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EFFECTS OF ENHANCED ACTIVITY ON SYNAPTIC TRANSMISSION IN MOUSE EXTENSOR DIGITORUM LONGUS MUSCLE

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SUMMARY

1. Transmitter release at neuromuscular junctions of extensor digitorum longus (EDL) muscle in mice was studied after 2–8 month periods of unforced running in wheels.

2. Intracellular recordings at 10 Hz stimulation revealed that the quantal content of endplate potentials (EPPs) in Mg^{2+}-blocked preparations was larger by 30% in trained (mean number of quanta, $m = 1{\cdot}75 \pm 0{\cdot}19$, $n = 7$) than in untrained control EDL muscles ($m = 1{\cdot}35 \pm 0{\cdot}35$, $n = 7$). Similarly the amplitudes of the first, maximum and plateau EPPs during tetanic stimulation (100 Hz for 1 s or 400 ms) in curare-blocked preparations were increased by 28% each; muscle fibre diameters did not differ while other postsynaptic effects were not excluded.

3. Training effects became particularly evident in two pairs of monozygotic twins, in which the time courses of facilitation and depression were changed as well: at 100 Hz stimulation the maximum EPP amplitude was reached on average at 2·6 impulses in controls but at 2·0 impulses in runners, and the following decline below the value of the first EPP at 5·0 and 3·8 impulses respectively.

4. Block resistance, as monitored by isometric tension measurements in different presynaptic (Mg^{2+}) and postsynaptic (curare) blocking solutions, was higher in trained than in control EDL muscles. Depression in a train of four nerve-evoked single twitches at 2 Hz was lower.

5. As expected from the unchanged fibre diameters (see above) isometric tetanic force was similar in trained and control EDL muscles. Muscle fatigue resistance was larger in trained animals and succinic dehydrogenase activity was higher in fibres of trained muscles indicating an endurance training of the EDL muscle.

6. It is concluded that besides changes in muscle fibre properties, prolonged elevated activity causes increased transmitter release in EDL muscles. As a consequence, the safety margin of transmission in trained EDL muscles is markedly elevated.

INTRODUCTION

The nerve–muscle junction is a dynamic structure, in which axonal sprouting with new synapse formation and axonal retraction and redistribution occur (reviewed by Wernig & Herrera, 1986). As a consequence of remodelling and growth, structural

and functional differences develop between young and old, exercised and unexercised animals, and in different seasons and hormonal statuses (for reviews of the literature see Wernig & Herrera, 1986; Atwood & Lnenicka, 1987; Cardasis & LaFontaine, 1987; Lichtman, Magrassi & Purves, 1987; Andonian & Fahim, 1988; Wernig & Dorlöchter, 1989; Herrera, Banner & Nagaya, 1990). In the present investigation, the effects on synaptic transmission of prolonged periods of running in wheels were studied in leg muscles of inbred mice and monozygotic twins. The effectiveness of the training procedure was apparent from an increase in fatigue resistance of extensor digitorum longus muscles (EDL) in the trained animals. Transmitter release capacity was determined from intracellular recordings of endplate potentials (EPPs) and from block resistance of transmission in isometric tension measurements. In addition, muscle fibre diameters and succinic dehydrogenase activity were established from frozen sections.

Training effects were studied only in EDL since soleus muscles suffer muscle fibre damage at the onset of running (Irintchev & Wernig, 1987) and it is not clear at present how this affects transmission (Wernig, Irintchev & Weisshaupt, 1990).

These results have previously been published in an abstract (Dorlöchter, Brinkers, Irintchev & Wernig, 1990).

METHODS

Animals and training procedure

By using the natural habit of mice to run several kilometres per night we designed two experimental groups: trained and untrained controls (see Irintchev & Wernig, 1987; Badke, Irintchev & Wernig, 1989). The animals were kept individually in plastic cages ($20 \times 30 \times 25$ cm) in a temperature- and light-controlled room (21–25 °C, 12 h light–12 h dark). In this study thirteen male and eight female mice from different batches of the inbred strain CBA/J were used. They were purchased from Savo-Ivanovar (Kisslegg, FRG) and Iffa Credo (L'Arbresle, France) at the age of 12 weeks and were randomly defined as runners or non-runners (seven male, four female runners and six male, four female non-runners). In addition, two pairs of monozygotic twins (NMRI females and SWS males) were bred in our own laboratory; they were defined as monozygotic twins from the presence of a single common placenta. One mouse of each pair was trained and the other used as a control. In the trained group each mouse was provided with a running wheel which could be used *ad libitum*. Wheels were connected to counters so that individual running activity could be controlled daily (see Irintchev & Wernig, 1987). Voluntary running amounted to 5–15 km (mean $= 9{\cdot}7 \pm 3{\cdot}8$ km) per day. Training periods were 240 days for animal No. 11 (curare block) and Nos 3–5 (see Table 1), and 60–160 days for the other animals.

