MINIREVIEW

Electrophysiology of Single Cardiac Cells

Hiroshi IRISAWA

National Institute for Physiological Sciences, Okazaki, Aichi, 444 Japan

The importance of using single myocytes isolated from the adult mammalian heart has lately received wide recognition, and many investigations on ionic currents using single ventricular cells have appeared from various laboratories. Cultured myocytes isolated from embryonic hearts have been a matter of interest for a long time, especially from a developmental standpoint, but their sizes are so small that most of the electrophysiological studies have been conducted on sheet, strand, or cluster specimens. In 1969, KONO [31] isolated single adult rat ventricular cells through the collagenase treatment; since then many methods for preparing viable cardiac cells have been reported, but few physiological studies were done using these single cells until POWELL, TERRAR, and TWIST [49, 50] and ISENBERG and KLÖCKNER [21] demonstrated the usefulness of the single cell for physiological study.

The heart has a functional syncytium and the excitation of a single cell is conducted to the adjacent cells through low resistance nexus [64]. Much traditional electrophysiological work on heart muscle has been carried out with multicellular muscle strands, such as Purkinje fibers and the papillary strands. Using these preparations, most basic properties of excitation and conduction of the heart muscle have been established. Voltage clamp experiments in multicellular specimens [for review, see Refs. 4, 9, 42] are not ideal because of the difficulty of the spatial clamp and the desire of most cardiac physiologists to design their experiments using single cardiac cells. Thus the work of POWELL and TWIST [50] stimulated researchers in many fields of cardiac electrophysiology to develop a method of obtaining viable cardiac cells and to devise a way of recording electrical activity from single cells.

Another development which triggered single cell physiology was the introduction of the patch clamp method [14], where single ionic channel activity and statistical analyses of channel activity became available. This newly developed method in the electrophysiology of the cardiac cell spurred increased interest among general physiologists and biophysicists, as well as cardiac physiologists.

Single myocytes isolated from the adult heart have the following unique features.

Received for publication March 9, 1984

入沢 宏

i) The diffusion of extracellular substance is more efficient in a single cell than in a multicellular preparation. In the latter, the central part of the specimen always suffers anoxia except in very small and thin preparations.

ii) In the multicellular specimens, the presence of extracellular clefts surrounding the cell causes complexities in experimental results. Thus the accumulation and depletion of the carrier ions at the cleft site are superimposed on the genuine change of membrane currents. In a single cell, these discussions can be limited to the T-system or caveolae which are inherent to the cell.

iii) Because the space constant of the cell is longer than the length of the single cell, better conditions for the spatial clamp in the single cell specimen can be expected. However, there are still many possibilities of an incomplete clamp due to point injection of the current when the size of the myocytes exceeds the appropriate size.

iv) In a multicellular specimen we cannot measure the exact number of cells in the preparation, thus the estimate of the surface area of the cell is not accurate. With a single cell, however, its dimension can be determined without a large error, although there remains a problem regarding the estimation of the surface folding.

v) Using a single cell, control of the intracellular medium is possible so that a biophysical experiment can be designed. This could not be done with the multicellular specimen.

vi) Only in the single cell can we measure single channel ionic currents.

Thus the development of the single cell study offers many merits of physiological importance. On the other hand, one should be somewhat cautious in these experiments and consider to what extent the results of the single cell can be extrapolated to the multicellular specimen. Because heart cells *in situ* are multicellular under normal conditions, a cell would lose its normal physiological nature after it has been isolated from adjacent cells. Also, it is possible that some unknown property of the membrane may be lost after enzymatic treatment.

A. METHOD OF DISPERSION OF THE SINGLE CELL

Most laboratories use the enzymatic dispersion method, but with many minor changes. Generally speaking, there are two methods of isolation, coronary perfusion and chopping methods.

1) *The coronary perfusion method*

Perfusion of the coronary artery is made through the aorta using the Langendorff method [6, 24, 49, 50, 58]. Isenberg and Klöckner introduced KB (Kraftbrühe) solution as a recovery solution, whereby after collagenase treatment the heart was perfused and the enzyme treated-heart was stored in the KB solution at least 1 hr before the experiments. The original KB solution has

been modified [59], but others [2, 3, 6, 49, 50] did not use that solution. BUSTAMANTE *et al.* [6] also perfused with the collagenase solution for only 10 min and prepared the specimen in a straightforward way.

2) *Chopping method*

This method has been employed mainly with the hearts of larger mammals (bovine or human) where the Langendorff perfusion is not effective. It has also been used in amphibian heart [15, 16] and mammalian Purkinje cell [56] where the special localization of the tissue is necessary before enzymatic dissociation. In most cases using this method, the heart tissue is initially cut into 2 mm thick cubic chunks and then incubated in an enzyme medium continuously bubbled with O₂. Then the supernatant is separated by using a 200 μ m mesh sheet and poured into the recovery solution. A similar method without the KB solution has been used [7]. The use of a recovery solution is still under discussion.

