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RECTIFICATION OF SYNAPTIC AND ACETYLCHOLINE CURRENTS IN THE MOUSE SUBMANDIBULAR GANGLION CELLS

BY HIROMU YAWO

From the Department of Physiology, Kyoto University Faculty of Medicine, Kyoto 606, Japan

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SUMMARY

1. Synaptic currents and responses to acetylcholine (ACh) were recorded from mouse submandibular ganglion (SMG) cells under whole-cell voltage clamp.

2. The peak amplitude of excitatory synaptic currents (ESCs) as well as the currents evoked by the ionophoretic application of ACh followed a unique non-linear current–voltage (*I–V*) relation. The chord conductance of the whole-cell currents decreased with depolarization of the membrane potential and became virtually 0 at 50 mV.

3. The decay of ESCs was described by two exponential functions. Both the fast (τ_f) and slow (τ_s) time constants were sharply decreased at depolarizing potentials beyond −40 mV, being insensitive to hyperpolarizing potentials more than −50 mV.

4. Single ACh receptor channels were characterized by the whole-cell current noise analysis. The single-channel currents followed Ohm's law at negative membrane potentials but tended to reach a plateau at positive membrane potentials. The mean slope conductance measured between −40 and −20 mV was 28·5 pS.

5. The product of the number of *functional* channels (N) and the probability of a channel *being open* (p) showed a steep voltage dependence. The value of Np at 20 mV was only 31 % of that at −20 mV.

6. The noise power spectrum was best fitted by a double-Lorentzian function. Both the fast (τ_f) and slow (τ_s) time constants were sharply decreased by depolarizations beyond −20 mV, being less sensitive to membrane potentials more negative than −30 mV.

7. The non-linear *I–V* relation of ESCs was attributed in part to the voltage dependence of p and in part to the voltage dependence of the single-channel conductance (γ) of ACh receptor channels.

INTRODUCTION

The nicotinic acetylcholine (ACh) receptor in skeletal muscle shows a relatively weak voltage dependence, and its peak synaptic current–voltage (*I–V*) relation conforms approximately to Ohm's law (Takeuchi & Takeuchi, 1960; Magleby & Stevens, 1972*a*). In contrast, the *I–V* relation of ACh receptor responses has been found to be non-linear in the rat sympathetic neurones (Mathie, Cull-Candy &

Colquhoun, 1987) and the rat adrenal chromaffin cells (Hirano, Kidokoro & Ohmori, 1987). A similar non-linear *I–V* relation has also been described for excitatory synaptic currents (ESCs) in rabbit sympathetic neurones (Selyanko, Derkach & Skok, 1979; Derkach, Selyanko & Skok, 1983). However, the mechanism underlying this non-linear behaviour has not been studied.

In the present experiments, the voltage sensitivity of ESCs and ACh responses were studied in the mouse submandibular ganglion (SMG) cells by the method of tight-seal whole-cell recording (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The mouse SMG cells are spherical in shape and lack dendrites (Snider, 1987). Most of the ganglion cells receive a single presynaptic input (Snider, 1987; see also, Lichtman, 1977) which activates the nicotinic ACh receptor on the somal membrane (Ascher, Large & Rang, 1979). This preparation thus provides favourable conditions for measuring synaptic currents under voltage clamp. The results show that both the ACh currents and ESCs are characterized by a striking non-linear current–voltage (*I–V*) relation. Whole-cell current noise analyses revealed that the single-channel conductance (γ) itself is reduced by depolarization. Also, depolarization shortened the apparent mean open time of ACh receptor channels, reducing the probability of a channel *being open* (p). It is concluded that the rectification of synaptic and ACh currents in the mouse SMG is due to the voltage dependence of both γ and p.

METHODS

Preparation

Adult mice (DDY strain; 25–35 g body weight) were anaesthetized with sodium pentobarbitone (250 mg/kg I.P.). The animal was perfused through the heart with oxygenated cold Krebs solution to remove the blood. The submandibular ganglia were identified along the salivary ducts (Purves & Lichtman, 1987; Purves, Voyvodic, Magrassi & Yawo, 1987). The preganglionic chorda tympani nerve was cut just proximal to the site where it merges with the hypoglossal nerve. The ganglia, excised with the salivary ducts and the preganglionic nerve, were placed in a recording chamber and superfused with modified Krebs solution equilibrated with 95% O_2 and 5% CO_2. The presynaptic nerve was prepared for stimulation with suction electrodes.

In order to visualize individual neurones, a small platinum plate was placed as a mirror underneath the ganglion (Purves & Lichtman, 1987; Purves *et al.* 1987). The ganglion was illuminated through the microscope objective (Zeiss 40× water immersion, NA 0·75) using a conventional epi-illumination system (Nikon, IGS filter unit).

The collagenous envelope was loosened by focal application of 0·1% collagenase (Sigma, type I) through a pipette of 20 μm tip diameter for 10–30 min at 25–30 °C. When individual ganglion cells were loosened from each other, collagenase was washed out rigorously with a Ca^{2+}-free saline containing 1 mM-EGTA and 0·5 units/ml heparin (Novo Industry A/S). After these treatments, the basal lamina on the ganglion cell was still left intact. Stronger enzymatic treatment that had disrupted the basal lamina usually resulted in depression of synaptic transmission. In a few experiments carried out without enzymatic pre-treatment, the mean amplitude and the voltage dependence of synaptic currents were similar to those observed after enzymatic treatment.

