Perfusion of a Strip of Mammalian Ventricle
Effects of K-Rich and Na-Deficient Solutions on Transmembrane Potentials

By JEAN DÉLEZE, M.D.

A preparation of sheep's or calf's myocardium is described: the m. transversus cordis of the right ventricle. This lends itself to perfusion of its capillary bed as well as to the recording of membrane potentials from single fibers. When the Na concentration in the perfusate is reduced, the action potentials undergo changes that suggest that the "sodium hypothesis" is applicable to cardiac ventricle.

It has been shown for a variety of tissues that Na ions are necessary to maintain excitability (see Hodgkin1). In contrast to this general finding, Coraboeuf and Ot-suka2 recently demonstrated that guinea pig ventricles continued to beat with an unaltered action potential for many minutes after the sodium in the bathing solution had been completely replaced by choline. Their experiments are open to the criticism that the choline probably did not actually enter the heart. It thus seemed desirable to perform similar experiments on a preparation which would lend itself to perfusion. Since it is thought that this preparation may be useful to other investigators, the method will be described in some detail.

Methods

In ungulates such as the calf and the sheep a bundle of myocardial fibers crosses the right ventricular cavity, running from the interventricular septum to the posterior wall. This bundle can easily be recognized and is known as m. transversus cordis.3 Its length is about 2 cm., and its diameter varies from 2 to 5 mm. in the sheep and from 4 to 8 mm. in the calf. The muscle fibers run parallel to one another and are about 15 μ thick. A blood vessel of 0.1—0.5 mm. in diameter is contained within the strip. By injection of dye it can be shown that this vessel is an anastomosis between the right and the left coronary systems, one end communicating with a branch of the a. coronaria dextra and the other with the ramus interventricularis anterior. For its blood supply, a large portion of the strip of muscle depends on this anastomotic artery, as can be shown by the infusion of dye into an excised preparation.

Heart ventricles were obtained at the slaughter house. To minimize the danger of intravascular clotting, they were transported to the laboratory in cool (4 C.) Tyrode solution. The strips of muscle were handled at room temperature until they were ready for perfusion. All experiments were then performed at 36 C., in the muscle chamber shown in figure 1.

Polyethylene tubes of 1.6 mm. o.d. were drawn out to various diameters. Under the microscope a cannula of a suitable size was introduced into the artery. A ligature was gently tightened until it became impossible to slide the knot back and forth on the cannula. In sheep hearts the vessel was often too thin for cannulation. It was therefore found advisable to obtain more than one heart at a time.

After cannulation of the artery, the polyethylene tube was plugged into a conical hole in the wall of the muscle chamber. This was connected, through a heating spiral, with a reservoir of Tyrode solution. In order to force fluid through the capillary system, it was necessary to ligate the noncannulated end of the anastomosis. In order to force fluid through the capillary system, it was necessary to ligate the noncannulated end of the anastomosis. At the beginning of each experiment, about 0.1 ml. of a 0.01 per cent solution of Evans blue was squeezed into the perfusion system through a side tube near the cannula (not shown in figure 1). The parts of the muscle that were actually irrigated from the arterial anastomosis retained some of the dye and were subsequently used for the experiment.

Test solutions were kept ready to flow into the tip of the cannula through the inner of two concentric polyethylene tubes. Change-over times were thus minimized.

In order to increase the flow rate, a number of substances recommended by various authors4-6...
were added to the perfusate: papaverine sulfate (10 mg./L.), thiamine HCl (50 mg./L.), khellin (1 mg./L.) and Recosen, a heart extract by Robopharm, Liestal, Switzerland (1 ml./L.). With these measures a flow rate of the order of 1 ml./Gm. of tissue/min. was reached, at a perfusion pressure of 50-100 cm. H₂O.

After a few hours of experimentation an increase of the exchange times was observed, and swelling of the muscle strip was obvious. All valid observations were therefore made during the first two hours.

If the bathing solution of a nonperfused preparation had to be changed, the muscle was held in a polyethylene spout (fig. 1, inset at upper right). Test solutions therefore reached the muscle surface in a rapid flow. Contractions were relatively weak. At the site of the electrode the preparation was held lightly in a small plastic clamp (not shown in figure 1), to reduce the movements of the muscle to a minimum in this region. In trial experiments it was shown that contractions of the whole muscle were sufficiently strong to operate a mechano—electrical transducer (RCA 5734).

As a rule there was no spontaneous activity. To stimulate the preparation, square pulses of current were applied between an indifferent electrode in the muscle bath and a thick-walled capillary tube touching the surface of the preparation.

Ling-Gerard electrodes filled with 3 M KCl were used to lead off transmembrane potentials. The use of a 3 M KCl microelectrode in the muscle bath had the advantage that changes in liquid junction potentials were relatively small. The cathode follower stages were of the type described by Copeland and were connected with one of the balanced amplifiers of a Dumont oscilloscope, model 333. Upstroke velocities of action potentials were measured by differentiating electrically the output voltage of the first amplifier and feeding this signal into the second amplifier of the Dumont double-gun oscilloscope.