B. MORPHOLOGICAL STUDY

Many morphological studies were reported during 1970, but most were not directly related to physiological experiments. Viability of the cell was determined by the ability of extrusion of trypan blue, oxygen consumption, and calcium tolerance. Investigations of the fine structure of the isolated cell where physiological experiments are conducted in parallel with morphological studies are especially important [15, 55]. Nakamura and Hama (unpublished) investigated the single cardiac cell isolated from rabbit heart morphologically [58]. They found that a cardiac cell isolated by our method and incubated 1 hr in a warm Tyrode solution retained ultrastructural characteristics which resembled those of myocytes in the intact heart. The dissociation of the cell inevitably results in separation of the intercalated discs by either uncoupling or tearing of the gap junction. Almost all previous reports agree that the adherent junctions (desmosome structures) separate easily along the extracellular plane between the two membranes but the gap junction complex remains with one of the cells and thus the resealing of the membrane occurs within seconds. During 1 hr incubation in the warm Tyrode solution, contact region of the cell loses its sharp edges with the smoothing and enrolling of the membrane.

One of the interesting findings that Nakamura and Hama made in their observations on the isolated pacemaker cell was that about 20% of the dispersed cells from the S-A node region of the rabbit became round in shape and had a smooth surface [19]. The fine structure of such a round cell reveals well preserved internal organelle, unorganized myofibrils situated at the periphery of the cell, and a large nucleus occupying the center of the cytoplasm. The surface area of the cell becomes smaller as the cell becomes round without changing its volume [19]. The fine structure of the round cell isolated from the S-A node region was

first reported by MASSON-PÉVET *et al.* [37], but the nodal cells under their study were not spontaneously active.

C. DEVELOPMENT OF THE METHODS

1) *Electrodes*

Three different kinds of electrodes were applied. The first one was the conventional 3 M KCl microelectrode. The sample and hold type voltage clamp experiments using one microelectrode [21, 40, 62, 63] as well as two microelectrodes were made [3, 32, 40]. Using the latter method, the activation and inactivation time courses of the calcium current have been much improved [59]. The second electrode was a large suction electrode (diameter 5 to 8 μm) [33] which was initially developed for the study of the *Aplysia* ganglion cell; later it was applied to heart cells [2, 3, 18, 34, 35]. It is difficult to obtain an absolute resting potential by this method, but full-blown, all-or-none action potentials are usually recorded when the cell is stimulated electrically. The third electrode [14] is a giga-sealed patch clamp micro-electrode, which is most promising. The electrode tip is less than 3 μm . It is fire-polished and with gentle suction the leak resistance to the extracellular fluid will increase up to $10^{10} \Omega$. Using this electrode, the whole cell clamp mode, inside out patch mode, and outside out patch mode are available.

2) *Intracellular dialysis method*

A method of changing intracellular milieu has been developed. Dialysis of the intracellular milieu was difficult in the multicellular specimen, although there have been several trials [22, 36]. In the single cell specimen, intracellular injection [59, 60] or dialysis became possible [18, 29, 35]. NOMA [57] devised a method which satisfies the internal perfusion of the cell (Fig. 1). Figure 2 gives an example of such an experiment which was conducted by Dr. H. Matsuda. Voltage clamp records under these conditions will be reported separately (Matsuda and Noma). This figure shows that within 2 to 3 min the intracellular milieu can be exchanged with the pipette solution. The diffusion of substance within the cell from the electrode was also evaluated by the diffusion of dye, LRB-200, which has a molecular weight of 680, readily diffuses into the cell within 2 min and is distributed rather evenly throughout the cell. Thus the intra-electrode solution diffuses freely into the cell using the patch clamp pipette, which is partly because the diameter of the electrode is large compared to the routine 3 M KCl microelectrode. We found that a considerable amount of substance diffuses into the cell from the pipette and affects the intracellular milieu of the cell even when a 3 M KCl electrode is used because the size of the single cell is small and there is no diffusion of the substance to the adjacent cells. Thus the introduction of a 3 M KCl microelectrode into a single ventricular cell often rapidly damages the cell, while the action potential is progressively prolonged with the presence of 1

Fig. 1. Method of dialysing intracellular milieu. A polyethylene (PE) tube was inserted into a suction pipette, and the tip of the PE tube was sharpened as small as $70\ \mu\text{m}$ and reached about $0.3\ \text{mm}$ from the tip of the pipette. The intraelectrode solution was placed in the reservoir. The solution flowed from the reservoir through the PE tube to the tip of the electrode and passed to the surplus container. The waste fluid was sucked out. (Figure obtained from A. Noma.)