Whole-cell current recording

Patch pipettes were made from thin-walled borosilicate glass capillaries (Hildenberg) and coated with silicon resin (KE106+CAT-RG, Shin-Etsu). The pipette had a resistence of about 3 MΩ when filled with a standard internal solution. The standard internal solution contained (mM): CsCl, 140; K_2EGTA, 10; and NaHEPES, 10 (pH 7·3 with KOH). The modified Krebs solution had the following composition (mM): NaCl, 138; $NaHCO_3$, 12; KCl, 4; KH_2PO_4, 1; $CaCl_2$, 2; $MgSO_4$, 1; HEPES/NaHEPES, 5; and glucose, 11 (pH 7·2 after equilibration with 95% O_2 and 5% CO_2). The basal lamina on the ganglion cell was ruptured by the patch pipette with positive pressure. The

pipette pressure was then immediately switched to negative in order to form a gigaohm seal (> 10 GΩ). The liquid junction potential (< 3 mV) was not corrected. For recording ACh currents, ACh was applied ionophoretically through another pipette filled with 2 M-ACh chloride (Nakarai). The pipette resistence was about 50 MΩ. Nicotine sulphate (40%, Nakarai) similarly applied in a few experiments gave qualitatively the same results as those with ACh. The experiments were made at 25–30 °C.

Recordings were made with an EPC-7 patch clamp amplifier (List electronic). The access resistence (R_s) of the pipette was usually less than 20 MΩ and compensated by 50–70%. The input resistence (R_{in}) of the cell was 2·3 GΩ on average (when R_{in} < 0·1 GΩ, the data were discarded). Whole-cell current records were low-pass filtered at 5 kHz (−3 dB, 8-pole Bessel filter), digitized at 10 kHz (ADX-98, Canopus) and stored on the RAM disk (BM-2000, MELCO) by a computer (PC-9801Vm21, NEC). The average whole-cell current was obtained from ten successive records.

Whole-cell current noise analysis

In order to record current fluctuations, AChCl was bath-applied at a concentration of 5–20 μM in the presence of 10 μM-neostigmine bromide (Nakarai). For the analysis of whole-cell current noise (Katz & Miledi, 1972; Anderson & Stevens, 1973) the signals were low-pass filtered at 500 Hz and high-pass filtered at 0·2 Hz (−3 dB, 8-pole Butterworth), digitized at 1000 Hz and stored on the RAM disk. The signal without high-pass filtering was also digitized at the same time in order to monitor DC shifts. The continuous digitized records were sampled for every 2048 points and replayed on the monitor. The record segments that contained obvious artifacts or spontaneously occurring synaptic currents were removed prior to the fast Fourier transformation. The power spectrum was averaged from the records spanning for 120 s. The ACh-induced noise power spectrum was then obtained by subtraction of the power spectrum obtained from the baseline noise of 120 s (60 s record before application of ACh and 60 s record after wash-out of the drug). The net spectral density was fitted by the sum of Lorentzian components $S_j(f)$ by the method of weighted non-linear least-squares (Colquhoun, Dreyer & Sheridan, 1979). The single-channel currents (i_1, i_2) were estimated by the following two methods (Anderson & Stevens, 1973; Rang, 1981; Cull-Candy, Howe & Ogden, 1988):

$$i_1 = \mathrm{var}^*(I)/\mu_I, \tag{1}$$

$$i_2 = (\pi/2) \sum_j S_j(0) f_j/\mu_I, \tag{2}$$

where var*(I) is the corrected variance of ACh-induced currents for loss by the band-pass filtering (Colquhoun *et al.* 1979), μ_I is the mean ACh-induced current, and $S_j(0)$ and f_j are the zero-frequency asymptote and half-power frequency of the ith Lorentzian component, respectively. The values, i_1 and i_2, estimated by the two methods showed an excellent agreement (Fig. 9). Therefore, the single-channel current was obtained by the algebraic mean, $i = (i_1 + i_2)/2$. The handling programme of the A/D converter and the programme for non-linear least-squares (Simplex method: Nelder & Mead, 1965) were provided by Dr Tetsuhiro Tsujimoto in our Department.

In order to improve the signal/noise (S/N) ratio, Cl^- in both the external and pipette solutions was replaced with SO_4^{2-}. The composition of the external solution was (mM): Na_2SO_4, 95; $NaHCO_3$, 12; Cs_2SO_4, 2; KH_2PO_4, 1; $CaSO_4$, 0·2; $MgSO_4$, 1; HEPES/NaHEPES, 5 (pH 7·2 with 95% O_2 and 5% CO_2). Tetrodotoxin (Sankyo; 0·6 μM) and atropine sulphate (Nakarai; 2 μM) were added to suppress the action potentials and muscarinic responses. The pipette solution was (mM): Cs_2SO_4, 90; K_2EGTA, 10; NaHEPES, 10 (pH 7·3). Sucrose (35 mM) was added to the pipette solution to increase its tonicity, since a hypertonic pipette solution kept the series resistence stable for long recording periods. The liquid junction potential (< 3 mV) was not corrected. The noise recording was performed at 25 °C.

RESULTS

The voltage dependence of synaptic currents

The mouse SMG neurones showed a peak inward current of 1·0–8·6 nA (2·87 ± 1·64, mean ± S.D., n = 22) at −50 mV in response to stimulation of the preganglionic nerve

at 0·5 Hz. At negative holding potentials, the peak ESCs were linearly related to the membrane potential and became null at about 0 mV (Fig. 1). However, the reversed outward currents were much smaller than those expected from extrapolation of the *I–V* relationship of the inward currents. Moreover, the outward currents was reduced

