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ION-SELECTIVITY OF SINGLE GLUTAMATE-GATED CHANNELS IN LOCUST SKELETAL MUSCLE

BY K. S. KITS* AND P. N. R. USHERWOOD

Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD, UK

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Summary

The ion-selectivity of the extrajunctional glutamate-gated ion channel in locust extensor tibiae muscle was studied using the patch-clamp technique. The alkali metal ions Li^+, Na^+, K^+, Rb^+ and Cs^+ were all highly permeant, with reversal potentials close to 0 mV. Both complete and partial replacement of Na^+ (180 mmol l^{-1} in standard saline) showed that conductance (γ) increased in the order $Li^+ < Na^+ < Cs^+ < Rb^+$ (approx. 70–125 pS), γ_K being close to γ_{Cs}. The channel was impermeable to the large organic monovalent ions tetramethylammonium, guanidinium and choline, and permeable to the smaller ammonium ion. Divalent cations (Ca^{2+} and Mg^{2+}) did not contribute measurably to the ionic current. Indications were obtained that high concentrations of Mg^{2+} or Ca^{2+} block the channel. The results suggest that the glutamate-gated channel combines a high conductance with a restricted ion-selectivity, based on ion charge and size, the conductance being dependent on the dehydration energy of the ionic species.

Introduction

The ion-selectivity of a membrane channel determines the nature of the current it conducts and, thereby, its role in the electrophysiological behaviour of an excitable cell. Glutamate-gated ion channels are the conducting elements of arthropod excitatory neuromuscular synapses (Usherwood, 1981) and a great number of excitatory synapses in the vertebrate central nervous system (Fonnum, 1984; Fagg, 1985). Insect skeletal muscle fibres provide a very accessible model system for the study of such synapses, and the L-glutamate (Glu-)-gated receptor–ionophore complex, in particular in locust extensor tibiae and retractor unguis muscle, has been extensively studied with regard to its ligand identification properties (Usherwood, 1978; Gration *et al.* 1979) and channel kinetics (Cull-Candy *et al.* 1980; Gration, 1982; Kerry *et al.* 1987).

The Glu-gated channel of locust metathoracic extensor tibiae muscle has been

* Present address: Department of Biology, Free University, P.O. Box 7161, 1007 MC Amsterdam, The Netherlands.

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shown to be a rather non-selective cation channel (i.e. a valence channel: Latorre & Miller, 1983), normally conducting K^+ and Na^+ ($p_{Na}/p_K = 0{\cdot}9$), by studying the effects of bath application of various salines on the excitatory postsynaptic currents and ionophoretically evoked excitatory junctional currents (Anwyl & Usherwood, 1975; Anwyl, 1977*a*,*b*).

In this paper we have used the patch-clamp technique (Neher *et al.* 1978; Patlak *et al.* 1979) to investigate the effects of various inorganic and organic ions on the conductance of the channel gated by extrajunctional glutamate receptors of the extensor tibiae muscle. One major advantage of this approach is that alterations of the extracellular medium can be restricted to the membrane area sealed by the tip of the patch pipette. Thus concomitant changes in transmembrane ion distributions, which might otherwise complicate data interpretation, are virtually eliminated.

Our results indicate that this Glu-gated channel combines a high single-channel conductance with a restricted ion-selectivity based on ion size and charge.

Materials and methods

Adult, laboratory-bred *Locusta migratoria* were used throughout this study. Recordings were obtained from midregional and proximal metathoracic extensor tibiae muscle fibres, with the preparation pinned down on a 184-silicone (Sylgard) layer in a 2 ml Perspex bath.

Standard locust saline had the following composition (in mmol l^{-1}): NaCl, 180; KCl, 10; $CaCl_2$, 2; Hepes, 10; pH 6·8; 20 ± 1 °C. The compositions of the various test salines are given in Table 1. In all experiments 10^{-4} mol l^{-1} L-glutamate was added to the pipette solution to activate glutamate-gated channels (see Patlak *et al.* 1979). To prevent desensitization, the muscle was bathed for 30 min in 2×10^{-6} mol l^{-1} concanavalin A prior to recording (Mathers & Usherwood, 1976; Mathers, 1981).

Muscle fibres from which patch-clamp recordings were taken were voltage-clamped with a standard two-electrode clamp to control membrane potential. Patch-clamp recordings employing mega-ohm seals were made using the technique of Neher *et al.* (1978) and Patlak *et al.* (1979). The current electrode was placed midway between the voltage pipette and the patch electrode (Fig. 1). Because of the spatial limitations of the voltage-clamp (space constant about 1 cm, Anderson *et al.* 1978), care was taken to ensure that the distance between the patch electrode and the current electrode (<100 μm) never exceeded the distance between the current and the voltage electrodes. At each site single-channel recordings were made at various membrane potentials. Single-channel currents were recorded with 0–3 kHz bandwidth and stored on tape (Racal store 4 DS). Prior to analysis the data were filtered at 1 kHz. From these data I/V plots were constructed to determine single-channel conductance and channel current reversal potential.

Since the test solutions were only applied in the patch pipette, the following experiments were undertaken to test whether the contents of the tip of the pipette

Table 1. *Saline compositions of the various test salines*

Na^+	K^+	Ca^{2+}	Foreign ion		Na^+	K^+	Ca^{2+}	Foreign ion	
180	10	2	(standard saline)		90	10		NH_4^+	90
	10		sucrose	390		10		NH_4^+	180
45	10		sucrose	300	135	10		TMA	45
90	10		sucrose	180		10		TMA	180
180	10				135	10		guanidinium	45
360	10					10		guanidinium	180
	40		sucrose	300	90	10		choline	90
	100		sucrose	180		10		choline	180
90	10		Li^+	90	135	10	30		
	10		Li^+	180	90	10	60		
90	10		Rb^+	90		10	120		
	10		Rb^+	180	135	10		Mg^{2+}	30
90	10		Cs^+	90	90	10		Mg^{2+}	60
	10		Cs^+	180		10		Mg^{2+}	120

All salines were buffered with 10 mmol l^{-1} Hepes; pH was set to 6·8.
Foreign ions were added as chloride salts.
Where possible, salines were made isotonic to standard saline. All values in mmol l^{-1}.