Fig. 2. Perfusion of Cs aspartate to the single ventricular cell. At the top is a slow speed record of the ventricular action potential. As soon as Cs aspartate was perfused through the pipette, the membrane depolarized progressively. At the second triangular sign, the intracellular perfusate was switched to the control K aspartate solution. The membrane progressively repolarized. The lower traces show the fast sweep of the action potentials. Numerals below the traces correspond to the same number of the action potentials. (Original record obtained by H. Matsuda.)

mm EGTA in the pipette. These experiments have been conducted in the past only in large invertebrate cells, but have now become possible in mammalian heart cells.

3) Recording technique

Patch clamp techniques for recording the high resolution of current have been

described in detail [14, 47].

D. RESTING AND ACTION POTENTIALS

Resting and action potentials in the single ventricular and Purkinje cell revealed a similar potential range as that measured in the intact tissue (Table 2). The zero current potential of the S-A node cell was -40 mV [41], which was also very close to those measured in the multicellular specimen. The dependence of the resting potential on K_o was also studied. It was 43 mV for a 10-fold change in K_o in frog atrium [15], whereas in mammalian ventricle it was approximately 60 mV between 5 and 100 mM K_o [6, 24, 58]. Noma and Gadsby (private communication) found Na-K pump mechanisms in the isolated ventricular cell.

E. PASSIVE PROPERTIES

The specific membrane resistance (R_m , $\Omega \cdot \text{cm}^2$) ranged between 2.4 and 7.3 in ventricular cells. SEVERS *et al.* [55] estimated the contribution of T tubules and surface folding and obtained the value of $4.3 \text{ k}\Omega \cdot \text{cm}^2$ and similarly C_m

Table 1.

Size (μm)	Surface area (μm^2)	R_m ($\text{k}\Omega \cdot \text{cm}^2$)	C_m ($\mu\text{F}/\text{cm}^2$)	τ_m (msec)	Input Z ($\text{M}\Omega$)	Loca- tion	Species	Ref.
$100 \times 20 \times 6$	6,000	2.4	1.9		40	Ventricle	Rat	[49]
$(95-140) \times$ $(11-17)$	4,742	2.6	1.0	2.6	55	Atrium	Guinea pig	[17]
204×7.1		7.9	2.2	19.7	50	Atrium	Frog	[15,16]
$94 \times 21 \times 6$		6.9	0.2	6.9	33	Ventricle	Rat	[23]
$98 \times 22 \times 16$		7.2	0.2	7.2	36	Ventricle	Guinea pig	[23]
$136 \times 35 \times 9$		7.3	0.18	7.3	41	Ventricle	Bovine	[23]
164×35	14,000	2.8	2.7	7.0	21	Purkinje	Dog	[56]
23×28	1,225	14.9	1.3	19.4	870	S-A node	Rabbit	[41]

Table 2. Action potential parameters.

Rest (mV)	Amp. (mV)	$V_{\max}$	Oversh. (mV)	Duration (msec)	Location	Species	Ref.
-80	120	230	40		Ventricle	Rat	[6]
-77	105	200-300	28	128	Ventricle	Rat	[4]
-60	99	—	39	196-349	Ventricle	Guinea pig	[32]
-92	134	200-300	42	305	Ventricle	Guinea pig	[24]
-80	124	200-300	40	400	Ventricle	Bovine	[24]
-79	116	263	37	93	Atrium	Guinea pig	[17]
-(70-85)				80-105	Atrium	Guinea pig	[1]
-40	88.6	8.5		100	S-A node	Rabbit	[41]
-88.6	124.4	42.2	35.8	722.5	Atrium	Frog	[15]

as $1.9 \mu\text{F}/\text{cm}^2$. Similar values have been reported in the bullfrog atrial cells as $R_m 7.9 \pm 1.2 \Omega \cdot \text{cm}^2$, C_m as $2.2 \pm 0.3 \mu\text{F}/\text{cm}^2$ [15]. These values are comparable to those reported in the multicellular preparations. The R_m and C_m were also estimated in the nodal pacemaker cells [41]. Table 1 summarizes the available data.

F. CURRENT SYSTEMS

1) i_{Na}

A voltage clamp with two suction pipettes enabled BROWN, LEE, and POWELL [2, 3] to analyze the i_{Na} in the rat ventricular cell. The i_{Na} elicited by single depolarizing pulse steps from a holding potential of -80 mV, has a threshold between -70 and -60 mV and a peak at -30 to -20 mV. The peak current was the order of 70 to 140 nA. Current densities at peak currents in the 145 mM Na were of the order of 0.9 – 1.8 mA/cm 2 , assuming an average cell surface area of $8,000 \mu\text{m}^2$. Maximum Na conductance ($\bar{g}_{Na}$) was calculated to be 25 mS/cm 2 in a 145 mM Na solution. Using a two microelectrode voltage clamp technique, the i_{Na} was greater than 50 nA which prevented adequate voltage control during the initial 2 msec [23]. In the cultured spherical cell of day 1, the peak amplitude of i_{Na} was less than 300 pA [8], which was probably due to the small size of the cell. Single channel analysis of i_{Na} has also been reported [8, 13], which may indicate a potentially important tool for studying the mechanisms of cardiac arrhythmia.