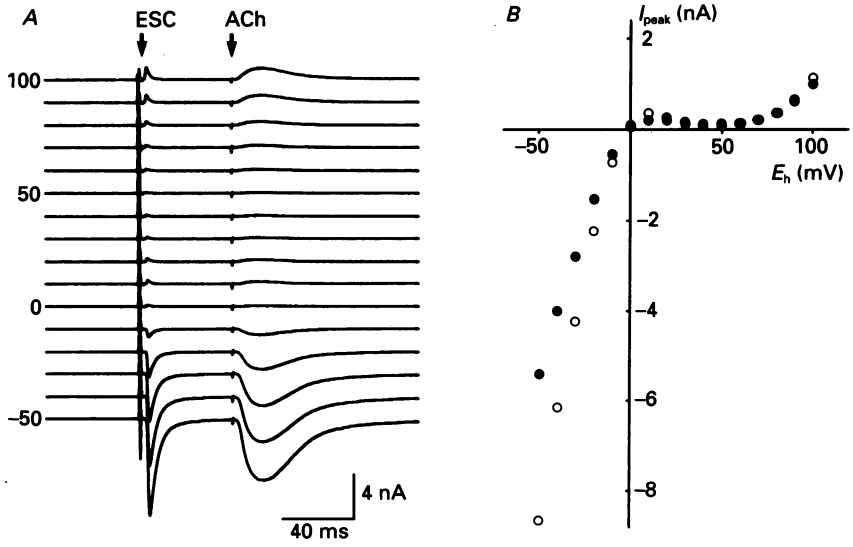

Fig. 1. Voltage dependence of synaptic currents (ESC) and acetylcholine (ACh) currents in a mouse submandibular ganglion cell. *A*, the holding potential (E_h) was changed from −50 to 100 mV with a 10 mV step. *B*, current–voltage relation of the peak synaptic current (○) and the maximum response to ionophoretically applied ACh (●) for the data shown in *A*. The reversal potential was −1 mV. The pipette was filled with CsCl. Temperature, 30 °C.

by a further depolarization, followed by a sharp increase beyond 50 mV. This behaviour is clearly different from that of end-plate currents (Takeuchi & Takeuchi, 1960; Magleby & Stevens, 1972*a*; Linder & Quastel, 1978) or the fast ESCs of frog parasympathetic neurones (Dennis, Harris & Kuffler, 1971). However, a non-linear *I–V* relation of fast ESCs has been reported at positive membrane potentials in bull-frog sympathetic (Kuba & Nishi, 1979) and rabbit sympathetic neurones (Selyanko *et al.* 1979; Derkach *et al.* 1983).

Rectification of the *I–V* relation of ESCs was consistently observed when the pipette was filled with CsCl, NaCl or KCl. Neither the ESCs nor the rectifying properties were affected by atropine (2 μM). The ESCs were reduced by 30% with *d*-tubocurarine (10 μM). Furthermore, the responses to ACh or nicotine applied focally onto the cell soma mimicked the rectifying properties of ESCs (Fig. 1). Therefore, the above properties of ESCs must reflect those of the nicotinic ACh receptor channels on the postsynaptic membrane.

A progressive decrement of the inward ESCs and a sudden increment of the inward current at strong depolarization (Fig. 1*B*) might be due to the presence of partial blockade of the channel by some cations (Bezanilla & Armstrong, 1972; Hagiwara, Miyazaki & Rosenthal, 1976; French & Wells, 1977; Ohmori, 1980; DiFrancesco,

1982; Hirano *et al.* 1987; Vandenberg, 1987; Matsuda, Saigusa & Irisawa, 1987; Horie, Irisawa & Noma, 1987). To test this possibility, attempts were made to examine whether rectification depends upon the direction of the ESCs. When the $[Na^+]_o$ was halved and the osmolarity was adjusted by mannitol, the ESCs were

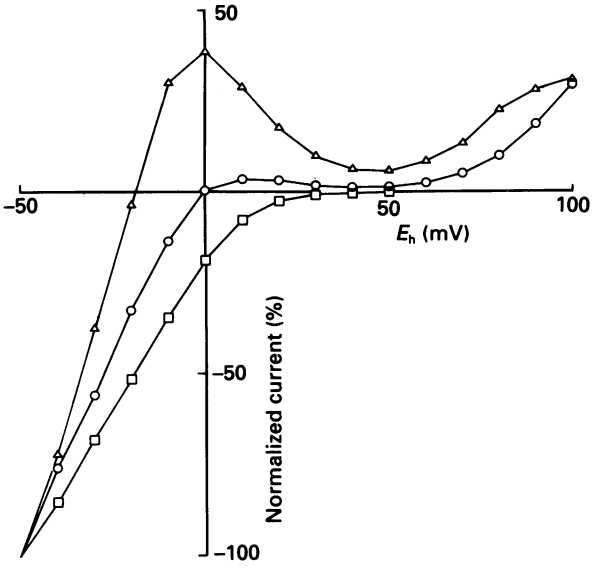

Fig. 2. Effects of different reversal potentials on the current–voltage profile of peak synaptic currents. The relative amplitudes of the mean synaptic currents from six cells observed in the standard solution (○) were plotted against holding potentials (abscissa). Ordinate, the normalized synaptic currents relative to the value at −50 mV (−100%). The reversal potential was $-0{\cdot}7 \pm 1{\cdot}5$ mV (mean ± S.D.). When $[Na^+]_o$ was halved and the osmolarity was adjusted by mannitol, the reversal potential was shifted to −19 mV (△). When internal cations were totally replaced with *N*-methyl-D-glucamine, the current–voltage profile shifted (□). Temperature, 25 °C.

reversed at −19 mV as predicted from the Nernst equation (Fig. 2, △). Similar results were observed when $[Na^+]_o$ was halved by replacing with *N*-methyl-D-glucamine. In another series of experiments, internal cations were totally replaced with *N*-methyl-D-glucamine. As expected, no outward currents were observed in this case during strong depolarizations up to 50 mV (Fig. 2, □). In all the three *I–V* curves, non-linear decrement of the ESCs was noted at membrane potentials between −10 and 50 mV regardless of the direction of currents (Fig. 2). Therefore, rectification appears to depend upon the membrane potential rather than upon the direction of the synaptic current.