In order to monitor the effectiveness of the training procedure, the O_2 consumption of a runner and a non-runner of approximately the same body weight (38·0 and 37·8 g, respectively) was determined. At rest (sleeping) animals consumed 109 ml O_2/h each. During night time, which is the main period of activity in mice, the O_2 consumption of the runner increased to $235{\cdot}8 \pm 69{\cdot}1$ ml O_2/h, and that of the non-runner increased to $153{\cdot}0 \pm 24{\cdot}1$ ml O_2/h (eight measurements from 20.00 to 06.00 h, $P < 0{\cdot}01$, t test; for methods see Badke, 1988). In another pair of mice the O_2 consumption of the runner was $224{\cdot}7 \pm 24{\cdot}4$ ml O_2/h as compared to $136{\cdot}5 \pm 22{\cdot}1$ ml O_2/h for the non-runner (three measurements, $P < 0{\cdot}005$, t test). During the interval from the start to the end of this experiment (about 4·5 h) the running activity of the trained animal amounted to a total of 2250 m.

Electrophysiological recordings

EDL muscles with their nerves were dissected from the animals under nembutal anaesthesia (40 mg/kg body weight); subsequently animals were killed by cervical dislocation. Nerve–muscle preparations were pinned out in a Sylgard-coated chamber and superfused with gassed (95% O_2, 5% CO_2) Tyrode solution containing (in mM): 125·0 NaCl, 24·0 $NaHCO_3$, 5·37 KCl, 2·75 $MgCl_2$, 0·4 $CaCl_2$ and 2·0 g/l glucose, or normal Tyrode solution containing (in mM): 125·0 NaCl, 24·0 $NaHCO_3$, 5·37 KCl, 1·0 $MgCl_2$, 1·8 $CaCl_2$ and 2·0 g/l glucose to which 2·16 μM-*d*-tubocurarine

chloride was added. Temperature was kept constant at 24·5–25·5 °C. The volume of the bath was about 3 ml; the rate of perfusion was about 5 ml/min. Endplate potentials were recorded with conventional glass microelectrodes from two to three superficial layers of muscle fibres distributed over the whole muscle surface. EPPs were accepted when their rise times were less than 2 ms.