2) i_{Ca}

In contrast to i_{Na} , the peak amplitude of i_{Ca} was smaller in the bovine ventricular cell. It was 8 ± 2 nA per cell; the current density was $42 \mu\text{A}/\text{cm}^2$ [23]. The current density of the i_{Ca} in the S-A and A-V node cells was $16.9 \pm 6.8 \mu\text{A}/\text{cm}^2$ and $21 \pm 13.1 \mu\text{A}/\text{cm}^2$ [41], respectively. Due to the restrictions of the method, no reliable value for the activation of i_{Ca} has appeared. The i_{Ca} peaked within a few msec, and the mean value estimated from a clamp step to 0 mV was 0.55 ± 0.15 msec [23], similarly the deactivation time constant was on the order of magnitude between 0.4 and 0.8 msec. These values indicate that activation of i_{Ca} is a fast process. The inactivation time course of i_{Ca} consists of two exponentials; the fast inactivation was 7 msec whereas the slow one was 30 msec. This time course has been repeatedly confirmed by others [18, 41] and is obviously slower than that of i_{Na} . There are several distinguishable differences between i_{Na} and i_{Ca} :

i) The run down process of i_{Ca} is more prominent than in i_{Na} [10, 11]. Using a large pore suction electrode we found the run-down process of i_{Ca} to be very rapid (within 5 min, Fig. 3). In this figure, the holding potential was -60 mV and the clamp pulse was $+40$ mV. Immediately after the test pulse, rapid

Fig. 3. Run down process of i_{Ca} . A: the slow speed oscilloscope tracings of potential (top curve) and currents (lower curves). Arrow indicates the decrease of i_{Ca} , from bottom to top. The i_{Na} is barely seen at the initial part of the trace. B: current (top) and potential traces (bottom) of i_{Na} , after i_{Ca} was abolished. (Original record obtained by Y. Kurachi.)

i_{Na} appeared and was followed by an activation of the i_{Ca} . The latter current gradually decreased (the arrow in the figure) and finally disappeared within 5 min. In B, after the i_{Ca} was abolished, i_{Na} at various clamp pulses was registered with a faster sweep. These figures show a rapid run-down of i_{Ca} whereas the i_{Na} remains. This observation gave the impression that some intracellular substances which might be important in preventing the run-down process of i_{Ca} might have been diffused out of the cell. We previously found the presence of ATP in pipette is essential to keep the i_{Ca} [18]. Recently, NOMA [41] found that the absence of ATP within the intracellular fluid causes an activation of the ATP-sensitive K channel.

ii) The i_{Ca} increased after intracellular injection of ATP [59]. Similarly, injection of the various adenine nucleotides other than ATP, *i.e.*, ADP, AMP, and CP, all enhanced the action potential amplitude and prolonged its duration [59]. Injection of cAMP caused an increase in the rate of spontaneous firing of the nodal cell and a prolongation of the ventricular action potential [60]. The prolongation or shortening of the action potential may suggest the concomitant reduction of i_{si} . The application of CN-Tyrode solution invariably caused a shortening of the action potential while a reduction of i_{si} was not so obvious, and an increase of the outward current was responsible for the shortening of the action potential duration [59]. It may be concluded that these adenine nucleotides influence the amplitude of i_{Ca} . The i_{Ca} is increased by an injection of a catalytic subunit of cAMP-dependent protein kinase [5, 48]. CACHELIN *et al.* [8] also found that 8-bromocyclic AMP increases the probability of opening of Ca^{2+} channels, confirming the hypothesis that the presence of AMP-dependent phosphorylation of Ca channels primarily promotes the forward rate constants which lead to the open state of a Ca^{2+} channel during depolarization. Thus the function of i_{Ca} is closely related to the metabolic state of the intracellular milieu.

iii) i_{Ca} is dependent on intracellular Ca^{2+} concentration. When Ca^{2+} is injected into the ventricular cell, the duration of the action potential rapidly

shortens, transient depolarization is elicited [39], and finally the action potential disappears. On the other hand, when EGTA is injected, prolongation of the action potential duration is observed [32]. Thus when the patch pipette is used for the purpose of internal dialysis, the interpipette solution should contain EGTA. KOKUBUN and IRISAWA [29] found i_{Ca} is abolished within 5 min when the concentration of Ca^{2+} within the pipette increases from 10^{-9} to 10^{-7} M, without changing inactivation kinetics. We also found that Ca^{2+} mediates inactivation of Ca^{2+} channel as has been found in many other excitable cells.