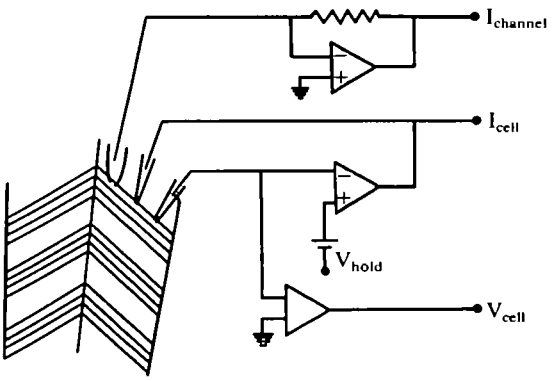

Fig. 1. Preparation and recording situation. Care was taken to place the voltage and the patch electrode at equal distances from the current electrode.

were influenced by dilution during recordings. With standard saline in the patch pipette and sucrose (390 mmol l^{-1}) saline (see Table 1) in the bath, channel openings of normal amplitude were recorded both at the resting membrane potential (−65 mV) and at more hyperpolarized membrane potentials (−70 to −140 mV) ($N = 3$). Conversely, with sucrose (390 mmol l^{-1}) saline or choline (180 mmol l^{-1}) saline in the pipette and standard saline in the bath, no channel openings were recorded, although good seals were obtained (three preparations;

more than 15 sites on each preparation). These observations suggest that dilution of the contents of the pipette tip was not a problem. Moreover, recordings were always made with slight positive pressure on the pipette solution.

For analysis, recordings were displayed on a Gould OS 1420 digital storage oscilloscope and then plotted on a YT plotter (JJ instruments CR 6505), with a final resolution of 1 or 2·5 ms mm^{-1} and 2 or 4 mm pA^{-1}. Amplitudes were measured by hand. The open channel current amplitude was defined as the distance between the mid-noise level during the closed periods preceding and following the opening and the mid-noise level during the opening. Recordings which were regarded as technically inferior, for example, because of drift or a poor signal to noise ratio (<3:1), were discarded. Distributions that were multimodal, due to double events, rim channels or, possibly, subconductance levels, were only used when the maximum peak clearly represented the single-channel current amplitude. From recordings of acceptable quality, 1–4 random samples of 2·5 s duration in total were taken, yielding a total of between 30 and 100 measurable opening events for a given membrane potential.

Frequency distributions of channel current amplitudes were constructed and modal amplitudes were determined from them. Mean amplitudes ± standard deviations (S.D.) were also determined from these data. Amplitude distributions were only used when they were normal, or nearly normal, i.e. some left-hand skewing due to clipping of fast events caused by the restricted bandwidth was accepted. Distributions where mean and mode differed by more than 0·5 S.D. were discarded. The modal values of the amplitude distributions of recordings at various holding potentials from one site were used in a linear least-squares fitting procedure to assess the conductance and the reversal potential (usually obtained by extrapolation). In general, each experiment was repeated three times.

Results

Alkali metal ions

The Glu-gated channel proved to be permeable to all ions from the alkali metal series that were studied, i.e. Li^+, Na^+, K^+, Rb^+ and Cs^+. An example of a typical experiment is illustrated in Fig. 2A, where channel openings at various holding potentials in Rb^+-saline are shown. It appears from these recordings that normal opening and closing behaviour of the channel occurs after complete substitution of Na^+ by Rb^+. This holds for all alkali metal ions. The amplitude distributions of the recordings at different holding potentials (from −60 to −140 mV) in Rb^+-saline and the resulting I/V plot are shown in Fig. 2B. Channel openings in Cs^+-saline are illustrated in Fig. 3C.

Table 2A gives the results for complete substitution of Na^+ by alkali metal ions. There is a clear effect of ion species on conductance, but much less on reversal potential. Single-channel conductance increases in the order $Li^+ < Na^+ < Cs^+ < Rb^+$ from 70 to 121 pS. The reversal potentials are all close to zero.