To quantify the voltage dependence of the peak amplitude of ESCs, the chord conductance was calculated at each membrane potential (Fig. 3). The conductance at 20 mV was about 10% on average ($n = 6$) of that at −20 mV and virtually 0 at 50 mV of the membrane potential. The conductance tended to increase again above 50 mV. A small decline of the chord conductance was observed by hyperpolarizations more than −60 mV although the extent of reduction varied from one cell to another.

The voltage dependence of the ESC decay

The ESC decay was best fitted by the sum of two exponential functions as illustrated in Fig. 4. Both the fast (τ_f) and slow (τ_s) time constants were decreased by depolarization beyond −50 mV, while these values were almost insensitive to

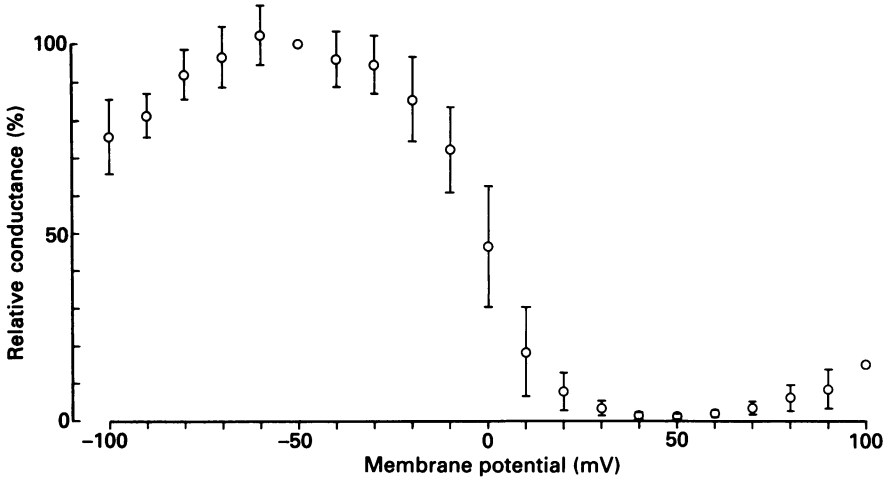

Fig. 3. Dependence of the whole-cell conductances of the postsynaptic acetylcholine receptor channels upon membrane potentials. The mean chord conductance at each holding potential was calculated from six ganglion cells and normalized to the mean chord conductance at −50 mV (○). Vertical bars, standard deviations. The average of two cells is plotted at 100 mV. CsCl-filled pipettes were used. Temperature, 25 °C.

membrane potentials more negative than −50 mV (Fig. 5*A*). The mean contribution of the slow components was 15%, regardless of the holding potentials. The reliable fitting of two exponential functions for the data at holding potentials more positive than −10 mV was difficult because of small S/N ratios. In these records, the decay from 90% to 20% of the ESC could be approximated by a single-exponential function (τ_1, Fig. 4*C*). The single-exponential time constant (τ_1) of the ESC decay was similarly estimated at a wide range of membrane potentials (Fig. 5*B*). The values of τ_1 were comparable to τ_f, and again τ_1 was insensitive to the membrane potential at hyperpolarizing potentials, steeply decreased by depolarizations between −40 and 30 mV, and increased at depolarizations above 40 mV. Quantitatively, an e-fold change of τ_1 for 38 mV on average ($n = 6$) was observed between −20 mV and 20 mV.

Since the chord conductance of ESCs and the ESC decay time constant depend upon the membrane potential in a similar manner, these two parameters appear to be mutually related. The voltage dependence of the ESC decay time constant has been attributed to the voltage-dependent property of the lifetime of individual open

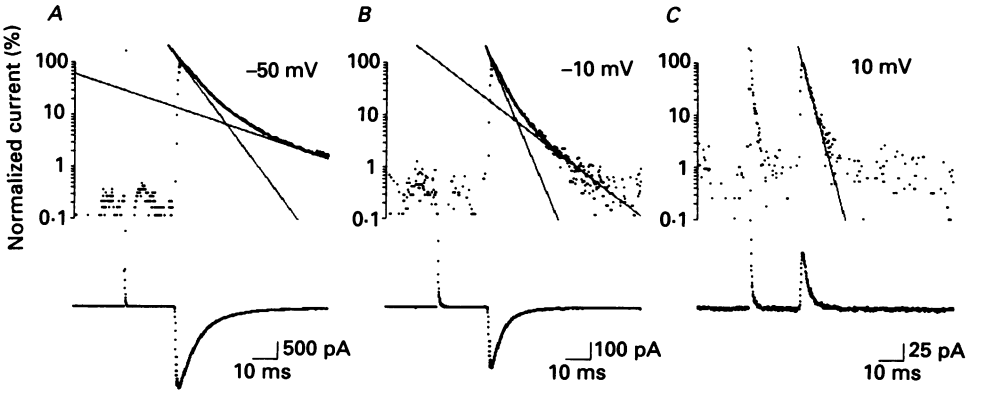

Fig. 4. Bi-exponential decay of synaptic currents. Upper traces are the semilogarithmic plots of the ESC decay, normalized using the peak synaptic current as 100%. Lower traces, sample record of the average of ten consecutive ESCs (0·5 Hz). *A*, the two exponential functions obtained by best-fitting to the ESC decay at a holding potential of −50 mV, using a non-linear least-squares method (see Methods), are drawn as straight lines. The fast (τ_f) and slow (τ_s) time constants were 6·7 and 26·9 ms, respectively. The sum of two exponential functions is also drawn. *B*, similar to *A*, but the holding potential was −10 mV. Estimated τ_f and τ_s was 3·8 and 11·8 ms, respectively. *C*, at a holding potential of 10 mV, the synaptic current decay was fitted with a single-exponential function (see text) because of relatively low S/N ratio. The time constant (τ_1) was 2·4 ms. All the data were obtained from the same cell. A CsCl-filled pipette was used. Temperature, 25 °C.

