The Chloride Conductance of Intermediate Fibres from Frog Muscles

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Abstract. Sheets of muscle fibres dissected from surface portions of frog ileofibularis and semitendinosus muscles were soaked in solutions with elevated K and Cl concentrations. The KCl-loaded muscles were then bathed in low [Cl^-] solutions, whereby the membrane potential became transiently inside positive. The repolarization of the twitch fibres from the tonus bundle ("intermediate fibres") was faster than that of the fibres adjacent to it ("fast fibres") when the preceding exposure to high KCl was brief (7—15 min), and it was slower than that of the fast fibres when KCl was applied for 4 hours. Measurements of the voltage displacement at constant current and of the current in a point voltage clamp showed that inwardly rectifying K channels were present in the membranes of both types of fibres. The ionic conductance ratio, g_K/g_{Cl}, was 191/523 in fast fibres and 335/230 in "intermediate" fibres. The different repolarization rates may thus be explained by differences in the chloride conductance of the fast and intermediate fibre membranes. The smaller diameter of the latter fibres may be another factor.

Key words: Membrane conductance — Chloride conductance — Potassium chloride — Frog muscle — Tonus bundle

Introduction

The common classification of skeletal muscle fibres differentiates between twitch and slow (tonic) fibres. Though most of the work in frog muscle physiology has been done on twitch fibres, tonic fibres have been studied extensively enough to permit a detailed description of their properties (for a review see Nasledov 1981; for electrical properties see Gilly and Hui 1980). In addition to twitch and slow fibres, the existence of a third class of muscle fibres has been claimed by some authors on the basis of physiological (Zhukov and Leushina 1948) and biochemical (Skorobovichuk and Chizhova 1976) data. In a detailed study, Smith and

Ovalle (1973) presented evidence for the existence of five fibre types in anura, three of which correspond to the twitch type and two to the tonic type of the binary classification. There seems to be little information on membrane characteristics of the transition type(s). In the work reported here, the membrane chloride conductance of one type of transition fibres was found to be higher than that of the tonic fibres but distinctly less than in typical fast twitch fibres. To avoid terminological confusion, which may arise because the examined fibres may not have covered the whole spectrum of "transition" fibres, the term "intermediate fibres" will be used in this paper. These fibres presumably belong to type 3 twitch fibres of the Smith-Ovalle classification.

Materials and Methods

Bundles consisting of 2—3 muscle fibre layers were dissected from superficial portions adjacent to the nerve entry of ileofibularis and either head of the semitendinosus muscles of male and female frogs (*Rana esculenta*). The animals (caught in the fall, 1985) were kept for 5—6 months at low temperature (5—7 °C) prior to sacrifice. In a typical preparation, 12—20 muscle fibres could be used for electrical measurements. In some cases, the preparation consisted of the "tonus bundle" alone, in others about one-half of the fibres belonged to areas outside the tonus bundle.

The preparations were fastened to the bottom of a shallow, about 3 mm wide, flow-through chamber and were superfused at 0.5—1.0 ml/min. Three types of experiments were performed. In the first, the muscles were exposed to a solution having a normal [K] × [Cl] product (solution A, Table 1) and then to a solution having an increased [K] × [Cl] product (solutions B or C, Table 1) for 7—15 or 240—300 min. The membrane potential of the muscle fibres was measured by the standard microelectrode technique. Membrane potential measurements were made at 15 min intervals following the application of a low-Cl solution containing 4 or 40 mmol/l K^+ (solutions D, E, F, Table 1). The solutions were hyperosmotic to compensate for the elevated K^+ and Cl^- content of the fibre interior. The elevated K concentration (40 mmol/l) was used to speed up the repolarization when the muscles were bathed in the low-Cl solution (Adrian 1960).