Partial substitution of Na^+ by alkali metal ions gives similar results. Again

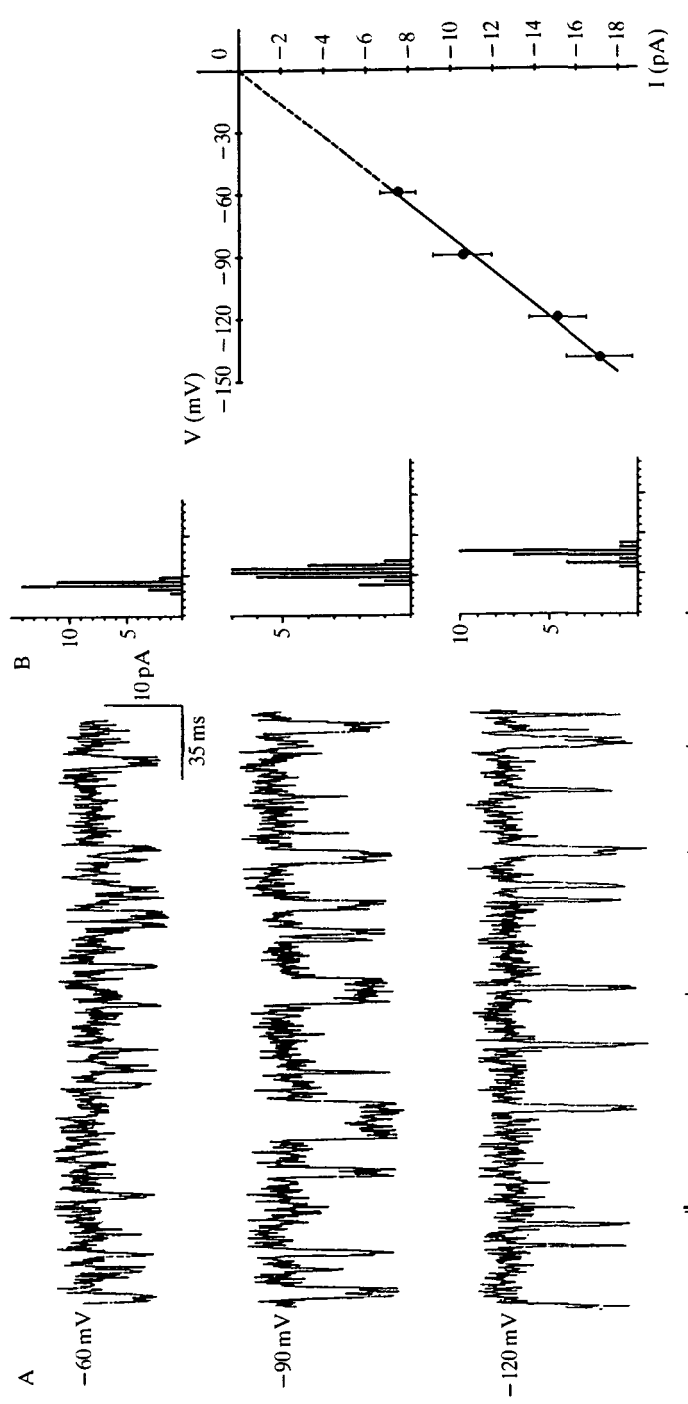

Fig. 2. (A) Single-channel recordings at different holding potentials (imposed by voltage-clamp) of the glutamate-gated channel. (B) Amplitude distributions and I/V plot of the recordings from A (modal values ± 1 S.D.). Saline (in mmol l^{-1}): RbCl, 180; KCl, 10; Hepes, 10; $\gamma = 122$ pS, $E_{rev} = 0$ mV.

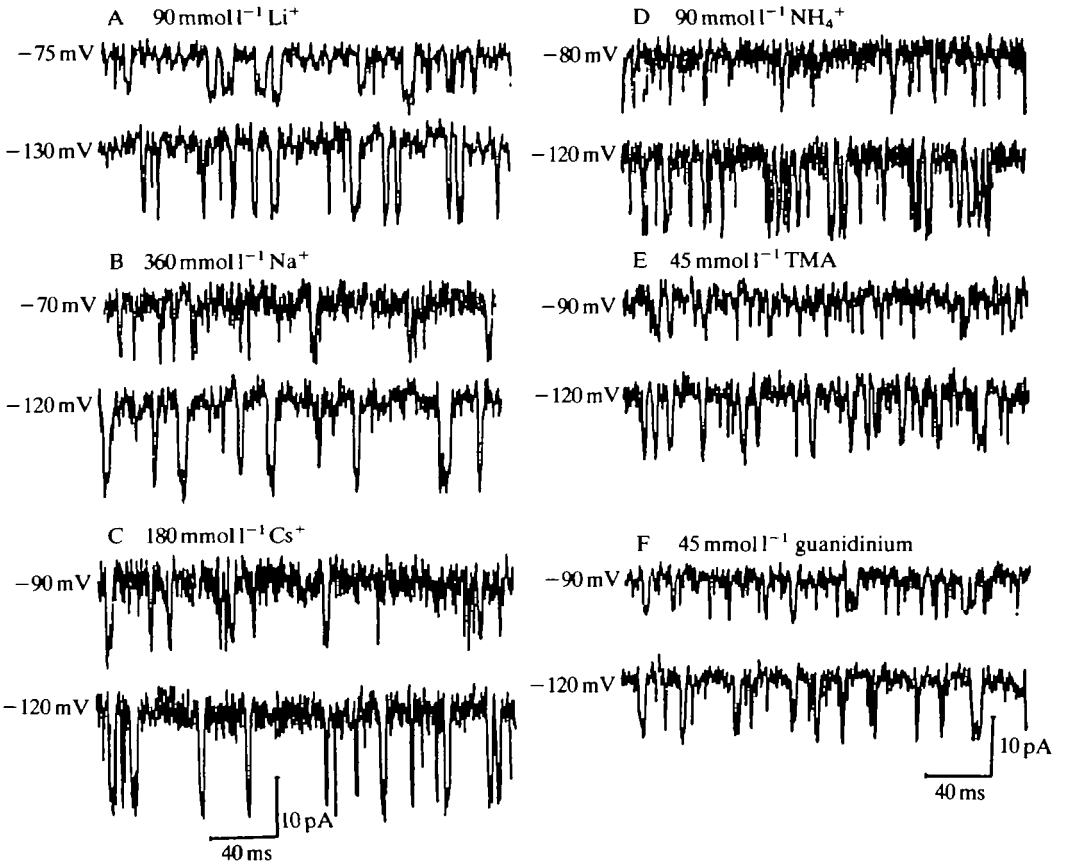

Fig. 3. Single-channel acitivity recorded in various salines. Holding potentials and concentrations of foreign monovalent cations are indicated. See Table 1 for complete saline compositions.

conductances increase in the same order from 80 pS with Li^+/Na^+-saline to125 pS with Rb^+/Na^+-saline, whereas the reversal potentials stay close to zero (Table 2B). Single-channel activity in Li^+/Na^+-saline is illustrated in Fig. 3A. An example of an I/V plot obtained in Cs^+/Na^+-saline is given in Fig. 4A.