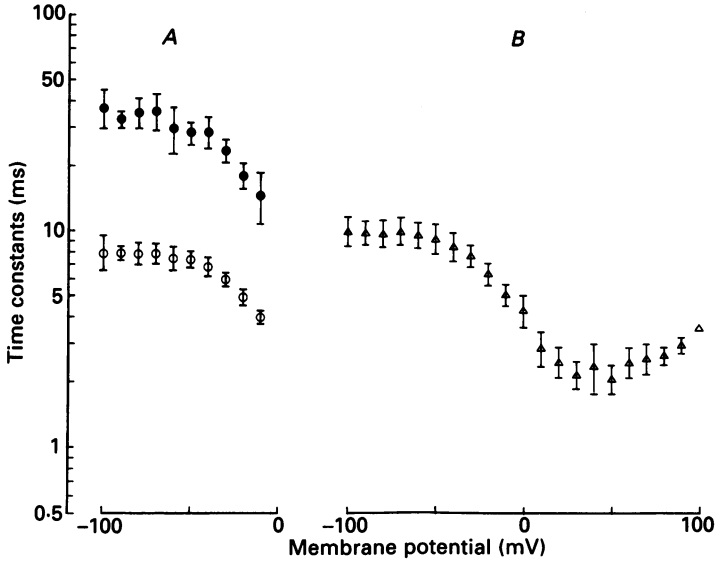

Fig. 5. Voltage dependence of the decay time constants of synaptic currents. *A*, the fast (τ_f, ○) and slow (τ_s, ●) time constants as a function of membrane potentials (averages of the six cells shown in Fig. 3; bars, S.D.). *B*, similar to *A*, but the early decay time constant (τ_1) fitted to a single-exponential function. △, the average τ_1 of the six cells in *A* (bars, S.D.). An e-fold change of τ_1 required 38 mV between −20 and +20 mV.

channels (Magleby & Stevens, 1972*b*; Anderson & Stevens, 1973). On the other hand, the whole-cell conductance (G) can be described by the relation:

$$G = N p \gamma, \tag{3}$$

where N is the number of *functional* channels, p is the probability of a channel *being open* (the opening probability times the mean duration of each opening) and γ is the single-channel conductance. It is then possible that p may be the parameter

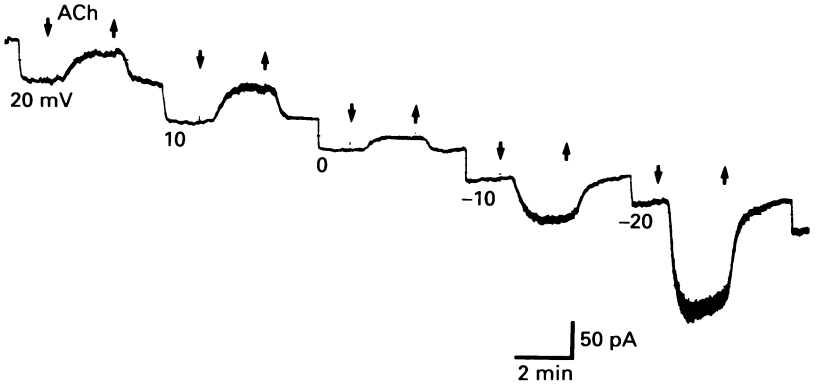

Fig. 6. Whole-cell currents in response to bath-applied acetylcholine. The holding potential was changed with a 10 mV step. The standard solution was switched to a solution containing 20 μM-acetylcholine and 10 μM-neostigmine, between downward and upward arrows. Cs_2SO_4-rich solution was used for the patch pipette (EDTA, 10 mM, replacing with EGTA). Temperature, 25 °C.

responsible for the similarity in voltage dependence between the ESC chord conductance and the ESC decay. To test this possibility, the voltage dependence of single-channel currents was investigated.

Single-channel currents

Initially, cell-attached single-channel recordings were attempted. However, this attempt failed presumably because of low densities of ACh receptors and/or because of localization of all the ACh receptors in the synaptic sites underneath presynaptic boutons (Loring & Zigmond, 1987, 1988). Therefore, single-channel properties were explored by the whole-cell ACh receptor current noise analysis.

Figure 6 illustrates the whole-cell currents elicited by the bath-application of 20 μM-ACh. The voltage dependence of macroscopic whole-cell currents was similar to that of the I–V relation of ESCs (Fig. 7 *vs.* Fig. 1). The inward currents at negative membrane potentials (beyond about −50 mV) had a tendency to reach a plateau or even decreased with a further hyperpolarization. This suppression was enhanced by increased duration of ACh application (e.g. Fig. 6, −20 mV) and by increased ACh concentrations. Therefore, this effect may be attributed to desensitization of ACh receptors, which is accelerated by hyperpolarization (Magazanik & Vyskocil, 1970, 1975; Magleby & Pallotta, 1981). Thus, noise analyses were limited to holding

potentials between −50 and 30 mV (at further positive potentials, the S/N ratio was insufficient for noise analysis).