In the second type of experiment the muscles were bathed in low-Cl solutions (Table 1, H) for at least 20 min. Two citrate-filled microelectrodes were inserted in a muscle fibre at a distance less than 100 μm and square current pulses were delivered through one of them while the resulting electrotonic potentials (ETP) were recorded with the other. The current amplitude was adjusted such that the final ETP amplitude was about 25 mV positive to the resting value. The polarity of the current pulse was then transiently switched to negative to enable fibre type identification (see Fig. 4). Solution G was then washed in for no longer than 15 s and the resulting change in the membrane potential level and the decrease of the ETP amplitude was read from the screen of a Tektronix Model D-11 storage oscilloscope or photographed with a Polaroid camera. Solutions containing 40 mmol/l K^+ were used in these experiments to avoid mechanical responses provoked by withdrawal of the Cl ions. Measurements of the membrane current-voltage relation were made using constant current pulses or voltage steps delivered by a conventional point voltage clamp.

In a third type of experiment the input resistance, R_o ($= V_o/I$, where I is the input current) was measured by inserting the potential-measuring microelectrode at distances 0.1; 1.0 and 2.0 mm from the current-passing microelectrode and by extrapolating the ETP value for zero mm microelectrode separation, V_o. The resistance of 1 cm of fibre membrane (r_m) was calculated from the relation $r_m = 2R_o\lambda$, where λ is the space constant of the muscle fibre obtained from the log plot of the ETP

Table 1. Ionic concentrations of the solutions (mmol/l)

Sol.	Na^+	G^+ *	K^+	Ca^{2+}	Mg^{2+}	Cl^-	Me^- **	ton.
A	115	—	2.5	1.5	—	119	—	1.00
B	105	—	93	1.4	—	201	—	1.68
C	96	—	169	1.3	—	268	—	2.24
D	140	—	4.0	1.5	—	3	144	1.23
E	137	18	40	1.4	—	3	195	1.65
F	127	91	40	1.4	—	3	258	2.18
G	105	—	40	—	1.5	148	—	1.24
H	105	—	40	—	1.5	3	145	1.24

* G = N-methyl-D-glucamine; ** Me = methanesulfonate; ton. = tonicity

data against microelectrode separation (Eisenberg and Gage 1969). The specific membrane resistance (R_m) was calculated from the following relation: $R_m = r_m 2\pi a$, where a is the average fibre radius found to be 51 $\pm$ 2.7 μm in 34 fibres outside the tonus bundle and 30 $\pm$ 1.9 (SEM) μm in 24 fibres within the tonus bundle (3 frogs). The muscle fibre diameters were measured with the help of a microscope eyepiece.

Results

When a twitch fibre is exposed to a solution containing K and Cl ions such that the product of their concentrations is higher than the $[K^+] \times [Cl^-]$ product inside the fibre, K and Cl ions will enter the muscle fibre until a new equilibrium is reached (Hodgkin and Horowicz 1959; Adrian 1960). Replacement of external Cl ions by an anion species which cannot pass the chloride channel of the fibre membrane will cause the equilibrium potential for Cl ions of the KCl-loaded fibres to be reversed with respect to the normal, i.e. to become inside positive. Since the chloride permeability of the muscle membrane is normally large, the measured membrane potential will also become positive under these conditions and may reach values exceeding + 60 mV. In an experiment of this kind, a frog iliofibularis muscle preparation containing fast twitch and tonus bundle fibres was used. For a few minutes following the replacement of the KCl solution by a low-Cl solution the membrane potential of all muscle fibres was positive. Within half an hour, however, all fibres within the tonus bundle had negative membrane potential values, while all those outside the tonus bundle were still positively polarized. This result is shown in Fig. 1. The measurements were made 55—57 min following the replacement of solution B (Table 1), which had been applied for 12 min, by a low-Cl solution (solution D). The group of muscle fibres having positive membrane potentials was clearly separated from the group having negative potentials, the boundary corresponding to the mi-

