correlates with the beginning of the early large birefringence signal after stimulation. The technique described above together with X-ray microanalysis is expected to reveal changes in the spatial distribution of Ca^{2+} during a single contraction relaxation cycle of a muscle fibre.

Electrogenicity of Na–Ca exchange and its alteration by antiarrhythmic agents

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A Na–Ca exchange mechanism has been described in heart cells (Reuter & Seitz, 1968). Such a countertransport which could exchange three or more Na for one Ca was reported to be voltage-dependent and possibly electrogenic (Horackova and Vassort, 1979). Recently, Cs-loading of frog heart cells together with the presence of external Cs (K-free) have been shown to abolish both the inward and outward rectifications thus making it possible to analyse precisely the remaining currents (Mentrard *et al.*, 1984). On applying a Na-poor solution (39.2 mM), under voltage clamp conditions on frog atrial preparations, an outward current was generated during both depolarizations and hyperpolarizations around the resting membrane potential. The current amplitude was sensitive to the external Ca and described a U-shaped function of the membrane potential. Such a relation could be expected for the exchange current I_{ex}, if one considers following Mullins' model (1977), that I_{ex} is a hyperbolic sinus function of $E_m - E_{ex}$ (E_{ex}, the equilibrium potential of the exchange $= 2E_{Na} - E_{Ca}$ assuming a 4 to 1 stoichiometry). Na_0 alterations did not induce variations in I_{ex} in the presence of La^{3+}, Co^{2+}. Variations in I_{ex} were also reduced by the addition of several antiarrhythmic agents. Most of the local anaesthetics (antiarrhythmic of class I), but lidocaine, reduced I_{ex} generally to a different extent than they reduced I_{Ca}, the slow inward current. The effect of amiodarone (class III) were limited to I_{Ca} and might be related to cyclic AMP reduction since in whole frog hearts treated with aminophylline and propanolol, amiodarone (2×10^{-5} M) decreased cAMP content by one third. Nifedipine has no effect on I_{ex} but D600 (class IV, 10^{-6} M) reduced both I_{ex} and I_{Ca}.

Such an exchange current might be involved both in the rhythmic activity of cardiac cells since, on the mathematical model, I_{ex} was shown to be of particular importance in controlling AP duration and re-excitability (Fischmeister & Vassort, 1981) and in the negative inotropic and chronotropic effects of some antiarrhythmic agents.

Action potentials of fast and slow fibres in rat skeletal muscles

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In this study single motor units were stimulated. It was possible to insert a micropipette in some fibres of the stimulated motor unit. Motor units of the soleus were indicated by the twitch contraction time. To determine the motor unit type in EDL the twitch contraction time and the histochemical characteristics were used. At the end of the experiment the fibres of the EDL motor unit were therefore glycogen depleted following the known method of Kugelberg. The intracellular recordings presented concern slow soleus and FTG (fast twitch glycolytic) EDL fibres. During the experiment the electromyogram and the force of the motor unit was recorded continuously to verify that the same motor unit was active.

The intracellular measurements were done with micropipettes. To avoid movement artefacts the micropipettes had a long flexible taper (a tip diameter about 0.2 μm; impedance < 55 MΩ). The pipettes were connected to a microprobe system of W.P.I. (mode M-707) by means of an Ag/AgCl electrode. The frequency band width of the system was checked in each penetrated fibre before the motor unit was stimulated. The intracellular recordings (digitally stored on disc; sample frequency 100 kHz) of slow and FTG fibres were compared using a set of characteristics.

Intracellular action potentials of slow and FTG fibres differed significantly in the mean values of all but one characteristic. The slow fibre action potentials showed less negative membrane potentials, smaller amplitudes and lower rates in the depolarization and repolarization phases in comparison with FTG fibres. The time courses of the intracellular action potentials of slow fibres are slow with respect to those of FTG fibres.

The differences described will influence the extracellular single action potential and consequently the motor unit action potential. In this way the results described contribute to the fundamental knowledge of the generation of the

electromyogram at single fibre and motor unit level.

Conductance kinetics and selectivity of sodium channels expressed in tonic muscle fibres of the frog after denervation

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The aim of the experiments was to characterize the new sodium channels in the muscle membrane of slow (tonic) muscle fibres of the frog, which appear only after denervation. The conductance kinetics parameters and selectivity of Na channels were compared with those in normal and denervated twitch muscle fibres from the same muscle. The measurements were performed under voltage-clamp conditions in cut muscle fibre segments in potassium-free internal and external environment. Sodium conductances (G_{Na}) as functions of both time and voltage were found to be described quantitatively by the Hodgkin–Huxley model for the sodium channel ($G_{Na} = \bar{G}_{Na} m^3 h$) with $\bar{G}_{Na} = 10$ mS cm^{-2}. The new sodium channels in tonic muscle fibres show slower kinetics in comparison with Na channels in normal phasic fibres ($\bar{\alpha}_m$, $\bar{\beta}_m$, $\bar{\beta}_h$). The other kinetic parameters are fairly similar in both the twitch and the slow muscle membranes. The selectivity of sodium channels appearing after denervation in tonic muscle fibres is very close to the selectivity of Na channels in normal twitch fibres from the same muscle ($P_{Na} : P_{Li} : P_{NH4} : P_K = 1 : 0.88 : 0.23 : 0.012$). The experiments represent a further positive test of the hypothesis stating that the new sodium channels are of the same genetic origin as the sodium channels present in twitch muscle fibres under physiological conditions. Synthesis of sodium channel proteins in innervated tonic fibres is suppressed by the action of innervating nerve cells. When this inhibitory effect is released by denervation the genetic information is expressed and new sodium channels become formed.

REGULATION IN SMOOTH MUSCLE AND SKELETAL MUSCLE

Multinuclear magnetic resonance study of smooth muscle regulation

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A considerable body of evidence indicates that phosphorylation of the P-light chains of myosin results in a significant increase of the Mg^{2+}-activated ATPase of vertebrate smooth muscle actomyosin. We are using ^{31}P and ^{1}H n.m.r. to study the structural aspects of phosphorylation of the P-light chain in purified chicken gizzard myosin as well as for the isolated light chain. Using fully phosphorylated, highly active myosin [Cole *et al.*, *FEBS Lett.* **158,** 17–20 (1983)] we are able to resolve and identify the ^{31}P signal deriving from the phosphoserine residue of the P-light chain at smooth myosin concentrations in the range 4–8 μM. The spectral characteristics of the signal (resonance energy and relaxation parameters) have been followed under varying solution conditions and at different temperatures in order to follow effects of pH, Mg^{2+}, nucleotides and urea. The hydrolysis of ATP can be followed simultaneously by monitoring the ^{31}P signals of ATP, ADP and inorganic phosphate. Analysis of the lineshapes of these resonances and preliminary data obtained with isolated myosin subfragment-1 provide kinetic information about product dissociation which reflects structural features of the enzyme–nucleotide interaction.

Complex formation with F-actin led to marked perturbation of the P-light chain signal of smooth myosin, in contrast to the spectral effects observed in the presence of G-actin. Studies on the isolated light chain, phosphorylated and dephosphorylated, by ^{31}P and ^{1}H n.m.r. show that complex formation with actin does not occur. It is likely therefore that the phosphorylation of the P-light chain enhances the Mg^{2+}-activated ATPase by altering the configuration of the domains of the myosin head. Studies are under way to define the disposition of the P-light chain relative to the myosin active site and the surfaces of contact with actin at different smooth myosin phosphorylation levels. These