ACh current noise spectra were best-fitted by two Lorentzian components at any holding potentials (Fig. 8). The single-channel currents (i_1, i_2) were calculated from

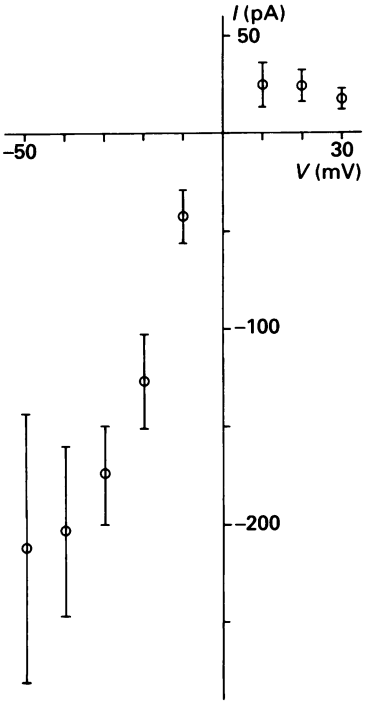

Fig. 7. Whole-cell currents in response to bath-applied acetylcholine (20 μM) at different holding potentials. Abscissa, the holding potential; ordinate, the mean current shifts by acetylcholine (○, mean; bars, S.D. in four cells). Cs_2SO_4-rich solution was used for the patch pipettes. Temperature, 25 °C.

eqns (1) and (2), respectively (see Methods). As illustrated in Fig. 9, these two values agreed very well at each holding potential between −60 and 30 mV. The inward single-channel currents were almost linearly related to the membrane potential (Fig. 10*A*). However, the outward single-channel currents had a tendency to reach a plateau. The mean slope of the *I–V* relation for the inward currents was about 42 pS (Fig. 10*A*). The reversal potential of the single ACh receptor channel was estimated from the nearest points as $-2{\cdot}5 \pm 2{\cdot}5$ mV (mean ± S.D., $n = 4$).

The *I–V* relation of single-channel currents (Fig. 10*A*) appears to be qualitatively similar to that of the ESCs shown by open circles in Figs 1 and 2. However, the chord conductance of the single channel at 20 mV was about 40% (average of four cells) of that at −20 mV in contrast to 10% calculated for the synaptic response (Fig. 3). Thus, the asymmetry of the single-channel conductance alone was not sufficient to account for the non-linear *I–V* relation of the whole-cell currents. Figure 10*B* plotted the product *Np* calculated from eqn (3) against the holding potential. *Np* had its peak at −20 mV and was decreased by a further depolarization. The mean *Np* at 20 mV

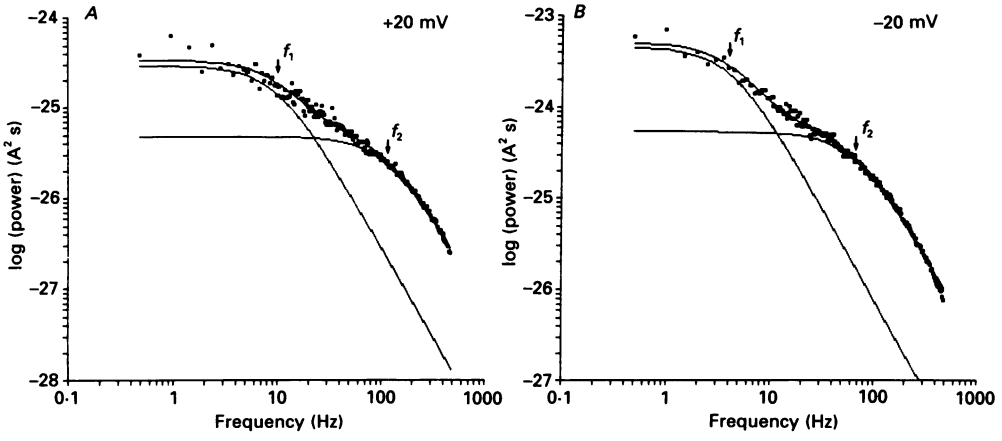

Fig. 8. Noise spectra of response to 20 μM-acetylcholine after subtracting baseline noise spectra for the cell shown in Fig. 6. *A*, the holding potential was 20 mV. The lines show optimal fitting of two Lorentzian components (f_1 = 10 Hz, f_2 = 117 Hz). The sum of two Lorentzian components is also drawn. *B*, similar to *A* but the holding potential was −20 mV (f_1 = 4 Hz, f_2 = 65 Hz).

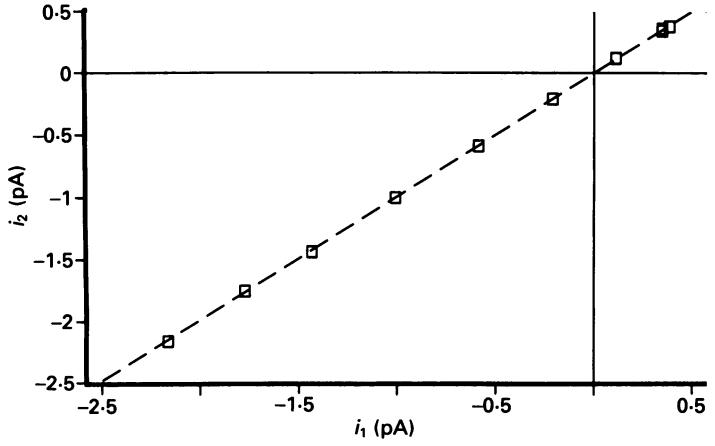

Fig. 9. Single-channel currents estimated from acetylcholine noise. Single-channel currents were calculated according to eqn (1) for i_1 and eqn (2) for i_2, for the cell shown in Fig. 6. The dashed line is the line of $i_1 = i_2$.

was 31% of the value at −20 mV (n = 4). *Np* also declined with hyperpolarization, and this may again be attributed to desensitization of ACh receptors. In fact, this decline of *Np* by hyperpolarization was reduced with reduced concentrations of ACh applied (5–10 μM)

