AN ATTEMPT TO INFER THE ELECTROPHYSIOLOGICAL FUNCTIONS OF SOME INTRACELLULAR STRUCTURES IN CARDIAC CELLS BY AN ELECTRONIC ANALOGUE

C. E. CHALLICE *and* T. A. CLARK
From the Department of Physics, University of Alberta, Calgary, Alberta, Canada

ABSTRACT A circuit which simulates the electrical conduction characteristics of the neuron has been modified by the addition of a feedback loop to simulate the electrical properties of some of the "specialized" tissues of the mammalian heart. It is suggested that there is similar electrical feedback in the muscle cells which is responsible for their electrical properties, and possible relationships between the feedback and observed structures are discussed.

INTRODUCTION

As early as 1899 (1) attempts were made to produce an electrical circuit which would behave in the same manner electrically as a neuron. Since the mathematical formulation of the electrical behavior of the neuron was published by Hodgkin and Huxley (2) a number of papers have appeared concerning electronic circuits which reproduce automatically, and more or less accurately, the electrical conduction of the nerve. A modified monostable multivibrator (3) and tunnel diodes (4) have been used in these circuits, and more recently, integrated solid-state circuitry has produced a successful continuous nerve analogue or "neuristor" (5). As Harmon (6) points out, the development of this type of analogue may be motivated by one of two causes: the use of a simulated nerve in studying the manipulation of information for the subsequent development of machines (e.g. computers); or for developing a system which will duplicate as closely as possible the physiological events which take place in vivo with a view to understanding more about those physiological or chemical events which are still not completely explained.

Although muscle cells have an active plasma membrane which is believed to act in a manner very similar to that of the mammalian neuron, little use has been made of electronic analogues in this area. For the second of Harmon's reasons, this possibility seemed of particular interest in the so called "specialized" tissues of the

mammalian heart, where the modified striated muscle cells perform a function of both conduction and contraction, but in which the conduction is probably more important than the contraction. In the pacemaker there are cells which are self-oscillatory. There are also latent pacemaker cells which can be self-oscillatory, but at a slower frequency, and which can be "driven" at a higher frequency when associated with the primary pacemaker cells. Then there are cells whose prime function is the delivery of contractile power upon receipt of an impulse, and which are not normally self-oscillatory. Morphological studies on these cells (7-14) show that there are characteristic differences in the degree of development of the cellular organelles in the different areas, and it was in the hope that some light could be shed on the electrical functions of these organelles that the present work was conducted.

EXPERIMENTAL

Several circuits have been used but the most illustrative so far has been the modified monostable multivibrator (Fig. 1). Variation of the feedback parameters (R and C) and the positive bias in this circuit in a manner indicated in Table I allows it to simulate the transmembrane action potentials of neuron, pacemaker, latent pacemaker, and ventricular muscle cells. When R is infinite and C zero (Fig. 1), then the circuit is a neuron analogue representing a short length of a nerve fiber. It

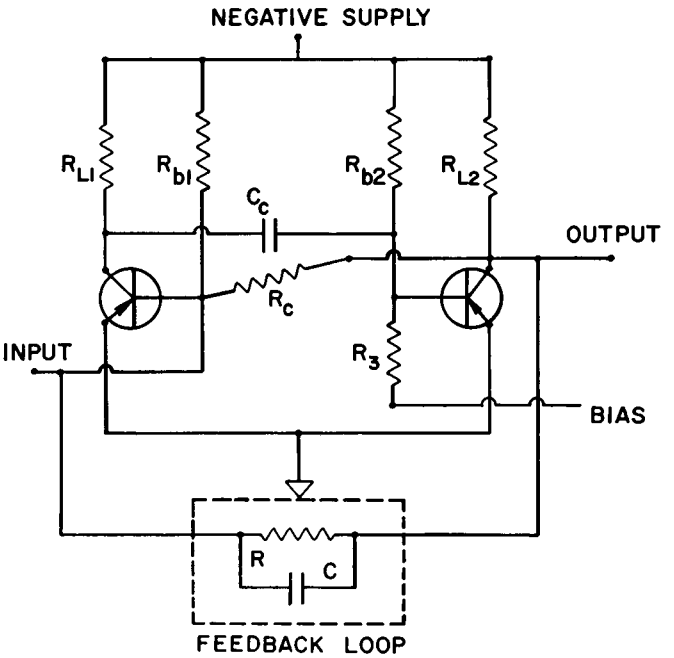

FIGURE 1 Electronic analogue of nerve and muscle. For discussion see text.