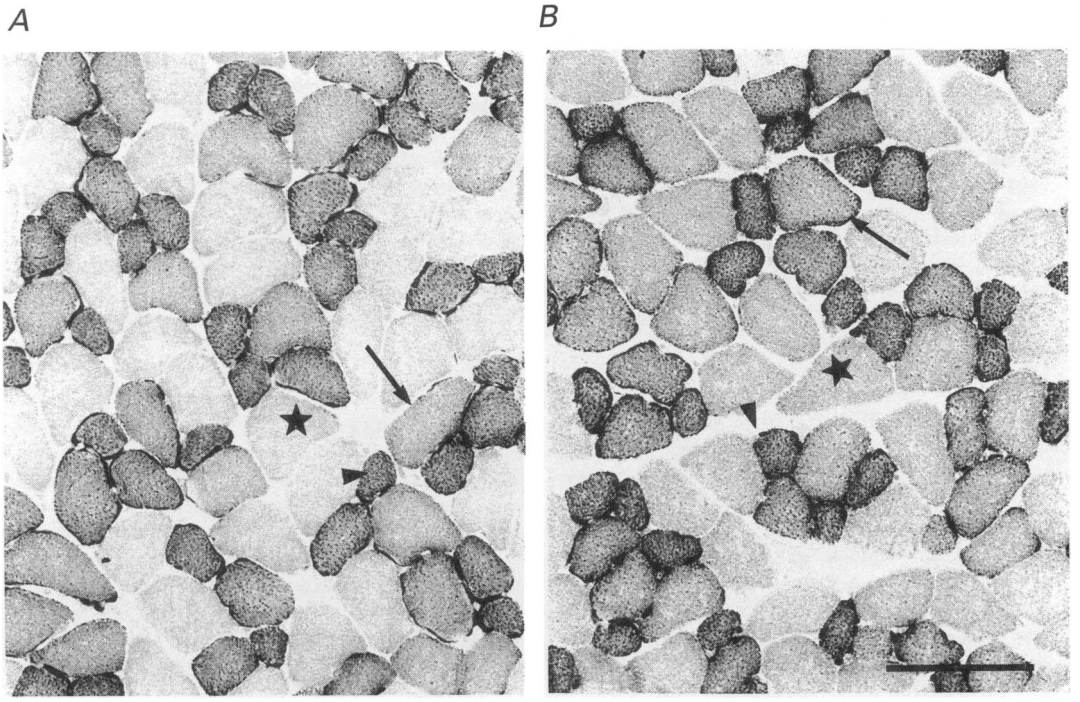

Fig. 1. Frozen cross-sections of untrained (*A*) and trained (*B*) EDL muscles after 2 months of voluntary running in wheels. Both sections were processed for succinic dehydrogenase (SDH) activity by staining them simultaneously on a single slide. Clearly, SDH activity was higher in low oxidative fibres (stars) of the trained muscles, whereas an increase was less evident in medium (arrows) and high (arrow-heads) oxidative fibres. Bar = 100 μm.

Stimulation frequencies were 10 Hz during Mg^{2+} block and 100 Hz for 1 s or 400 ms during curare block. In each blocking solution between twenty and sixty-three cells per muscle were recorded, and in Mg^{2+} block between 100 and 150 EPPs per cell. The sequence of investigations was usually kept constant with Mg^{2+} block preceding curare block. When solutions were changed, an equilibration time of at least 40 min was maintained throughout. In some muscles recordings were performed in one blocking solution only.

Analysis of the electrophysiological recordings was performed as described by Hinz & Wernig (1988). Signals were stored on magnetic tape, digitized and processed with a PDP11 computer. Numbers of quanta were determined from the EPP amplitude distributions directly when these showed peaks at regular intervals and quantal release was low ($m < 3$); junctions with apparently low quantal output but without regular peaks in their EPP amplitude distribution were discarded. In junctions with high quantal content, m was calculated by dividing the mean EPP amplitude by the mean amplitude of miniature EPPs. Since EPP amplitudes were small (maximum about 5 mV) non-linear summation was unlikely to affect the amplitude measurement. In curare-blocked preparations the absolute amplitudes of the first, maximum and plateau (average of the last five) EPPs in the 100 Hz train were measured. In addition, as indices of tetanic facilitation and depression, the ordinal locations of the maximum EPP (*position of the maximum*) and of the first EPP which fell below the magnitude of the initial EPP (*position of the decline*) were determined; obviously these measures are independent of absolute amplitudes and provide information on presynaptic events.

Isometric tension measurements

In order to measure the safety margin of transmission and fatigue resistance, the contralateral muscles from animals used for the intracellular recordings were taken for simultaneous isometric tension measurements *in vitro* at 24·5–25·5 °C. Measurements were made with strain gauges (Höttinger Baldwin Messtechnik, Darmstadt). The nerve–muscle preparations were superfused with gassed normal Tyrode solution (see above), or Tyrode solution containing 0·5 mM-Ca^{2+} and 1·4, 1·75 or 2·0 mM-Mg^{2+}, or with Tyrode solution to which 0·6 or 1·2 μM-*d*-tubocurarine chloride was added. To evaluate susceptibility of the preparations to the blocking agents (*block resistance*) absolute forces after nerve stimulation in the blocking solutions were directly compared to those in Tyrode solution and given as percentages of the values in normal Tyrode solution ('nerve index' in Mg^{2+} or curare). Block resistance was determined with single stimuli as well as 50 or 100 Hz tetani for 2 s (compare with Badke *et al.* 1989). As a measure of *depression*, the ratio (in per cent) of the last to the first twitch amplitude in a train of four nerve stimuli at 2 Hz was used. For determination of fatigue resistance twenty consecutive tetanic contractions at 3 s intervals were evoked by direct muscle stimulation at 100 Hz for 0·2 s. Fatigue resistance was expressed as the ratio of the last to the first amplitude of tetanic responses. The amplitudes of muscle twitch and tetanic responses were read from the screen of a Hameg Digital Storage Scope HM 208 or reproduced from a magnetic tape.