Many references suggest the existence of Ca^{2+} activated K conductance [12, 22]. However, in a single cell preparation, injection of Ca^{2+} into the cell invariably causes shortening of the action potential which is due either to a Ca^{2+} mediated inactivation or the activation of the non-selective cation channel [11]. We have not been able to detect the Ca^{2+} controlled K^+ conductance in the ventricular cell [38, 60]. MATSUDA [39] considers the non-selective increase in membrane conductance to cations, which has been postulated to account for both the Ca-induced depolarization in gland cells and oscillatory inward currents in Ca-loaded cardiac cells [11, 28, 39].

iv) pH: Alterations of the cytoplasmic proton concentration are known to affect the i_{Ca} [32]. The shortening of the action potential plateau was found when an acidic solution was injected into the ventricular cell, whereas an alkaline caused prolongation. A slight reduction of the peak of i_{Ca} was observed.

v) Acetylcholine (ACh): In the atrial and nodal cells, ACh hyperpolarizes the membrane and shortens the action potential duration. A negative inotropic effect was observed concomitantly. The mechanisms of this shortening have not been clearly settled, because in the mammalian atrial tissue and S-A node cell, ACh is effective on the outward K current, whereas in the frog atrial tissue ACh is effective mainly on the i_{Ca} . In a single atrial cell [17], ACh in a very low dose increased the outward current without appreciably affecting the i_{Ca} . In higher doses, the amplitude of the i_{Ca} decreased markedly because of the coexistence of a large amount of outward current. Presence of ACh activated K current as well as the relaxation of the ACh effect have been demonstrated [44].

vi) Inotropic action: The most striking difference between i_{Ca} and i_{Na} is the commitment of i_{Ca} to the excitation and contraction coupling, whereas i_{Na} is only responsible for excitation. Inotropic action of adrenaline has been proved in a single channel level [10, 51, 52]. KOKUBUN and REUTER [30] found dihydropyridine derivatives prolong the open state of Ca channels in cultured cardiac myocytes.

3) K currents

There are many K channels in the heart cell [9, 42].

i) i_{K1} : The inward rectifier channel (i_{K1}) has been investigated in the ventricle [27, 54] and atrium, but was rarely found in the nodal cell [45]. This dif-

ference has been attributed to the large difference in the resting potential between nodal and ventricular cells. The time-dependent inactivation of i_{K1} has been thought to be due to the accumulation of K^+ ions within a narrow cleft between the cell, but now it is attributed to the presence of the T tubule as well as to an intrinsic inactivation mechanism.

ii) i_K : In the atrial and nodal cells, the presence of i_K is demonstrated. The kinetics of i_K in the nodal cell appear very similar to those observed in the multicellular specimen. In the single channel recording, KAKEI and NOMA [25] analyzed the K current in the A-V node cell, and they found most of it was related to the ATP sensitive K channel. Yet to be completed is the single channel analysis of the time-dependent K current of the pacemaker cell.

4) i_h

In the nodal cell, the hyperpolarization activated current, i_h , has been observed in the single cell [41] as well as in the multicellular preparation [61]. However, some nodal cells lack i_h , even when showing a regular pacemaker activity before the voltage clamp experiment. This observation led us to doubt the contribution of i_h to the cause of the spontaneous activity. In a preliminary experiment, we did not observe any single channel activity corresponding to i_h . The significance of i_h on the pacemaker current has been discussed recently [20, 44]. We believe the major physiological roll of i_h in the nodal cell is to ensure the area of the pacemaker, by reducing the negative resting potential of the follower cell [61].

5) i_{ATP}

This current is in a closed state in the normal cell, where ATP concentration within a cell is above 2 mM. It opens when ATP concentration within a cell is below 2 mM [25, 43]. Thus this channel only opens during a pathological condition such as hypoxia or ischemia, and the physiological significance of this channel needs to be discussed. If there is a compartmentalization of ATP in the cell, and if it occurs near the membrane, this channel may become active even when overall concentration of the cell ATP remains at a normal level. Also, it is possible when the local cell groups only suffer a severe ATP deficit, due to a microischemia in the small coronary artery, the overall concentration of ATP may not change much, but locally a severe ATP deficiency may occur. In this case, this channel may become activated and local shortening of an action potential duration will occur, which will lead to an arrhythmia.

6) i_{K-Na}

A similar K channel sensitivity to the increase in the intracellular Na concentration has been found [26]. This channel opens when the Na concentration inside the cell increased to 20 to 30 mM, and has no relation to the intracellular Ca concentration. The physiological significance of the Na activated K channel

may be similar to the ATP activated K channel.

7) i_{ACh}

As described before, i_{ACh} is observed in the atrial cell as well as in the nodal cell, and is definitely different from the i_{K1} channel [46, 53, 57].

