a voltage-clamp run was less than or equal to ±5 mV, and (3) the individual currents had a stable baseline.

Fast excitatory postsynaptic currents were elicited by constant preganglionic stimulation at a frequency of ~ 0.4 Hz. Individual currents were filtered (2 kHz) and digitized with a PDP 11/03 computer (Digital Equipment Corp., Marlboro, MA). Four to eight individual currents were averaged to improve the signal to noise ratio. However, under all experimental conditions, each individual e.p.s.c. was similar to the averaged e.p.s.c. Each mean current was analysed for peak current amplitude and rate of decay. The e.p.s.c. decay in control cells was well described as a single exponential function. However, in many barium-treated cells the e.p.s.c. decay was not adequately fitted as a single exponential but was adequately fitted by the sum of two exponential components. The following procedure was established to determine the appropriate fit; i.e. single or double exponential, for the decay time course. The decay phase of e.p.s.cs from barium-treated cells was fitted both as a single exponential or as the sum of two exponential components. A computer generated function was then superimposed on the e.p.s.c. decay. A RMS error between the actual and theoretical traces was determined to estimate the goodness of fit. E.p.s.cs were considered more adequately fitted by the sum of two exponentials if the double exponential curve fit reduced the RMS values obtained with the single fit by at least 50%. The current decay was analysed by a weighted linear regression of log (amplitude) versus time which compensated for the bias of noise (Connor *et al.*, 1983). Double exponential fits were obtained by successive peeling of each exponential component from the decay as described previously (Connor *et al.*, 1983).

The decay time course of spontaneous miniature excitatory postsynaptic currents (m.e.p.s.cs) and evoked e.p.s.cs were compared in some of the barium-treated preparations. The frequency of m.e.p.s.cs is very low in ganglia, but can be enhanced by repetitive preganglionic stimulation (Blackman *et al.*, 1963; McLachlan, 1977). Consequently, for these experiments, m.e.p.s.cs and e.p.s.cs were recorded after a 10 s period of 15 Hz preganglionic stimulation. In separate experiments the influence of 8 mM barium was determined on m.e.p.s.c. decay in cells exposed to an elevated potassium concentration. M.e.p.s.c. frequency is also increased in high potassium (McIsaac, 1971). In these experiments, 15 mM KCl was added directly to the control Ringer solution and m.e.p.s.cs were recorded from different cells before and during exposure to 8 mM barium. There are two cell types (B cells and C cells) in the ganglion. However, the B cells are consistently larger than C cells (Dodd & Horn, 1983) so that we only chose the largest cells for these particular experiments.

Total charge movement during the e.p.s.c., calculated for control cells and barium-treated cells voltage-clamped to − 50 mV and − 90 mV was determined by computerized integration.

Average data values obtained from several cells are consistently presented as mean ± s.e.mean.

Results

Postganglionic cell action potentials are prolonged in barium

In the initial series of experiments we studied the influence of 4 mM barium on the action potential configuration of postganglionic neurones. Indirectly stimulated action potentials were recorded in a number of control cells; barium was then added to the bathing solution and recordings were made in a number of other cells. Adams *et al.* (1982) have demonstrated that brief exposures to barium (1–4 mM) selectively inhibited the 'M' current but with longer durations of treatment, barium in the same concentration range also blocked the delayed rectifier. As a consequence, postganglionic cell action potentials progressively increased in duration. This was observed in the present experiments. Action potential prolongation developed gradually until 30–40 min of exposure. In addition to an altered action potential configuration, barium-treated cells gradually became depolarized so that in many of these cells, trains of spikes were recorded immediately following impalement. These diminished gradually as the cell depolarized further following impalement or could be stopped abruptly when hyperpolarized by the injection of current. Typical results are shown in Figure 1. Example (a) is a control cell action potential elicited by preganglionic stimulation and recorded at a membrane potential of − 55 mV. Record (b) shows the configuration of spontaneous spikes recorded in another cell maintained in the 4 mM barium solution for approximately 90 min. The resting membrane potential of this cell was − 46 mV when first impaled and a train of these spontaneous spikes was recorded. Record (c) is an indirectly stimulated spike recorded in the same cell after being hyperpolarized to − 57 mV. These results demonstrate the effect of barium on action potential configuration following a reduction of the voltage-dependent potassium current.

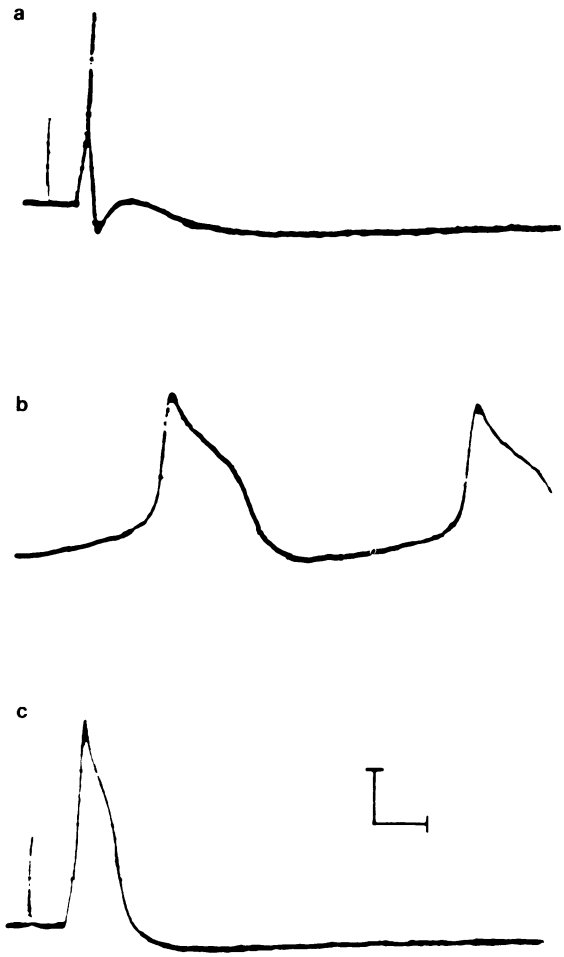

Figure 1 The influence of barium on ganglion cell action potentials. (a) An indirectly-stimulated action potential recorded in a control cell (resting potential was − 53 mV). (b) Spontaneous action potentials recorded immediately following impalement of a cell exposed to 4 mM barium for approximately 90 min (resting potential was − 48 mV). (c) An indirectly stimulated action potential recorded in the same cell as in (b) with the membrane potential increased to − 57 mV to stop the spontaneous activity. For all traces, the calibration equals 20 mV (vertical axis) and 20 ms (horizontal axis).

