followed by a membrane hyperpolarization and inhibition of cell firing (biphasic responses, fig. 2). The latent period of responses was 0.1–2 sec and the time to peak of the first phase 0.5–25 sec. The responses of identified neurones to cAMP and cGMP injection varied in different ganglions (table).

The amplitude of depolarization became larger with membrane hyperpolarization (fig. 3). The extrapolated reversal potential of the effect was about −10 mV. Replacement of the standard Ringer solution by a Na^+-free one led in 15–20 min to the disappearance of the depolarizing response (not shown).

The phosphodiesterase inhibitor theophylline applied extracellularly in the concentration 0.5 mM, or injected intracellularly, increased the amplitude and duration of both cAMP and cGMP responses approximately 1.5-fold in 15 neurones out of 23 (fig. 1). At a higher concentration (1 mM) theophylline itself elicited apparent membrane depolarization.

It can be assumed that the main ionic mechanism of cAMP and cGMP depolarization is an increase in potential-independent Na^+-conductance. The following data support this suggestion; R_{in} is decreased during depolarization, the reversal potential of the effect is near −10 mV and finally, the effect is eliminated by the removal of Na^+ from the extracellular solution. The value of the reversal potential also allows us to suggest an involvment of the changes in K^+-conductance, but of course as an additional (not primary) component. At the same time the potential-independence of the effect excludes the participation of Ca^{2+}-channels.

The results of the present study, together with data from the literature[1-9] show that in different cells cyclic nucleotides may regulate the activity of different ionic channels, in different ways. Moreover, the effect of cyclic nucleotide injection into the same identified neurone of different preparations is not always the same. Obviously, cell responses depend on the experimental conditions and the state of the animal.

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Calcium uptake by sarcoplasmic reticulum from nerve-intact and standard skeletal muscle grafts

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Summary. A freely grafted rat soleus muscle exhibits a decrease in velocity and capacity of SR calcium uptake. This deficit is not prevented by maintaining neural connections (nerve-intact graft) during grafting. Thus the greater mechanical capability of nerve-intact grafts, relative to standard grafts, is not accompanied by any enhancement of the SR tubules.
Key words. Rat soleus muscle; muscle, rat soleus; sarcoplasmic reticulum; muscle graft; calcium uptake.

The sarcoplasmic reticulum (SR) of fast-twitch extensor digitorum longus (EDL) muscles has a faster rate of calcium uptake and a greater capacity for accumulating calcium than does the SR from slow-twitch soleus (SOL) muscles[1,2]. Cross-innervation can reverse the SR calcium uptake properties[3].

When a muscle is severed from its neurovascular supply, it first degenerates due to ischemia, then myogenic cells arise and bring about the regeneration of new muscle fibers[4,5]. If the nerve is not cut during grafting, the resulting nerve-intact graft will also undergo degeneration and regeneration but ultimately possesses a greater muscle mass and can generate a stronger force of contraction than can a standard graft in which both vessels and nerves are severed[6,7]. In view of the neural influence on SR, it was decided to compare calcium uptake by SR in nerve-intact and standard grafts to determine if differences in SR characteristics could be contributing to the observed contractile differences.

Materials and methods. Male Sprague-Dawley rats, weighing an average of 404 ± 10 g when sacrificed, were used in these experiments. Under chloral hydrate anesthesia (40 mg/100 g b.wt) the SOL muscle was exposed and the proximal and distal tendons cut. In one group of rats the vessels to the SOL muscle

Muscle weight and SR calcium uptake in normal and grafted soleus muscles

	Muscle weight (mg)	Calcium uptake Velocity (μmol/mg/min)	Capacity (μmol/mg)	Velocity-capacity ratio (min^{-1})
Control SOL (6)	172.0 ± 7.0[a]	0.089 ± 0.008[a]	0.261 ± 0.014[a]	0.344 ± 0.037[a]
Standard SOL Graft (8)	83.9 ± 14.9[b]	0.052 ± 0.001[b]	0.158 ± 0.010[b]	0.326 ± 0.036[a]
Nerve-intact SOL Graft (9)	128.4 ± 16.2[a,b]	0.052 ± 0.004[b]	0.158 ± 0.007[b]	0.327 ± 0.016[a]

Values are means ± SE. The number of muscles per group is given in parenthesis. Within each column, means with different superscripts are significantly different.