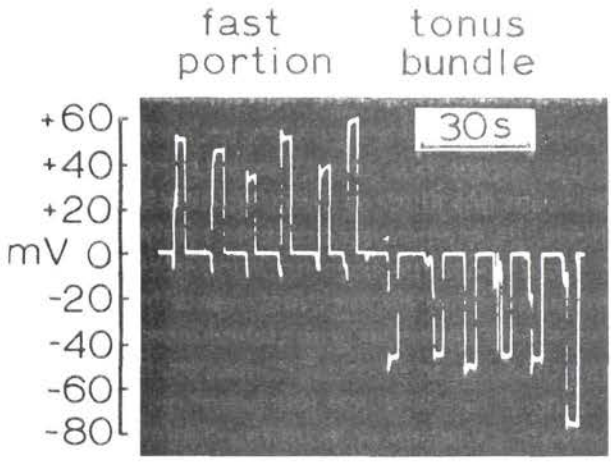

Fig. 1. Continuous record of membrane potential in a frog muscle preparation containing portions from the tonus bundle and adjacent fast fibres. The preparation had been soaked in solution B (91 mmol/l K, 201 mmol/l Cl) for 12 min; the recording was started 55 min after the replacement of solution B by solution D (4 mmol/l K, 3 mmol/l Cl).

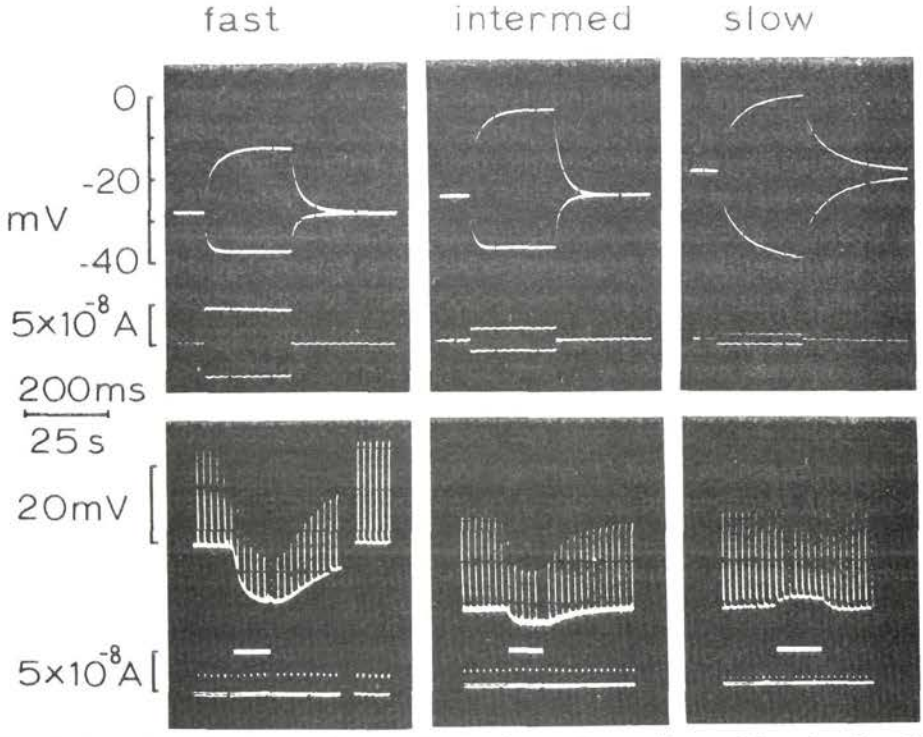

Fig. 2. Upper 3 panels: electrotonic potentials in a fast, an intermediate, and in a slow fibre. Note the difference in the amplitudes of the corresponding current pulses. Solution H (40 mmol/l K, 3 mmol/l Cl). Lower panels: ep recorded at a slow sweep speed in solution H; bars under the potential record: application of solution G (40 mmol/lK, 148 mmol/l Cl). To estimate the hyperpolarization in solution G, a value of −5 mV had to be added to the observed potential change (= junctional potential difference for solutions G and H).

croscopically recognizable boundary of the tonus bundle. Similar results were obtained in an earlier work (Lorković 1963).