E.p.s.c. decay time course is altered in barium-treated cells

In all control cells, the e.p.s.c. rose to a peak value within a few ms and then decayed as a single exponential function over most of its time course (Kuba & Nishi, 1979; MacDermott *et al.*, 1980; Connor *et al.*, 1983). In eight control cells from three preparations voltage-clamped to − 50 mV, the time constant of decay (τ) was 4.7 ± 0.1 ms. Exposure to barium (2–8 mM) produced a concentration-dependent prolongation of the e.p.s.c. This is illustrated in Figure 2 which shows averaged e.p.s.cs obtained in four different cells from the same preparation, each voltage-clamped to − 50 mV. Record (a) was recorded from a control cell before barium treatment and records (b), (c) and (d) were recorded during exposure to 2, 4 or 8 mM barium. In the presence of barium, e.p.s.c. decay was not just simply prolonged in many cells but also could no longer fit as a single exponential function. In these cases the decay was more accurately described as the sum of two exponential decay

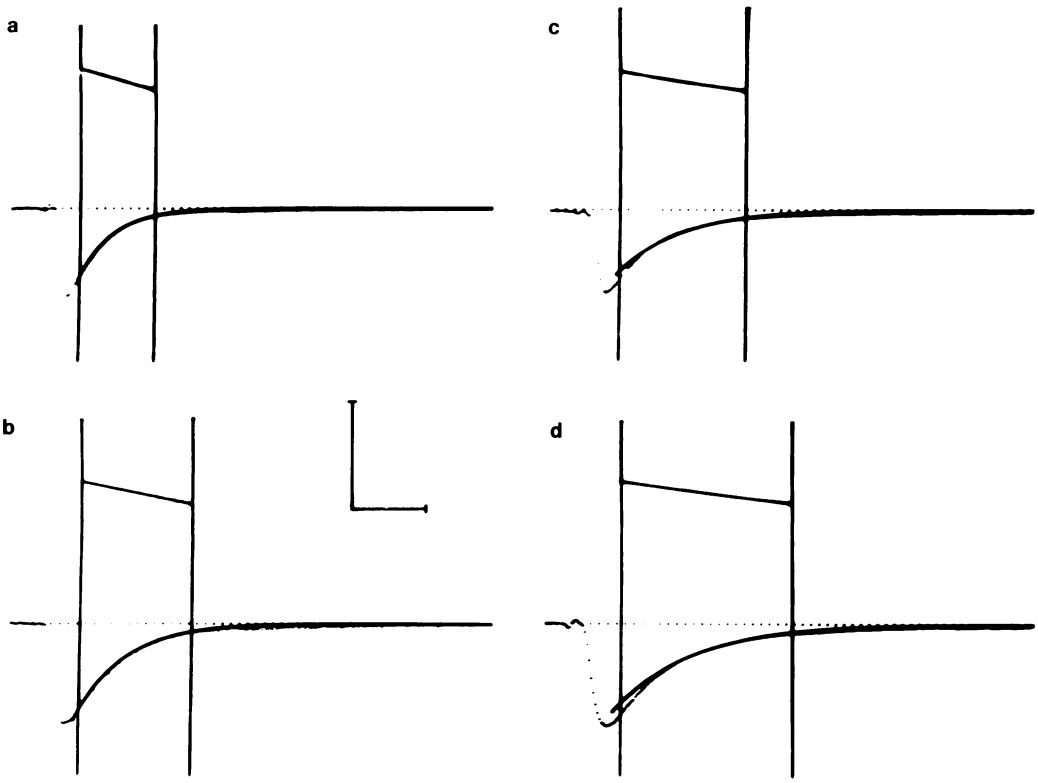

Figure 2 Averaged e.p.s.cs from different cells in one ganglion preparation illustrating the e.p.s.c. decay prolongation and deviation from a single exponential in the presence of barium. E.p.s.cs were obtained at −50 mV. (a) An averaged e.p.s.c. obtained in the absence of barium. (b–d) averaged e.p.s.cs recorded in 2 mM barium for 74 min, 4 mM for 63 min, or 8 mM for 51 min. A linear fit to the ln of the decay phase between the vertical cursor lines is shown above each trace. A computer generated exponential function with the τ derived from the component between the cursors (approx. 80–20% decay) is superimposed on the e.p.s.c. decay to illustrate the initial deviation from a single exponential and the residual tail component of e.p.s.cs recorded in barium. The vertical calibration bar equals 5 nA and the horizontal calibration equals 10 ms.

Table 1 Summary of decay characteristics in barium-treated preparations

Barium conc.	*Membrane voltage*	τ_1	τ_2	*Number of cells studied*	*% of cells exhibiting complex decay*
0	−50	4.7±0.1	—	8	0
0	−90	5.3±0.2	—	8	0
2	−50	4.1*	13.6*	4	50
2	−90	4.8±0.2	14.3±1.4	4	75
4	−50	3.9±0.3	13.3±0.2	5	80
4	−90	4.9±0.1	17.5±1.2	5	100
8	−50	5.1±0.2	17.9±1.0	11	100
8	−90	6.1±0.6	25.2±1.9	10	100

The results are expressed as mean ± s.e.mean except * mean of 2 experiments.

components. Because of this deviation from a single exponential decay, we have estimated the extent of e.p.s.c. prolongation by comparing the e.p.s.c. duration at the 50% decay time point in control cells and in cells exposed to barium. For cells voltage-clamped to − 50 mV, the duration was increased by approximately 25, 34 and 57% in 2, 4 or 8 mM barium over that of the control cell e.p.s.cs. In barium-treated cells the deviation of the e.p.s.c. decay phase from a single exponential time course was often subtle. It appeared as an initial period of rapid decay (τ_1) and as a residual tail component (τ_2). The pattern of this deviation can be seen by inspection of the records in Figure 2. In all of these examples a computer generated exponential is superimposed on the averaged e.p.s.c. decay phase. The time constant of this exponential was derived from a ln current versus time plot determined between the vertical cursor lines and shown above each record. In examples (c) and (d) the current trace deviated noticeably at the initial phase and tail component of the current decay time course. The percentage of cells with e.p.s.c. decays better fitted with two exponential components increased with increasing concentrations of barium. For those cells voltage-clamped to − 50 mV and exposed to 1.8 mM calcium Ringer solution containing either 2, 4 or 8 mM barium, the percentage of cells requiring two components for adequate fitting was 50, 75 and 100%, respectively. The two exponential component decay time constants were similar for cells treated with 2 or 4 mM barium, but were considerably greater in 8 mM barium-treated cells. These values are summarized in Table 1.

