Electrophysiology of the Normal-to-Hypoxic Transition Zone

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SUMMARY. The "normal-to-hypoxic" transition zone was modeled after the chamber technique for perpendicular and parallel orientation with respect to the direction of fiber axis. The evidence obtained from the recording of the transmembrane action potentials suggests the presence of a preservation phenomenon in the hypoxic area, based on the utilization of energy stores of the normoxic area. The preservation phenomenon is enhanced with improvement of the intercellular communication. Better intercellular coupling in longitudinal than in transverse fiber direction results in the anisotropic properties of the preservation phenomenon. The preservation phenomenon provides a basis for the existence of critical size of the viable hypoxic area compared to the size of the transitional zone. The crucial role of electrotonic coupling was demonstrated, as well as the possible contribution to the preservation phenomenon mechanism of the cell-to-cell diffusion of metabolites. (Circ Res 51:321-329, 1982)

THE transitional zone between the normal and ischemic myocardium is of great interest in terms of the development of the myocardial infarction zone, the survival of the boundary area, and the onset of ischemic arrhythmias. Considerable progress in getting insight into the biochemical properties of the transitional zone has been made in recent years (Hearse et al., 1977; Janse et al., 1979).

Thus, by taking minute local specimens, it was possible to show that there exists a difference in biochemical properties, with a clear-cut 2- to 4-mm wide boundary between the normal and injured zones (Hearse et al., 1977). The employment of histochemical and fluorescent techniques demonstrated that the boundary area was still narrower (Barlow and Chance, 1976; Harken et al., 1978, Janse et al., 1979). However, the mechanisms underlying the development of the transitional zone still remain unclear. Because of the importance of intercellular junctions in the interaction between the normal and ischemic myocardium, we focused on the role of cell-to-cell coupling in the formation of the transitional zone.

In the present study, we used hypoxia with no glucose in the medium as a model of ischemia. This model reflects the biochemical and electrophysiological properties of the ischemic tissues (Morena et al., 1980).

Methods

In all, 49 experiments were performed on isolated trabecular preparations from rabbit atrium. Conventional microelectrode technique was employed to record transmembrane action potentials (AP) of the myocardial fibers. Cathodal electric pulses of twice threshold intensity and 2 msec long were delivered via a bipolar Ag-AgCl electrode. Normally, the preparations were perfused with normoxic Tyrode’s solution of the following composition (mm): NaCl, 136; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 0.5; NaH₂PO₄, 4.6; NaHCO₃, 14.0; glucose, 5.0 (at 37°C and pH 7.35). To maintain hypoxic conditions, the saline was gassed continuously with 95% N₂ + 5% CO₂ without glucose. The PO₂ of the normoxic saline was 500 mm Hg, whereas that of the hypoxic one was only 20–30 mm Hg.

Hypoxic conditions were achieved either by producing general hypoxia of the whole preparation, or by using the chamber technique. All experiments commenced with perfusion of a preparation with normoxic Tyrode’s solution for at least 1 hour. The experimental arrangements used in the chamber technique for modeling the normal-hypoxic (N-H) transition zone are shown, for the two directions of fiber axis, in Figure 1, A and B. Preparations were pulled through a rectangular scoop in the chamber partition (1), which was then closed by letting a movable fluoroplastic rectangular plate about 200 μm thick (2) drop through grooves in the partition. A and B show the respective arrangements with fibers running perpendicular and parallel with respect to the partition. The fiber direction is shown by the dashed lines. The left compartment of the chamber was bathed with normoxic Tyrode’s solution, while the right one was perfused with hypoxic saline.

Transmembrane action potentials were recorded simultaneously from both sides of the preparation with two microelectrodes at varying distances from the partition and varying durations of hypoxia. Experiments were carried out on preparations with intact intercellular junctions in the normal-hypoxic zone as well as on those with injuries by deliberately squeezing the zone with the movable rectangular plate [(2) in Fig. 1B]. In the latter case, for a better recovery of the preparation after crushing, 1.5 hours were allowed to elapse, and the experiments were started after the action potential was restored, in the area of the partition. Squeezing usually would bring about a complete conduction block between the zones. The criteria for absence of intercellular interaction were as follows: (1) lack of the electrotonic spread between the zones, linked to the action potentials generated asynchronously in both zones, (2) lack of the electrotonic spread between the zones at imposed
hyperpolarization of one by a 100-msec pulse [for a
detailed description of the technique of intracellular polariza-
tion of normoxic and hypoxic solutions, the perfusion rates
were set so that diffusion was negligible. A polaro-
graphic technique using platinum electrodes was used to
to the partition. 1—partition with rectangular scoop, 2—a movable
rectangle partition.