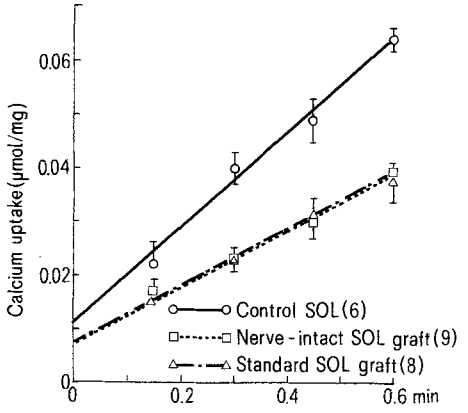
were cut but the nerve was left intact (nerve-intact graft). The muscle was then rotated 180° so that its distal end could be sutured to the proximal tendon left in situ and vice versa. In another group of rats, both the nerve and the vessels to the SOL muscle were cut (standard grafts). The muscles were then rotated and sutured to the tendon stumps in a similar fashion. These nerve-intact and standard grafts were compared to normal SOL muscles from a third group of rats.

60 days after the surgery, the animals were again anesthetized and the grafts and control muscles were removed, weighed and homogenized. The homogenization was carried out in ice cold 10 mM imidazole (pH 7, with 1.0 ml of buffer per 100 mg of tissue) for 15 sec using a Tekmar (SDT) homogenizer.

Calcium uptake by an aliquot of each homogenate was measured in a 10 ml bath being continuously stirred, at a pH of 7.0 and maintained at 37 °C. The bathing medium consisted of 100 mM KCl, 20 mM imidazole, 10 mM K-oxalate, 10 mM Na azide (to inhibit mitochondrial calcium uptake), 5 mM ATP, 5 mM $MgCl_2$, 0.1 mM $CaCl_2$ and 0.8 μc/ml of $^{45}CaCl_2$. The experimental procedure consisted of adding 0.1 ml of the muscle homogenate (1.0 mg muscle per ml bath) to the incubation bath already at 37 °C but containing only the KCl, imidazole, K-oxalate and Na-azide. After a 30 sec incubation period, calcium uptake was initiated by adding a solution of ATP, MgCl, $CaCl_2$ and $^{45}CaCl_2$ which had also been preincubated at 37 °C and which brought the components to their final concentrations. Samples of the bath were taken at 0.15, 0.30, 0.45 and 0.60 min to measure the initial velocity of calcium uptake; and at 30 min to determine the total calcium accumulation which would occur. With each sampling, calcium uptake was stopped by filtration through 0.45 μm Millipore filters to remove the SR vesicles. The ^{45}Ca remaining in the filtrate was assayed by liquid scintillation counting and the amount of calcium removed by the SR vesicles was then calculated. The results are reported in terms of μmol of calcium taken up per mg of protein. Protein concentration was determined by the Lowry method[8].

Statistical analysis of the data was done by performing an analysis of variance followed by Tukey's studentized range test. A value of $p < 0.05$ was accepted as indicating a significant difference.

Results. Figure 1 shows the early oxalate-supported calcium uptake by the muscle homogenates which occurred in these experiments. The average calcium uptake was calculated and plotted for each sampling time. With the incubation conditions employed, calcium uptake was essentially limited to vesicles of the fragmented sarcoplasmic reticulum[9]. The slope of each resulting linear regression line represents the velocity of calcium uptake. Table 1 gives the numerical data, including the weights of the grafts and control muscles. Each velocity value is the average of all the velocities of calcium uptake which occurred during the first 0.6 min of each experiment and is represented by the slope of the corresponding linear regression line plotted in figure 1. The average total calcium uptake which occurred during the 30 min experimental period is given as the capacity value. The data show that both the velocity and capacity of calcium uptake are reduced in standard and nerve-intact SOL grafts when compared to normal SOL muscles. Leaving the nerve intact during the grafting had no apparent beneficial effect on the SR.