The increase of e.p.s.c. duration was more pronounced at hyperpolarized values of membrane potential. For cells voltage-clamped to − 90 mV the % increase in duration over the control cell e.p.s.c. was 33, 43 and 75% for cells exposed to 2, 4 or 8 mM barium. The percentage of cells which exhibited a complex decay also increased with hyperpolarization. Consequently, the percentage of cells voltage-clamped to − 90 mV which exhibited a complex decay was 75, 100 and 100% in the presence of 2, 4 or 8 mM barium, respectively. This influence of hyperpolarization on e.p.s.c. decay time course for a control cell and a barium-treated (8 mM) cell is shown in Figure 3. For the control cell, the e.p.s.c. duration is increased slightly with hyperpolarization. In contrast, the duration of the e.p.s.c. from the barium-treated cell was obviously more prolonged with hyperpolarization and the deviation from a single exponential decay accentuated at the more negative membrane potential. An illustration of the more adequate fit of the e.p.s.c. decay in barium-treated cells as the sum of two exponential components is shown in panel B_3. The e.p.s.c. shown in panel B_2 is fitted in panel B_3 as the sum of two exponential components having time constants of 6.0 and 21.3 ms. The computer-generated two component exponential function superimposed on the actual current clearly provides a good fit to the decay phase.

For the eight control cells the decay time constant (τ) showed a negative dependence on voltage in the range of − 30 to − 90 mV (MacDermott *et al.*, 1980; Connor *et al.*, 1983). As shown previously, the τ versus voltage relationship could be adequately fitted by the expression:

$$\tau(V) = a \exp(AV)$$

where a and A are constants. The average value of the voltage-sensitive coefficient A for eight control cells was $-0.0027 \pm 0.0003\ mV^{-1}$ (21–23°C). The voltage dependence of the two decay components was quantitated in ten cells exposed to 8 mM barium. In these cells both τ_1 and τ_2 increased with hyperpolarization; the coefficient of voltage dependence for τ_1 being -0.0049 ± 0.0011 and for τ_2 being -0.0086 ± 0.0022.

The alteration in e.p.s.c. decay by barium treatment was reversible. The characteristics of e.p.s.cs recorded from cells during the recovery period gradually returned close to those obtained from cells before barium treatment. Results from one of these experiments are presented in Figure 4. Record (a) shows an e.p.s.c. recorded at − 50 mV from a control cell. Record (b) was obtained at − 50 mV from another cell maintained in a 4 mM barium containing solution for 53 min. Record (c) shows an e.p.s.c. obtained from a third cell 165 min after this preparation was returned back to the barium-free Ringer solution. For this preparation the mean e.p.s.c. peak size and decay τ for four control cells voltage-clamped to − 50 mV was -3.3 ± 0.3 nA and 8.0 ± 0.5 ms. The values for e.p.s.c. size and τ recorded at − 50 mV from four other cells which were first exposed to 4 mM barium for 60 min and then collected during a 16–212 min wash period were -4.0 ± 0.6 nA and 7.8 ± 0.6 ms.

M.e.p.s.cs are also prolonged in barium-treated ganglia

In separate experiments we compared the time courses of m.e.p.s.cs and e.p.s.cs recorded in barium-treated preparations. Even though the frequency of m.e.p.s.cs is normally low in ganglia (Blackman *et al.*, 1963), the m.e.p.s.c. frequency can be increased transiently by a brief period of repetitive preganglionic stimulation. This post-stimulation facilitation of m.e.p.s.c. release is greatly enhanced in barium-treated preparations (McLachlan, 1977; Silinsky, 1977). Consequently, we recorded e.p.s.cs and m.e.p.s.cs from 4 and 8 mM barium-treated cells after a 10 s period of preganglionic stimulation of 15 Hz. For these experiments the cells were voltage-

