most of Spain and Portugal has little accumulation of sediment; the sedimentary rocks of the narrow continental terrace are folded and faulted, and the continental slope is steep and of tectonic or faulted origin. A basement high, or a possible structural bench of basement rock, may occur at the foot of the slope off France; there the upper slope is an undissected thick section of slope sediments.

The reflection profiles west of the English Channel trough show that the bedding of the uppermost kilometer of Tertiary rock (*3*) is parallel with the general surface of the continental slope (Figs. 2 and 3); thus, successive southwesterly dipping beds have been deposited on the continental slope, building it seaward. Lines 11 (Figs. 1 and 3) and 12 (Fig. 1) also have suggestions of outcrop of older basement rocks near the foot of the continental slope west of Brittany. Submarine canyons apparently were subsequently incised in the Tertiary rock, and slumping from the walls of the canyons was caused by oversteepening. This structural form resembles that found recently off parts of the eastern United States (*6*) and many other parts of the world.

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Muscle Postjunctional Membrane: Changes in Chemosensitivity Produced by Calcium

Abstract. *Increases in the extracellular concentration of calcium ions above 1.8 millimoles per liter caused a reversible decrease in the sensitivity of the muscle postjunctional membrane to carbamylcholine. A quantitative study of the inhibitory effect of calcium ions on membrane depolarization produced by carbamylcholine indicates that calcium ions compete with carbamylcholine for some common binding sites on the postjunctional membrane. Calcium ions (20 millimoles per liter) caused a neuromuscular block wherein prolonged endplate potentials were produced after nerve stimulation. Calcium ions applied ionophoretically to the postjunctional membrane decreased the amplitude and prolonged the time course of the transient depolarization produced by ionophoretically applied carbamylcholine.*

Calcium has a considerable influence on neuromuscular transmission, and its important role in facilitating the release of acetylcholine from presynaptic terminals is well known. However, many investigators who have studied the effects of Ca^{2+} at the neuromuscular junction have tended to minimize the action of this ion on the postjunctional membrane (PJM). Such a position is generally based on the work of del Castillo and Stark (*1*), who concluded that Ca^{2+} has no significant effect on the depolarization of the PJM caused by the application of acetylcholine in bulk. In our view, the action of Ca^{2+} on the PJM is appreciable. Our reasons for reemphasizing the postjunctional effects of Ca^{2+} are as follows: (i) work from our laboratory (*2*) has shown that Ca^{2+} has an important influence on the "desensitization" of the PJM which develops during sustained application of quaternary ammonium agents such as carbamylcholine (Carb); (ii) the amplitude of miniature endplate potentials is significantly reduced when the concentration of Ca^{2+} is raised to 7.2 m*M* (*3*); (iii) increased concentrations of extracellular Ca^{2+} decrease the conductance of the PJM during neuromuscular transmission (*4*); and (iv) the techniques used by del Castillo and Stark, although the best available at the time, do not give results as definitive as can now be achieved.

We studied (in vitro) the effect of changes in extracellular concentrations of Ca^{2+} on sensitivity of the postjunctional membrane of frog sartorius muscle to Carb. (Carb is an analogue of acetylcholine; it can depolarize the PJM, but is resistant to hydrolysis by acetylcholinesterase.) By means of intracellular recordings at the neuromuscular junction, the sensitivity of the PJM was determined by measuring the maximum reduction in membrane potential achieved during microperfusion of the junction with carbamylcholine in various concentrations (*5*).

Maximum membrane depolarization was attained after 10 to 20 seconds of perfusion with Carb. Where Carb was applied in such high concentration that the fiber gave a mechanical response, an additional set of experiments was conducted in which Carb was applied in bulk and 2 minutes later, when the muscle movements had ceased, postjunctional membrane potentials were determined during the following 3 minutes. The maximum values of depolarization thereby obtained agreed reasonably well with those obtained when intracellular recordings were made continuously during Carb perfusion.

The Ringer solution we used was buffered with 1.0 m*M* tris (hydroxymethyl) aminomethane (Tris) to prevent precipitation of Ca^{2+}. The *p*H of each bathing solution was adjusted to 7.0 to 7.3 by the addition of HCl. Control experiments indicate to us that Tris itself has no detectable effect on neuromuscular transmission. When the concentration of Ca^{2+} in the bathing solution was changed from 1.8 m*M* (control) to a new value, the preparation was allowed to equilibrate at the new concentration of Ca^{2+} for 1 hour before any tests with Carb were run.

The resting potential and the minimum value of the membrane potential produced by application of Carb ($3.2 \times 10^{-5} M$) are given for muscles equilibrated with various concentrations of extracellular Ca^{2+} (Fig. 1). The depolarizing action of Carb is considered to be measured by the term ΔV which represents the difference between the

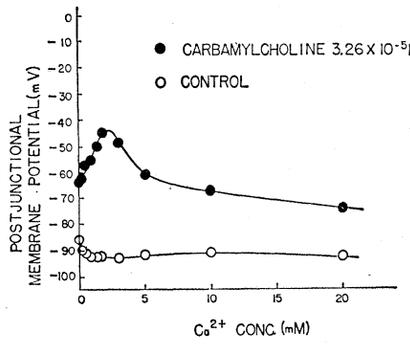

Fig. 1. Average postjunctional membrane potential of control fibers and muscle fibers treated with Carb, equilibrated at various concentrations of extracellular Ca^{2+} as shown.