SUMMARY

Whatever techniques of isolation one may use, it is certainly true that the isolated single heart cells are very useful in various physiological experiments. For electrophysiology, the application of the single cell serves to test findings previously obtained in the multicellular preparation. Therefore, summary and comparison of the data will become necessary in the near future. Furthermore, the application of the single cell opens new areas for electrophysiology of the heart, because when using only the single cell preparation, one can dialyse intracellular milieu and can also measure single channel analyses.

Key Words: isolated ventricular cell, collagenase, voltage clamp, patch clamp, ionic current.

The author wishes to thank his coworkers for permitting him to use their original figures. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. BELARDINELLI, L. and ISENBERG, G. (1983) Isolated atrial myocytes: Adenosine and acetylcholine increase potassium conductance. *Am. J. Physiol.*, **244**: H734-H737.
2. BROWN, A. M., LEE, K. S., and POWELL, T. (1981) Voltage clamp and internal perfusion of single rat heart muscle cells. *J. Physiol. (Lond.)*, **318**: 455-477.
3. BROWN, A. M., LEE, K. S., and POWELL, T. (1981) Sodium current in single rat heart muscles. *J. Physiol. (Lond.)*, **318**: 479-500.
4. BROWN, H. (1982) Electrophysiology of the sinoatrial node. *Physiol. Rev.*, **62**: 505-530.
5. BRUM, G., FLOCKERGI, V., HOFMANN, F., OSTERIEDER, W., and TRAUTWEIN, W. (1983) Injection of catalytic subunit of cAMP dependent protein kinase into isolated cardiac myocytes. *Pflügers Arch.*, **398**: 147-154.
6. BUSTAMANTE, J. O., WATANABE, T., and McDONALD, T. F. (1982) Single cells from adult mammalian heart: Isolation procedure and primary electrophysiological studies. *Can. J. Physiol. Pharmacol.*, **59**: 907-910.
7. BUSTAMANTE, J. O., WATANABE, T., MURPHY, D. A., and McDONALD, T. F. (1982) Isolation of single atrial and ventricular cells from the human heart. *Can. Med. Assoc. J.*, **126**: 791-793.
8. CACHELIN, A. B., PEYERS, J. E., KOKUBUN, S., and REUTER, H. (1983) Ca^{2+} channel modulation by 8-bromocyclic AMP in cultured heart cells. *Nature*, **304**: 462-464.
9. CARMELIET, E. and VEREECKE, J. (1979) Electrogenesis of the action potential and automaticity. In: Handbook of Physiology, Sec. 2, The Cardiovascular System, Vol. 1, The