This result may suggest that the tonus bundle contains only tonic fibres known to have membranes which are sparsely permeable to Cl ions. Another property of these membranes is that they normally contain no Na channels (Gilly and Hui 1980). To test the above suggestion, muscle fibres of the tonus bundle were stimulated with 1 ms depolarizing pulses delivered through a second microelectrode. In three such experiments (3 frogs) 33 out of 37 (89%) fibres proved to be excitable twitch fibres; their action potentials had amplitudes between 102 and 126 mV (not shown). The inexcitable fibres had resting potentials of about −50 mV and, judging from the large negative-going ETPs obtained with low-amplitude current pulses, were true tonic fibres.

Another possible explanation for the unusually fast repolarization, after KCl loading, of the twitch muscle fibres from the tonus bundle was that the K channel of their membranes did not possess the inward rectifying property which prevents K ions taken up in the presence of Cl ions to be released at a rate comparable to that of their uptake (for a review of the inward rectifier property in muscle see Stefani and Chiarandini 1982). To test this point, the amplitudes of the electrotonic potentials (ETP) provoked by current pulses having the same amplitude and opposite polarities were measured. The results presented in the upper part of Fig. 2 show that the ratios of the ETP amplitudes provoked by positive and negative currents were about the same in twitch fibres from the tonus bundle and in those outside the bundle. More complete information on the properties of the K channel in the two kinds of twitch fibres is presented in Fig. 3. The current-voltage curves for the two fibre types were similar; if the current values for the tonus bundle are multiplied by 1.5 the resulting curve nearly overlaps that obtained for the fibres outside the tonus bundle. This result suggests that the properties of the K channel in the two fibre types are the same, the lower current values for a particular voltage level reflecting the smaller diameter of the twitch fibres from the tonus bundle as compared to that of the fast fibres.

A third possibility was that the twitch fibres of the tonus bundle have a lower Cl permeability than the extra-bundle fibres. Evidence in favour of this possibility is presented in Fig. 2.

Application of a Cl-containing solution (sol. G) to a muscle equilibrated in a Cl-free solution (sol. H) provoked a large hyperpolarization and a marked diminution of the ETP amplitude in fast fibres. These changes were less in the twitch fibres of the tonus bundle ("intermediate fibres") and nearly absent in tonic fibres. The average values for the membrane potential, for the amplitude of the ETP in solution H (Cl-free), for the hyperpolarization provoked by applying the Cl-containing solution G, and for the ratio of the ETP amplitudes

measured in solutions H and G are presented in Table 2. These results, as well as those obtained by measuring the specific membrane resistance, R_m (Table 3) shows that the Cl permeability of the muscle membrane is indeed higher in fast than in intermediate fibres. The variability of the data obtained in a single animal was low enough to permit identification of the fibre type from the measurement of g_{Cl} alone.

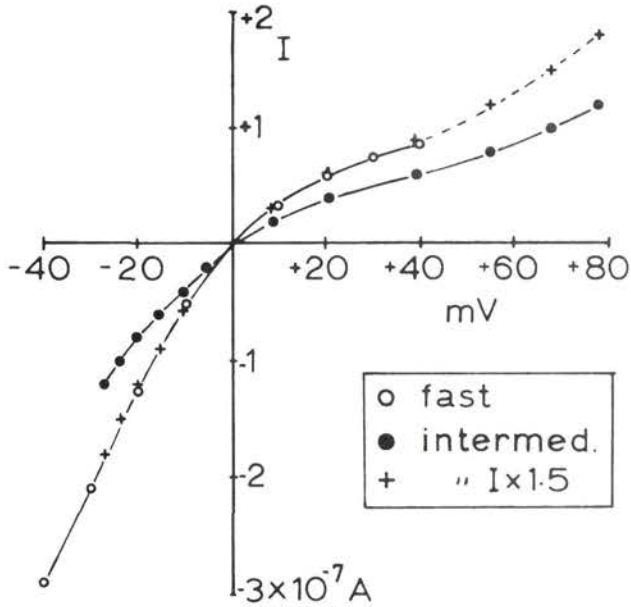

Fig. 3. Current-voltage curves in fast and intermediate frog muscle fibres bathed in solution H (40 mmol/l K, 3 mmol/l Cl). Crosses denote values obtained when the ordinates of the points for intermediate fibres (filled circles) were multiplied by 1.5. Four values per point.