Results

Impact of General Hypoxia on Physiology of the
Rabbit Atrium

There are quite a number of reports available about the
effects of hypoxia on action potentials of the
myocardial units (Trautwein et al., 1954; Girardier,
1971-72). However, we felt it was necessary for us to
run a series of these experiments ourselves, so that
we could determine the most appropriate electrophysiolo-
gical parameter for evaluating the effect of hypoxia,
and the relationships between such a parameter and the
duration of hypoxia, in order to serve later as a reference.

In this series, 12 experiments were carried out. Transmembrane action potentials were recorded from
both the epicardial and endocardial sides of a prepa-
rating. Sample recordings showing action potential
changes under hypoxic conditions are presented in
Figure 2A. Only a slight hypoxic effect was evident in
the resting potential (RP) and the action potential
upstroke slope; however, there was a marked decrease
in the action potential height and duration. After 40–
60 minutes of hypoxia, response conduction was
blocked and excitability faded. The averaged data for
the resting potential values and the action potential
durations and heights are displayed in Figure 2, B–D,
as a function of the duration of hypoxia. It can be
seen that 1 hour of hypoxia had almost no effect on
the resting potential, whereas there was a marked
decrease in the action potential height and duration.
Thus, the parameters most sensitive to hypoxic
conditions were the action potential height and du-
ration. The action potential height strongly depends
upon the resting potential and the quality of a cell's
impalment with the microelectrode, which gives
scattered values, and hence causes certain difficulties
in evaluation of this parameter. In contrast, the action
potential duration is considerably less affected by the
above-mentioned factors. The AP shape and duration
display negligible distortions even when the recording
is performed with suction electrodes (Olsson, 1971).
On the basis of this evidence, we have selected, for
this study, the action potential duration (APD) as the
index of the effect of hypoxia.

Electrophysiology of the Normal-to-Hypoxic
Transition Zone: Chamber Technique

The chamber technique was used to study the
longitudinal and transverse position of the axis of the
fibers in the N-H transition zone to assess the role of
myocardial anisotropy in the formation of the transi-
tional zone.

This approach enabled us to evaluate the impor-
tance of cell-to-cell coupling for individual param-
eters of the transitional zone, since, depending on
fiber positioning, one deals either with good (longi-
tudinal positioning—along trabeculae direction) or
poor (transverse positioning) intercellular interaction
(Bukauskas et al., 1976).
The two cases are considered in detail below.

Case 1. The Normal-to-Hypoxic Transition Zone is
Perpendicular to the Fiber Axis (Fig. 1A)

The experiments were performed with intact (ten
experiments) as well as damaged (eight experiments)
intercellular contacts in the transition zone. The rep-
resentative recordings of the action potential shape as
a function of the distance from the partition on the
30th minute are shown in Figure 3. As one can see,
the action potential displays only minor changes in
the vicinity of the partition, but rapidly shortsens with
increasing distance. For simplicity, the phenomenon
of a slower decrease of the action potential duration
will henceforth be referred to as "preservation phe-
nomenon." The averaged action potential duration
data as a function of the duration of hypoxia and the
remoteness of the hypoxic area with respect to the
partition, are plotted in Figure 4, for intact (A, C) and
damaged (B, D) cell-to-cell communication. There is
a decline in the action potential duration with increas-
ing duration of hypoxia, with the least changes re-
corded nearest the partition (x = 0). Yet, at a distance
as small as 4 mm, the relationship is much like that
general hypoxia (Fig. 4A, B, dashed line). It is
obvious, from a comparison of Figure 4, A and B, that
a violation of cell-to-cell communication brings about
a clear-cut increase in the rate of action potential
shortening and broadens the action potential duration
range in the transition zone. In the control zone, the
action potential shortening occurs to a lesser degree
(the data for curve N in Figure 4, A and B, were
obtained at the point beside the partition in the nor-
The families of curves in Figure 4, C and D, were derived from the curves in A and B, and they show the action potential duration as a function of the distance from the partition at fixed time intervals from the onset of hypoxia. It can be seen that deterioration of cell-to-cell coupling results in accentuation of action potential duration changes in the hypoxic area and in stabilization of the parameter in the control zone. Although diminished, the preservation phenomenon persists (see Fig. 5), and the possibility of a contribution to the phenomenon by the extracellular space cannot be excluded. In the cases of intact intercellular contacts, a prolonged bathing (for 3–4 hours) of a preparation in the hypoxic solution suppresses excitability (Figure 5, A and B). Delineated is a boundary for alteration of electrophysiological properties of the zone which is characterized by a drastic change of the resting potential from −70 to −80 mV up to −8 to 0 mV, over as small a distance as 0.5 mm. Such a phenomenon is observed only in the hypoxic area farther from the partition, and is possibly due to the injury of intercellular junctions. A further prolongation of hypoxia causes the narrowing of the transitional zone and its subsequent shift.
In nine experiments in which 2,4,6-chloromethoxy carbonyl cyanide phenylhydrazone was used instead of hypoxia the action potential shortening occurred about 30 times more rapidly than with hypoxia (Figure 6C). In Figure 6A, averaged data are presented, showing the character of dependence of the action potential duration on the 2,4,6-chloromethoxy carbonyl cyanide phenylhydrazone action duration for the first minute of incubation at four separate recording sites shown in Figure 6B. The action potential duration vs. the incubation in the 2,4,6-chloromethoxy carbonyl cyanide phenylhydrazone time plot shows no difference, in qualitative terms, between the respective action potential duration changes at individual recording sites under hypoxic conditions and with 2,4,6-chloromethoxy carbonyl cyanide phenylhydrazone (cf. Figure 4A). A prolonged incubation (to 100 minutes) in 2,4,6-chloromethoxy carbonyl cyanide phenylhydrazone leads to a complete suppression of excitation and a very marked depolarization in 20–30 minutes (Figure 7B). In the control zone, the action potential is shortened by 60–80% in 20 minutes of 2,4,6-chloromethoxy carbonyl cyanide phenylhydrazone action, thereafter starting to expand gradually to the control level (Figure 7A), vs. the background of negligible decrease of the resting potential. The action potential duration restoration to the control level starts at the onset of complete suppression of the excitation in the 2,4,6-chloromethoxy carbonyl cya-
FIGURE 5. The action potential shape as a function of the recording site and duration of hypoxia. Note the build up, with increasing duration, of a non-excitable zone (see A, 150 and 300 minutes; B, 90 and 220 minutes) characterized by a tendency to extend toward the normal area. A and B stand for recording sets from two experiments. The bottom of the action potential upstrokes were positioned to match respective recording sites (heavy dots) on the bottom diagrams.