Na^+ and K^+

Under physiological conditions, Na^+ and K^+ are the primary charge carriers through the Glu-gated channel, a permeability ratio p_{Na}/p_K of 0·9 having been proposed in previous studies (Anwyl, 1977*a*,*b*). Our experiments with various concentrations of Na^+ and K^+ have confirmed the high permeability of Na^+ and K^+ (Table 3; Fig. 3B). With $[K^+]_o$ at 100 mmol l^{-1}, inward currents were recorded at holding potentials of −60 mV and less, yielding a mean conductance of 101 pS and a mean reversal potential of −4 mV (Fig. 4B; Table 3B). The extrapolated reversal potentials obtained in various Na^+- and K^+-salines (except in

Table 2. *Conductances and reversal potentials with alkali metal ions completely (A) or partially (B) replacing Na^+*

	Alkali metal ion	$[x]_o$ (mmol l^{-1})	N	Conductance (pS)	E_{rev} (mV)	$\gamma_x \pm$ S.D. (pS)	$E_{rev} \pm$ S.D. (mV)	E_{exp}* (mV)
A	LiCl	180	5	84	−5			
			5	60	2	70 ± 12·7	0 ± 4	3·6
			4	65	3			
	NaCl	180	6	83	3			
			5	86	−6			
			3	95	2	89 ± 4·5	−3 ± 7·3	3·6
			4	91	4			
			4	93	−12			
			5	88	−11			
	RbCl	180	3	124	−8			
			5	112	−5	121 ± 6·2	−6 ± 4·7	3·6
			4	126	−11			
			4	122	0			
	CsCl	180	3	125	0			
			3	100	0	103 ± 20·7	3 ± 4·6	3·6
			5	84	8			
B	LiCl	90	4	103	−27			
			4	66	5	80 ± 10·3	−6 ± 17·9	3·6
			5	70	3			
	RbCl	90	5	125	−8			
	CsCl	90	5	110	2			
			7	113	−6	116 ± 8·5	−1 ± 4·6	3·6
			7	126	2			

* Theoretically expected reversal potentials, assuming $[Na^+]_i = 10$ mmol l^{-1} (see Anwyl, 1977*a*), $[K^+]_i = 140$ mmol l^{-1} (see Leech, 1986), $p_{Na}/p_K = 0{\cdot}9$ (see Anwyl, 1977*a*) and $p_x = p_{Na}$.

Columns 3–5 give the data for the separate experiments, columns 6 and 7 give the means and standard deviations of the experiments at each condition.

x denotes ion species replacing Na^+; N = number of holding potentials successfully tested in a given experiment, γ_x = mean conductance; E_{rev} = mean reversal potential.

Same conventions apply to Tables 3–5.

90 mmol l^{-1} Na) agree reasonably well with those expected for a p_K/p_{Na} of 0·9, assuming internal Na^+ and K^+ concentrations of 10 and 140 mmol l^{-1}, respectively (see Leech, 1986). The extrapolated reversal potential in 90 mmol l^{-1} Na^+-saline differs from the expected value. However, since the conductance in 90 mmol l^{-1} Na^+ is rather low (38 pS), a slight error in the calculated value of this parameter would have introduced a large error in the estimate of the reversal potential.

A clear effect of $[Na^+]_o$ on the single-channel conductance was obtained: the conductance increasing from 38 pS in 90 mmol l^{-1} to 106 pS in 360 mmol l^{-1} Na^+

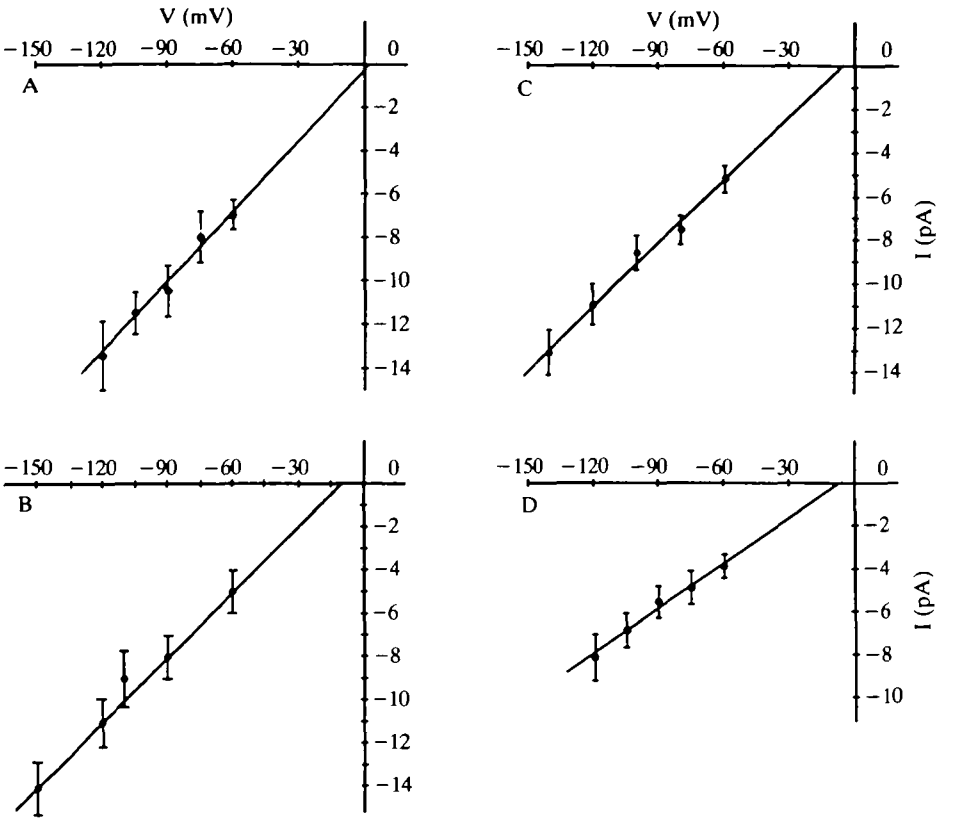

Fig. 4. I/V plots of the glutamate-gated channel in various salines (modal values ± 1 S.D.). (A) CsCl, 90; NaCl, 90; KCl, 10; Hepes, 10; $\gamma = 110$ pS, $E_{rev} = 2$ mV. (B) KCl, 100; sucrose, 180; Hepes, 10; $\gamma = 100$ pS, $E_{rev} = -10$ mV. (C) NH_4Cl, 90; NaCl, 90; KCl, 10; Hepes, 10; $\gamma = 97$ pS, $E_{rev} = -6$ mV. (D) Guanidinium chloride, 45; NaCl, 135; KCl, 10; Hepes, 10; $\gamma = 69$ pS, $E_{rev} = -7$ mV. All concentrations in mmol l^{-1}.