Muscle fibre diameters and histochemistry

At the end of the electrophysiological recordings or tension measurements some of the muscles (see Table 1) were frozen at resting length in isopentane pre-cooled with liquid nitrogen. Cross-sections (10 μm) were cut from a defined region of EDL (most proximal endplate region and largest muscle width) and stained with Toluidine Blue. From these sections video prints were taken at a final magnification of $\times 580$ for fibre diameter measurements. One muscle fibre in the upper right part of every fascicle in the muscle was chosen (between 25 and 69 fascicles per muscle cross-section, mean $= 44 \pm 10$) and its diameter measured by a modification of the method of Song, Shimada & Anderson (1963) (Schmitt, 1976); accordingly muscle fibre diameter was defined as the mean of both othogonal diameters, i.e. the largest diameter and the minor axis running perpendicular through the centre of the largest diameter (Wernig, Irintchev & Weisshaupt, 1990).

Adjacent frozen sections were stained for the demonstration of succinic dehydrogenase (SDH) activity (Nachlas, Tsou, DeSouza, Chang & Seligman, 1957).

Statistical analysis

Throughout this paper the results are presented as mean values with standard deviations. The data were subjected to analysis of variance (including analysis for possible interactions) followed by Student's t test. Furthermore the Mann–Whitney U test or a combination of tests for significance was employed (Birnbaum, 1954). A significance level of 5% was accepted as the basis for a genuine difference.

RESULTS

Several investigations were performed to compare synaptic transmission in trained *versus* control (untrained) EDL muscles: in isolated nerve–muscle preparations mean quantal content at 10 Hz continuous stimulation in Mg^{2+} block, EPP amplitudes during 1 s or 400 ms trains of 100 Hz stimulation in curare, and block resistance of transmission in isometric tension measurements on whole muscles were monitored. The effectiveness of the training procedure was independently determined from the increase in SDH activity in trained EDL muscles; Fig. 1 shows that SDH activity was clearly higher in low oxidative fibres of the runners, while the increase was less obvious in medium and high oxidative fibres. Furthermore, no differences were found in tetanic force between trained and control animals (trained, $376 \pm 87{\cdot}6$ mN; control, $362 \pm 99{\cdot}6$ mN, $n = 11$, not significant) but fatigue resistance upon direct muscle stimulation was higher in runners. After twenty consecutive tetanic

contractions, in the trained muscles 65·6 ± 7·0 % ($n = 8$) of the initial tetanic force was maintained as compared to 53·6 ± 10·5 % in controls ($n = 8$, $P < 0·025$, Mann–Whitney U test) (data not shown).

Endplate potential measurements

In Mg^{2+} block (2·75 mM-Mg^{2+}, 0·4 mM-Ca^{2+}) synapses of trained EDL muscles had, on average, a 30 % higher quantal content than those in control muscles (1·75 ± 0·19, $n = 7$, *versus* 1·35 ± 0·35, $n = 7$, $p < 0·05$, Mann–Whitney U test, Table 1). It is clear, therefore, that there is an increase in transmitter release due to prolonged training. In curare block, the first, maximum and plateau amplitudes in a train were on

TABLE 1. Quantal content, EPP amplitudes, membrane potentials and muscle fibre diameters in EDL muscles of trained and control animals