Discussion

The data obtained point out that, with cell-to-cell coupling of good quality, there is a gradual change in values of electrophysiological parameters between one zone and another. A slower time-course of the APD decrease in the hypoxic zone near the partition, as compared with that observed with general hypoxia, was termed a "preservation phenomenon." In order to understand the mechanism of this, some factors of major importance should be given proper concern. They are (1) electrotonic coupling, (2) cell-to-cell diffusion of ions and molecules due to a concentration gradient, and (3) diffusion in the extracellular compartment.

Electrotonic Coupling

In 2,4,6-chloromethoxy carbonyl cyanide phenylhydrazone experiments, no phase shifts were found between the curves for the action potential duration as a function of the duration of the drug's action, at
any of the recording sites used. This finding suggests that the time needed for triggering the mechanism underlying the preservation phenomenon should be less than the time for the action potential duration decrease, i.e., it should be less than 10 seconds. Since the time for electrotonic coupling in the myocardium is within the range of 5–20 msec, it thus meets the above requirement and points to the involvement of electrotonic processes in the mechanisms underlying the preservation phenomenon. This is favored by quite a large decay length constant (1 mm on the average) for electrotonic spread along the atrial trabeculae (Bukauskas et al., 1976). With increasing duration of hypoxia, the decay length constant decreases (Bredikis et al., 1976; Wojtczak, 1979) and this should diminish the preservation phenomenon. This is probably one of the major causes of the "narrowing" of the normal-to-hypoxic transition zone with sustained hypoxia.

Cell-to-Cell Diffusion

Hypoxia inhibits the pumping ability of the electrogenic membrane and thus leads to a potassium loss and Na⁺ accumulation by the cell. Concurrently, as a result of suppression of the oxidative phosphorylation and due to activation of the glycolysis, there builds up an intracellular deficiency in high-energy compounds and a redundancy of products of the glycolysis (Williamson et al., 1977). Thus an intracellular gradient for a variety of compounds arises, as well as the diffusional forces directed toward diminishing this gradient. Weidmann (1966) has shown the intercellular junctions to be highly permeable to K⁺ and other ions. Intercellular exchange with macromolecules (ATP, creatine phosphate, lactates) is also possible (Imanaga, 1974; Pollack, 1976). By utilizing the
chamber technique, these investigators were able to obtain evidence for intercellular diffusion of compounds with large molecules (with molecular weight up to 700) several millimeters from the site of their application. Recently, there has been growing evidence that the specialized junctions (nexuses), with pores of up to 10-15 Å, are the sites at which the overwhelming majority of the diffusional macromolecular exchange occurs (McNutt and Weinstein, 1973). It remains to be determined however, how powerful and far-reaching the intercellular diffusional flow of metabolites is. By utilizing the equation for free intercellular diffusion (Weingard, 1974), we can find that a compound with a diffusion coefficient of 10^-7 cm/sec takes 2 minutes to travel 1 mm. Temporal parameters for diffusion are 3 or 4 orders of magnitude larger than those for electrotonic processes. For this reason, in 2,4,6-chloromethoxy carbonyl cyanide phenylhydrzone experiments, only the electrotonic processes had enough time to make their contribution to the preservation phenomenon, whereas, with the agents that bring about injury after more than ten minutes (e.g., hypoxia), the diffusional phenomena also should have had an effect.