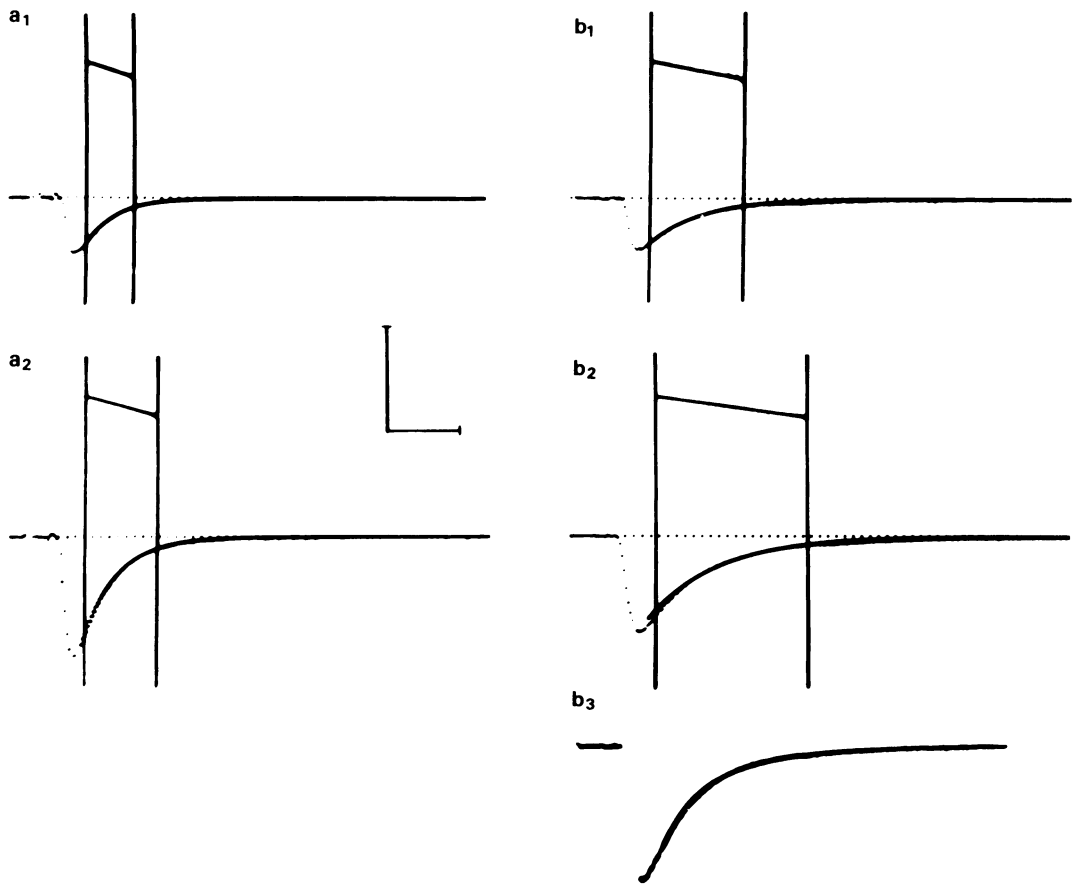

Figure 3 Averaged e.p.s.cs from two different cells which illustrate the voltage dependence of the alteration in the e.p.s.c. decay time course by barium. (a) Averaged e.p.s.cs obtained at − 50 mV (a_1) or − 90 mV (a_2) in a control cell. (b) Averaged e.p.s.cs obtained in another cell from the same preparation during exposure to 8 mM barium at − 50 mV (b_1) or − 90 mV (b_2). The control cell e.p.s.c. decay remained well fit by the computer generated single exponential function superimposed on decay phase of the current at both voltages. The e.p.s.c. decay in this barium-treated cell voltage-clamped to − 50 mV deviated only slightly from the superimposed exponential function. However, the prolongation of the e.p.s.c. and deviation from a single exponential was more marked when the cell was voltage clamped to − 90 mV. The decay phase of the e.p.s.c. in (b_2) is fitted in (b_3) as the sum of two exponential components. The decay phase of the current was fit with a computer generated two component exponential function. The superimposed function had two decay component time constants of 6.0 and 21.3 ms. The vertical calibration equals 5 nA in (a_1, a_2 and b_1); 10 nA in (b_2); and 7 nA in (b_3). The horizontal calibration equals 10 ms in (a_1, a_2, b_1 and b_2) and 14 ms in (b_3).

clamped to − 50 mV during the initial period of tetanic stimulation and then quickly taken to − 120 mV to record m.e.p.s.cs and e.p.s.cs (at 0.4 Hz stimulation). Unfortunately, in the control preparations voltage-clamped to − 120 mV the m.e.p.s.cs were still not of sufficient size to be distinguished accurately from the baseline noise. In many of the barium-treated preparations, individual m.e.p.s.cs could be readily discerned from the baseline noise. In all of these experiments we found that the time course of the spontaneous m.e.p.s.cs was prolonged to an extent very close to that of e.p.s.cs recorded at the same voltage from the barium-treated cells. Examples of records from one of these experiments are shown in Figure 5. Example (a) is an averaged e.p.s.c. recorded from a control cell voltage-clamped to − 120 mV. Records (b_1) and (c_1) are averaged e.p.s.cs recorded at − 120 mV in two other cells exposed to a Ringer solution containing 4 mM barium. Records (b_2) and (c_2) are averaged m.e.p.s.cs recorded from these same two cells just before collecting the e.p.s.cs. The amplitude of the averaged

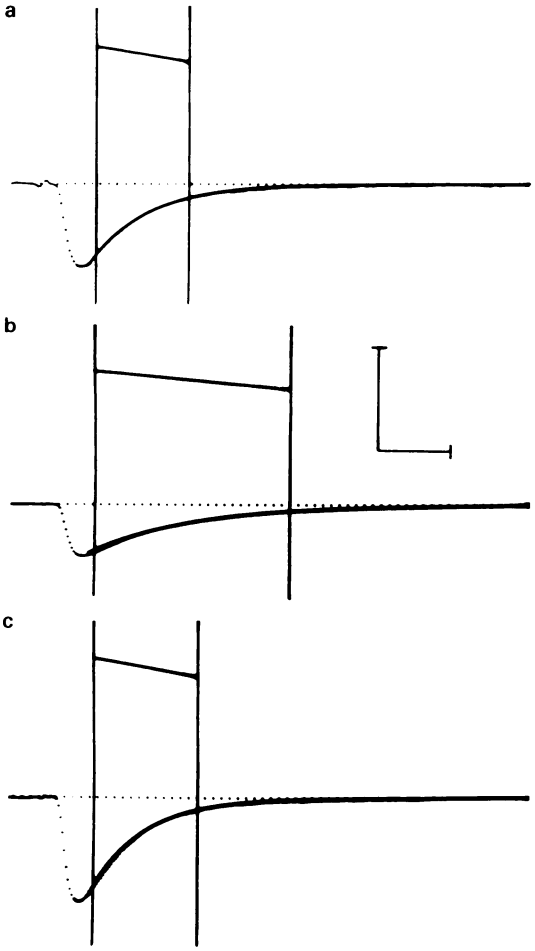

Figure 4 The alteration in e.p.s.c. decay time course by barium was reversible. (a) An averaged e.p.s.c. in a control cell voltage-clamped to − 50 mV. (b) An averaged e.p.s.c. recorded from another cell maintained in 4 mM for 53 min and voltage-clamped to − 50 mV. (c) An averaged e.p.s.c. recorded at − 50 mV from a third cell in the same ganglion preparation after 165 min of recovery in the control Ringer solution. Vertical calibration equals 5 nA in traces (a and c) and 10 nA in record (b). The horizontal calibration equals 10 ms.

m.e.p.s.cs even at − 120 mV was too small to accurately fit the decay time course. Therefore, we used the half decay time to compare the duration of e.p.s.cs and m.e.p.s.cs recorded in the same cells. For the examples shown in Figure 5, the half-decay time for the e.p.s.c. was 9 ms in (b) and 13 ms in (c) and the half-decay time of the m.e.p.s.c. was 9 ms in (b) and 12 ms in (c).