Initial rapid calcium uptake compared in grafts and control soleus muscles. Linear regression lines are shown for the data collected from 0 to 0.6 min with the slopes representing the velocity of calcium uptake. Points and associated bars represent means ± SE, respectively. The number of muscles used is indicated by numbers in parenthesis.

Discussion. Following skeletal muscle transplantation, the muscle undergoes degeneration and then regeneration. Carlson et al.[4,5] have described in detail the morphological events of muscle regeneration. Though muscle regeneration is an impressive feat, it is not surprising that the muscle graft does not regain its full mechanical force[6,7]. The current study shows that transplantation results in a similar incomplete restoration of the SR capabilities. This deficit is not prevented by maintaining neural connections (nerve-intact grafts) during transplantation.

The advantage which nerve-intact grafts have over standard grafts is that reinnervation can occur along preserved Schwann cell channels[10] resulting in a greater number of innervating nerve fibers[6]. Otherwise the nerve-intact graft degenerates and regenerates as does a standard graft. Muscle mass as well as twitch and tetanic tension are clearly greater in nerve-intact grafts than in standard grafts. For example, in another study (unpublished data) twitch tensions were found to be 25 ± 1 g for control SOL muscles, 20 ± 2 g for nerve-intact SOL grafts and 12 ± 5 g for standard SOL grafts. Similarly, tetanic tension was highest (109 ± 5 g) in control SOL muscles, intermediate (84 ± 7 g) in nerve-intact SOL grafts and lowest (41 ± 13 g) in standard SOL grafts. Similar relative tensions have been reported for control EDL muscles, nerve-intact EDL grafts and standard EDL grafts[6,7]. The enhanced contractile performance, especially in small muscle grafts, is produced mainly because the muscle fibers in nerve-intact grafts have considerably greater cross-sectional areas than those of standard grafts[6,7]. The current study showed that there was no enhancement of SR calcium uptake characteristics in rat nerve-intact grafts when compared to standard grafts. Thus the greater mechanical capabilities of nerve-intact grafts, relative to standard grafts, cannot be attributed to any enhancement of the SR.

Calcium uptake by the SR initiates relaxation in vivo, yet the relationship between half relaxation time (HRT) and the rate of calcium uptake by SR in vitro is dubious. We found no difference in the HRT of nerve-intact SOL grafts (0.18 ± 0.04 sec), standard SOL grafts (0.23 ± 0.05 sec) and control SOL muscles (0.20 ± 0.02 sec). These data are consistent with other reports[7] but inconsistent with the observed decreases in SR calcium uptake rates reported in this study for SOL grafts. There may be an interplay between the magnitude of the tension generated and the rate of calcium uptake which determines HRT. Thus, though SR calcium uptake is slower in SOL grafts than control SOL muscles, their HRT's could be similar since the SOL grafts are beginning relaxation from a lower peak tension.

Since the velocity of calcium uptake is related to the number of pumps per mg of muscle protein and the capacity to accumulate calcium is related to the volume of SR per mg muscle protein, then the velocity-capacity ratio is an indicator of the pump density, i.e. the number of pumps per unit volume of SR. While both nerve-intact grafts and standard grafts exhibited SR calcium uptake velocities and capacities that were about 60% of control values, pump densities (velocity-capacity

ratios) were not decreased in either type of graft (table). Thus the qualitative SR characteristics in SOL grafts were not different from those typical of slow-twitch SOL muscles. Assuming the qualitative properties of the SR are similar in both types of grafts, the quantity of SR per unit of muscle protein must also be the same in nerve-intact and standard grafts. Since nerve-intact grafts have fewer but larger fibers per mg of muscle protein, the quantity of SR would appear to be more closely related to muscle protein concentration rather than the concentration of fibers.