**RESULTS**

**Time Required for Extracellular Exchange.**

Figure 2 shows 12 successive action potential images on the oscilloscope, photographed during one minute while the electrode was kept
Xa-DEFICIENCY ON CARDIAC POTENTIALS

in the same fiber. The perfusate had been changed from Tyrode solution to a test solution with a potassium concentration of 15.6 mM instead of 2.6 mM. The effects of increasing the K concentration are well known: (1) a decrease of the resting potential, (2) a decrease of the 'overshoot' of the action potential, (3) a lengthening of the rising phase, and (4) a shortening of the falling phase of the action potential. With 6x K Tyrode, the propagated action potential disappeared after a perfusion time of 45 seconds (nine experiments, standard deviation ± 18 seconds). The time needed to reach inexcitability by merely changing the bathing solution was 12 minutes (four experiments, standard deviation ± 4 minutes). With 4x K Tyrode, excitability was maintained, the shortening of the action potential had a half time of about 45 seconds.

Lowering the Na Concentration. Experiments were performed with 17 sheep and 5 calf hearts. The Na concentration was lowered either by substituting choline Cl for NaCl, atropine (20 mg./L.) being added, or by using isosmotic saccharose as a NaCl substitute. Both methods of lowering the Na content gave essentially the same results. Quantitative data were collected for Na concentrations of 0, 10, 20, 25, and 50 per cent. A detailed table may be obtained from the author. Table 1 of the present paper and figure 3 convey the essential results.

Upon switching to Xa-deficient perfusion (fig. 3) the amplitude and the duration of the action potential decreased rapidly; a new steady state was almost reached at the end of one minute.

Within the limit of experimental error the resting potential was independent of the Na concentration. This is to be expected if, as the "sodium hypothesis" predicts, the resting membrane is relatively impermeable to Na ions.

When the extracellular Na concentration was lowered to one quarter of the normal level, the height of the action potential was reduced by 21 mv. (table 1). The simplest version of the Na hypothesis (active mem-

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<th>TABLE 1.—Effect of Reducing NaCl to 25 per cent (5 sheep hearts, 18 impalements)</th>
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<td>Control</td>
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<td>Resting potential (mv.)</td>
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<td>Amplitude of action potential (mv.)</td>
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<td>Maximal rate of rise of action potential ( percentage of initial value)</td>
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* ± standard error.
tential until, after a period of about 20 minutes, both the resting potential and the action potential decreased rapidly.

Furthermore, evidence could be obtained that at least part of the Na ions in the interspaces were rapidly replaced by choline ions: (1) contractions of the hearts became stronger, which is a well known symptom of Na-deficiency, and (2) in 25 per cent Na the upstroke velocities of the action potentials decreased reversibly to about one third of their control values.

The results were the same when NaCl was replaced by sucrose, a finding which would exclude the possibility that choline takes the place of sodium.

DISCUSSION

For a variety of excitable cells it has been shown that electric activity is due to a permeability increase of the surface membrane to sodium ions, allowing positive charge (Na+) to enter the cytoplasm. The present results permit an extension of the "sodium hypothesis" to the ventricular muscle of the sheep and of the calf. They are in line with similar findings for frog ventricle and for mammalian Purkinje fibers.

No progress has been made toward an explanation as to why the guinea pig ventricle seems to be an exception to this general rule. By adding to the evidence that Na ions can actually escape from the interspace while the amplitude of the action potential does not change, the situation has become, if anything, more confusing.

SUMMARY

Membrane potentials were recorded by means of Ling-Gerard electrodes from a piece of right ventricular muscle of the sheep or the calf. The capillary bed of the muscle was perfused by various test solutions. Changes of the action potentials occurred about 10 times more rapidly when the perfusate was changed than when the bathing solution of a non-perfused preparation was altered. In completely Na-free solutions excitability was lost, but the resting potential was maintained. Relative Na-deficiency resulted in a lowering of the amplitude and upstroke velocity, as well as in a shortening of the action potentials. It is concluded that the "sodium hypothesis" can be applied to sheep and calf ventricle.

SUMMARY IN INTERLINGUA

Potenzialles membranal esseva registrate per medio de electrodos de Ling-Gerard in pecias de musculo dextero-ventricular ab oves e vitellos. Le capillatura del musculo esseva perfundite con varie solutiones experimental. Alterationes del potentiates de action occurreva circa 10 vices plus rapidemente quando le perfusato esseva alterate que quando le solution del banio de non-perfundite preparatos esseva alterate. In solutiones complete-mente libere de natrium, le excitabilitate esseva perdite, sed le potential de reposo esseva mantenite. Deficientia relative de natrium resultava in un reduction del amplitude e del velocitate in le ascendita e etiam in plus curte potentiates de action. Es concludite que le "hypohese de natrium" pote esser applicate al ventriculo de oves e vitellos.

REFERENCES

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JEAN DELEZE

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