(Fig. 5). The above results were confirmed for *Schistocerca* extensor tibiae muscle: salines with 100 mmol l^{-1} K^+ and 0 or 90 mmol l^{-1} Na^+ yielded conductances of 70·5 and 114 pS and reversal potentials of +10 and +9·5 mV, respectively (C. Kerry, unpublished data).

Monovalent organic ions

The permeability of the Glu-gated channel to large monovalent ions was tested using the organic compounds ammonium (NH_4^+), guanidinium [$C(NH_2)_4^+$], tetramethylammonium [$N^+(CH_3)_4$; TMA] and choline [$C_2H_4OH\text{-}N^+\text{-}(CH_3)_3$]. No channel activity was seen after complete replacement of Na^+ by choline guanidinium or TMA. Table 4 shows the quantitative results obtained after partial replacement of Na^+. Only NH_4^+ substitution gives normal channel current

Table 3. *Conductances and reversal potentials in salines with different concentrations of Na^+ (A) and K^+ (B)*

A $[Na^+]_o$ ($mmol\,l^{-1}$)	N	Conductance (pS)	E_{rev} (mV)	$\gamma_x \pm$ S.D. (pS)	$E_{rev} \pm$ S.D. (mV)	E_{exp}* (mV)
90	3	38	11†	38 (−)	8 ± 4·2	−12·5
	3	38	5‡			
135		See Table 4§		72 ± 4·6	−9 ± 6·1	−3·2
180		See Table 2A		89 ± 4·5	−3 ± 7·3	3·6
360	5	101	3			
	5	105	17	106 ± 13·9	13 ± 8·1	20·4
	5	92	9			
	3	125	21			

B $[K^+]_o$ ($mmol\,l^{-1}$)	N	Conductance (pS)	E_{rev} (mV)	$\gamma_x \pm$ S.D. (pS)	$E_{rev} \pm$ S.D. (mV)	E_{exp}* (mV)
40	4	93	−38			−33·3
100	3	100	3			
	5	100	−10	101 ± 1·2	−4 ± 6·7	−10·0
	4	102	−6			

* Assuming $[Na^+]_i = 10\,mmol\,l^{-1}$, $[K^+]_i = 140\,mmol\,l^{-1}$ and $p_{Na}/p_K = 0{\cdot}9$.

† $90\,mmol\,l^{-1}$ Na^+/$90\,mmol\,l^{-1}$ choline-saline.

‡ $90\,mmol\,l^{-1}$ Na^+/$180\,mmol\,l^{-1}$ sucrose-saline.

§ Data shown are the overall means from the experiments with $135\,mmol\,l^{-1}$ Na^+ and $45\,mmol\,l^{-1}$ TMA or guanidinium.

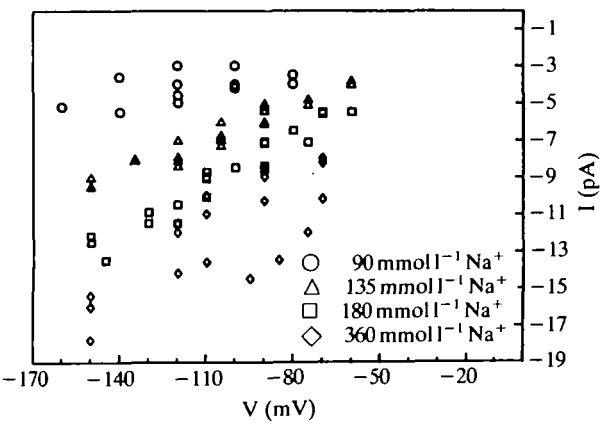

Fig. 5. Modal amplitudes at various holding potentials using different concentrations of Na^+ in the pipette. Each point represents the modal value of the current amplitude distribution at the given holding potential. Pooled data of all experiments.

Table 4. *Conductances and reversal potentials after partial replacement of Na^+ by monovalent organic ions*

Organic ion	$[x]_o$ ($mmol\,l^{-1}$)	N	Conductance (pS)	E_{rev} (mV)	$\gamma_x \pm$ S.D. (pS)	$E_{rev} \pm$ S.D. (mV)	E_{exp}* (mV)
NH_4^+	90	5	97	−6			
		3	98	−8	95 ± 4·9	−4 ± 4·7	3·6
		3	89	1			
TMA	45	3	83	−16			
		4	74	−5			
		3	70	1	73 ± 5·5	−10 ± 7·2	−3·2
		3	73	−9			
		3	67	−15			
		5	71	−17			
Guanidinium	45	4	72	−5			
		5	71	−5	70 ± 2·2	−8 ± 4·8	−3·2
		5	69	−7			
		3	67	−15			
Choline	90	3	38	5			−12·5

* Assuming $[Na^+]_i = 10\,mmol\,l^{-1}$, $[K^+]_i = 140\,mmol\,l^{-1}$, $p_{Na}/p_K = 0{\cdot}9$, $p_{NH_4} = p_{Na}$, and TMA, guanidinium and choline are impermeant.