For a qualitative evaluation of a contribution to the preservation phenomenon by the diffusion of metabolic intermediates across the intercellular junctions, we used an "end-to-end" model for two, "normal" and "hypoxic", cylindrical cells. We can estimate the amount of creatine phosphate (CP) flow across the junctional membrane and the molecular weight of the junctional membrane and the molecular weight (Pollack, 1976). The evidence obtained made it possible for us to evaluate the formation of the transitional zone at three different levels of intercellular interaction: (1) good (longitudinal direction), (2) mediocre (transverse direction), and (3) no interaction, due to a mechanical damage of the transitional zone. As one can see from Figures 4, A and B, and 5A, with the intercellular interaction impaired, the preservation phenomenon is considerably weakened, yet not abolished. Since the cells are electrotonically uncoupled in this paradigm, only the extracellular diffusion of molecules should be given concern. The extracellular space, which makes up as much as 25% of the total volume of the tissue (Prasad et al., 1974), apparently provides the basis for the diffusion of oxygen molecules and reequilibration of electrolyte levels.

The Role of Intercellular Coupling in the Interaction between Hypoxic and Normal Myocardium

Cardiac structures, especially atrial trabeculae, are known to display marked anisotropic electrical properties. The transversal intercellular coupling in the rabbit atrial trabeculae is 7 to 10 times less effective than the longitudinal coupling (Bukauskas et al., 1976). The evidence obtained made it possible for us to evaluate the formation of the transitional zone.
Critical Size of Hypoxic Area

Electrical and diffusional cell-to-cell links make it possible for a healthy myocardium to compensate for the energy deficit of the hypoxic area. It is possible that there is a critical size for the hypoxic area which is still sufficient to safeguard the tissue from necrosis by virtue of compensatory processes. It should be much like the size of the normal-to-hypoxic transition zone.

From a qualitative analysis of the transition zone, one can infer that the area of local hypoxia, still capable of functioning due to the preservation phenomenon, would not exceed 2–3 mm in size. The corollary is that, the stronger the links keeping a cell built in a whole system, the easier it is for it to endure various abnormal conditions, and the larger the critical size of hypoxic area.

If a hypoxic area is large enough, this will ultimately bring about a gradual exhaustion of energy stores around the area. Owing to a permanently existing “source of leakage,” the injured area should gradually expand. However, due to impaired cell-to-cell coupling under hypoxia, as has been shown in earlier work (Bredikis et al., 1976; Wojtczak, 1979). Since cell-to-cell coupling in the longitudinal direction is far more efficient than that in the transversal direction, the transitional zone should be elongated in the direction of the fiber axis and therefore the hypoxic area will display anisotropic properties. This can be observed only in relatively small hypoxic areas; i.e., their size must be the same order as that of the transitional zone.

Onset of Hypoxia-Induced Arrhythmias

In a search for mechanisms of hypoxia-induced arrhythmias, the emphasis usually has been placed on findings of marked nonhomogeneity of the action potential duration, which, according to Sano et al. (1972) and Krinskij et al. (1976), can give rise, through a reexcitation, to a focus of ectopic beats.

We were not able to observe reexcitation in rabbit atrium preparations even though, in the hypoxic area, a clear-cut depolarizing “hump” was found during the action potential repolarization phase induced by electrotonic interference from the normal zone. To make the excitation mechanism operable, some special requirements may have to be met; those concerning cell-to-cell coupling and accommodation properties are two of the most important (Arita et al., 1976; Sakson et al., 1976). On one hand, intercellular coupling should be strong enough to give rise to effective stimulating local currents; on the other hand, effective intercellular communication is an unfavorable condition for development of considerable action potential duration gradients over short distance. These contradictory effects make reexcitation impossible with either very effective intercellular coupling or complete uncoupling. There is evidently a certain optimum extent in intercellular electrical coupling for the reexcitation mechanism to be put into effect.

Normally, atrial and ventricular fibers of the working myocardium exhibit clear-cut accommodative properties. This also hinders reexcitation. In Purkinje fibers, which have poor accommodative properties, and in guinea pig papillary muscle fibers with impaired accommodative properties, due to the action of low-potassium medium and catecholamines, repetitive firing has been reported in response to prolonged injections of a depolarizing current (Arita et al., 1976; Katzung et al., 1975). Therefore, one may suggest that, under ischemic conditions, the impairment of the cell-to-cell coupling and the increased catecholamine release (Valori et al., 1967; Griffits et al., 1971) are the prerequisites due to which the inherent nonhomogeneity of the action potential duration is transformed via the reexcitation mechanism into a source of extrasystoles.

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