The lower g_{Cl} of the membrane of intermediate fibres may explain the fast repolarization of these fibres following a brief exposure to high KCl. It may also be used to predict their rate of repolarization (relative to that in fast fibres) following a prolonged exposure to high KCl. If the quick repolarization of the less Cl-permeable intermediate fibres was due to an incomplete KCl loading during a brief exposure to KCl the prolonged exposure to KCl should cause the differences in the rates of repolarization of the two fibre types to diminish and, provided the KCl concentration is high enough and the differences between the average diameters of the muscle fibres are not excessively large, to make the repolarization rate for the intermediate fibres even slower than that for the fast fibres. The results presented in panels B and C of Fig. 4 confirm this prediction. Panel D shows that the reversal of the repolarization rates for fast and inter-

mediate fibres was not due to some irreversible damage caused by the prolonged KCl application: another, brief exposure of the repolarized muscle to high KCl caused the fast fibres to repolarize more slowly than the intermediate fibres, as in a fresh preparation.

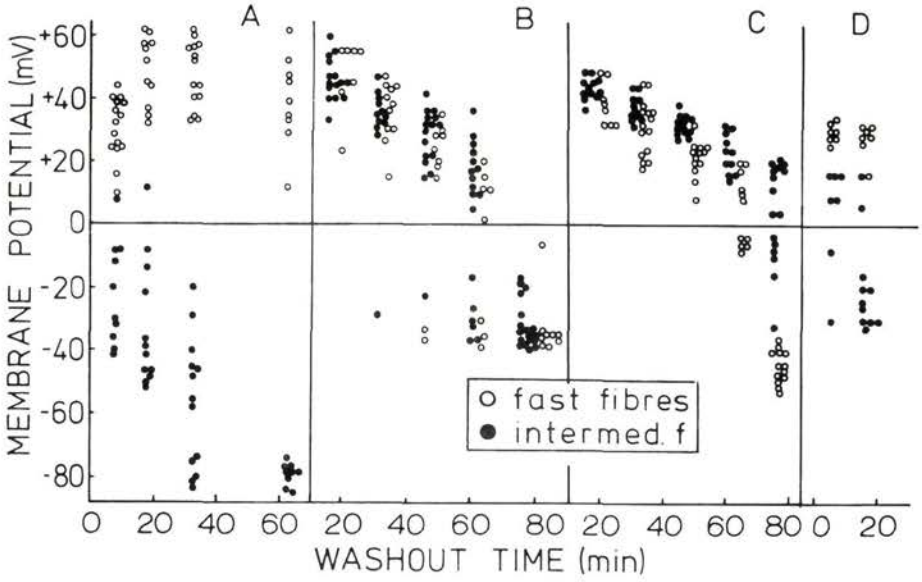

Fig. 4. Membrane potentials of individual fast and intermediate muscle fibres measured approximately at the KCl-washout time indicated on the abscissa. *A*: prior to washout in solution D (4 mmol/l K, 3 mmol/l Cl) solution B (93 mmol/l K, 201 mmol/l Cl) was applied for 15 min. *B*: prior to solution E (40 mmol/l K, 3 mmol/l Cl) solution B (93 mmol/l K, 201 mmol/l Cl) was applied for 3 h. *C*: prior to solution F (40 mmol/l K, 3 mmol/l Cl) solution C (169 mmol/l K, 268 mmol/l Cl) was applied for 3.5 h. *D*: prior to solution F (40 mmol/l K, 3 mmol/l Cl) solution C (169 mmol/l K, 268 mmol/l Cl) was applied for 7 min. C and D were obtained with the same muscle, A and B with different muscles.