In other experiments we investigated the influence of 8 mM barium on the duration of m.e.p.s.cs recorded in potassium-stimulated cells. For these experiments the ganglion cells were maintained in a solution containing an elevated potassium chloride solution (15 mM). M.e.p.s.cs were recorded from different cells voltage-clamped to − 100 mV before and during exposure to 8 mM barium. From simple inspection of the current traces, it was apparent that the m.e.p.s.c. size was bigger and duration longer in the barium-treated cells than those recorded from the untreated cells. However, quantitation of this difference was difficult because in many control cells most of the m.e.p.s.cs were too small to analyse accurately the decay time course. In addition, even in the presence of an elevated potassium concentration their frequency was very variable (McIsaac, 1971). Further, it was much more difficult to voltage-clamp the control cells (in high K^+) for the time required to collect m.e.p.s.cs than those cells maintained in the high potassium solution containing barium. In spite of these difficulties, m.e.p.s.c. decay was determined for the largest events obtained in eight control cells from three different ganglion preparations. The average decay time constant was 7.2 ± 0.6 ms. In 14 other cells from these same three ganglia exposed to 8 mM barium the m.e.p.s.c. decay phase was markedly longer. For comparison, the decay phase was fitted as a single exponential; the average value of τ being 13.7 ± 1.0 ms. However, for many of the largest m.e.p.s.cs in the barium-treated cells, the decay was not exponential; an observation similar to that observed with m.e.p.s.cs recorded in the barium-treated cells following repetitive preganglionic stimulation. Examples of currents recorded from a control cell and from another cell following exposure to 8 mM barium are presented in Figure 6.

A voltage-dependent potentiation of peak e.p.s.c. amplitude occurs in barium-treated cells

The peak e.p.s.c.-voltage relation of control cells increased linearly with hyperpolarization in the voltage range between − 20 and − 100 mV (Kuba & Nishi, 1979; MacDermott *et al.*, 1980; Connor *et al.*, 1983). In most of the barium-treated cells, the peak e.p.s.c.-voltage plot became progressively non-linear with hyperpolarization. This non-linearity, which was particularly marked in preparations exposed to 8 mM barium, is illustrated in Figure 7. This figure shows the peak e.p.s.c.-voltage plot for a control cell and another cell from the same preparation exposed to 8 mM barium. As illustrated in this example, there was a marked progressive increase in peak current amplitude in barium-treated cells as the membrane potential was increased from − 50 to − 100 mV.

The data in Figure 8 summarize the voltage-dependent potentiation of e.p.s.c. amplitudes by barium recorded in different cells voltage-clamped to

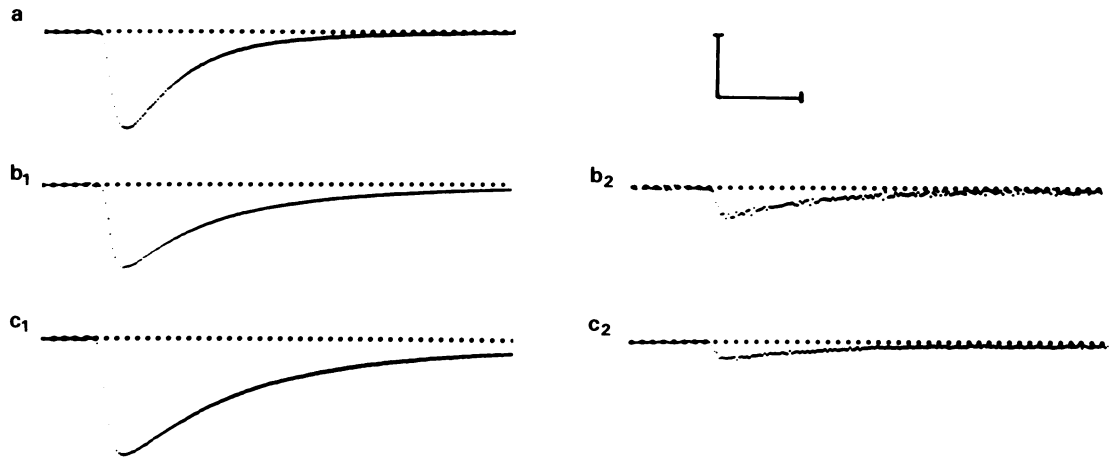

Figure 5 Both e.p.s.cs and m.e.p.s.cs exhibit a prolonged decay time course in barium-treated preparations. (a) An averaged e.p.s.c. recorded at − 120 mV in a control cell. (b_1 and c_1) Averaged e.p.s.cs from two different cells in the same ganglion exposed to 4 mM barium and voltage-clamped to − 120 mV. (b_2 and c_2) Averaged m.e.p.s.cs recorded at − 120 mV in these same two barium-treated cells. Both the e.p.s.c. and m.e.p.s.c. decay is slower in the barium-treated cells than that of the control e.p.s.c. The vertical calibration equals 5 nA in (a), 10 nA in (b_1 and c_1) and 1.0 nA in (b_2 and c_2). The horizontal calibration equals 10 ms.

− 50 mV and − 90 mV. When cells were voltage-clamped to − 50 mV, the amplitude of the e.p.s.c. was not markedly changed by the addition of barium (2–8 mM). In contrast, when these same cells were voltage-clamped to − 90 mV, the e.p.s.c. amplitude increased dramatically as a function of the barium concentration.

The e.p.s.c. reversal potential determined by interpolation was shifted to a more negative value in the barium-treated cells. The reversal potential (E_r) for a group of 12 untreated cells was -6.3 ± 1.6 mV whereas in three cells exposed to 4 mM barium the average E_r was -11.8 ± 1.0 mV and for five other cells exposed to 8 mM barium the E_r value was -11.4 ± 2.1 mV.