			Trained						
	Mg^{2+} block		Curare block					Histology	
No.	N	m	N	First EPP (mV)	Max. EPP (mV)	Plateau EPP (mV)	RP (mV)	N	Fibre diameter (μm)
1	37	1·68 ± 1·26	37	1·54 ± 0·75	1·64 ± 0·76	0·58 ± 0·34	80 ± 7	56	47 ± 8
2	34	1·71 ± 0·83	31	1·20 ± 0·55	1·36 ± 0·54	0·48 ± 0·19	77 ± 7	—	—
3	30	1·45 ± 0·67	36	1·04 ± 0·49	1·20 ± 0·51	0·44 ± 0·21	75 ± 5	59	45 ± 6
4	31	2·09 ± 1·08	36	1·36 ± 0·57	1·43 ± 0·58	0·45 ± 0·21	76 ± 7	48	50 ± 7
5	37	1·71 ± 0·84	39	1·32 ± 0·47	1·40 ± 0·46	0·46 ± 0·18	80 ± 6	38	43 ± 5
6*	24	1·79 ± 0·88	39	1·29 ± 0·60	1·37 ± 0·59	0·55 ± 0·27	83 ± 11	—	—
7*	20	1·85 ± 0·54	45	2·06 ± 1·07	2·11 ± 1·04	0·65 ± 0·29	73 ± 7	—	—
8	—	—	60	1·35 ± 0·63	1·37 ± 0·63	0·54 ± 0·23	77 ± 6	46	53 ± 8
9	—	—	60	1·15 ± 0·54	1·20 ± 0·56	0·37 ± 0·19	74 ± 9	—	—
10	—	—	61	1·24 ± 0·48	1·30 ± 0·49	0·50 ± 0·22	79 ± 10	37	44 ± 8
11	—	—	39	1·42 ± 0·62	1·56 ± 0·68	0·55 ± 0·28	82 ± 8	55	48 ± 7
Mean ± S.D.		1·75 ± 0·19		1·36 ± 0·27	1·45 ± 0·26	0·51 ± 0·08	78 ± 3		47 ± 4
(n)		(7)		(11)	(11)	(11)	(11)		(7)
			Control						
1	36	0·92 ± 0·66	40	0·56 ± 0·20	0·65 ± 0·98	0·25 ± 0·13	77 ± 8	36	46 ± 7
2	35	1·04 ± 0·39	42	0·78 ± 0·27	0·92 ± 0·35	0·34 ± 0·16	86 ± 10	—	—
3	35	1·92 ± 0·98	37	1·38 ± 0·82	1·42 ± 0·83	0·34 ± 0·21	75 ± 12	42	50 ± 8
4	33	1·50 ± 0·69	35	1·16 ± 0·47	1·26 ± 0·57	0·43 ± 0·21	79 ± 8	53	45 ± 6
5	34	1·31 ± 0·56	32	1·18 ± 0·60	1·34 ± 0·65	0·50 ± 0·30	79 ± 9	39	41 ± 7
6*	22	1·60 ± 0·66	40	1·19 ± 0·63	1·25 ± 0·62	0·38 ± 0·21	71 ± 9	—	—
7*	20	1·16 ± 0·64	57	1·08 ± 0·59	1·13 ± 0·62	0·44 ± 0·15	75 ± 8	—	—
8	—	—	57	1·32 ± 0·76	1·36 ± 0·77	0·50 ± 0·26	76 ± 7	69	43 ± 7
9	—	—	40	0·73 ± 0·33	0·78 ± 0·37	0·33 ± 0·16	75 ± 8	—	—
10	—	—	63	1·19 ± 0·54	1·22 ± 0·54	0·45 ± 0·20	74 ± 7	25	47 ± 5
Mean ± S.D.		1·35 ± 0·35		1·06 ± 0·27	1·13 ± 0·26	0·40 ± 0·08	77 ± 4		45 ± 3
n		(7)		(10)	(10)	(10)	(10)		(6)
$P <$		0·05		0·05	0·01	0·01	n.s.		n.s.

N is the number of junctions recorded in each muscle; m is the mean number of quanta released per impulse at 10 Hz. In curare block EPPs were recorded in a train of 100 Hz for 1 s or 400 ms. Max. EPP is the maximum EPP in the train; RP is the resting potential. Fibre diameters were determined from frozen sections. Values for each parameter are compared in trained and control groups (Mann–Whitney U tests); n is the number of muscles studied.