TABLE I

VALUES OF THE VARIABLE PARAMETERS NECESSARY TO SIMULATE NEURON AND HEART MUSCLE ACTION IN THE CIRCUIT OF FIG. 1

Analogue	Neuron	Ventricular muscle	Latent pacemaker	Pacemaker
C($\mu\mu$F)	0	6800	2000	1500
R($k\Omega$)	10	10	6.5	10
Bias at base of transistor 2 (v)	+0.3	0	−1.3	−5.5
Corresponding output waveform in Fig. 2	(a) Triggered	(b) Triggered	(c) Oscillatory (d) Triggered	(e) Oscillatory

then has a purely resistive feedback network and on receipt of an input potential above the threshold produces a narrow output pulse (Fig. 2*a*). Addition of a large leaky capacitance into the feedback network with little bias change results in a broadening of the period of depolarization (phases 1 and 2 of the action potential), simulating more closely the ventricular muscle action potential (Fig. 2*b*). If the

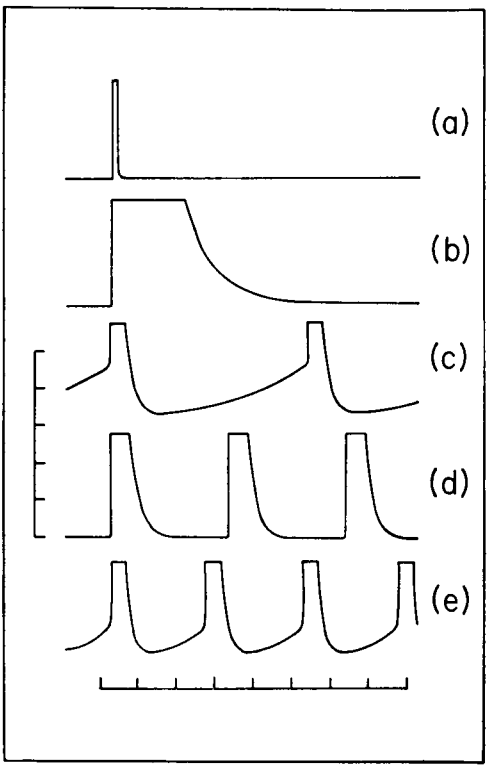

FIGURE 2 Traces or recorded action potentials measured with the nerve and muscle analogue circuit of Fig. 1 under the conditions listed in Table I. (*a*) triggered neuron; (*b*) triggered ventricle muscle; (*c*) self-oscillatory latent pacemaker of the heart; (*d*) latent pacemaker triggered by an external stimulus at a frequency greater than its natural oscillation frequency; (*e*) stable self-oscillatory pacemaker. Scales: 2 v per division and 10 μsec per division.

capacitance is now reduced and at the same time the positive bias decreased, the system becomes a somewhat unreliable slow oscillator, its period being dependent upon both the bias and the feedback parameters. Further change of the variables in this direction (i.e. bias and capacitance reduced) results in a faster, more reliable oscillation. The output under these conditions shows a period of slow spontaneous depolarization (phase 4 of the cardiac transmembrane potential) which leads to a rapid depolarization (phase 0); i.e., the circuit acts like a latent pacemaker. The slope of the slow depolarization (and hence of the period of oscillation) is proportional to the change in bias from that minimum value which will produce a horizontal diastolic period, and is also proportional to the capacitance in the feedback circuit. At this stage the circuit may be triggered at a rate faster than its natural frequency by applying a suitable input signal with a higher frequency (Fig. 2*c* and *d*). Still further reduction of bias and capacitance results in a faster oscillation producing a waveform which resembles the action potential of the pacemaker of the heart (Fig. 2*e*). It is perhaps noteworthy that a condition of oscillation may be reached such that the circuit is difficult to trigger at a faster frequency by the application of a faster input frequency. However, the spike (phase 1) of the triggered action potential is not reproduced by this circuit, neither is the rounded form of the pacemaker potential, nor the enhanced potential difference of the neuron refractory period. Circuit refinements can produce these properties but have been omitted from the present work for the sake of simplicity.