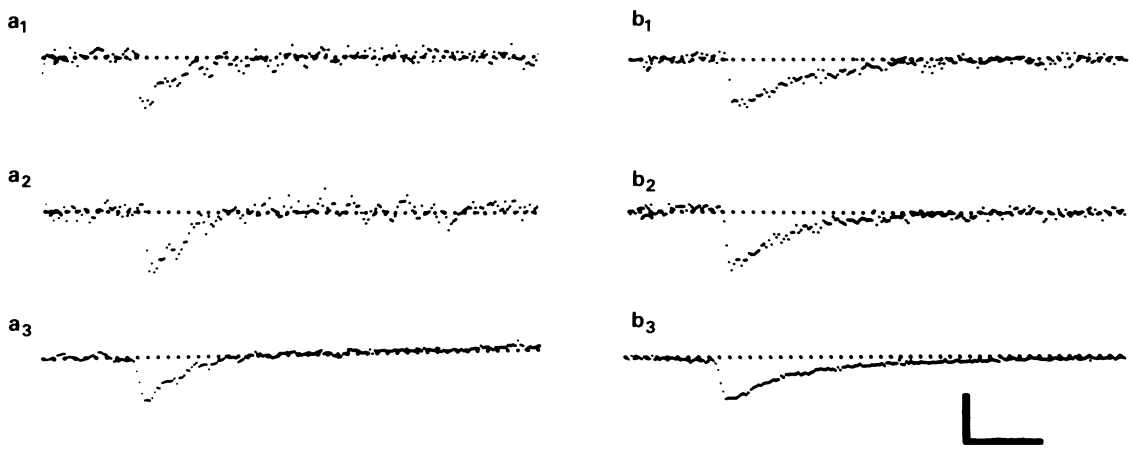

Figure 6 Influence of barium on m.e.p.s.cs recorded from potassium-stimulated (15 mM potassium) ganglia. (a) Current traces obtained from a control cell voltage-clamped to − 100 mV. (b) M.e.p.s.cs recorded from another cell also voltage-clamped to − 100 mV and exposed to 8 mM barium for 23 min. In both (a) and (b), traces (1 and 2) show single m.e.p.s.cs and (3) is an averaged m.e.p.s.c. (11 events in a_3 and 9 events in b_3). The vertical calibration equals 0.35 nA and the horizontal calibration equals 12 ms.

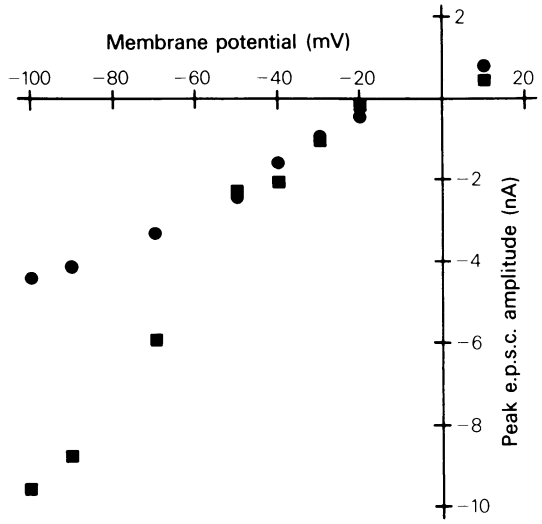

Figure 7 The peak e.p.s.c. amplitude-voltage relation for a control cell (●) and another cell from the same ganglion exposed to 8 mM barium (■).

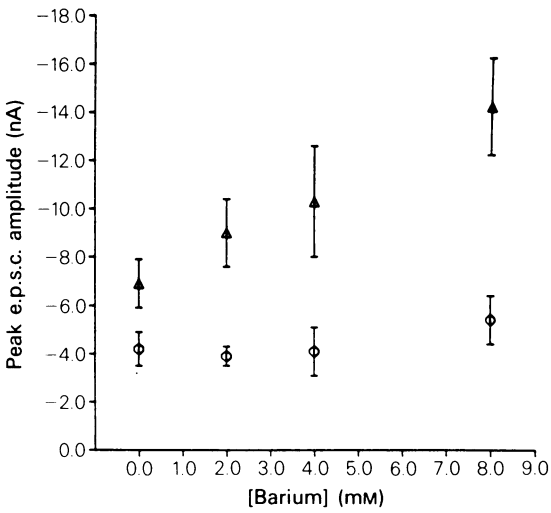

Figure 8 The influence of barium concentration on e.p.s.c. amplitude in cells voltage-clamped to − 50 mV (○) and − 90 mV (△). Each point is the mean of results from at least five cells; vertical lines show s.e.mean.

Table 2 Influence of barium on charge movement during the e.p.s.c.

Barium conc. (mM)	*Charge moved at − 50 mV (P coul)*	*Charge moved at − 90 mV (P coul)*	*Ratio of charge at − 90/− 50 mV*	*Number of cells*
0	− 30.6 ± 4.7	− 52.4 ± 6.7	1.7	7
2	− 31.7 ± 4.3	− 85.4 ± 9.7	2.2	5
4	− 32.3 ± 6.0	− 137.7 ± 32.5	4.3	5
8	− 74.9 ± 17.0	− 257.2 ± 41.7	3.4	10

Total charge moved during the e.p.s.c. was voltage-dependent in barium-treated cells

The amount of charge transferred during the e.p.s.c. was determined by computerized graphical integration in cells voltage-clamped to both − 50 and − 90 mV. At − 50 mV total charge moved was similar for control cells and cells exposed to either 2 or 4 mM barium. However, there was a greater total charge movement during e.p.s.cs recorded in cells voltage-clamped to − 50 mV and exposed to 8 mM barium. At − 90 mV, there was a progressively greater average charge movement for cells exposed to increasing barium concentrations. These observations are summarized in Table 2.

Discussion

The results of this study demonstrate that the ganglionic nicotinic fast e.p.s.c. is altered by barium treatment. We suggest that all of the observed changes in e.p.s.c. characteristics can be attributed to a direct postsynaptic effect of barium. First of all, the observation that both m.e.p.s.cs and e.p.s.cs recorded in barium-treated cells were prolonged (Figures 5 and 6) indicates that the alteration of e.p.s.c. decay by barium is not due to an alteration in the kinetics of phasic transmitter release. Secondly, the voltage-dependent nature of the barium-induced change in peak e.p.s.c. amplitude (Figure 8) and total charge movement (Table 2) is more consistent with a postsynaptic rather than presynaptic site of action.