- Heart, Am. Physiol. Soc., Bethesda, Maryland, pp. 269-334.
10. CAVALIE, A., OCHI, R., PELZER, D., and TRAUTWEIN, W. (1983) Elementary currents through Ca^{2+} channels in guinea pig myocytes. *Pflügers Arch.*, **398**: 284-297.
11. COLQUHOUN, D., NEHER, E., REUTER, H., and STEVENS, C. F. (1981) Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature*, **294**: 752-754.
12. GOTO, M., HYODO, T., and IKEDA, K. (1983) Ca-dependent outward currents in bullfrog myocardium. *Jpn. J. Physiol.*, **33**: 837-854.
13. GRANT, A. O., STARMER, C. F., and STRAUSS, H. C. (1983) Unitary sodium channels in isolated cardiac myocytes of rabbit. *Circ. Res.*, **53**: 823-829.
14. HAMILL, O. P., MARTY, A., NEHER, E., SACKMANN, B., and SIGWORTH, F. J. (1981) Improved patch-clamp techniques for high-resolution current recordings from cells and cell-free membrane patches. *Pflügers Arch.*, **391**: 85-100.
15. HUME, J. R. and GILES, W. (1981) Active and passive electrical properties of single bullfrog atrial cells. *J. Gen. Physiol.*, **78**: 19-42.
16. HUME, J. R. and GILES, W. (1983) Ionic currents in single isolated bullfrog atrial cells. *J. Gen. Physiol.*, **81**: 153-194.
17. IJIMA, T., KAMEYAMA, M., and IRISAWA, H. (1984) Membrane current in single guinea-pig atrial cells. *J. Physiol. Soc. Jpn.*, **45**: 429 (1983).
18. IRISAWA, H. and KOKUBUN, S. (1983) Modulation by intracellular ATP and cyclic AMP of the slow inward current in isolated single ventricular cells of the guinea-pig. *J. Physiol. (Lond.)*, **338**: 321-337.
19. IRISAWA, H. and NAKAYAMA, T. (1984) Isolation of a single pacemaker cell from rabbit S-A node. *Jikeikai Med. J.*, **30**: 65-70.
20. IRISAWA, H. and NOMA, A. (1984) Pacemaker currents in mammalian nodal cells. *J. Cell. Mol. Cardiol.*, in press.
21. ISENBERG, G. and KLÖCKNER (1980) Glycocalyx is not required for slow inward calcium current in isolated rat heart myocytes. *Nature*, **284**: 358-360.
22. ISENBERG, G. (1977) Cardiac Purkinje fibres $[\text{Ca}^{2+}]_i$ controls steady state potassium conductance. *Pflügers Arch.*, **371**: 71-76.
23. ISENBERG, G. and KLÖCKNER, U. (1982) Calcium currents of isolated bovine ventricular myocytes are fast and of large amplitude. *Pflügers Arch.*, **395**: 30-41.
24. ISENBERG, G. and KLÖCKNER, U. (1982) Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium." *Pflügers Arch.*, **395**: 6-18.
25. KAKEI, M. and NOMA, A. (1984) Adenosine 5'-triphosphate-sensitive single potassium channel in the A-V node cell of the rabbit heart. *J. Physiol. (Lond.)*, in press.
26. KAMEYAMA, M., KAKEI, M., SATO, R., SHIBASAKI, T., MATSUDA, H., and IRISAWA, H. (1984) Intracellular Na^+ activates a K^+ channel in the mammalian cardiac cells. *Nature*, **309**: 354-356.
27. KAMEYAMA, M., KIYOSUE, T., and SOEJIMA, M. (1983) Single channel analysis of the inward rectifier K current in the rabbit ventricular cells. *Jpn. J. Physiol.*, **33**: 1039-1056.
28. KASS, R. S., TSIEN, R. W., and WEINGART, R. (1978) Ionic basis of transient inward current induced by strophanthidin in cardiac Purkinje fibres. *J. Physiol. (Lond.)*, **281**: 209-226.
29. KOKUBUN, S. and IRISAWA, H. (1984) Effects of various intracellular Ca ion concentrations on the slow inward current of guinea-pig isolated single ventricular cells. *Jpn. J. Physiol.*, **34**: in press.
30. KOKUBUN, S. and REUTER, H. (1984) Dihydropyridine derivatives prolong the open state of Ca channels in cultured cardiac cells. *Proc. Natl. Acad. Sci. U.S.A.*, in press.
31. KONO, T. (1969) Role of collagenases and other proteolytic enzymes in the dispersal of animal tissues. *Biochim. Biophys. Acta*, **178**: 397-400.
32. KURACHI, Y. (1982) The effects of intracellular protons on the electrical activity of single

- ventricular cells. *Pflügers Arch.*, **394**: 264–270.
33. LEE, K. S., AKAIKE, N., and BROWN, A. M. (1978) Properties of internally perfused voltage-clamped isolated nerve cell bodies. *J. Gen. Physiol.*, **71**: 489–507.
 34. LEE, K. S. and TSIEN, R. W. (1982) Reversal of current through calcium channels in dialysed single heart cells. *Nature*, **297**: 498–501.
 35. LEE, K. S. and TSIEN, R. W. (1984) High selectivity of calcium channels in single dialyzed heart cells of the guinea pig. *J. Physiol. (Lond.)*, in press.
 36. MARBAN, E. and TSIEN, R. W. (1982) Enhancement of calcium current during digitalis inotropy in mammalian heart: Positive feed-back regulation by intracellular calcium? *J. Physiol. (Lond.)*, **329**: 589–614.
 37. MASSON-PÉVET, M., JONGSMA, H. J., BLEEKER, W. M., TSJERNINA, L., VAN GINNEKEN, A. C. G., TREIJTEL, B. W., and BOUMAN, L. N. (1982) Intact isolated sinus node from the adult rabbit heart. *J. Mol. Cell. Cardiol.*, **14**: 295–299.
 38. MATSUDA, H. (1983) Effects of intracellular calcium injection on steady state membrane currents in isolated single ventricular cells. *Pflügers Arch.*, **397**: 81–83.
 39. MATSUDA, H., NOMA, A., KURACHI, Y., and IRISAWA, H. (1982) Transient depolarization and spontaneous voltage fluctuations in isolated single cells from guinea-pig ventricles. Calcium-mediated membrane potential fluctuations. *Circ. Res.*, **51**: 142–151.
 40. MITCHELL, M. R., POWELL, T., TERROR, D. A., and TWIST, V. W. (1983) Characteristics of the second inward current in cells isolated from rat ventricular muscle. *Proc. R. Soc. Lond. (Biol.)*, **219**: 447–469.
 41. NAKAYAMA, T., KURACHI, Y., NOMA, A., and IRISAWA, H. (1984) Action potential and membrane currents of single pacemaker cells of the rabbit heart. *Pflügers Arch.*, in press.
 42. NOBLE, D. (1979) *The Initiation of the Heart Beat*, Clarendon Press, Oxford.
 43. NOMA, A. (1983) ATP-regulated K^+ channels in cardiac muscle. *Nature*, **305**: 147–148.
 44. NOMA, A., MORAD, M., and IRISAWA, H. (1983) Does the “pacemaker current” generate the diastolic depolarization in the rabbit SA node cells? *Pflügers Arch.*, **397**: 190–194.
 45. NOMA, A., NAKAYAMA, T., KURACHI, Y., and IRISAWA, H. (1984) Resting K conductances in pacemaker and non-pacemaker heart cells of the rabbit. *Jpn. J. Physiol.*, **34**: 245–254.
 46. NOMA, A. and TRAUTWEIN, W. (1978) Relaxation of the ACh-induced potassium current in the rabbit sinoatrial node cell. *Pflügers Arch.*, **377**: 193–200.
 47. OHARA, M., KAMEYAMA, M., NOMA, A., and IRISAWA, H. (1983) Giga-seal suction electrode and its application to single cardiac myocytes. *J. Physiol. Soc. Jpn.*, **45**: 629–639 (in Japanese).
 48. OSTERRIEDER, W., BRUM, G., HESCHELER, J., and TRAUTWEIN, W. (1982) Injection of subunits of cyclic AMP-dependent protein kinase into cardiac myocytes modulates Ca^{2+} current. *Nature*, **298**: 576–578.
 49. POWELL, T., TERRAR, D. A., and TWIST, V. W. (1980) Electrical properties of individual cells isolated from adult rat ventricular myocardium. *J. Physiol. (Lond.)*, **302**: 131–153.
 50. POWELL, T. and TWIST, V. W. (1976) A rapid technique for the isolation and purification of adult cardiac muscle cells having respiratory control and a tolerance to calcium. *Biochem. Biophys. Res. Commun.*, **72**: 327–333.
 51. REUTER, H. (1983) Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature*, **301**: 569–573.
 52. REUTER, H., STEVENS, C. F., TSIEN, R. W., and YELLEN, G. (1982) Properties of single calcium channels in cardiac cell culture. *Nature*, **297**: 501–504.
 53. SAKMANN, B., NOMA, A., and TRAUTWEIN, W. (1983) Acetylcholine activation of single muscarinic K^+ channels in isolated pacemaker cells of the mammalian heart. *Nature*, **303**: 250–253.
 54. SACKMANN, B. and TRUBE, G. (1984) Conductance properties of single inwardly rectifying