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Stimulatory effect of methyl jasmonate on the ethylene production in tomato fruits

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Summary. Methyl jasmonate at a concentration of 0.5% in lanolin paste was applied to detached mature green and red ripe tomatoes cv. Tempo. One to 10 days after treatment, slices were cut at a depth of about 2 mm for ethylene determination. It was found that methyl jasmonate strongly stimulated ethylene production both in green and red fruits, production was about 1.6 to 7.9 times higher than in control tissue.
Key words. Tomato; *Lycopersicon esculentum;* methyl jasmonate; ethylene production.

Methyl jasmonate (JA-Me) or jasmonic acid have been identified in many plants[2-8] and were found to be powerful promotors of leaf senescence[8-11]. Saniewski and Czapski[12] showed that methyl jasmonate almost totally inhibited lycopene accumulation and stimulated β-carotene accumulation during the ripening of tomatoes. Recently Edwards et al.[13] found that inhibitors of ethylene biosynthesis – aminoethoxyvinylglycine (AVG), aminooxyacetic acid (AOA), and α-aminoisobutyric acid (AIBA) – inhibited lycopene and ethylene biosynthesis in 1.5 cm disks of excised pericarp tissue of tomato cv. Michigan Ohio Hybrid. Application of external ethylene promoted normal lycopene synthesis but did not stimulate ethylene synthesis. The authors suggest that ethylene production is not essential for lycopene synthesis in tomato fruit. The aim of this work was to study the effect of methyl jasmonate, which inhibits lycopene accumulation, on the ethylene production in tomato fruits.

Materials and methods. Mature green and ripe tomatoes, *Lycopersicon esculentum* Mill. cv. Tempo, grown in a garden frame and picked on September 9–27, 1983 were used. Five to 10 fruits were treated with (±) -methyl jasmonate at a concentration of 0.5% (w/w) in lanolin paste (prepared by mixing lanolin with ⅓k part of distilled water). This was applied on one side of the fruit over an area 2.5 cm^2. The other side was treated with lanolin paste without JA-Me as a control. In the course of the experiment, fruits were kept at room temperature (about 20 °C) in natural light conditions. After 1–10 days from the beginning of the treatment samples were cut off at a depth of 2 mm and used for determination of ethylene production. Slices, ranging from 150 to 350 mg, were placed in 10-ml glass vials and sealed tightly. After 1–3 h 0.5-ml gas samples were withdrawn and analyzed by gas chromatography. Ethylene production is expressed in nl of ethylene per g of fresh tissue per h. There were 5–7 replications for each treatment and all experiments were repeated 3–5 times. Differences among means were evaluated using the test of Dean and Dixon[14].

Results and discussion. Methyl jasmonate applied to detached mature green tomatoes caused the formation of a yellow colored epidermis and pericarp at a depth of 2 mm at the place of treatment, as observed previously[12].

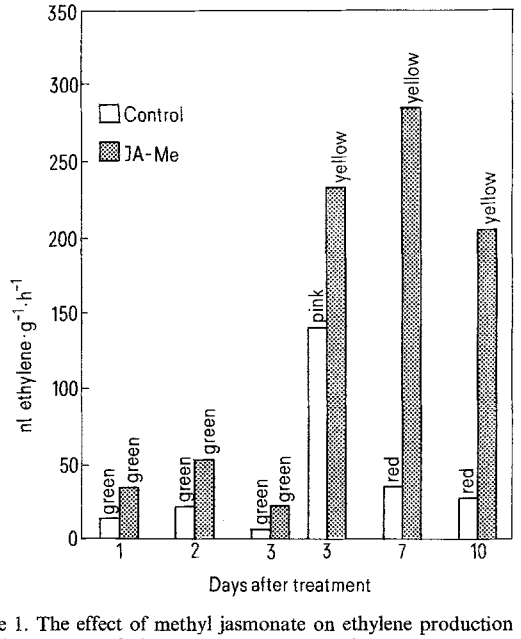

Figure 1. The effect of methyl jasmonate on ethylene production from slices from tomato fruits. Treatments were made on green mature fruits starting on day 0. Each bar represents the means for that particular day of measurement. LSD 1% values are as follows; day 3, 12.4 (green); day 7, 130.1; day 10, 145.3. LSD 5% values are as follows; day 1, 17.2; day 2, 30.2; and day 3 (pink, yellow), 76.2.