Table 2. Membrane potential (mp), amplitude of electrotonic potentials (+ ETP and − ETP), input current (*I*), and hyperpolarization by Cl^- applied after Me (hyp), in frog muscles. The subscripts Cl and Me denote values measured in the presence and in the absence of Cl ions.

	1 mp (mV)	2 $+ETP_{Me}$ (mV)	3 $\frac{+ETP_{Me}}{-ETP_{Me}}$	4 I ($\times 10^{-7}$A)	5 $\frac{+ETP_{Me}}{+ETP_{Cl}}$	6 hyp (mV)
A) intact muscles						
fast fibres	28.9 ± 1.3	25.4 ± 1.1	2.05 ± 0.26	2.04 ± 0.52	3.10 ± 0.34	14.5 ± 1.1
intermed. f.	24.9 ± 1.7	26.5 ± 1.1	2.16 ± 0.18	1.21 ± 0.32	1.73 ± 0.12	7.0 ± 0.6

The listed values are means ± SEM of measurements in 5—8 muscles (= *n*); 8—13 fibres per muscle were tested.

Table 3. Membrane resistance parameters (r_m, R_m), potassium (g_K) and chloride (g_{Cl}) conductances of frog muscles

fibre type	anion	r_m (Ω)	R_m (Ωcm^2)	g_K (μS)	g_{Cl} (μS)	%
fast	Cl	0.43×10^5	1.63×10^3	191	523	27:73
	Me	1.62×10^5	6.11×10^3			
intermed.	Cl	0.96×10^5	2.41×10^3	335	230	59:41
	Me	1.62×10^5	4.06×10^3			

the coefficient of variation for the listed r_m values (top to bottom) was 23, 40, 12, and 21%, resp. The number of fibres examined was 4, 7, 7, and 13, resp.

Discussion

The results of this work show that the twitch fibres contained in tonus bundles of frog muscles (intermediate fibres) differ from those of the areas adjacent to the tonus bundle with respect to the repolarization pattern following exposure to elevated [K] and [Cl]. The faster repolarization rate after a brief exposure to KCl of the intermediate fibres could not be explained by the properties of the potassium channel of the muscle fibre membranes but rather by a lower Cl conductance in these, as compared to the fast fibre membranes. With respect to the data on g_K and g_{Cl} a word of caution is in order. The measurement of changes in ETP amplitude caused by brief application of Cl-containing solutions to muscles equilibrated in a low-Cl, high-K solution has several important advantages over the customary method employing fully equilibrated cells at normal resting potential, such as a full reversibility of the effects and the rate at which many fibres may be sampled. However, the method does not lend itself to exact determinations of the membrane ionic conductances because, among other things, voltage-dependent alterations of the membrane properties are neglected. Since these alterations were found to be similar in fast and intermediate muscle fibres, valid statements on relative chloride conductance values may still be made on the basis of the results. Thus it could be shown that g_{Cl} of the outer muscle fibre membrane is 2—3 times less in intermediate than in fast muscle fibres.

The twitch muscle fibres of the tonus bundles contained in thigh muscles of the frog, which were the object of this investigation, were derived from the surface portions of the tonus bundles. This location, together with the fact that true tonic fibres were often found in the preparations, and that the intermediate fibres had a thickness which was visibly smaller than that of the fast fibres all indicate that the latter belonged to type 3 of the Smith-Ovalle classification. The properties of the deeper lying muscle fibres of the tonus bundle which had a similar ("milky") appearance as the surface fibres were not examined systemat-

ically. The $+ ETP_{Me} + ETP_{Cl}$ ratio in the superficial fibres and in those from the opposite side of one preparation (the latter presumably containing some of the thicker, type 2 fibres) gave a larger value for the superficial than for the deeper lying fibres (1.62 vs. 1.39; 12 and 11 fibres, respectively). Thus it does not seem that type 2 fibres have a higher Cl conductance than type 3 fibres.

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