Previously, McLachlin (1977) showed for mammalian ganglia that barium failed to exert either a partial agonist or antagonist action on phasic transmitter release. Given that the duration of the action potential in the presynaptic nerve terminal would be expected to increase following prolonged exposure to barium (see Figure 1), one might anticipate that the kinetics of transmitter release might also be altered. In the present study, we have not directly estimated

quantal content such as from a determination of mean e.p.s.c. amplitude/mean m.e.p.s.c. amplitude before and during barium treatment. In the ganglion preparation the frequency of m.e.p.s.cs is very low and their amplitude is too small even at hyperpolarized membrane voltages to obtain an accurate estimate of size (Connor & Parsons, 1983b). Consequently, we cannot exclude the possibility in amphibian ganglia that there is some alteration in the kinetics of phasic release during prolonged barium treatment. However, in the present study there was no consistent increase in e.p.s.c. size in the presence of barium when the cells were voltage-clamped to -50 mV. Further, other recent observations obtained with the presynaptic potentiating drug, 4-aminopyridine (4-AP) (Thesleff, 1980) suggest that any small change in transmitter release kinetics which might occur in the barium-treated cells would not be responsible for the barium-induced prolongation of the e.p.s.c. With 4-AP, the e.p.s.c. size was significantly increased without a change in e.p.s.c. duration comparable to that occurring in barium. For instance, in 13 control cells voltage-clamped to -90 mV, the peak e.p.s.c. size and τ was -4.1 ± 0.4 nA and 5.2 ± 0.1 ms, whereas in seven other cells also voltage-clamped to -90 mV and exposed to 10 μM 4-AP, the e.p.s.c. size and decay τ were -22.2 ± 2.6 nA and 5.9 ± 0.3 ms. We conclude therefore that the prolongation of e.p.s.c. decay by barium cannot be attributed to an alteration in the kinetics of transmitter release.

Although we have not demonstrated directly that some of the alterations in e.p.s.c. decay characteristics in barium were not due to partial cholinesterase inhibition, we consider it unlikely that this is a primary mechanism of action. To our knowledge there is no report in the literature of an anticholinesterase action of barium.

Barium is a potent blocker of most potassium channels (Sperelakis *et al.*, 1967; Standen & Stanfield, 1978; Armstrong & Taylor, 1980; Adams *et al.*, 1982). As shown in Figure 1, the action potential duration was markedly increased in barium-treated ganglion cells. Further, cells were depolarized in barium and generally required less holding current to be voltage-clamped over the range of membrane potentials studied. However, we suggest that the alteration in e.p.s.c. characteristics in barium-treated preparations is not due to any of its already well established pharmacological actions. The e.p.s.c. alteration in barium increased with hyperpolarization and was more pronounced at voltages between -80 and -100 mV; i.e., membrane potentials close to the potassium equilibrium potential, than at -50 mV. Consequently, we suggest that barium directly alters the kinetics of nicotinic receptor-channel gating in sympathetic ganglion cells rather than indirectly influencing e.p.s.c. characteristics through an alteration of the non-synaptic membrane conductance.

The molecular mechanism(s) by which barium alters ganglionic e.p.s.c. characteristics is not clear at present. However, the strong voltage-dependence suggests that the site of action may be within the synaptic channel. Although not demonstrated as completely in the case of ganglion cells, it is well known that endplate channels are quite permeable to many divalent cations (Adams *et al.*, 1980). Furthermore, Adams *et al.* (1980) have presented evidence which suggests that divalent cations may interact with chemical groups within endplate channels. The pattern of complex decay of e.p.s.cs recorded in barium-treated cells is not similar to that observed with e.p.s.cs recorded from either atropine- or procaine-treated ganglion preparations (Connor & Parsons, 1983a; Connor *et al.*, 1983). Consequently, we suggest that a simple open channel block type mechanism by itself is not sufficient to explain the complex e.p.s.c. decay in barium-treated cells (Adams, 1976; Ruff, 1977; 1982). There have been a number of previous studies which demonstrate that miniature endplate current characteristics as well as mean channel lifetime and single channel conductance of endplate channels are influenced by the addition of many different divalent cations (Bregestovski *et al.*, 1979; Lewis, 1979; Magleby & Weinstock, 1980; Miledi & Parker, 1980; Takeda *et al.*, 1982). However, to our knowledge there has been no report, at the motor endplate, of a voltage-dependent potentiation of peak amplitude similar to that produced by barium nor any mention of a complex pattern of decay such as observed with barium-treated ganglion cells. In preliminary experiments on snake twitch muscle fibres, neither miniature endplate current decay nor the m.e.p.c.-voltage relationship (between -60 to -140 mV) was consistently changed by exposure to 4 mM barium (Neel & Parsons, unpublished observations). These preliminary observations suggest that these barium-induced alterations observed in ganglion cells may not occur at the motor endplate; thus supporting the view that some of the basic biophysical and pharmacological characteristics of the nicotinic receptor-channel complex differ in the two preparations.

Kuba & Nishi (1979) showed previously that ganglionic e.p.s.c. decay also is slowed by raising calcium. We have observed that e.p.s.c. decay is slowed and often complex in preparations maintained in either elevated calcium (7.2 mM) or strontium (8 mM). However, the peak e.p.s.c. amplitude-voltage relationship remains linear between -20 to -100 mV in these calcium or strontium-treated cells (Connor & Parsons, 1981). Therefore, the voltage-dependent increase in e.p.s.c. amplitude and charge movement appears to be unique to barium-treated ganglion

preparations. This suggests that the different alterations in ganglionic e.p.s.c. characteristics by barium may not be due to a single action, but rather may reflect the summed effect of more than one intervention. We speculate, therefore, that in the barium-treated ganglion cells both the single channel conductance and mean channel open time may be altered via some voltage-dependent mechanism(s).

Recently, Schwindt & Crill (1980) observed that excitatory postsynaptic potentials (e.p.s.ps) were prolonged and increased in amplitude in barium-treated motoneurones; an effect suggested to be due to an enhancement of transmitter release from afferent terminals. However, barium does not appear to influence evoked transmitter release from motoneurone terminals and preganglionic fibres (Miledi, 1966; Silinski, 1977; McLachlan, 1977). Consequently, we suggest as an alternative explanation that a postsynaptic action may be responsible for the potentiation of e.p.s.ps recorded in barium-treated motoneurones. The synaptic potential is dependent on both the amplitude and duration of the synaptic current so that an alteration in the e.p.s.c. similar to that produced by barium in ganglion cells would be expected to increase the amplitude and duration of the excitatory postsynaptic potential.