DISCUSSION

If the electrical behavior of the nerve membrane is accepted as established, and if the mode of action of the muscle fiber membrane is essentially similar, then the observation that the nerve analogue may be modified to simulate the specialized muscle by varying parameters suggests that it should be possible to find the analogues of these parameters in the muscle cell. The micromorphology of the specialized muscle cells can then be re-examined with a view to suggesting possible structural sources of these parameters.

Morphological studies have shown that in skeletal striated muscle the fibrils are surrounded by an array of vesicles or tubules (Fig. 3). This is the structure known as the sarcoplasmic reticulum (15, 16) and it is believed to exercise control over the availability of calcium ions (Ca^{++}) which are commonly believed to represent an essential factor in the chemistry of the muscular contractile process (17-20). Some years ago it was suggested that the limiting membrane of this structure was a conducting membrane, and that it might even act in a manner similar to that of the active plasma membrane (21, 22). It was also suggested that all membrane systems in all cells should be regarded in this way (23). Another membrane system known as the T system (24, 25) has been shown to be essentially invaginations of the cell plasma membrane. This forms a transverse link across the fibrils in a fiber

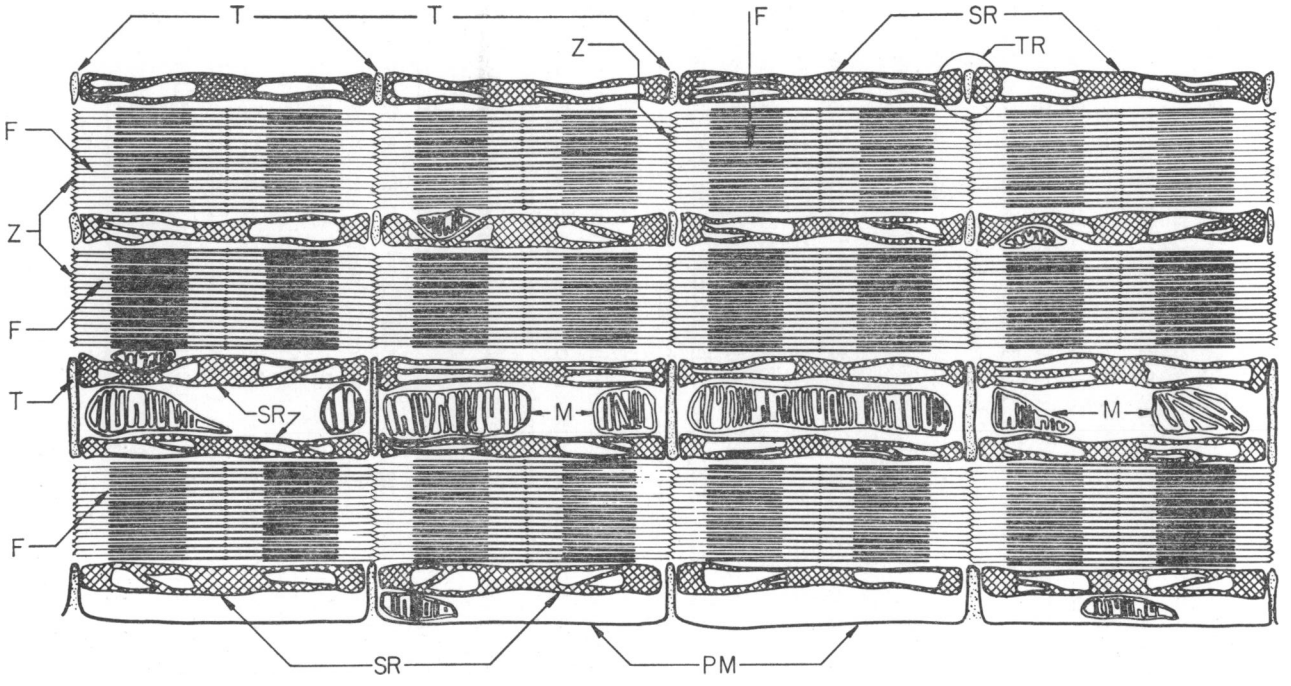

FIGURE 3 Schematic diagram of longitudinal section of skeletal muscle. *PM* — plasma membrane; *T* — T system; *SR* — sarcoplasmic reticulum; *F* — Fibril; *Z* — *Z* band of fibril; *M* — mitochondrion; *TR* — triad. The close apposition of the sarcoplasmic reticulum and the T system at the triads can be seen. In transverse section at a region other than the *Z* band, the sarcoplasmic reticulum would appear as a series of vesicles surrounding the fibrils. In cardiac muscle the sarcoplasmic reticulum is similarly placed but less abundant.

at Z band level, coming into close proximity with the sarcoplasmic reticulum at the triad (15) (Fig. 3). Recent studies have suggested that there are membranelike (26) or tubular (27) structures connecting the sarcoplasmic reticulum with the T system at these points and that there may be electrotonic spread of current from the invaginations of the plasma membrane (T system) to the sarcoplasmic reticulum at these points (26). There is, therefore, evidence at this time that the sarcoplasmic reticulum receives electrical charge from the plasma membrane at periodic points. Leaving aside for the moment the possibility that the potential thus received might be self-propagating as is the case with the plasma membrane, one might expect the electrical action of the sarcoplasmic reticulum to be like a leaky capacitor across segments of the plasma membrane[1] (Fig. 1). From the ionic point of view, the excitation of the plasma membrane would, through the membrane of the sarcoplasmic reticulum, be able to allow the release of Ca^{++} and thus control the contraction (or relaxation) of the fibril, provided the supply of Ca^{++} ions in the sarcoplasmic reticulum sacs were sufficient.