* Data in Mg^{2+} and curare block have been obtained from different animals.

average larger in runners than in non-runners by about 28% each (from Table 1), which is in line with the observation of quantal content. Postsynaptic effects have not been excluded though the similarity of muscle fibre diameters (Table 1) in general indicates unchanged fibre input impedance; furthermore, resting potentials, and

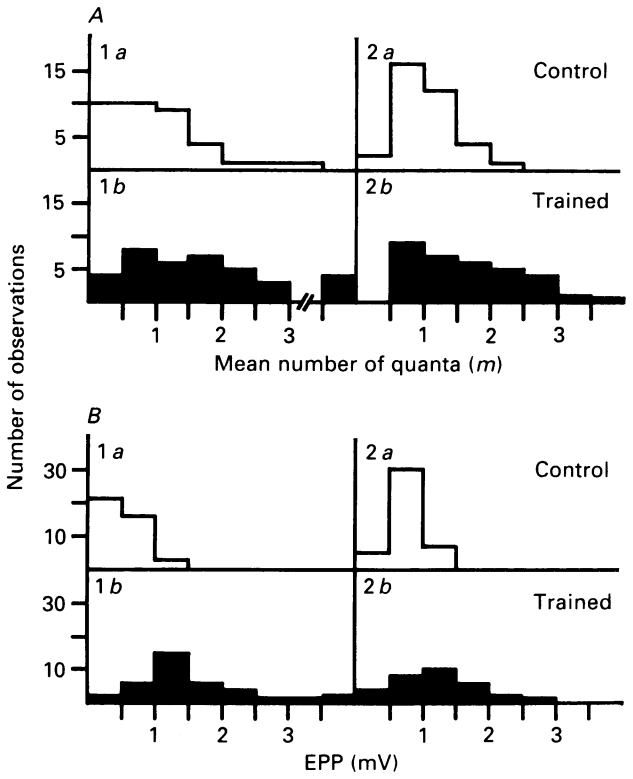

Fig. 2. Mean number of quanta (m; A) and the amplitude of the first EPP in a train of 100 Hz (B) in junctions of EDL muscles of untrained control (a), and trained (b) monozygotic twins (animals 1 and 2; see also Table 1). Number of observations indicates the number of junctions recorded in a muscle.

thus presumably the driving forces for EPPs, were unchanged (Table 1; see also Discussion).

Training effects were even more obvious in the two pairs of monozygotic twins studied (animals 1 and 2 in Table 1; Figs 2 and 3). In Mg^{2+} block m was higher in the runners by some 73% ($1{\cdot}70 \pm 0{\cdot}02$ *versus* $0{\cdot}98 \pm 0{\cdot}08$, $P < 0{\cdot}005$, t test; data from animals 1 and 2 in the upper and lower part of Table 1; Fig. 2). This is due to a general increase in release since a reduction in the numbers of junctions with small values for m was paralleled by an increase in junctions with large values (Fig. 2A).

During curare block a similar shift to the right was observed for amplitudes of the first EPP (Fig. 2B) as well as for the maximum and plateau EPPs in a train of 100 Hz for 1 s (not shown). On average, amplitudes of the first, maximum and plateau EPPs were about two times higher in runners than in non-runners ($1{\cdot}37 \pm 0{\cdot}24$ *versus*

0·67 ± 0·16 mV, 1·50 ± 0·20 *versus* 0·79 ± 0·19 mV, and 0·53 ± 0·07 *versus* 0·30 ± 0·06 mV, respectively, $P < 0·05$, t test; Fig. 3). Muscle fibre diameters and resting potentials did not differ significantly (Table 1).

In addition, there were differences in the time courses of the facilitation and depression of EPP amplitudes. In the trained animals, not only were the EPPs larger