The single-channel conductance estimated in the above study (42 pS) was a little larger than the value reported by others on ACh receptors of mammalian autonomic ganglia (31 pS, Rang, 1981; 35 pS, Mathie *et al.* 1987; however, 44 pS, Fenwick,

Marty & Neher, 1982; 20 and 50 pS, Derkach, North, Selyanko & Skok, 1987; 20–30 and 50–60 pS, Adams, Fieber & Konishi, 1987). This could be attributed to the higher cationic concentration used for improving the S/N ratio and/or the low Ca^{2+} in the bathing solution. With the standard cationic concentration (155 mM; $[Ca^{2+}]_o$

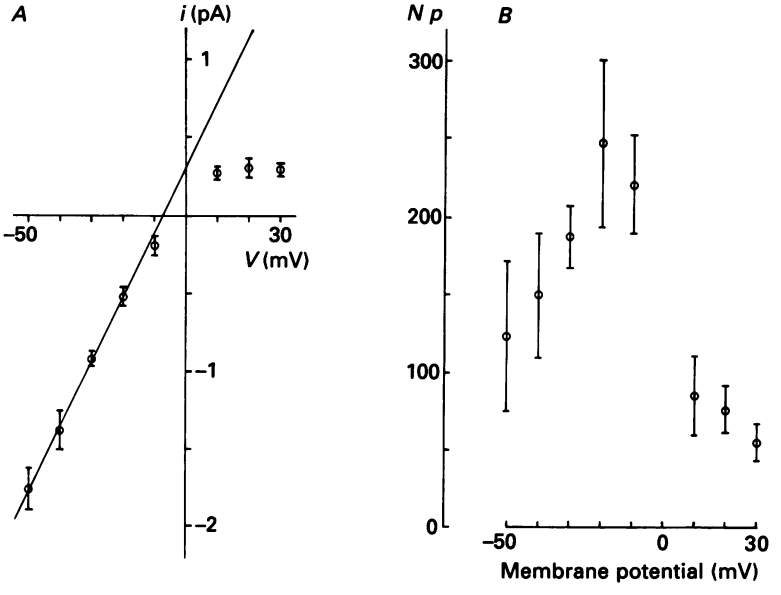

Fig. 10. Effects of membrane potential (abscissa) on single-channel currents (*A*) and the products *Np* of the number of *functional* channels, *N*, and the probability of a channel *being open*, *p* (*B*). Each point is the mean and S.D. of the four cells shown in Fig. 7. In *A*, the straight line indicates a slope conductance of 42 pS obtained by the least-squares fitting of the data between −50 and −20 mV.

= 0·2 mM), the single-channel conductance between −40 and −20 mV was 28·5 ± 4·3 pS (mean ± S.D., $n = 5$). A reduction in single-channel conductances of ACh receptor channels has been observed with increased concentrations of divalent cations, e.g. Mg^{2+}, Ca^{2+}, Sr^{2+} or Ba^{2+} (Colquhoun & Sakmann, 1985; Imoto, Methfessel, Sakmann, Mishina, Mori, Konno, Fukuda, Kurasaki, Bujo, Fujita & Numa, 1986; Mathie *et al.* 1987). This possibility was not tested in the present study.

Voltage dependence of the time constants of the ACh noise

Both the slow and fast time constants ($\tau_s = 1/2\pi f_1$, $\tau_f = 1/2\pi f_2$) had a similar voltage dependence. As shown in Fig. 11, the two time constants decreased with depolarizations, and were less sensitive to membrane potentials more negative than −30 mV. At 20 mV, both τ_s and τ_f were about 45% (average of four cells) of the values at −20 mV. These values correspond to an e-fold change by about 46 mV which is comparable to the voltage sensitivity of the ESC decay time constant (e-fold change by 38 mV). The fast and slow time constants of ACh noise (Fig. 11) were similar to but not exactly the same as the fast and slow time constants of the ESC decay (Fig. 5*A*). For example, at −50 mV, τ_f and τ_s obtained by the noise analysis were 2·9 ± 0·35 ms and 50 ± 5·9 ms, respectively (mean ± S.D., $n = 4$), while these

values estimated from the ESC decay were 7·3 ± 0·68 ms and 28 ± 3·4 ms, respectively (mean ± S.D., $n = 6$). The reason for this discrepancy is not clear.

Effects of intracellular Mg^{2+}

The inward rectification of certain K^+ channels was attributed to the block by Mg^{2+} from the inside (Vandenberg, 1987; Matsuda *et al.* 1987; Horie *et al.* 1987). To

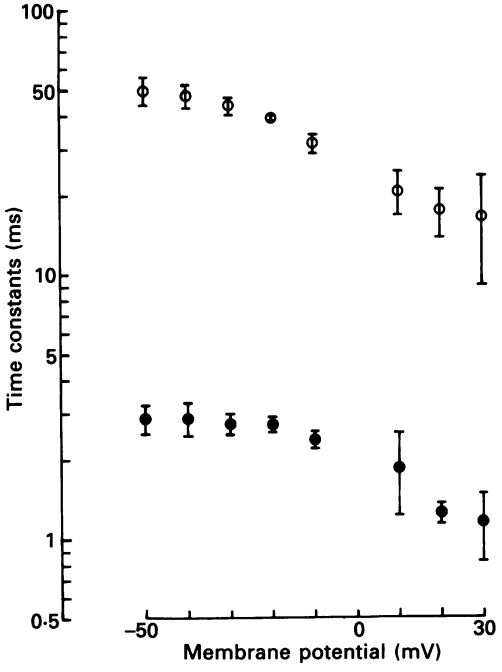

Fig. 11. Voltage dependence of τ_s (○) and τ_f (●) of the ACh noise spectra. Abscissa, the holding potential; ordinate, time constants plotted on a semilogarithmic scale. Data are the mean from the four cells shown in Fig. 7 (bars, S.D.).

see the effects of $[Mg^{2+}]_i$ on ACh receptor channels, 10 mM-EDTA was used, instead of EGTA. This treatment did not affect the voltage dependence of whole-cell currents nor single-channel properties (e.g. Fig. 6). Also, neither the addition of Mg^{2+} to the pipette solution (1 mM) nor the deprivation of extracellular Mg^{2+} or Ca^{2+} altered the *I–V* relation of the macroscopic currents. Also, the *I–V* profile of ESCs was not significantly altered by changes in the intracellular pH (5·4–9·4) or by addition of ATP (0·5–2 mM) to the pipette solution.