- potassium channels in ventricular cells from guinea-pig heart. *J. Physiol. (Lond.)*, **347**: 641–657; Voltage dependent inactivation of inwardly-rectifying single-channel currents in the guinea-pig heart cell membrane. *J. Physiol. (Lond.)*, **347**: 659–683.
55. SEVERS, N. J., SLADE, A. M., POWELL, T., TWIST, V. W., and WARREN, R. L. (1982) Correlation of ultrastructure and function in calcium-tolerant myocytes isolated from the adult heart. *J. Ultrastruct. Res.*, **81**: 222–239.
 56. SHEETS, M. F., JANUARY, C. T., and FOZZARD, H. A. (1983) Isolation and characterization of single canine Purkinje cells. *Circ. Res.*, **53**: 544–548.
 57. SOEJIMA, M. and NOMA, A. (1984) Mode of regulation of the K channel by the muscarinic receptor in rabbit atrial cells. *Pflügers Arch.*, **400**, 424–431.
 58. TANIGUCHI, J., KOKUBUN, S., NOMA, A., and IRISAWA, H. (1981) Spontaneously active cells isolated from the sino-atrial and atrio-ventricular nodes of the rabbit heart. *Jpn. J. Physiol.*, **31**: 547–558.
 59. TANIGUCHI, J., NOMA, A., and IRISAWA, H. (1983) Modification of the cardiac action potential by intracellular injection of adenosine triphosphate and related substances in guinea pig single ventricular cells. *Circ. Res.*, **53**: 131–139.
 60. TRAUTWEIN, W., TANIGUCHI, J., and NOMA, A. (1982) The effect of intracellular cyclic nucleotides and calcium on the action potential and acetylcholine response of isolated cardiac cells. *Pflügers Arch.*, **392**: 307–314.
 61. YANAGIHARA, K. and IRISAWA, H. (1980) Inward current activated during hyperpolarization in the rabbit sinoatrial node cell. *Pflügers Arch.*, **385**: 11–19.
 62. YATANI, A. and GOTO, M. (1983) The effect of extracellular low pH on the plateau current in isolated, single rat ventricular cells—A voltage clamp study. *Jpn. J. Physiol.*, **33**: 403–415.
 63. YATANI, A., IMOTO, Y., and GOTO, M. (1984) The effects of caffeine on electrical properties of isolated single rat ventricular cells. *Jpn. J. Physiol.*, **34**, 337–349.
 64. WEIDMANN, S. (1969) Electrical coupling between myocardial cells. *Prog. Brain Res.*, **31**: 275–281.