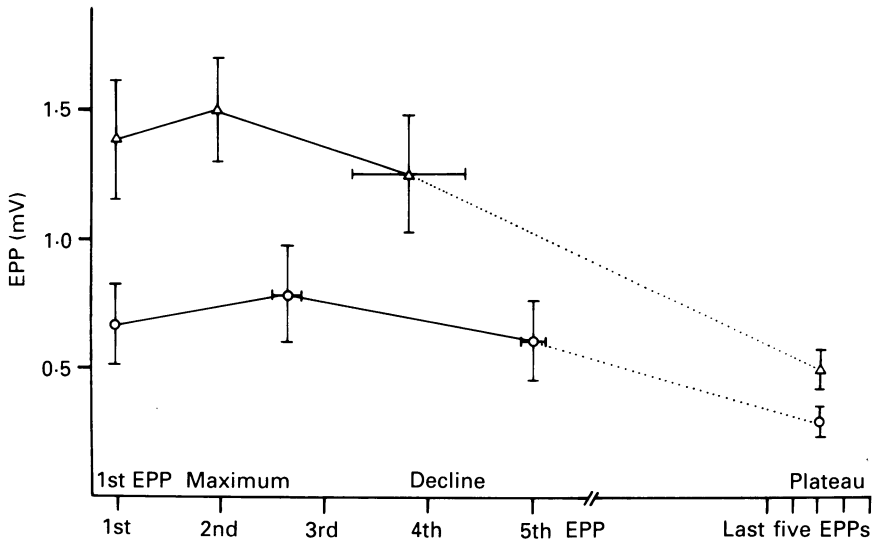

Fig. 3. EPPs in trains of 100 Hz for 1 s in trained (△) and untrained (control, ○) EDL muscles of monozygotic twins. Two pairs of twins were studied (animals 1 and 2; see also Table 1); data are given as means ± S.D., and scatter bar is omitted when S.D. is smaller than the symbol. Amplitudes of the first, maximum, decline (EPP below value of the first EPP) and plateau (mean of last five EPPs) EPPs are indicated, as are the positions of maximum and decline values.

but they reached their maximum values earlier in the train (by impulse number 2·0 (± 0) *versus* 2·6 (± 0·11) in the untrained animals; $P < 0·01$; t test). Furthermore, the decline in amplitude below the value of the initial EPP began earlier in the train in the exercised mice (by impulse number 3·8 (± 0·57) *versus* 5·0 (± 0·11) in the controls; $0·1 > P > 0·05$, t test). This result is shown graphically in Fig. 3.

Safety margin of transmission (block resistance)

Using suitable concentrations of pre- and postsynaptic blockers (Mg^{2+} and curare) the amount of the overall safety margin of transmission in a muscle was determined from isometric tension measurements. Nerve-evoked isometric force in different blocking solutions normalized with values in normal Tyrode solution (block resistance) was significantly higher in trained than in control EDL muscles ($P <$ 0·001, combination of tests, Birnbaum, 1954; Fig. 4*A* and *B*). Thus trained EDL muscles revealed a higher safety margin of transmission, which is in line with the finding of enhanced transmitter release.

Low-frequency nerve stimulation (four pulses at 2 Hz, trains of four; Fig. 4*C*) caused little depression with consecutive stimuli in normal Tyrode solution. Marked differences between trained and control EDL muscles were revealed in the blocking solutions: depression in trained EDL muscles was less pronounced in curare block or in Mg^{2+} block, and even absent as compared to untrained control animals (Fig. 4*C*).