amplitudes (Figs 3D, 4C), yielding a mean conductance of 95 pS and a mean reversal potential of −4 mV, both close to the values obtained in standard saline. Single-channel activities in TMA/Na^+-saline and in guanidinium/Na^+-saline are shown in Fig. 3E,F. Partial substitution of Na^+ by TMA or guanidinium reduced both the single-channel conductance and the reversal potential. Fig. 4D illustrates this for guanidinium/Na^+-saline. Quantitatively the decrease in reversal potential to −10 mV with 45 $mmol\,l^{-1}$ TMA and to −8 mV with 45 $mmol\,l^{-1}$ guanidinium is consistent with a permeability of the channel for TMA and guanidinium that is zero (in which case the expected reversal potential is −3·2 mV, assuming $[Na^+]_i = 10\,mmol\,l^{-1}$ and $[K^+]_i = 140\,mmol\,l^{-1}$) (cf. Leech, 1986) or close to zero (the expected reversal potential is −2·3 mV for $p_x/p_K = 0{\cdot}1$). This bears out the absence of channel activity in TMA- or guanidinium-salines. The results with choline/Na^+-saline did not yield reliable reversal potentials. However, a large decrease in conductance was obtained. Finally, no differences were found between salines in which 90 $mmol\,l^{-1}$ Na^+ was replaced by either 90 $mmol\,l^{-1}$ choline or 180 $mmol\,l^{-1}$ sucrose (Fig. 5; Table 3A). These data strongly suggest that choline is impermeant.

Divalent cations

$MgCl_2$ and $CaCl_2$ were used to assess the permeability of the Glu-gated channel to divalent cations. No channel activity was detected with either 120 $mmol\,l^{-1}$

$MgCl_2$ or $CaCl_2$ to replace 180 mmol l^{-1} NaCl ($N = 3$ preparations; various sites per preparation). Partial replacement of Na^+ by Mg^{2+} or Ca^{2+} substantially reduced the unitary current amplitude (Figs 6, 7). The resulting I/V plots showed a decreased conductance compared with standard saline for 30 and 60 mmol l^{-1} Mg^{2+}-salines and 30 mmol l^{-1} Ca^{2+}-saline (Table 5), suggesting that Ca^{2+} and Mg^{2+} are impermeant, or do not contribute measurably to the ionic current. The experimentally obtained reversal potentials are in agreement with zero or low permeancy of Ca^{2+} and Mg^{2+}. In general, it was observed that recordings obtained

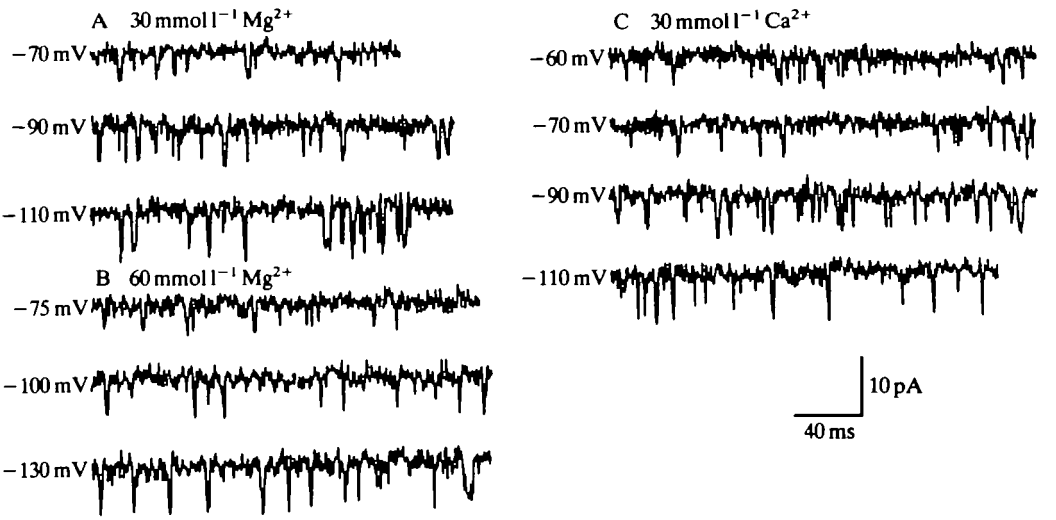

Fig. 6. Single-channel activity in high-Mg^{2+} and high-Ca^{2+}-salines. Holding potentials and concentrations of divalent cations indicated. See Table 1 for complete saline compositions.

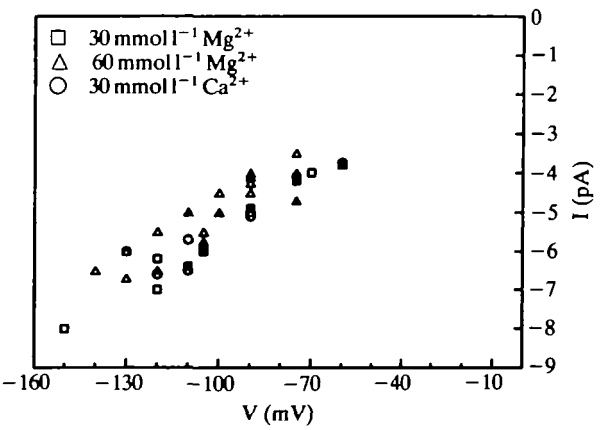

Fig. 7. Modal amplitudes plotted against holding potentials for 30 and 60 mmol l^{-1} Mg^{2+} and 30 mmol l^{-1} Ca^{2+}. Pooled data of all experiments.