Electron microscopical observations on heart ventricular muscle show that the sarcoplasmic reticulum is much less abundant in these cells than is generally the case in skeletal muscle cells (14). Here a sustained contraction is not required, only a regular periodic response to a received impulse with only slight changes in frequency. However, the muscle must respond to the receipt of an impulse rather than oscillate on its own. It must also be well synchronized and develop a reasonable amount of power, thus some amount of Ca^{++} must be made available in a controlled manner. The electron microscopical studies on the pacemaker and latent pacemaker areas in the atrium show that the sarcoplasmic reticulum is even more sparse in these areas, particularly in the true pacemaker, where fibrillar organization is particularly poor (14). In these tissues it seems difficult, if not impossible, to maintain sustained contraction by electrical depolarization, which is consistent with the idea that the electrical depolarization of the plasma membrane communicates with the sarcoplasmic reticulum, thus releasing the essential Ca^{++}. In these pacemaker and latent pacemaker cells there is very little calcium contained in sarcoplasmic reticulum to be released, the supply thus becoming quickly exhausted following depolarization.

Analyses of Table I and Fig. 2 show that the electronic analogue is consistent with the idea that the capacitance is proportional to the amount of sarcoplasmic reticulum present in the fibers. The width (or duration) of pulse is wider for the larger capacitances, and for a given bias the period is longer for the higher values of C. However, the analogue of the bias factor must now be found. A change in bias would appear to be equivalent to changing the transmembrane ionic concentrations or the membrane ionic permeability characteristics, which can be accomplished experimentally by use of drugs (28).

[1] If the action of the membrane were self-propagating, the analogue would need two monostable multivibrators in parallel with differing circuit constants.

It will be noted that the analogue breaks down if applied to skeletal muscle. Here, although there is a larger amount of sarcoplasmic reticulum than in any cardiac cells, the action potential does not normally have a flat phase 2 (cardiac action potential nomenclature). Rather, phases 2, 3, and 4 are thought to be almost exponential in shape (see reference 29 for review). This would tend to suggest the action of the sarcoplasmic reticulum to be something more complex than simply that of a leaky capacitor, possibly being another active membrane phenomenon as has been suggested (21, 22). Attempts are being made to extend the analogue to include skeletal muscle, but the electronics clearly become considerably more complex.