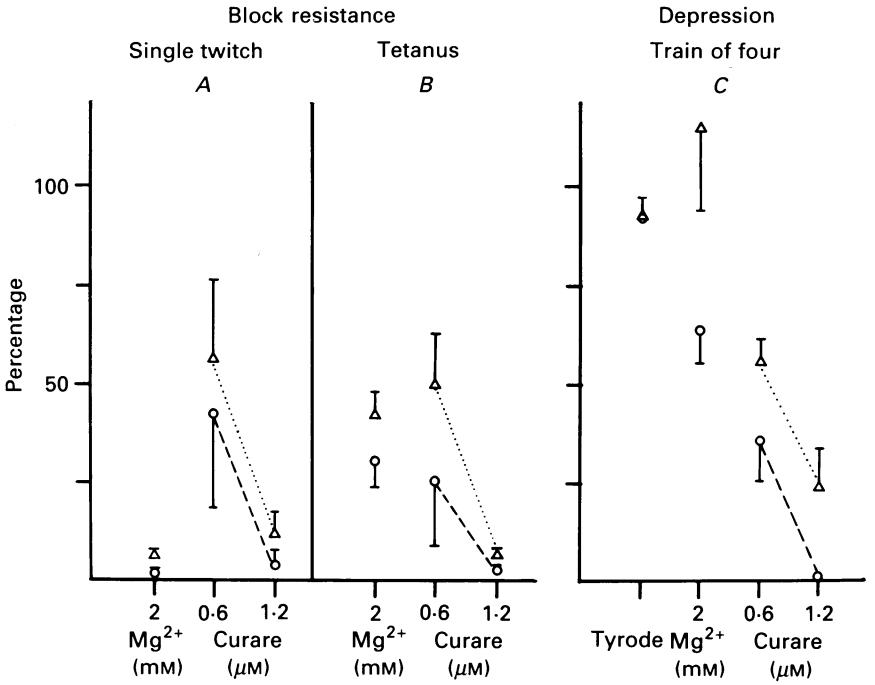

Fig. 4. Block resistance (*A* and *B*) and synaptic depression (*C*) determined from *in vitro* isometric tension measurements in trained (△) and untrained (control, ○) EDL muscles. Block resistance is given as muscle forces upon single (*A*) or tetanic (100 Hz, *B*) nerve stimulation in blocking solutions as a percentage of the values in normal Tyrode solution. Depression is expressed as the ratio (in per cent) of the last to the first twitch amplitude in a train of four nerve stimuli at 2 Hz (*C*). Combining the independent tests of significance for each group (Birnbaum, 1954) shows that differences between trained and control EDL in block resistance are highly significant ($P < 0{\cdot}001$). Differences between trained and control EDL in depression are significant at every single point ($P < 0{\cdot}05$, Mann–Whitney *U* test) except for the experiments in normal Tyrode solution when there was no apparent difference. Results are presented as means and S.D.; scatter bars are omitted when S.D. is smaller than the symbol. Number of muscles tested was three or four each for trained and control EDL.

DISCUSSION

Extensor digitorum longus muscles typically work against little load; in this study there was no increase in muscle fibre diameters or tetanic muscle force after several weeks of unforced running but an increase in SDH activity, which is a typical outcome of endurance training. Accordingly, fatigue resistance of muscles upon

direct stimulation was higher in the trained animals. Voluntary running in wheels for 12 months (4–10 months longer than in the present experiments) has been shown to lead to changes in fibre type composition and a decline in tetanic force in EDL muscles (Wernig, Irintchev & Weisshaupt, 1990).

A number of different experimental conditions indicate that junctions in EDL muscles increase their transmitter release capacity after prolonged periods of elevated activity. The most direct evidence is a 30% increase in quantal transmitter release in Mg^{2+} block. Also the increases in block resistance and EPP amplitudes in curare indicate enhanced transmitter release; since no significant differences in muscle fibre diameters were observed, changes in fibre input impedance are unlikely causes of increased EPPs (see Grinnell & Herrera, 1980). The increased capacity of terminals to release transmitter might best be explained by an increase in terminal size. Enlarged EDL junctions in mice after prolonged exercise were, indeed, recently observed by Andonian & Fahim (1988). With the monozygotic twins, the effects of enhanced running activity were much more obvious. The additional changes in facilitation and depression apparent in these animals warrant further discussion. The finding that maximum EPP amplitudes and declines are reached earlier in the trained EDL muscles resembles the smaller degree of facilitation in muscles with enhanced transmitter release caused by elevated Ca^{2+} concentrations (see Mallart & Martin, 1968; Rahamimoff, 1968). This change in time course suggests an increase in release probability, i.e. fractional release resulting in a faster decline in the available pool of transmitter, which in addition appears to be enlarged. It is noteworthy that the parameters describing the time course of the EPPs in a train presumably provide presynaptic measures of synaptic functioning independent of quantal content measurements.

It has been reported previously that transmitter release and junctional size increase with ageing in some muscles but not in others (Kelly & Robbins, 1983; Robbins & Fahim, 1985; Cardasis & LaFontaine, 1987); interestingly, continuously active muscles like the diaphragm showed no differences in either parameter throughout adulthood indicating that maintained activity might maintain junctional characteristics. Effects on endplate structure and function of abnormal patterns of activity achieved with electrical stimulation of motoneurons have previously been reported (Atwood & Lnenicka, 1987; Hinz & Wernig, 1988; Dorlöchter, Braden, Langenfeld & Wernig, 1989; see also Lömo & Waerhaug, 1985); elevated activity with a tonic stimulus pattern imposed over several days onto phasic muscles apparently caused depression in initial transmitter release but increased facilitation, or tetanic potentiation. In the present experiments elevated natural activity of the fast EDL muscle over several months caused an increase in initial release and early plateau values emphasizing that the time span of training and/or the activity pattern of a neuron are important factors in regulating functional and structural characteristics of junctions.

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