Table 5. *Conductances and reversal potentials after partial replacement of Na^+ by divalent cations*

Organic ion	$[x]_o$ (mmol l^{-1})	N	Conductance (pS)	E_{rev} (mV)	$\gamma_x \pm$ S.D. (pS)	$E_{rev} \pm$ S.D. (mV)	E_{exp}* (mV)
Mg^{2+}	30	4	63	−10			
	30	3	60	−4	58 ± 6·8	−2 ± 9·2	−3·2
	30	6	50	8			
Mg^{2+}	60	4	50	−10			
	60	4	59	−16	56 ± 4·9	−12 ± 3·8	−12·5
	60	5	58	−9			
	120	†	–	–	–	–	−68·3
Ca^{2+}	30	4	60	−1	55 ± 7·1	4 ± 6·4	−3·2
	30	3	50	8			
	120	†	–	–	–	–	−68·3

* Assuming $[Na^+]_i = 10$ mmol l^{-1}, $[K^+]_i = 140$ mmol l^{-1}, $p_{NA}/p_K = 0{\cdot}9$, $p_{Ca} = 0$, $p_{Mg} = 0$.
† Three preparations, various sites and holding potentials per preparation.

in salines containing high concentrations of Mg^{2+} or Ca^{2+} were less stable than those obtained in standard saline. Instability was particularly marked with 60 mmol l^{-1} Ca^{2+} and this made it impossible to construct I/V plots. Also with high Ca^{2+} concentrations, channel openings were clearly less frequent and of shorter duration than in standard saline. These findings might imply that high concentrations of Ca^{2+} or Mg^{2+} have a blocking action on the channel. In this respect it should be noted that the conductance in saline with 30 mmol l^{-1} Mg^{2+} or Ca^{2+} and 135 mmol l^{-1} Na^+ is below that in saline with 135 mmol l^{-1} Na^+ and 45 mmol l^{-1} TMA or guanidinium, again indicating a possible inhibitory action of high concentrations of divalent cations, causing a decrease in channel lifetime, that results, at 1 kHz recording bandwidth, in reduced current amplitudes.

To test the patch-clamp results, we performed three experiments using ionophoretic application of L-glutamate in isotonic Ca^{2+}-saline (see Gration *et al.* 1979, for experimental details). It was found that stable junctional Glu-potentials slowly decreased to zero amplitude upon replacement of all Na^+ by 120 mmol l^{-1} Ca^{2+}. Block of Glu-potentials persisted for 10–12 min upon washing with standard saline, after which the Glu-potentials were gradually restored to the original amplitude (Fig. 8).

The qualitative conclusion from these results is that Mg^{2+} and Ca^{2+} are impermeant or, at best, poorly permeant, and possibly Glu-receptor channel blockers in this system (see also Duce & Usherwood, 1986).

Discussion

The extrajunctional Glu-gated channel in locust extensor tibiae muscle was

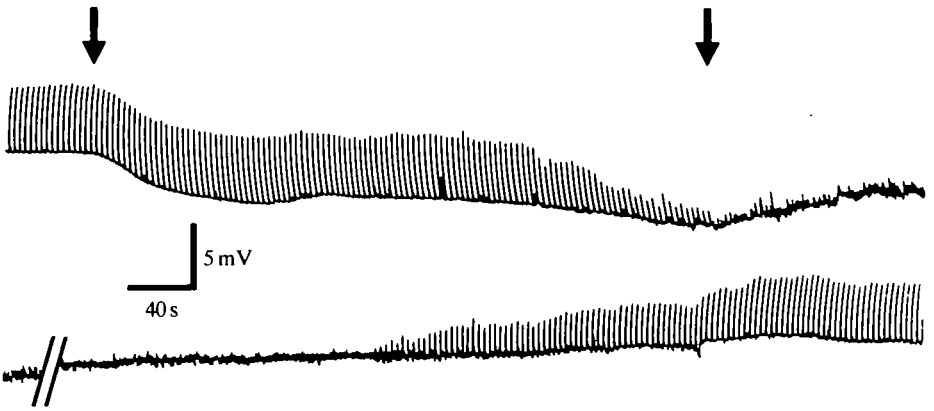

Fig. 8. Glutamate potentials evoked by ionophoretic stimulation of junctional glutamate receptors. The potentials are abolished in isotonic Ca^{2+}-saline and slowly reappear upon washing with standard saline. Arrows indicate onset and end of application of isotonic Ca^{2+}-saline. //marks omitted interval of 7 min during which potentials were absent.

found to have a high permeability for all alkali metal ions and the ammonium ion, but to be virtually impermeable for the organic monovalent cations (TMA, guanidinium and choline). The divalent cations, Ca^{2+} and Mg^{2+}, could not replace Na^{2+} as charge carrier, either because of low or zero permeancy or a blocking action when applied in high concentrations.

The single-channel conductance in standard saline was between 80 and 100 pS. This is lower than the values reported in previous studies (approx. 125 pS: Patlak *et al.* 1979; Cull-Candy *et al.* 1980; Gration, 1982). We do not have an explanation for this difference.

The relative conductances of the alkali metal ions were found to be as follows: $Rb^+ > Cs^+ > Na^+ > Li^+$. Furthermore, since the conductance in 100 mmol l^{-1} K^+ (101 pS) was greater than in 180 mmol l^{-1} Na^+ (89 pS), it follows that $\gamma_K > \gamma_{Na}$. Since γ_{Cs} (103 pS in 180 mmol l^{-1} Cs^+) and γ_K (101 pS in 100 mmol l^{-1} K^+) are very close, the most likely order seems to be either $Rb^+ > Cs^+ > K^+ > Na^+ > Li^+$ or $Rb^+ > K^+ > Cs^+ > Na^+ > Li^+$, which are identical to Eisenman sequences II and III, respectively, suggesting that the selectivity of the channel is primarily determined by the cation's dehydration energy and interactions of the ion with anionic groups of relatively weak electrostatic field strength (Eisenman & Horn, 1983; Hille, 1984). These conclusions are supported by results with partial substitution of Na^+, where the same order of relative conductances was found.

At present, our data do not allow conclusions on the order of relative permeabilities of the Glu-gated channel, as deduced from the reversal potentials. This is because the reversal potentials obtained in these experiments are to some extent inaccurate, since they were obtained by extrapolation and were, therefore, more sensitive to noise in the data than the conductances, which were calculated

directly from the data. (In this respect it should be noted that the most obvious discrepancies between experimental data and expected values of the reversal potential occurred when γ was small, i.e. when the data were obtained from recordings with a lower signal to noise ratio due to reduced current amplitudes.) Thus, conclusions on the ion-selectivity in terms of relative permeabilities await future giga-seal recordings of the channel, providing a better signal to noise ratio, and a larger range of holding potentials, possibly allowing direct measurement of the reversal potential.