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References

ADAMS, D.J., DWYER, T.M. & HILLE, B. (1980). The permeability of endplate channels to monovalent and divalent metal cations. *J. Gen. Physiol.*, **75,** 493–510.

ADAMS, P.R. (1976). Drug blockade of open end-plate channels. *J. Physiol.*, **260,** 531–552.

ADAMS, P.R., BROWN, D.A. & CONSTANTI, A. (1982). Pharmacological inhibition of the M-current. *J. Physiol.*, **332,** 223–262.

ARMSTRONG, C.M. & TAYLOR, S.R. (1980). Interaction of barium ions with potassium channels in squid giant axons. *Biophys. J.*, **30,** 473–488.

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

BREGESTOVSKI, P.D., MILEDI, R. & PARKER, I. (1979). Calcium conductance of acetylcholine-induced endplate channels. *Nature*, **279,** 638–639.

CONNOR, E.A., LEVY, S.M. & PARSONS, R.L. (1983). Kinetic analysis of atropine-induced alterations in bullfrog ganglionic fast synaptic currents. *J. Physiol.*, **337,** 137–158.

CONNOR, E.A. & PARSONS, R.L. (1981). External calcium influences the characteristics of fast synaptic currents at sympathetic ganglion. *Neurosci. Abstracts*, **7,** 444.

CONNOR, E.A. & PARSONS, R.L. (1983a). Procaine alters fast excitatory post-synaptic current decay in amphibian sympathetic ganglia. *Br. J. Pharmac.*, **78,** 293–299.

CONNOR, E.A. & PARSONS, R.L. (1983b). Analysis of fast excitatory postsynaptic currents in bullfrog parasympathetic ganglion cells. *J. Neurosci.*, **3,** 2164–2171.

DODD, J. & HORN, J.P. (1983). A reclassification of B and C neurones in the ninth and tenth paravertebral sympathetic ganglia of the bullfrog. *J. Physiol.*, **334,** 255–269.

GORMAN, A.L.F. & HERMANN, A. (1979). Internal effects of divalent cations on potassium permeability in Molluscan neurones. *J. Physiol.*, **296,** 373–410.

HAGIWARA, S. & BYERLY, L. (1981). Calcium channels. *Ann. Rev. Neurosci.*, **4,** 69–125.

HAGIWARA, S. & OHMORI, H. (1982). Studies of calcium channels in rat clonal pituitary cells with patch electrode voltage clamp. *J. Physiol.*, **331,** 231–252.

KRNJEVIC, K., PUMAIN, R. & RENAUD, L. (1971). Effects of Ba^{2+} and tetramethylammonium on cortical neurons. *J. Physiol.*, **215,** 223–245.

KUBA, K. & NISHI, S. (1979). Characteristics of fast excitatory postsynaptic current in bullfrog sympathetic ganglion cells. *Pflügers Arch.*, **378,** 205–212.

LEWIS, C.A. (1979). Ion-concentration dependence of the reversal potential and the single channel conductance of ion channels at the frog neuromuscular junction. *J. Physiol.*, **286,** 417–445.

MACDERMOTT, A.B., CONNOR, E.A., DIONNE, V.E. & PARSONS, R.L. (1980). Voltage-clamp study of fast excitatory synaptic currents in bullfrog sympathetic ganglion cells. *J. Gen. Physiol.*, **75,** 39–60.

MAGLEBY, K.L. & WEINSTOCK, M.M. (1980). Nickel and calcium ions modify the characteristics of the acetylcholine receptor-channel complex at the frog neuromuscular junction. *J. Physiol.*, **299,** 203–218.

MCISAAC, R.J. (1971). The influence of sodium, potassium and calcium on spontaneous miniature potentials in frog sympathetic ganglion neurons. *Neuropharmacology*, **10,** 649–659.

MCLACHLAN, E.M. (1977). The effects of strontium and barium ions at synapses in sympathetic ganglion. *J. Physiol.*, **267,** 497–518.

MILEDI, R. (1966). Strontium as a substitute for calcium in the process of transmitter release at the neuromuscular junction. *Nature*, **212,** 1233–1234.

MILEDI, R. & PARKER, I. (1980). Effects of strontium ions on end-plate channel properties. *J. Physiol.*, **306,** 567–577.

REUTER, H., STEVENS, C.F., TSIEN, R.W. & YELLIN, G. (1982). Properties of single calcium channels in cardiac cell culture. *Nature*, **297,** 501–504.

RUFF, R. (1977). A quantitative analysis of local anaesthetic

alteration of miniature end-plate currents and end-plate current fluctuations. *J. Physiol.*, **264,** 89–124.

RUFF, R.C. (1982). The kinetics of local anesthetic blockade of end-plate channels. *Biophys. J.*, **37,** 625–631.

SCHWINDT, P.C. & CRILL, W.E. (1980). Effects of barium on cat spinal motoneurons studied by voltage clamp. *J. Neurophysiol.*, **44,** 827–846.

SILINSKY, E.M. (1977). Can barium support the release of acetylcholine by nerve impulses. *Br. J. Pharmac.*, **59,** 215–217.

SPERELAKIS, N., SCHNEIDER, M.F. & HARRIS, E.J. (1967). Decreased K^+ conductance produced by Ba^{2+} in frog sartorius muscle. *J. Gen. Physiol.*, **50,** 1565–1583.

STANDEN, N.B. & STANFIELD, P.R. (1978). A potential and time-dependent blockade of inward rectification in frog skeletal muscle fibres by barium and strontium ions. *J. Physiol.*, **280,** 169–191.

TAKEDA, K., GAGE, P.W. & BARRY, P.H. (1982). Effects of divalent cations on toad end-plate channels. *J. memb. Biol.*, **64,** 55–66.

THESLEFF, S. (1980). Aminopyridines and synaptic transmission. *Neuroscience*, **5,** 1413–1419.

TILLOTSON, D. (1979). Inactivation of Ca conductance dependent on entry of Ca ions in Molluscan neurons. *Proc. natn. Acad. Sci.* U.S.A., **76,** 1497–1500.

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