membrane potential before Carb application and the minimum value of the membrane potential achieved shortly after addition of Carb. As the test concentration of Ca^{2+} was increased from 0 to 1.8 mM, the depolarizing action of Carb increased until the maximum in ΔV was reached at a concentration of Ca^{2+} equal to 1.8 mM. When the concentration of Ca^{2+} was increased still further, ΔV diminished, and thus the inhibitory action of Ca^{2+} was revealed. When the Ca^{2+} concentration was increased beyond 1.8 mM, this inhibitory effect of Ca^{2+} reached full development relatively slowly. For example, when the concentration of Ca^{2+} was increased to 10 mM, maximum inhibition was reached only after 30 minutes of soaking. If the concentration was increased to 30 mM, peak inhibition appeared to be reached in about 5 minutes, and thereafter neuromuscular transmission was blocked. When

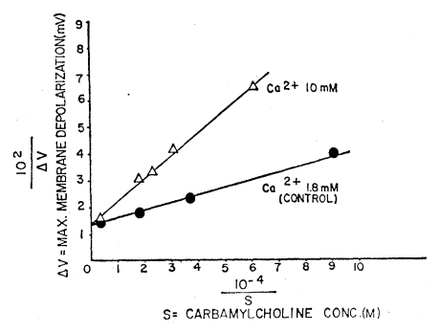

Fig. 2. Lineweaver-Burk plots of $10^2/\Delta V$, the reciprocal of the maximum change in postjunctional membrane potential against $10^{-4}/S$, the reciprocal of the concentration of carbamylcholine which produces ΔV, for two values of extracellular concentration of Ca^{2+} equal to 1.8 and 10 mM.

exposed to 30 mM Ca^{2+} for longer periods, the muscle fiber membrane slowly depolarized.

The inhibitory effect of high concentrations of Ca^{2+} was reversed if the preparation was returned to Ringer solution containing 1.8 mM Ca^{2+}. This reversal was a slow process, completion of which required 60 minutes or more depending on the number of times the bath was drained and refilled with fresh solution. After exposure to 20 mM Ca^{2+}, recovery of PJM sensitivity was hastened if the preparation was washed in hypertonic Ringer solution in which the concentration of Na^{+} was raised to 240 mM by the addition of solid NaCl.

The inhibitory action of Ca^{2+} was also demonstrated when neuromuscular transmission was blocked after the Ca^{2+} concentration had been increased to 20 mM. Under these conditions, with intracellular recordings made at the neuromuscular junction, nerve stimulation produced only endplate potentials in many muscle fibers. These endplate potentials, which did not initiate muscle action potentials, were 15 to 30 mv in amplitude and were moderately prolonged as regards time course. We interpret the marked diminution in the amplitude of the endplate potential to mean that inhibition by Ca^{2+} occurred at both pre- and postjunctional sites.

We determined the maximum depolarization (ΔV) produced by various concentrations of Carb(S) when the concentration of Ca^{2+} was held at 1.8 or at 10 mM (Fig. 2). Each point represents average determinations from ten junctions taken from fibers of two or three muscles. It can be seen that plotting $10^2/\Delta V$ against $10^{-4}/S$ gives straight lines for both concentrations of Ca^{2+}. The fact that the same Y intercept is obtained for experiments done in 1.8 and 10 mM Ca^{2+} indicates that Ca^{2+} and Carb are competing for a common binding site on the PJM.

We attempted to localize the sites of action of Ca^{2+} by means of the ionophoretic technique (*6*, *7*), using micropipettes filled with 2M $CaCl_2$ to apply Ca^{2+} to the external surface of the PJM. Approximately 5 to 10 μm away, a second externally placed micropipette containing Carb (1M) served for rapid transient ejection of this agent. The membrane depolarizations produced thereby were recorded with an intracellular electrode also placed at the neuromuscular junction. Spots on the PJM sensitive to Carb were first located by repeated trial placements of the Carb pipette; then with the $CaCl_2$-filled pipette placed very close to the Carb pipette, Ca^{2+} ions were driven onto the PJM. Within approximately 2 seconds, ionophoretically ejected Ca^{2+} decreased the amplitude and slowed the time course of the transient Carb-produced depolarizations. With time, the amplitude of the "Carb potentials" decreased to undetectable levels. The above effects of Ca^{2+} were found to be reversible to a considerable extent. These data indicate that Ca^{2+} inhibits the depolarizing action of Carb and that the onset of Ca^{2+} inhibition is greatly accelerated if Ca^{2+} is applied directly to the PJM near the membrane site activated by Carb.

Because high concentrations of Ca^{2+} block neuromuscular transmission and appear to antagonize Carb competitively, one might assume that the sites which bind Ca^{2+} in the PJM are the same as those which react with acetylcholine during neuromuscular transmission. As has already been mentioned, excess Ca^{2+} decreases the changes in the conductance of the PJM which occur during neuromuscular transmission (*4*). Furthermore, we have demonstrated that UO_2^{2+} ions inhibit depolarization of the PJM produced by Carb (*8*). These results appear to indicate that the receptive sites on the PJM contain phosphoryl ligands. If so, then it is possible that the acetylcholine liberated from terminals of the motor nerve also acts on phosphoryl ligands of the PJM to increase the ionic conductance of this membrane.

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