Theoretical arguments (see Eisenman & Horn, 1983; Hille, 1984) have pointed out that profound differences between relative permeabilities and conductances of a channel will occur when ion permeation involves interaction with binding sites (energy wells) within the channel pore, rather than a simple molecular sieving mechanism. Recent studies using giga-seal recordings of glycine-gated channels (Bormann, 1987) and Ca^{2+} channels (Coronado & Smith, 1987) have confirmed these predictions. It is very possible that the Glu-gated channel also displays such differences between relative conductance and permeability, as is suggested by some apparent differences between our data on conductance and data on permeability obtained by Anwyl (1977*a*,*b*). With regard to the alkali metal ions, Anwyl reported a high permeability of the junctional channel to Li^+, Na^+ and K^+, but a low permeability to Cs^+, indicating an order of relative permeabilities almost the inverse of the order of conductances. Other differences concern guanidinium, TMA and Ca^{2+} (see later). However, apart from the p_{Na}/p_K ratio of 0·9, no quantitative data on relative permeabilities were given by Anwyl.

Our data on the relationship between $[Na^+]_o$ and the reversal potential agree with a ratio for p_{NA}/p_K of 0·9, independent of $[Na^+]_o$, as reported by Anwyl (1977*a*). We also found a clear relationship between $[Na^+]_o$ and γ_{Na}. A similar concentration-dependence of the single-channel conductance has been reported for inward rectifier K^+ channels (Sakmann & Trube, 1984; Payet *et al.* 1985). Anwyl (1977*a*) suggested that p_K is reduced in low-Na^+ saline and even abolished in Na^+-free saline since the shift in reversal potential upon lowering of $[Na^+]_o$ was less than theoretically expected and no Glu-current was seen in Na^+-free salines. Our data clearly show that in Na^+-free, high-K^+ saline inward currents are still recorded, yielding a single-channel conductance of about normal value. This discrepancy may be explained by assuming strong inward rectification of the channel in low-Na^+ saline, resulting in normal inward currents with low-Na^+, high-K^+ saline and less than expected shifts of the reversal potential in low-Na^+, low-K^+ saline. This hypothesis would also explain why no outward single-channel currents are recorded in Na^+-free salines (with either 180 mmol l^{-1} choline or 390 mmol l^{-1} sucrose) at holding potentials positive to E_K.

Of the organic ions tested, only NH_4^+ proved to be permeant. The crystal ionic radius of NH_4^+ is about the same as that of Rb^+ and Cs^+ (see Hille, 1984), whereas the other ions, TMA, guanidinium and choline are (much) larger. This might suggest that for the latter ions size is the limiting factor in channel permeation. In this respect the Glu-gated channel appears to have a higher selectivity than the

cholinergic endplate channel that is highly permeable to guanidinium and even slightly to choline (Adams *et al.* 1980; Edwards, 1982). Our results partly agree with those of Anwyl (1977*b*) who, using ionophoretically evoked Glu-currents, reported a high permeability of the junctional Glu-channel to NH_4^+ but also to guanidinium, a low permeability to TMA and no permeability to choline.

With regard to the divalent cations our results seem to differ from those of Anwyl (1977*b*) and Cull-Candy & Miledi (1980), who claimed a high Ca^{2+} permeability of the junctional channels, whereas our results, both the patch-clamp recordings and the ionophoresis experiments, did not reveal any contribution of high-Ca^{2+} or Mg^{2+} to the Glu-induced currents.

It should be stated, however, that our results do not exclude some contribution of Ca^{2+} to the channel current at low Ca^{2+} concentrations. Our results with 30 and 60 mmol l^{-1} Ca^{2+} or Mg^{2+} would, alternatively, be explained by a blocking action of Ca^{2+} and Mg^{2+} that becomes evident at high concentrations. Such blocking action is consistent with results obtained by Cull-Candy & Miledi (1980), who reported a decrease in mean open time of the junctional channel in isotonic Ca^{2+} to about one-third of the control value. In this respect it is of interest that the cholinergic endplate channel too, though permeable to Ca^{2+} (see Edwards, 1982), is affected by a high $[Ca^{2+}]_o$, in that the single-channel conductance is reduced (Kuba & Takeshita, 1983; Lewis, 1984). Refined giga-seal experiments should elucidate the possible contribution of Ca^{2+} to the single-channel current through the Glu-gated channel.

It is noteworthy that many of our experiments were performed in salines without Ca^{2+} (no EGTA added). Apparently, the presence of Ca^{2+} is not a prerequisite for channel opening. This contrasts the findings of Franke *et al.* (1987), who reported that Ca^{2+} is necessary for opening of Glu-gated channels in crayfish muscle. Our data are supported by the recent findings that channel openings can be recorded from *Schistocerca* muscle using saline with (in mmol l^{-1}) Na^+, 148; K^+, 10; Ca^{2+}, 0; and EGTA, 1 (C. Kerry, unpublished data).

In evaluating our results and those of Anwyl (1977*b*) and Cull-Candy & Miledi (1980), the following should be considered. First, Anwyl reports that in Ca^{2+}- or guanidinium-saline a rapid block of the postsynaptic current and only a temporary response to ionophoretic glutamate pulses is seen, the response being abolished after 20–60 min. Thus, the report that Ca^{2+} and guanidinium are permeant does not apply to the steady-state condition. The slow abolition of Glu-potentials in isotonic Ca^{2+}, however, is to some extent confirmed by our ionophoresis experiments, which yielded a complete block of Glu potentials only after 5–10 min. Second, there might be a difference between the extrajunctional channels probed in our patch-clamp recordings and the junctional ones, used by the other authors. Particularly, ion concentrations in the restricted extracellular space of the synaptic cleft may well be influenced by ionic pumps.

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