CONCLUSION

The consistency of the performance of this analogue with the electrical behavior of several types of muscle in the heart suggests the electrical effect of the structures present in these cells but not present in the neuron. It is hoped that improvements in the analogue will reproduce even more faithfully the action of the various muscle fibers, allowing a more detailed analysis of the electrical action of the components and possibly indicating the causes of abnormalities in function.

Part of this work was supported by a grant from the National Research Council of Canada.
Received for publication 28 June 1965.

REFERENCES

1. HERMANN, L., *Arch. ges. Physiol.*, 1899, **75**, 574.
2. HODGKIN, A. L., and HUXLEY, A. F., *J. Physiol.*, 1952, **117**, 500.
3. HARMON, L. D., *Science*, 1959, **129**, 962.
4. NAGUMO, J., ARIMOTO, S., and YOSHIZAWA, S., *Proc. Inst. Radio Engrs.*, 1962, **50**, 2061.
5. AMBROZIAK, A., *Solid-State Electron.*, 1964, **7**, 259.
6. HARMON, L. D., *Kybernetik*, 1961, **1**, 89.
7. MUIR, A. R., *J. Anat., London*, 1957, **91**, 251.
8. CAESAR, R., EDWARDS, G. A., and RUSKA, H., *Z. Zellforsch. und Mikroskop. Anat.*, 1958, **48**, 698.
9. VIRAGH, S., and PORTE, A., *Compt. rend. Acad. Sc.*, 1960, **251**, 2086.
10. VIRAGH, S., and PORTE, A., *Z. Zellforsch. und Mikroskop.*, 1961, **55**, 263.
11. RHODIN, J. A. G., DEL MISSIER, P., and REID, L. C., *Circulation*, 1961, **24**, 349.
12. TRAUTWEIN, W., and UCHIZONO, K., *Z. Zellforsch. und Mikroskop.*, 1963, **61**, 96.
13. CHALLICE, C. E., and TATTON, W. G., *Proc. European Regional Conf. Electron Microscopy*, 1964, Prague, Czechoslovakia Academy Science, 101.
14. CHALLICE, C. E., Muscle, (W. M. Paul, E. E. Daniel, C. M. Kay, and G. Monckton, editors), 1965, London, Pergamon Press, 71.
15. PORTER, K. R., and PALADE, G. E., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.
16. PORTER, K. R., *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, part 2, 219.
17. EBASHI, S., and LIPMANN, F., *J. Cell. Biol.*, 1962, **14**, 389.
18. SEIDEL, J. C., and GERGELY, J., *J. Biol. Chem.*, 1963, **238**, 3648.
19. HASSELBACH, W., *Proc. Roy. Soc. London, Series B*, 1964, **160**, 501.
20. CONSTANTIN, L. L., FRANZINI-ARMSTRONG, C., and PODOLSKY, R. J., *Science*, 1965, **147**, 158.

21. PORTER, K. R., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, suppl. 163.
22. EDWARDS, G. A., RUSKA, H., SOUZA SANTOS, P., and VALLEJO-FRIERE, A., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, suppl., 143.
23. RUSKA, H., EDWARDS, G. A., and CAESAR, R., *Experientia*, 1958, **14**, 117.
24. FRANZINI-ARMSTRONG, C., and PORTER, K. R., *J. Cell. Biol.*, 1964, **22**, 675.
25. HUXLEY, H. E., *Nature*, 1964, **202**, 1067.
26. WALKER, S. M., and SCHRODT, G. R., 1965, Ninth Annual Meeting Biophysical Society Abstracts, 124.
27. BIRKS, R. I., Muscle, (W. M. Paul, E. E. Daniel, C. M. Kay, and G. Monckton, editors), London, Pergamon Press, 199.
28. HOFFMANN, B. G., and CRANEFIELD, P. F., Electrophysiology of the Heart, 1960, New York, McGraw-Hill Book Company, Inc.
29. FALK, G., Biophysics of Physiological and Pharmacological Actions, American Association for Advancement of Science, Washington, D. C., 1961, 259.