DISCUSSION

Non-linear I–V relation of ESCs

The results presented in this paper show that the ESCs of mouse SMG are characterized by a non-linear *I–V* relation. Membrane depolarization (e.g. from −20 to +20 mV) had several effects on ACh receptor characteristics: (1) decreased whole-

cell conductance (90% reduction), (2) decreased fast and slow time constants of the ESC decay (60% reduction), (3) decreased single-channel conductance, γ (60% reduction), (4) decreased product (Np; 70% reduction), of the number of *functional* channels at any given voltage (N; note, this does not reflect the actual number of channels in the cell) and the probability of a channel *being open* (p; the opening probability times the mean duration of each opening), and (5) decreased fast and slow time constants of ACh noise (55% reduction). From these results, it seems reasonable to conclude that the non-linearity of whole-cell conductance is due to the voltage dependence of γ and Np in single channels of ACh receptors and that the voltage-dependent changes in the ESC decay reflect changes in the mean open time of the single channel.

In the rat SMG, Rang (1981) has reported that the peak ESCs followed a linear relationship with membrane potentials. However, he has also noted the difficulty in reversing the ESCs by depolarization. In addition, he has reported that two time constants of the ESC decay have a weak sensitivity to the membrane potential as observed at the frog neuromuscular junction (Magleby & Stevens, 1972*b*). However, Rang (1981) did not study the voltage dependence of the time constants at potentials more depolarizing than −30 mV. Thus, the general properties described in the present results are not inconsistent with those reported for the rat SMG (e.g. compare Fig. 5*A* in the present paper with Fig. 3 of Rang, 1981).

The voltage dependence of mean channel lifetime

Generally, two possibilities could be considered to account for the bi-exponential ESC decay kinetics and the double-Lorentzian representation of the ACh noise spectra: (1) the presence of at least two distinct types of ACh-gated channels with a similar single-channel conductance but with different mean open times (Rang, 1981); (2) a single population of ACh-gated channels with complex open–close kinetics (Colquhoun & Sakmann, 1985; Mathie *et al.* 1987). At present, these two possibilities cannot be distinguished. However, the fast and slow time constants estimated from both the ESC decay and ACh noise showed a similar voltage sensitivity. Therefore, there is little doubt that depolarization shortens the mean channel lifetime (Anderson & Stevens, 1973; Colquhoun & Hawkes, 1977; Fenwick *et al.* 1982; Howe, Colquhoun & Cull-Candy, 1988). Since p is defined as the product of the opening probability of a channel and the mean duration of each opening, the voltage sensitivity of p may be due at least in part to the voltage sensitivity of mean channel lifetime. In the present study, N and p could not be measured independently. Therefore, it remains unknown whether N is also dependent on the membrane potential.

The voltage dependence of single-channel conductance

Inward rectification of whole-cell ACh receptor currents observed in cultured rat sympathetic ganglion cells does not appear to be associated with rectification of single-channel currents in the excised membrane (Mathie *et al.* 1987). This is in contrast with the present results that the I–V relation of single-channel currents shows an inward rectification. It is possible that different configurations of recording (whole-cell *vs.* outside-out) may affect the properties of the ACh receptor channel (see Ballivet, Nef, Couturier, Rungger, Bader, Bertrand & Cooper, 1988). Another

possible explanation is that because the noise data of the present study were filtered at 500 Hz, the single-channel current may have been underestimated since the open state would have been frequently interrupted by transient closures. In fact, single-channel currents recorded from cultured rat sympathetic neurones were interrupted by brief shut periods (Mathie *et al.* 1987), as were those in the frog end-plates (Colquhoun & Sakmann, 1985). However, Mathie *et al.* (1987) have not reported whether the frequency of transient closures is increased by depolarization. They have also noted that the burst length of the ACh receptor channels decreased with depolarization (e-fold change by 110–120 mV). However, this voltage sensitivity is smaller than that of the time constants observed in the present results (e-fold change by 38–46 mV).

The origin of rectification

The non-linear *I–V* relation in chromaffin cells has been attributed to channel block by internal Cs^+ ions (Hirano *et al.* 1987). Apparently, this was not the case in the present study, since the non-linear *I–V* relation was still observed with other cations (Na^+, K^+). Also, removal of internal or external Mg^{2+}, intracellular pH changes or addition of ATP to the internal solution did not affect the non-linear *I–V* profile of synaptic or ACh currents. Furthermore, rectification of the ESCs was independent of the direction of the current. Thus, the macroscopic conductance of ACh receptor channels of mouse SMG seems to be controlled only by the membrane potential. One puzzling observation was an additional increment in the macroscopic conductance at membrane potentials more positive than 50 mV (Figs 1, 2 and 3). This behaviour was not analysed in the present study and remains unexplained. With the depolarization-induced rectification, synaptically evoked action potentials may not be curtailed in mammalian autonomic ganglion cells in contrast to the 'short-circuit' effects observed in neuromuscular junctions (Fatt & Katz, 1951).

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